Green Energy and Technology

# Andrea Monti Editor

# **Switchgrass** A Valuable Biomass Crop for Energy



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# Switchgrass

A Valuable Biomass Crop for Energy



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### Foreword

Switchgrass (*Panicum virgatum* L.) is a warm-season ( $C_4$ ) perennial grass that is being developed internationally into a biomass energy crop. It is native to the prairies and steppes of North America. Switchgrass has been used in pastures and for conservation plantings in the Great Plains and the Midwest, USA, since the 1940s. The research supporting its use as a pasture and conservation species was initiated in the mid-1930s and was largely conducted by U.S. Department of Agriculture (USDA) research programs, most notably the Agricultural Research Service (ARS) project located at the University of Nebraska-Lincoln and USDA Plant Materials Centers which are located throughout the United States. It was often used in mixtures with other native warm-season grasses.

Interest in switchgrass as a bioenergy crop began with potential biomass energy species screening trials conducted in the 1980s which were funded by the U.S. Department of Energy (DOE) via the Oak Ridge National Laboratory. Switchgrass was among the top two or three species for biomass yield in the majority of the trials. In 1991, switchgrass was selected as the model perennial grass biomass species by DOE. In addition to being a native species, other desirable attributes were its known soil conservation benefits, it could be propagated by seed, there was an existing seed industry, and it could be grown and harvested with available forage equipment. Since 1992, there has been a steadily increasing amount of research conducted on all aspects of switchgrass production and use as a biomass energy crop both in the USA and in Europe. This funding was largely by government agencies until the early 2000s when commercial companies also began investing in switchgrass research. Over \$1 billion has been allocated in the USA to biomass energy research since 2006 by both government and commercial companies with much of the emphasis on perennial grass energy crops such as switchgrass. Because of the investments that have been made in switchgrass research, a large amount of information has and is emerging from the switchgrass research pipeline and the pipeline itself is increasing in volume and delivery rate.

This book is the first devoted entirely to switchgrass. International authorities on the development and use of switchgrass for bioenergy have reviewed and summarized by major topic areas all the past and current scientific literature on switchgrass. Information is provided about the evolution of switchgrass as an energy crop, its breeding, genetics, genomics, physiology, and management including harvest and storage. The book also provides information about the economics of switchgrass production for bioenergy and information about switchgrass biomass biochemical and thermochemical conversion into biofuels. In addition, the potential environmental impacts of switchgrass grown for bioenergy are reviewed. This book, *Switchgrass: a Valuable Biomass Crop for Energy* is very comprehensive and should prove to be an invaluable resource.

Kenneth P. Vogel University of Nebraska, Lincoln

### Preface

When Mr. Anthony Doyle suggested I put together a book on energy crops, I immediately thought of switchgrass, followed by two questions: is our current knowledge on switchgrass-for-energy substantial enough, and mature enough, to merit such a publication? Is it appropriate that this initiative be undertaken by a European with much less experience than his North American colleagues? The first answer was certainly positive, while the second can only be answered by the authors of the book and its future readers.

The reasons that led me to accept the challenge can be summed up in two closely connected objectives. In the first place, the desire to help bring the "scholar" closer to the "teacher", in other words to combine the recent but significant knowledge acquired in Europe with the more substantial and consolidated North American knowledge. The other, more ambitious objective is to use the "energy" of switchgrass to create "synergy" in multidisciplinary communications among scientists and stakeholders, as well as in parallel R&D programs in Europe, North America, and elsewhere.

Undoubtedly, there are still many questions regarding science and technology associated with the production and utilization of switchgrass, and the ambition of this book is to offer a state-of-the-art overview on the knowledge and prospects of switchgrass as a raw material for energy use, as well as suggestions for future research programs. Nearly all the areas of lively current research which have received ample attention, such as crop management, physiology, genetics and genomics, logistic, economic and environmental assessment, and transformation processes, are touched upon here.

In the introductory chapter, David Parrish and co-authors provide a fascinating description of the evolution of switchgrass from its prehistoric origins to the latetwentieth century efforts to develop it into an energy crop. In Chap. 2, Michael Casler presents a brilliant review on the genetics and genomics of switchgrass, showing how this species is still greatly undomesticated with a vast potential for improvement of biofuel traits. Crop physiology is extensively discussed in Chap. 3 by Walter Zegada-Lizarazu and co-authors, who emphasize the considerable use efficiency of natural resources by this crop, which indeed could be significantly improved through developing permanent switchgrass physiology programs. In Chap. 4, Matt Sanderson and co-authors provide an in-depth overview on the agronomy of switchgrass, and the specific management characteristics it requires if vield and quality are to be maximized. Rob Mitchell and Marty Schmer reviewed the importance of harvest and storage methods in providing consistent and highquality biomass to conversion plants. In Chap. 5, the authors clearly point out the critical effects of harvest and storage management on feedstock characteristics as landscape scale deployment of switchgrass for bioenergy moves forward. Whilst switchgrass undoubtedly has potential advantages, it could have ecological or environmental weaknesses in some situations. These issues are analyzed and discussed in Chap. 6 by Howard Skinner and co-authors, who reviewed the major environmental impacts of growing switchgrass as an energy crop, including the current debate on land use change which has led to the fuel versus food debacle. Several challenges still have to be overcome to produce biofuels and chemicals from biomass in an economic and sustainable manner. Different processing steps within the biochemical and thermochemical platforms are accurately discussed in Chap. 7 by Venkatesh Balan and co-authors, while the analysis of economic viability and conditions under which switchgrass becomes cost-competitive are skilfully examined in Chap. 8 by Anthony Turhollow and Francis Epplin.

I am extremely grateful to the authors for preparing the excellent contributions to this book of which I have the honor of being the editor, and to those whom I could not invite due to the size of the book, I offer my apologies.

Andrea Monti University of Bologna

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### Chapter 1 The Evolution of Switchgrass as an Energy Crop

#### David J. Parrish, Michael D. Casler and Andrea Monti

Abstract This chapter discusses the prehistoric origins of switchgrass, its mid-twentieth century adoption as a crop, and late-twentieth century efforts to develop it into an energy crop. The species probably first appeared about 2 million years ago (MYA) and has continued to evolve since, producing two distinct ecotypes and widely varying ploidy levels. We build the case that all existing switchgrass lineages must be descended from plants that survived the most recent glaciation of North America and then, in just 11,000 years, re-colonized the eastern two-thirds of the continent. Moving to historic times, we discuss how switchgrass was first considered as a crop to be grown in monoculture only in the 1940s. Based on scientific reports indexed in a well-known database, interest in switchgrass grew very slowly from the 1940s until it began being considered by the US department of energy (DOE) as a potential energy crop in the 1980s. The history of how switchgrass became DOE's 'model' herbaceous energy crop species is recounted here. Also chronicled are the early research efforts on switchgrass-for-energy in the US, Canada, and Europe and the explosive growth in the last decade of publications discussing switchgrass as an energy crop. If switchgrass-still very much a 'wild' species, especially compared to several domesticated grasses-truly attains global status as a species of choice for bioenergy technologies, it will have been a very remarkable evolution.

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#### **1.1 Introduction**

Beating swords into plowshares and spears into pruning hooks (Isaiah 2:4) is a lofty goal. Not so noble but still laudable might be a figurative reshaping of plows into oil derricks and coal tipples. In such a scenario, energy crops would be grown on marginal or non-cropland for conversion into energy forms that reduce dependence on petroleum and coal. The benefits would be multifold. Energy cropping could offset fossil fuel use, thereby extending the supply of non-renewable forms and reducing greenhouse gas emissions. Furthermore, a mature, sustainable, biomass-based energy supply could offer economic and social renewal to many rural areas. We do not reckon that making fuel from the grain of maize (*Zea mays* L.) provides a sustainable path, but we hope that practice is paving the way for a truly sustainable second generation of biomass-based energy crops [1, 2].

Switchgrass (*Panicum virgatum* L.) has garnered much attention as an energy crop in the past few years. This book was commissioned because the body of knowledge on switchgrass-for-energy is implicitly now substantial enough and mature enough to merit such a publication. We think that analysis will hold up to scrutiny, but in this chapter we invite readers to consider that switchgrass is still a very new crop—one that was not planted in monocultures until the mid twentieth century. In a few decades, though, the species has catapulted from obscurity to being the focus for a wealth of good science and to being frequently cited as the feedstock of choice for a second generation biofuels industry.

The rest of this book will deal with the wealth of good science focused on switchgrass; this chapter will explain how switchgrass came to be that focus. We will discuss first the species' biological origins—its evolutionary relationship to other members of the grass family. Then we will document its very recent 'birth' as a crop. Finally, we will discuss how this very new crop has come to be considered the energy crop of choice by so many. The explanation is related partially to the species' biology and agronomy; but it also involves serendipity, a bureaucratic decision, political and economic exigencies, and a 'model' that may have been forgotten.

#### **1.2 The Evolution of Switchgrass**

Based on deposits of their distinctive pollen in the fossil record, grasses originated 55 to perhaps 70 million years ago (MYA) [3]. Since then, the grass family (Poaceae or Gramineae) has evolved from rather humble beginnings into forms that dominate significant portions of the planet. Since the much more recent appearance of humans, the grass family's connections to us have become extensive

and, in some cases, essentially symbiotic. Human use and selection in the last 10,000 years have clearly reshaped some grass forms and functions [4], but grasses may have had an even bigger impact on human development. The environment in East African tall-grass savannas 2 MYA may have fostered the evolution of bipedalism, tool-holding hands, and increased intellect in hominids [5]. That, of course, is speculative, but we know without doubt that we are very highly dependent on the grasses today. A few grasses-most notably those that became domesticated during a hundred centuries of interaction with humans [6]-now produce the great majority of the caloric energy consumed in human diets. These might be considered the Olympians within the pantheon of valuable grasses. Some other grasses are not so vital from a human perspective, but they could still be described as belonging within the pantheon of valued grasses because they provide feed for livestock and, hence, additional human dietary components as well as draft power; and still other grasses are valued as sources of fiber, turf, and ornamentation. In this anthropocentric context, switchgrass is clearly already within the pantheon, but it might be poised to join the Olympians-not as food from the gods but as fire brought down from the sky.

#### 1.2.1 Taxonomic and Phylogenetic Relationships Within the Grasses

The approximately 10,000 grass species have been grouped into 600 to 800 genera [7]. The number of genera is in some flux as taxonomists and systematists work to make classification schemes more natural, i.e., to better reflect evolutionary relationships—a task made perhaps especially difficult in the grasses by numerous cases of parallel or convergent evolution [8]. Using morphological and cytogenetic comparisons, the grass genera have been divided into six subfamilies, with various numbers of tribes and subtribes in each [9]. In this classification scheme, *Panicum* is the type genus of both its subfamily (Panicoideae) and its tribe (Paniceae). Fellow subfamily members include *Zea*, *Sorghum*, and *Miscanthus* (each in a different tribe), while the tribe Paniceae consists of about 100 C<sub>3</sub> and C<sub>4</sub> genera, some with familiar names such as *Echinochloa*, *Paspalum*, and *Setaria*—all within the same Setariinae subtribe as *Panicum* [9].

The *Panicum* genus is large and cosmopolitan, with over 450 rather heterogeneous species. The unifying trait within the genus is a distinctive spikelet morphology, but little else seems to hold the taxon together; for example, five different base chromosome numbers, ranging from 8 to 15, occur within the genus [10]. To alleviate the unwieldiness—and likely unnaturalness—of the genus, some taxonomists have subdivided *Panicum* into six or more subgenera and numerous sections [10]. Systematics underwent a revolution in the last quarter of the twentieth century with the advent of technologies that allow comparisons between and within species at the gene level. Instead of focusing on morphological features, such as inflorescences or embryos, this approach looks at DNA sequences. The logic is straightforward: plants share genes that hark back to some putative original plant; transcriptional 'accidents' infrequently but inevitably generate new, enduring base-pair sequences within those genes (and non-coding DNA regions); during and following speciation, varying sequences evolve into different taxa; and, over evolutionary time, unique, new sequences are added to each evolving lineage. Hence, when examining the variations in base-pair sequences of a gene, the more similar the sequences are between two species, the more closely related are they assumed to be. Increasing variation in DNA sequences for a gene indicates greater evolutionary divergence. If molecular studies incorporate large enough stretches of DNA and sufficient numbers of organisms, phylogenetic relationships generally emerge [3].

In the late 1990s, a consortium of systematists from several institutions formed the Grass Phylogeny Working Group [11]. They looked at 62 species representing the breadth of the grass family. The 62 included switchgrass and several other crops, as well as some lesser known but taxonomically important species. Basepair variations for six nuclear genes as well as chloroplast restriction site data were compiled and analyzed to produce a 'family tree' that showed the most likely genetic relationships of all 62 species. The findings suggested that the grass family might reasonably be divided into 12 subfamilies, including the Panicoideae [11]. The GPWG phylogeny placed switchgrass as a close relative (adjacent branch) of pearl millet (*Pennesetum alopecuroides* (L.) Spreng.), and that pair was next most closely related to maize and *Miscanthus japonicus* Andersson. Of course, these were comparisons only within the 62 species examined, but they suggested a more natural classification for the grass family overall and retained switchgrass firmly within its previously recognized taxa.

Molecular phylogenetic comparisons can be used to deduce when specific taxa or traits first appeared in evolutionary time. The notion of a molecular clock is now well appreciated [12]. Variations in base-pair sequences are assumed to accumulate at some inexorable-but deducible-rate, which may vary among angiosperm families [13]; and the number of variants observed can provide an indirect measure of when they began to accumulate. The grass family is considered to be monophyletic, i.e., all grasses are related to a putative original grass species [3]. The molecular/genetic changes that led to the first event of grass speciation caused a first branching point, or node, and each new branch since has followed its own path of incremental changes and accrual of variations in base-pair sequences. If the origin of the first grass can be placed in geological time, phylogeneticists can reason when in evolutionary time various nodes appeared. As noted above, the grass time line is reasoned from the fossil record to have begun 55 to 70 MYA. The molecular clock suggests that the primordial grass lineage must have undergone a total duplication of its genome almost immediately and then remained rather stable until dividing into several subfamilies beginning about 50 MYA [14, 15]; although some fossil evidence reveals morphological differences equating to some sub-families may have appeared as early as 65 MYA [16].

For agronomists, one of grasses' most important traits is the ability of species to withstand drought and thrive in full sunlight. Interestingly, that is thought to be a derived trait—not the 'wild type'. Evidence suggests the early grasses were adapted to forest margins and shade, just as some grass species still are [3]. Based on molecular phylogenies, a tolerance of or preference for open habitats evolved in grasses in at least two subfamilies but perhaps only after 20 million years as shade-preferring plants [3, 11, 16].

The family connections between switchgrass and other grass species have been investigated with molecular clock methods. One study looked at inter- and intraspecies variation in the DNA sequence of Acc-1, the gene that codes for plastid acetyl-CoA carboxylase [17]. That study and more recent work [13, 18] conclude that present-day switchgrass and maize diverged from a shared ancestor about 22 to 23 MYA. Switchgrass and pearl millet shared a common ancestor until about 16.5 MYA [13]. In a Huang et al. [17] study, while looking at Acc-1 in various switchgrass cultivars, the authors concluded that the gene pool associated with the species now recognized as switchgrass was assembled by about 2 MYA-initially as a diploid—and that the now widespread polyploidization of the species has occurred within the last 1 million years [17]. Based on chloroplast DNA (cpDNA) sequences, Zhang et al. [18] estimated switchgrass diverged from two of its diploid ancestors, P. hallii and P. capillari, about 5 to 10 MYA, suggesting that the evolution of this tall-growing polyploid required many millions of years to evolve out of a highly heterogeneous mix of diploid ancestors. This divergence occurred as hybridization, natural selection, and polyploidization allowed switchgrass to evolve into unique forms and habitats.

The appearance of switchgrass about 2 MYA was in the midst of the Pleistocene Era, which began about 2.5 MYA and is often described as the 'Last Ice Age'. The new species perhaps originated during an interglacial period, but it would have to endure many glacial episodes over the next 2 million years. We will come back to this point soon.

#### 1.2.2 Evolution of $C_4$ Pathways

Because it is so important for both productivity and drought tolerance, the evolution of  $C_4$  photosynthesis, which layers  $CO_2$ -concentrating mechanisms onto the archetypical  $C_3$  pathway, is of particular interest. Based on phylogenetic observations and species-specific differences in  $C_4$  biochemistry [19], we can conclude immediately that 'the  $C_4$  pathway' is actually multiple  $C_4$  pathways, which have arisen multiple times within the angiosperms, i.e., their appearances exemplify parallel or convergent evolution. A closer look at the grasses in particular suggests that development of  $C_4$ ness occurred multiple times just within that family.

#### 1.2.2.1 Where do C<sub>4</sub> Pathways Appear Phylogenetically?

 $C_4$  pathways occur in at least 7,500 angiosperm species found in 19 families—16 eudicot and three monocot [19, 20]. Within taxa where the pathways occur, they are not uniformly present—even within genera. *Panicum*, for example, has  $C_3$ ,  $C_4$ , and intermediate  $C_3/C_4$  members. However, the very large *Panicum* genus is thought by many to be polyphyletic, i.e., not to arise from a single *Panicum* progenitor [3, 10, 20].

The number of separate convergent appearances of  $C_4$  pathways has recently been tallied at 62 [20], with the majority of those lineages in the eudicots. But the grasses have clearly capitalized most on the pathways, with at least 4,500 of the 7,500  $C_4$  species being grasses [19, 20]. The molecular studies by the Grass Phylogeny Working Group [11] revealed at least five separate appearances of the  $C_4$  pathway just within the 62 species they examined [3]. A systematic comparison of all 10,000 grasses suggests  $C_4$  pathways must have evolved at least 11 separate times in the Poaceae (to include 7 times in just the Paniceae tribe), because  $C_4$ pathways appear in that many different tribes or subtribes with  $C_3$  ancestry [8, 19].

With the multiple, convergent appearances of  $C_4$  photosynthesis, there clearly must be strong selective pressure to develop these pathways. The pathways may provide survival value, or greater fitness, through increased CO<sub>2</sub> fixation rates especially under brighter, warmer, drier, or saline conditions—and greater water use efficiency. Nitrogen use efficiency also accrues to  $C_4$  species, since they require less RuBisCO, the rather inefficient, sole CO<sub>2</sub>-fixing enzyme in the  $C_3$ pathway. Some have noted that  $C_4$  plants might be less subject to herbivory because of their less favorable (for herbivores) protein content and C/N ratio [21]. In a very interesting review of the topic, Sage [19] suggests that the greatest survival value of  $C_4$  pathways—and the reason they developed in so many disparate families—may simply be the relief provided from photorespiratory C losses, which stem from the nature of RuBisCO's kinetics, especially its dual carboxylation/oxygenation proclivity.

#### 1.2.2.2 When did C<sub>4</sub> Pathways Appear?

The distinctive <sup>12</sup>C:<sup>13</sup>C isotopic fingerprint associated with C<sub>4</sub> species begins to be evident in geologic strata dating to 20 MYA; and, by 5 to 8 MYA, C<sub>4</sub> species apparently had become dominant producers in some parts of the globe [19]. This supports the notion that C<sub>4</sub> species (probably dominated by grasses, since they were the pioneer C<sub>4</sub>s) were already common in some mid-Miocene ecosystems [19]. Based on molecular clock methods, the first C<sub>4</sub> pathway(s) appeared in the grass family from 24 [14] to 30 [11] or 34 [14, 19] MYA. Based on the presence of intermediate C<sub>3</sub>/C<sub>4</sub> species and on genomic evidence from C<sub>3</sub> plants where key genes appear to be evolving in a direction that might favor C<sub>4</sub>ness [14], additional species could be only a few steps, albeit perhaps many eons, away from C<sub>4</sub> status. But when did C<sub>4</sub>ness first appear in switchgrass' ancestry? We know, for example, maize and *Sorghum spp*. are in the all-C<sub>4</sub> Andropogoneae tribe and have a presumptive shared C<sub>4</sub> ancestor that lived 12 to 15 MYA [14]. The origin of C<sub>4</sub>ness is not so clear with *Panicum*'s ancestry. Because *Panicum* has species with C<sub>3</sub>, C<sub>4</sub>, and C<sub>3</sub>/C<sub>4</sub> intermediate pathways, we might assume that C<sub>4</sub> evolved within the genus sometime after the original *Panicum* appeared. This makes very relevant the earlier speculation that the genus—as it is usually constituted—is not monophyletic, i.e., it does not arise from a single original *Panicum*.

A consensus would appear to be developing that *Panicum*, as currently constituted with its 450+ species, is polyphyletic [3, 10]—even 'highly' polyphyletic [20]. By definition, then, it is impossible to place its various species into definitive lineages and date the origin(s) of the C<sub>4</sub>ness. Based on molecular data, it has been proposed that *Panicum virgatum* be retained within the 'true' *Panicum (sensu stricto)* along with a few other strictly C<sub>4</sub> *Panicum spp.* [10]. Still open to question is whether C<sub>4</sub>ness developed de novo within a smaller, truly monophyletic *Panicum* genus or was inherited from a non-*Panicum* progenitor. The GPWG survey of base-pair sequences in six genes within 62 grass species suggested switchgrass and pearl millet arose from a shared C<sub>4</sub> ancestor [3], but the GPWG analysis was admittedly limited—not surveying any other species within *Panicum* or *Pennisetum*.

In short, we do not know when  $C_4$  first appeared in switchgrass's lineage. The answer to that question must await a suitable parsing of the lineage, which must include a sorting out of the *Panicum* genus.

#### 1.2.3 Center of Switchgrass's Origin and Spread Across North America

Switchgrass is a New World species. Its range when Europeans arrived included Central America and eastern North America [22]. It could be found in a wide range of habitats nearly anywhere east of the 100th meridian. After the species arose some 2 MYA [17], it likely radiated and adapted across major portions of the North American continent. However, a priori reasoning suggests that periods of glaciation in the last 2 million years would have driven most of those lineages into extinction or into more southern, ice-free climates. The survivors would presumably have followed the ice northward during interglacial periods, only to repeat the retreat/re-colonize cycle again and again [18, 23].

McMillan [24] posited switchgrass (and other prairie grasses) retreated to refugia during the ice ages and then moved poleward again as the climate warmed. He posited more specifically three regional refugia arose during the most recent glaciation: Lowland (or Southern) Great Plains, Eastern Gulf Coast, and Upland Plains. Recent molecular marker studies examining simple sequence repeats (SSRs) of 18 switchgrass cultivars and accessions [25] have provided tantalizing

support for this three-refugia theory. The latter work provides strong evidence that most—perhaps all—of today's cultivars can be sorted into three groups based on SSRs, with each group harking back to one of the three putative refugia. To follow the line of reasoning, we must first look more closely at the notion of switchgrass 'ecotypes'.

#### 1.2.4 Upland and Lowland Origins, Distinctions, and Connections

Essentially all cultivars, lines, or accessions of switchgrass can be placed into one of two categories: upland or lowland. A few 'intermediate' or 'ambiguous' types, which are not readily assigned to one of these two categories, may represent archaic natural hybrids [23]. The upland and lowland groups are usually described as 'ecotypes', a term from evolutionary ecology connoting genetic variations within a species that allow the ecotypes to be better adapted to particular geographies or habitats. Ecotypes—sometimes also described as subspecies—typically differ in morphology or physiology in ways that make them better suited for different environments, but they are able to interbreed and produce fertile offspring—meeting that classical criterion for a species. More recently, these groupings have also been termed upland and lowland 'cytotypes', referring to the diagnostic DNA sequence data carried in their plastids [26].

Within Panicum virgatum, genotypes belonging to the upland ecotype are typically finer stemmed and shorter than those identified as lowlands. As the upland designation might imply, these lines are also generally better adapted to drier and colder habitats, while the lowland ecotype tends to thrive in warmer, wetter habitats. Indeed, most of the lowland lines, e.g., Alamo, are derived from accessions from the southern USA; and the upland genotypes are more generally associated with the northern Great Plains. All identified cultivars from the lowland ecotype are tetraploid (2n = 4x = 36), whereas the upland ecotype consists of genotypes that are both tetraploid and octoploid (2n = 8x = 72) [25]. Only recently have possible octoploid lowland plants been discovered in a small number of accessions [18, 23]. The two ecotypes, which were initially distinguished by their phenotypes, can now also be grouped into upland and lowland genetic clusters, or cytotypes, using various molecular markers [25, 27]. While crosses between octoploid (upland) and tetraploid (lowland) genotypes are incompatible, tetraploid cultivars from each of the two ecotypes have been crossed and produced fertile offspring, exhibiting significant hybrid vigor [27].

Using molecular clock calculations based on cpDNA sequences, estimates of the upland–lowland divergence range from 0.5 to 1.3 MYA [18, 28]. Because octoploids are extremely rare within the lowland ecotype, it is likely that polyploidization from 4x to 8x occurred after the upland–lowland divergence. Indeed, there is evidence for multiple polyploidization events within the upland lineage, suggesting that this process has occurred frequently. Clear separation of tetraploid and octoploid lineages within the upland ecotype suggests that some of these

octoploid lineages are indeed very ancient [18]. Because 2n gametes are very common in the Poaceae, polyploidization from the 4x to 8x level could have occurred many times in many different lineages of switchgrass. It must be noted that 2n gametes have not specifically been identified in switchgrass, so the mechanism of polyploidization is still unknown.

Key to understanding the evolution of switchgrass is the massive impact that Ice Age cycles have had on habitats that we tend to think of as permanent and immobile. During the past 2 million years, there have been approximately 16 to 20 continental glaciation events in North America, each sufficient to force the complete relocation of the tall-grass prairie and savanna habitats toward warmer climates, e.g., the Gulf Coast. Individual lineages of switchgrass that had evolved to become adapted to more northern areas would have survived by migrating southward (via pollen or seed), or they would have gone extinct. The polyploid nature of switchgrass would have been a key factor in helping lineages to survive, preserving vast amounts of genetic variability within populations, individual plants, seeds, and even individual pollen grains.

Lineages of switchgrass that would have survived the Ice Ages would be those that were endemic to or immigrated southward to areas that allowed their survival during many centuries of glaciation. As suggested by McMillan [24], in the most recent period of glaciation, three areas may have provided ice-free and sufficiently warm growing seasons to serve as refugia for many grassland species. McMillan's logic, which was built on an understanding of climatic geography during the last glacial period, suggested the Lowland (or Southern) Great Plains, the Eastern Gulf Coast, and an area in the Upland Plains were three places that—even in the midst of the glaciation—would have had growing seasons suitable for many of the plants that eventually re-colonized the Great Plains.

Casler and colleagues have looked carefully at the distribution of North American populations of the two switchgrass ecotypes and the morphological and genetic similarities and differences between and within those populations [18, 23, 25, 29, 30]. Other labs (e.g., [31]) have provided similar or additional evidence that the current populations of North American switchgrasses can be placed into a few groups based on molecular markers and that those groups are associated with particular geographies, or provenances.

Zalapa et al. [25] examined SSRs in 18 switchgrass cultivars: 7 lowland (all tetraploid) and 11 upland (two tetraploid and the remainder octoploid). The work found alleles unique to, i.e., diagnostic for, each ecotype and also found alleles that distinguished tetraploid from octoploid members of the upland populations. The analysis revealed also clusters of allelic similarities, or genetic pools, within each of the ecotypes; and, perhaps not surprisingly, those groupings reflected geography of origin. Accordingly, lowland cultivars were grouped by allelic similarities into two clusters; cultivars in one cluster all came from the Eastern Gulf Coast region, and those in the other were all from the Southern Great Plains. The nine octoploid upland cultivars fell into three allelic clusters, or genetic pools, each with a unique provenance: those associated with the Central Great Plains, the Northern Great Plains, and the Eastern Savannah [25]. Zalapa et al. [25] suggest their findings may

provide support for the three Ice Age refugia posited by McMillan [24].<sup>1</sup> Zalapa et al. [25] hypothesized that each of the two lowland allelic (and geographic) genetic pools noted above is descended from McMillan's similarly named refugium, i.e., Lowland/Southern Great Plains and Eastern Gulf Coast. They suggested also that at least two of the upland genetic pools may be the descendants of plants that survived in the Upland Plains refugium. The Zalapa et al. [25] work also offers a reasonable model for arriving at the current situation where octoploids are the more frequent ploidy level for upland cultivars. It builds on the notion that the duplicated genome offers more grist for the evolutionary mill, a notion reflected in the writings of others (e.g., [13, 16]).

More recent studies have identified multiple upland and lowland lineages within the eastern USA [18, 23]. The observation of obvious geographic patterning among upland lineages in the northern USA, combined with a general lack of patterning among lowland lineages in the southern USA, suggests that evolutionary forces have acted on the nuclear genomes of migratory switchgrass, allowing these populations to adapt to a wide range of habitats and climates during the 11,000 years since the last glacial period. Indeed, the allelic patterns of SSR markers identified by Zalapa et al. [25] are sufficiently specific to geographic regions that Zhang et al. [18] were able to identify two 8x upland accessions that were inadvertently transported by the US Army to remote regions of the USA, eventually becoming established and many years later incorrectly identified as 'local' switchgrass accessions.

One more evolutionary consequence of the Ice Ages was the periodic juxtaposition (in refugia) of upland and lowland lineages for tens of thousands of years, resulting in upland-lowland matings and the establishment of mixed or hybrid lineages, some of which completely defy simple classification [23]. These hybrid lineages are an additional mechanism by which switchgrass enriches and preserves genetic variability to be utilized during and after post-glacial migrations, creating phenotypic variations in flowering time, cold tolerance, and heat tolerance [30, 32] that have allowed it to adapt to such a wide range of habitats.

In sum, we can suggest that our 'modern' switchgrasses, i.e., those that emerged from and radiated after the last Ice Age, may have come from a relatively small number of survivors. Those survivors included a few—maybe only two—groups representing the lowland genetic pool and perhaps a few more groups carrying the upland gene set. What we see today reflects the rather remarkable ability of those few survivors/pioneers to radiate, adapt, and re-colonize two-thirds of a continent in a scant 11,000 years; but 2 million years of switchgrass evolution (which included repeated winnowings and forgings on the anvil of continental glaciation) and development of two ecotypes (with some representatives possessing a quadrupled genome size) clearly set the stage well for a rapid reclaiming of the North American landscape once it was again habitable.

<sup>&</sup>lt;sup>1</sup> Casler et al. [30, 32] had adumbrated earlier the colonization of prairie ecosystems by remnants from southern refugia.

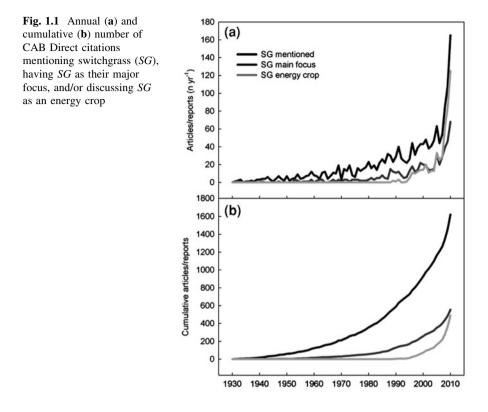
#### 1.3 The Agronomic History of Switchgrass

Switchgrass has been a 'crop' in the usual sense of that word for only a few decades. Unlike maize, wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and some other grasses that prehistoric humans co-opted into domestication [6], switchgrass has only very recently even been planted or studied in monoculture. *Panicum virgatum* preexisted *Homo sapiens*, of course, but only recently have we begun to take note of it and adapt it to human purposes.

One way to document the rise of switchgrass into human consciousness-or human technology—is to survey the history of publications about the species. We have done that using CAB Direct, the bibliographic database of Commonwealth Agriculture Bureau, which indexes over 9 million entries from applied life sciences fields-entries from 1900 to the present. We searched it for the occurrences of Panicum virgatum, switchgrass, or switch grass in 'all fields', i.e., title, abstract, key words, or CAB's coding descriptors and identifiers. As a result, some articles indexing to switchgrass mention it rather coincidentally, e.g., not a host for an aphid, or as one among many species in mixed swards or in multi-species screenings. Along with refereed journal publications, the canvass returns a number of brief abstracts and non-refereed proceedings from agronomy, animal science, and weed science conferences, as well as agricultural experiment station bulletins. On the other hand, some published reports that deal with switchgrass rather extensively (e.g., [1, 33]) do not index to switchgrass, because they do not mention switchgrass in their abstract and the indexer has not included the species as an 'organism descriptor'. Or, in other cases, switchgrass reports are published in a source-often a book or monograph such as this one-that is not cataloged by CAB (e.g., [34, 35]). So, we know our survey is not an exhaustive or comprehensive list of publications dealing with switchgrass, but we feel confident that it provides a good indication of the overall trend or trajectory for such publications.

As part of our survey, we perused each abstract (and a few full articles) to determine in what context switchgrass was discussed. Was switchgrass a primary focus of the work? For what use/purpose was it being considered? Figure 1.1 plots the total number of CAB-indexed reports referring to switchgrass, the number looking only at switchgrass (or comparing it with only one other species), and the number mentioning switchgrass as a potential energy crop. Accordingly, it documents the 'birth' of switchgrass as a crop: first appearing as a subject in scientific investigations about a century ago, exhibiting a long 'lag phase', and then entering a vigorous 'growth phase' in just the last 20 years.

The volume of work on switchgrass is still very small compared to many other crops. For example, canvassing CAB Direct for citations in 1940 produces 713 hits for *Zea mays*, 395 for *Avena sativa* (oats), and only six for *Panicum virgatum*. The number of publications in 2010 indexing to switchgrass is 165, but that barely outdistances the number of hits for maize in 1929 (and maize provides 5,610 hits in 2010). On the other hand, the 165 switchgrass citations in 2010 compare with just 15 for big bluestem (*Andropogon gerardii* Vitman), a tall-grass prairie species



that has much in common with switchgrass historically and ecologically; and in only two of those 15 citations is big bluestem a primary focus of the work.

#### 1.3.1 'Prairie Grass' Origins

The first indexed occurrence of switchgrass in the CAB database comes in 1914, where the species is mentioned as not being a host for the aphid about which the article was written. The next appearance is in 1931, in the quaintly named 'Who's who among the prairie grasses' [36], where switchgrass is mentioned as occupying 'less desirable lowland soils'. That publication and most of those few that followed over the next 20 years allude to switchgrass as one of the species in 'tall-grass prairie', 'prairie grasses', 'prairie hay', 'native grasses', 'range grasses', 'mixed grasses', 'warm-season grasses', etc.

Those early papers discussing switchgrass's contribution to grass mixtures include a few peer-reviewed articles and numerous agricultural experiment station bulletins and annual reports. Also appearing at this time are reports on the natural occurrence of switchgrass in various ecosystems. One such report, coming

in 1932 from Massachusetts, is the first CAB-indexed citation where switchgrass is the primary or sole species of interest [37].

# 1.3.2 Early Studies and Uses as a Monoculture, and the Growth of Reports on Switchgrass

Switchgrass begins to emerge from the anonymity of being 'just' a prairie grass in the 1940s. An article in 1941 looks at differences among various accessions in susceptibility to rusts and is the second paper published with switchgrass as the primary or sole subject of the investigation [38]. A 1947 agricultural experiment station report refers to studies of switchgrass and other prairie grasses done on pure stands established in 1937 [39]. During the late 1940s and 1950s, reports on selection and breeding studies with switchgrass appear in a few agricultural experiment station. Indeed, through 1960, a total of 123 CAB-indexed reports mention switchgrass, and many of those simply mention its occurrence in various ecosystems.

The first CAB-indexed paper dedicated solely to switchgrass physiology (and only the third where the species is the primary focus) appears in 1947; it sought relationships between ploidy level and winter survival (but found none) [40]. The total number of indexed studies with switchgrass as their major focus grows slowly. By 1960, 14 such studies have accumulated. By 1970, there are 30; and by 1980, 55. Those reports focusing on switchgrass as a monoculture, i.e., a 'true' crop, deal with a range of topics. Some are reports of cultivar releases, e.g., Blackwell, Caddo, Summer, Pathfinder, and then Kanlow. A 1953 publication provides pioneering data on chemical composition [41]. Some as early as the 1940s discuss switchgrass for erosion control in waterways, and several in the 1960s considered the species' value in reclamation. However, most of the switchgrass-focused reports deal with the species as a forage crop either from an agronomic perspective or from an animal nutrition perspective. All of the cultivar release reports noted above discuss forage value.

Beginning in the 1980s, we observe an up-tick in the study of switchgrass. In that decade, 65 indexed reports appear dealing primarily or solely with switchgrass—more than doubling the previous 50 years' cumulative for this statistic. The focus is still heavily on forage value and breeding, but a few reports deal with reclamation, erosion control, and diseases. At the close of the decade comes the first peer-reviewed article written on switchgrass as an energy crop [42]. Some more background on that publication and further discussion of the trajectory in research studies on switchgrass as an energy crop will be given in Sect. 1.4.1.1.

After a plateau in the early 1990s, interest in switchgrass (as conveyed by indexed publications at least) increases noticeably in the second half of the decade. Reports dealing solely or primarily with switchgrass average eight per year from

1990 through 1994, nearly the same rate as in the 1980s; but the second half of the decade sees an average of 16 articles per year focused on switchgrass. As will be discussed below, that burst of activity is driven largely by the increasingly frequent appearance of reports on switchgrass as an energy crop, but the species continues to be studied for forage and other purposes as well.

In sum, for this section, based on indexed reports in the scientific literature, the history of switchgrass as a crop is very short. Only during the second half of the twentieth century did the species move clearly from being one of the 'prairie grasses' to being a crop grown in monoculture. For the first 40 years of its very short agronomic history, the volume of work on switchgrass was small, averaging only about five CAB-indexed mentions per year and averaging less than one report per year dedicated primarily or solely to it. From 1930 to 2010, more than 1,600 reports that index to switchgrass have been published, with more than half of those appearing after 1997. This might suggest that the crop is in the process of joining the Olympian list of 'most useful grasses', but let us hold that judgment in abeyance until we have looked at some other matters.

#### 1.3.3 Current and Proposed Uses

The caveat about 'most useful' status notwithstanding, we can say without reservation that switchgrass now serves us very well in several roles, i.e., it belongs in the grass pantheon. Its initial adoption as a forage species was probably a logical extension of its millennia-long role as food for ungulates on the Great Plains of North America. In addition to that use, though, it has been adopted or is under consideration for a broad range of other purposes [43], which we will simply summarize:

Established roles/uses for switchgrass:

- Forage for grazing, hay, or haylage;
- Erosion control in waterways, levees, stream margins, etc.;
- Vegetative filter strips (to reduce runoff of soil and nutrients);
- Reclamation/stabilization of sand dunes and disturbed areas;
- Wildlife habitat.

Other roles/purposes under study (or in early adoption):

- Energy feedstock for:
  - Combustion;
  - Conversion to liquid or gaseous forms.
- Fiber or pulp for paper;
- Phytoremediation to include smelter and mining sites;
- Pharmaceuticals, biomaterials, plastics, etc.;
- Value-added 'by-products' from biorefineries;
- Substrate for mushroom culture.

#### 1.4 The Origins of Switchgrass as an Energy Crop

A few published articles have discussed the brief history of switchgrass as an energy crop. One [44] is by individuals in the US Department of Energy (DOE) funding agency that initiated studies on switchgrass as an energy crop, but its account begins essentially after the decision has been made to focus on switchgrass. An internal DOE report [45] and a subsequent journal publication [46] provide more of the 'back story' of how switchgrass came to be essentially the sole focus in DOE's efforts on herbaceous energy crops. One of us (DJP) was a participant in the discussions that first brought switchgrass [34, 43, 47]. We will summarize and expand on all of these sources in the sections that follow; and some personal observations are provided because we hope they will show how serendipity, pragmatic decision-making, and politics—as well as science, of course—have shaped the narrative of the switchgrass story.

We shall take up our account of the switchgrass-for-energy 'story' beginning in 1984 with DOE's early work on herbaceous biomass species. However, interest in biomass as an energy source certainly predates that. Indeed, biomass use for energy is prehistoric, with wood remaining a primary energy source until the mid nineteenth century. Since then, we have become increasingly dependent on fossil fuels, but many countries—especially in war time—revert to biomass energy sources. In one of the more recent occurrences, the 'energy crisis' following the embargo imposed by oil producing and exporting countries (OPEC) in 1973 spurred interest and work on energy cropping and biomass as a feedstock in the USA, the EC [48], and the UN [49]. Out of those efforts came many reports, including one in the USA that mentions switchgrass as a possible biomass source [50]. However, in our view, there is a loss of continuity (or certainly of momentum) in the biomass-for-energy narrative, when interest in biofuels flagged in the late 1970s, as oil prices returned to 'pre-crisis' levels.

#### 1.4.1 In North America

It is fitting that the first studies of switchgrass as an energy crop were done in North America, but not every energy crop candidate has been first studied in its region or country of origin. For example, miscanthus from southern Asia was first studied as a possible energy crop in England (see Sect. 1.4.2.2). But for switchgrass, the impetus to consider it as an energy crop was as native as the species itself.

#### 1.4.1.1 DOE Extramural Screening Studies

In 1982, the Oak Ridge National Laboratory (ORNL) of the US DOE assumed control of a young program looking at woody species for energy purposes [46].

In 1984, ORNL expanded their biomass-for-energy program and issued a request for proposals (RFP) to screen herbaceous species as energy crops, i.e., species that might produce significant amounts of lignocellulosic biomass. The RFP further stipulated that the work must be done on 'marginal croplands' [46]. In 1985, the first five subcontracts were awarded for what became known as the Herbaceous Energy Crops Program (HECP); and, in the first few years of the HECP, both the 'woody' and 'herbaceous' subcontractors met together periodically to compare biomass production data.

After the five initial HECP subcontractors were identified, ORNL called them together in April 1985. They came from Alabama (Auburn), Indiana (Purdue), Ohio (a private research firm), New York (Cornell), and Virginia (Virginia Tech). Two more subcontractors—Iowa State and North Dakota State—were added to the screening study in 1988 [45]. At that April meeting, each of the five groups shared their list of species to be screened. Each list was appropriate to the region in which the work was to be done, but no species was common to all lists. No benchmark species was there to allow cross-region comparisons of biomass productivity of the over 30 disparate species that would be grown at over 30 disparate locations, each of which was marginal for disparate reasons.<sup>2</sup>

The eight species proposed by Virginia Tech included switchgrass. Their proposal noted that switchgrass is a native that will 'produce better growth and cover on droughty, infertile, eroded soils [which characterized the marginal sites proposed for studies in Virginia] than most introduced grasses'. Dale Wolf, the forage scientist who chose switchgrass for Virginia Tech's proposal, suggested to those present at the 1985 meeting of subcontractors and administrators that the wide natural occurrence of switchgrass should allow it to serve well as the desired benchmark species. His suggestion was adopted, and he subsequently supplied Cave-in-Rock seed from a single source for all subcontractors. For the later-added subcontractors, switchgrass was stipulated as a candidate/benchmark species. So, switchgrass appeared in all seven subcontractors' screening studies—but only after it was added to most. By contrast, 17 candidates from the screening studies were on only one of the seven lists.

The initial round of subcontracts called for a 5-year study to allow each of the screened species to come to full production and to experience a range of growing seasons.<sup>3</sup> With the addition of two more subcontractors in 1988, the total number of species screened grew to 36 plus two polycultures, and the total number of sites was 31 [45]. When the final reports of the screening studies were compiled, switchgrass had proven itself to be one of the most prolific producers of biomass across most of the locations.<sup>4</sup> It, in fact, did well soon enough in the 5-year cycle

 $<sup>^2</sup>$  Each group would plant their list of candidates at from two to eight sites. (Data in this and the next few paragraphs were compiled from Wright [45]).

<sup>&</sup>lt;sup>3</sup> Most were perennials, but that was not a requirement of the RFP.

<sup>&</sup>lt;sup>4</sup> Switchgrass did not fare so well in Ohio perhaps because the marginal sites there were poorly drained.

that some of the subcontractors did switchgrass-specific studies looking at management techniques (Virginia Tech) and screening cultivars (Auburn) [45].

Partly because of the long-term nature of the screening studies and perhaps because biomass production for energy purposes was not yet a 'standard' topic for editors of agronomic publications, the first papers on switchgrass as a model species were slow to appear. Only two switchgrass-based and DOE-credited reports appeared during that first 5-year cycle. Both came out of Purdue and included the paper mentioned above [42]. However, annual reports and final reports from all of the subcontractors and ORNL HECP staff were submitted in a timely fashion.<sup>5</sup>

#### 1.4.1.2 Intramural Efforts at ORNL and Other DOE Agencies

In the early 1990s, HECP was subsumed into the Bioenergy Feedstock Development Program (BFDP), reflecting some reorganization within ORNL and merging woody and herbaceous biomass programs under this new name and management. As of this writing, feedstock development efforts in DOE remain based in ORNL's Environmental Sciences Division (part of ORNL's Energy and Environmental Sciences Directorate) with a program name of Renewable Energy Systems. In addition, work on microbial conversion of biomass into biofuels is housed in the directorate's Biosciences Division as the Bioconversion Science and Technology Program.<sup>6</sup>

A very significant body of work on switchgrass has and continues to come from ORNL scientists. Besides intramural studies on microbial conversions of switchgrass biomass, ORNL staff have examined molecular markers and basic physiology of switchgrass and the species' potential for sequestering carbon. Several on the ORNL staff have also looked at the economics of large-scale switchgrass production. Much of that body of work—as well as annual and final reports from subcontract work—can be accessed at ORNL's website.<sup>7</sup>

Other DOE laboratories outside of Oak Ridge are also engaged in work on switchgrass as well as other biomass species. Much of the biomass conversion work has been done at the Solar Energy Research Institute (SERI), which was formed in 1977 and was reorganized and renamed the National Renewable Energy Lab (NREL) in 1991. NREL is the home of the National Bioenergy Center; and, along with ORNL and three other national DOE laboratories, it supports the efforts of DOE's umbrella Biomass Program. As the name implies, NREL deals with more than biofuels, but their portfolio includes efforts aimed at development and commercialization of biomass conversion technologies, i.e., biorefineries.

<sup>&</sup>lt;sup>5</sup> http://www.ornl.gov/info/reports/

<sup>&</sup>lt;sup>6</sup> http://www.ornl.gov/sci/ees/organization.shtml

<sup>&</sup>lt;sup>7</sup> http://www.ornl.gov/info/reports/

#### 1.4.1.3 DOE Extramural 'Model Species' Studies

Switchgrass, which was somewhat serendipitously chosen as the benchmark species for HECP's initial 5-year, 7-state, 36-species screening studies, did so well in those studies that ORNL's BFDP subsequently invited proposals to study only switchgrass. The RFP described switchgrass as a 'model species' [44-46]-perhaps an implicit reservation or caveat that switchgrass might ultimately prove less productive than some of the thousands of species that had not been screened. However, it was a very reasonable notion that lessons learned from studies of a model species could be applied or adapted to other promising biomass species when they might appear. The decision to focus on a single herbaceous species was made at a time when ORNL's budgets for biofuels work were shrinking, suggesting that the decision to focus on a single herbaceous species was perhaps at least partially a pragmatic one, based on fiscal constraints [45, 46]. ORNL narrowed their focus on woody biomass to a single model species at this time also [45]. The upshot of the switchgrass-as-a-model-species decision was that DOE essentially stopped looking for new herbaceous energy crops after the first 5-year screening study.

A second and then a third 5-year round of DOE-funded extramural subcontract work—now focused solely on switchgrass—began in 1992 and 1997. In 1992, several long-term varietal and management studies were initiated, some of which ultimately received 10 years of DOE support (e.g., [51, 52]). Field studies on cultivar selection, improving establishment, and management for biomass production (especially fertilization and harvest management when grown for biomass) were performed at Auburn, Iowa State, Texas A&M, and Virginia Tech [44]. During DOE's 'model species' funding cycles, switchgrass breeding efforts were supported at Oklahoma State and the University of Georgia, as were tissue culture and transgenic work at the University of Tennessee [44]. Also included in these rounds of DOE/ORNL funding were collaborations with USDA personnel based at various public universities and USDA facilities, included the very productive program at the University of Nebraska [44].

During this era, papers discussing switchgrass as an energy crop authored by DOE subcontractors, ORNL scientists, and collaborating USDA personnel began to appear with increasing frequency (Fig. 1.1). These papers represented the bulk of papers being published on herbaceous biomass species at the time, and they typically discussed switchgrass as a 'model species', 'energy crop candidate', 'potential energy crop' etc.; but the 'model' designation seemed to fade from consciousness (along with 'marginal croplands') as more and more reports appeared on switchgrass—especially as non-DOE efforts increased. Essentially all of the first several papers dealing with switchgrass as an energy crop can be traced to DOE/ORNL efforts and support, but that would change quickly at the beginning of the twenty-first century.

# 1.4.1.4 Transition of Support from DOE to DOT, USDA, and the Private Sector

Shifting US politics and administrations cause the switchgrass story to take a righthand turn in 2002. The DOE/ORNL/BFDP program had issued a new RFP for switchgrass studies in 2001 and was in discussion with potential subcontractors when funding was withdrawn based on 'decisions made within DOE' [44]. Work continued within DOE, but no more funding went to outside parties. Interestingly, towards the end of the same administration, switchgrass was given a boost into the public consciousness when it was mentioned in the 2006 State of the Union message to the US Congress and citizens. That reference triggered much interest from the news media, resulting in a flurry of telephone calls and e-mails to the relatively small fraternity of scientists then working on switchgrass; and it probably brought first knowledge of the species and its bioenergy potential to millions. It was almost certainly the impetus for a spate of magazine and newspaper articles.

Following the loss of DOE funding for extramural research on switchgrassfor-energy, the US Department of Ariculture (USDA) began slowly and then more vigorously to assume leadership. For example, the USDA Agricultural Research Service (ARS) developed a national intramural program on Bioenergy and Energy Alternatives that includes major studies with switchgrass at several USDA facilities.<sup>8</sup>

Some of the initial post-DOE funding for efforts on switchgrass came through the Sun Grant Program, which was enacted legislatively in 2002 and overseen by the US Department of Transportation (DOT) with substantial inputs from both USDA and DOE. Various regional studies on switchgrass and other bioenergy species were developed and funded (and continue to be funded) by Sun Grant.<sup>9</sup>

Also stepping into the biofuels arena increasingly in the first decade of the twenty-first century has been the private sector. Some major petroleum companies have invested in biofuels research, in some cases via centers established at universities. A number of new companies that hope to capitalize on switchgrass's and other species' bioenergy potential have also appeared. Most of them have their own cadre of research scientists, but they have also contracted work out to scientists at various public and private institutions. Another major participant in switchgrass-for-energy studies has been the private, not-for-profit Noble Foundation in Oklahoma, which has expanded its long-standing efforts on forages into studies aimed specifically at the energy crop potential of switchgrass.<sup>10</sup>

During this time of change in funding sources and administrative oversight of bioenergy efforts, there were also quantitative and qualitative changes in the trajectory of publications on switchgrass. Since the first two switchgrass-for-energy citations in 1989, the number of reports dealing with that topic has grown rapidly.

<sup>&</sup>lt;sup>8</sup> http://www.ars.usda.gov/research/programs/programs.htm?np\_code=307

<sup>&</sup>lt;sup>9</sup> http://www.sungrant.org/

<sup>&</sup>lt;sup>10</sup> http://www.noble.org/Research/Biofuels/index.html

In the 1990s, 57 appear; and from 2000 to 2010, another 429 are added to the CAB database. Not all of those are dealing solely with switchgrass; some only compare it briefly with another species of interest; but it seems in many cases that switchgrass is the standard—the benchmark again—against which other biomass species are being compared. Interest in switchgrass for other purposes certainly does not go away during this time, but the great majority of reports with switchgrass as the main focus are looking at it for its bioenergy potential. For example, as of this writing, over 100 CAB Direct entries in 2011 index to switchgrass, and three-quarters of them mention it as an energy crop.

#### 1.4.1.5 Canadian Efforts

Switchgrass is native to southern portions of Canada, and Canadian workers became involved in switchgrass-for-energy studies early on, but it was largely a one-institution project based at McGill University. In 1993, workers at McGill planted a screening study that looked at five species of warm-season grasses, to include 12 cultivars or lines of switchgrass [53]. They followed phenology and yields for two post-establishment seasons and reported biomass on a per-plant basis. They concluded that several cultivars of switchgrass and one of cord grass (*Spartina pectinata* L.) were the most productive. A simultaneous study at the same location looked at phenology and allometry of nine switchgrass cultivars and one line of big bluestem [54]. It showed again potential for these warm-season grasses in a short-season locale. Those screening studies were followed by several studies focusing specifically on switchgrass: looking at management, seed physiology, energy yield, and chemical composition. Taken together, these works represent a significant body of knowledge on the potential of switchgrass-for energy (or other) purposes in southern Quebec.

After the flurry of studies done at McGill, Canadian work on switchgrassfor-energy was taken up and expanded to an international scale by scientists at Resource Efficient Agricultural Production (REAP) Canada, which is located on the McGill campus. That organization has championed in Canada and elsewhere the adoption of switchgrass as an energy crop. They are particularly interested in—and are fostering commercial ventures that employ—the concept of growing switchgrass for conversion into densified units (e.g., pellets) that can be used for heating [55]. Their web site<sup>11</sup> has a comprehensive list of their work and recommendations, which include growing guides and information about pelletizing and burning switchgrass.

<sup>20</sup> 

<sup>11</sup> http://www.reap-canada.com/

#### 1.4.2 In Europe and Elsewhere

#### 1.4.2.1 A Brief History of Switchgrass in Europe

If the North American history of switchgrass as an energy crop can be considered brief, the same is all the more true in Europe. It is notable that the first European studies on switchgrass-for-energy were done at the famed Institute of Arable Crops Research (IACR)—formerly known as Rothamsted Experimental Station—when scientists there planted plots of upland and lowland switchgrass in 1993 [56, 57]. One year later, A. Biotec, an Italian private research institute located in Cervia (southern Italy) undertook pioneering studies on switchgrass in the Mediterranean region. These English and Italian studies revealed an adaptability of switchgrass to both northern and southern European conditions. However, because of lower productivity compared to other biomass crops, switchgrass was initially deemed to be a less suitable crop for energy conversion than some other species. In southern Europe, switchgrass produced significantly lower biomass yields than giant reed (*Arundo donax* L.) and sorghum (*Sorghum bicolor* L.), while in northern Europe it was less productive than miscanthus and some short-rotation coppices such as poplar (*Populus spp.*) and willow (*Salix spp.*).

Nevertheless, the promising results with switchgrass beginning to come from North America in the 1990s paved the way for a 1998 to 2001 European project specifically focused on switchgrass: 'Switchgrass as an alternative energy crop in Europe' [58]. This project effectively coordinated research activities on switchgrass in Europe and extended trials over a wide range of European latitudes, soils, and climates. In addition to trials in the Netherlands and UK, studies were established in southern Europe: Trisaia, Italy (40.09' N, 16:38' E), Aliartos, Greece (38.22' N, 23.10' E) [59], and Bologna, Italy (44.43' N, 11:47' E) [60].

In general, the results from this first pan-European switchgrass project confirmed a yield advantage of lowland over upland ecotypes; but, in some cases, it also revealed lowlands' susceptibility to cold stress, which considerably limited their productivity. Some lowland plantings failed, especially in Germany, western UK, and Ireland. This was likely due to a particularly harsh winter, since there were other cases of successful lowland plantings with high yields in more northern countries.

Other studies carried on as part of this first European switchgrass project assessed economic and environmental impacts of switchgrass [61, 62], variety choice [63], nutrient composition [58], modeling [64], thermal conversion (combustion, gasification, and pyrolysis), ethanol production [61, 65], and industrial non-energy uses [66, 67]. Studies by Monti et al. [68] and Minelli et al. [69] examined tillage and weed control methods for improving switchgrass establishment and provided sustainability strategies aimed at the use of machinery and herbicides.

Collectively, these projects led to several conclusions: (1) switchgrass can be grown over a wide range of European latitudes, although the level of biomass

production may not always be satisfactory; (2) production of up to 25 Mg biomass  $ha^{-1}$  is possible, with higher yields generally seen when planting lowland cultivars in more southern latitudes; (3) seed propagation is valued by farmers, as it significantly reduces their investments compared to vegetatively propagated grasses and short rotation coppices; (4) switchgrass is particularly vulnerable during establishment, but, once established, it requires relatively low use of chemicals and farm resources.

A weakness of the first European (and American) switchgrass projects was the absence of farm-scale studies under truly operational conditions. Nearly all production data—and subsequent economic and environmental analyses—were in fact extrapolated from plot trials, which it is generally conceded often produce inflated yields. Studies at farm and field scales were therefore undertaken in a subsequent 2001 to 2005 project, 'Bio-energy chains from perennial crops in southern Europe' [70], which compared switchgrass, miscanthus, giant reed, and cardoon (*Cynara cardunculus* L.) grown in the Mediterranean region. The University of Bologna led the work on switchgrass and planted Europe's first field-scale experiment (ca. 9 ha), where interdisciplinary studies were carried out, e.g., geo-statistical [71], economic [72], agronomic [73], and agro-environmental [74].

One of the main findings of this Italian work was that, under real-world operational conditions (using common farm machinery), up to 30% of switchgrass biomass can be left in the field. However, much of that loss could be reduced through simple machinery adjustments. Therefore, switchgrass generally achieved acceptable biomass yields, and its management required very few investments in terms of additional machinery or implements. Along with these important operational advantages, switchgrass could also provide significant environmental and economic benefits compared to other energy crops [74]. The advantages seen in Italy, however, were not always seen in other Mediterranean countries. For example, in Greece the productivity of switchgrass was much lower than that of giant reed, and in Spain the productivity of cardoon was clearly higher than switchgrass. Therefore, no clear picture emerged on which crop should be used for energy in the Mediterranean area. Indeed, just as in the USA, it may well be that some species will out-perform switchgrass as biomass producers in specific locales.

Following the first two multi-year European projects, a number of new national (e.g., LignoGuide in France, and BioSeGen in Italy) and multi-country projects were launched (Fig. 1.2) to look at switchgrass—in many cases along with other species. Agronomic studies at different scales or levels increased, especially in western Europe, but they moved eastward as well (Fig. 1.2). Some European projects that have emphasized the possibilities of switchgrass as an energy crop are: (1) On-Cultivos (2005–2012), which has set out to define, promote, and develop a sustainable market of energy crops; (2) Babilafuente (2007–2022), which hopes to demonstrate the commercial feasibility of a second-generation ethanol plant in Spain; (3) 4FCROPS (2009–2011), which has analyzed potential land allocation and prospects for switchgrass in Europe; (4) BIOLYFE (2010–2013), which has addressed second-generation cellulosic ethanol, including



**Fig. 1.2** Switchgrass trials in Europe. Data gathered from the literature and by personal communications. Numbers inside each country identify which of the following European projects that country has participated in: *I* Switchgrass (1998–2001); *2* Bioenergy chains (2002–2005); *3* On-cultivos (2005–2012); *4* Babilafuente (2007–2022); *5* 4FCrops (2009–2011); *6* BIOLYFE (2010–2013); *7* Pellets-for-Power (2010–2013); *8* OPTIMA (2011–2014)

upstream and down-stream processes, such as pretreatment steps; (5) Pelletsfor-Power (2010–2013), which is aimed at developing switchgrass on 1 to 5 million currently underutilized hectares in the Ukraine and other countries of eastern Europe: (6) OPTIMA (2011–2014), which is dealing with the development of perennial grasses in the Mediterranean basin, particularly on marginal lands.

These continuing research efforts and the reports coming from them have 'raised the profile' of this non-native species in Europe, such that switchgrass has become an increasingly frequent subject for scientists, farmers, and entrepreneurs. By virtue of its frequent appearance in scientific and popular reports, switchgrass has come to be considered as one of the most important energy crops in Europe—much as happened in the US.

To summarize, the results to date from various European studies suggest that switchgrass is broadly adapted to many of Europe's countries. However, there is still great uncertainty on whether lowland or upland ecotypes should be used in northern European countries. Biomass productivity is clearly the most important determinant in selecting energy crops in Europe. For this reason, the expectations for switchgrass as an energy crop are still significantly lower compared to other perennial and annual grasses which may out-yield it: giant reed, sorghum, and miscanthus in southern Europe and miscanthus in northern Europe. The advantage of switchgrass compared to other competing perennial grasses mainly lies in its integrated assessment, i.e., by weighing all the operational, economic, and environmental aspects. In its favor, switchgrass is propagated by seed and requires very little investment in terms of farm machinery and agricultural inputs. In comparing several biomass crops, Monti et al. [74] and Fazio and Monti [75] found that the environmental loads and the annual equivalent cost per unit biomass were the lowest in switchgrass. The ongoing projects will likely contribute to raising the awareness of switchgrass benefits in Europe by emphasizing the integrated assessment in terms of farming systems and economic and environmental sustainability.

#### 1.4.2.2 Switchgrass Studies in Other Countries

The number of non-North American and non-European countries in which switchgrass has been studied is growing. In a tally done in 2005, the species had been investigated or was reported as in use in 11 countries [43]. The list now stands at more than 20. Among the first reports of adoption of the species outside of North America was one from Australia considering switchgrass-for soil conservation uses [76]. Besides the North American and European nations already noted, the countries producing studies on switchgrass include: Argentina, Australia, China, Colombia, Japan, Korea, Mexico, Pakistan, Poland, Sudan, and Venezuela. In most cases, especially for the more recent citations, the studies deal with switchgrass-for-energy.

#### 1.5 Conclusions

The story of switchgrass, which began 2 MYA in the first quarter of the Pleistocene epoch (the Ice Ages), does not intersect with human science and technology until the middle of the twentieth century. Initially the species was of interest to us primarily as a member of prairie ecosystems, but it began slowly to gain attention as a potential forage crop and then for other uses when grown in monoculture. Less than three decades ago, we began to consider it for bioenergy purposes.

Switchgrass came out of the Ice Ages' climatic upheavals and into our scientific era as two distinct, polyploid ecotypes, each possessing a range of morphologic,

physiologic, and genetic differences. The species' legacy of having endured repeated glacial and interglacial episodes combined with the greater genetic plasticity afforded by its polyploidy likely explain switchgrass's ability to rapidly re-colonize North America after the last glacial episode; and those same factors likely produced a deeper, wider genetic pool from which we can now draw traits to serve us for a variety of purposes today.

The history of switchgrass-for-energy begins in 1985 with its selection as the benchmark species for a US DOE herbaceous energy crop screening study. Switchgrass proved to be among the top biomass producers of the 36 crops considered, and in 1991 DOE designated it a 'model species' for further study. Since DOE was the primary US agency supporting biomass-for-energy work at that time, the next decade produced a significant amount of work on switchgrass as a model energy crop, while no other herbaceous lignocellulosic species were being considered in a systematic way. When DOE funding for extramural work on switchgrass ceased in 2002, other public, as well as private, organizations kept the work moving and even accelerating. But in the transition, the notion of 'model' may have become blurred or even lost altogether. As a result, switchgrass has perhaps become a de facto biomass species of choice. At the very least, it is regularly held up as a benchmark against which other energy crop candidates are being considered.

Incontrovertible data show a remarkable rise of interest in switchgrass as a bioenergy crop in the last quarter century. If that trajectory is maintained, switchgrass could well become what some have already ascribed to it—the biomass species of choice for many systems. We feel that its biology, especially its phenotypic and genetic variability, may well make it more than just a good model species. In time, it may be grown over millions of hectares to serve as a transformer of the sun's energy into forms that reduce our dependency on fossil fuels. If that happens, it should, indeed, be allowed to join the handful of species that constitute the Olympians within the grass pantheon.

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# **Chapter 2 Switchgrass Breeding, Genetics, and Genomics**

Michael D. Casler

**Abstract** Switchgrass was one of the dominant species of the North American tallgrass prairie and savanna ecosystems that once dominated a large portion of the continent. It is currently used for pasture, hay production, soil conservation, and biomass production for conversion to energy. Switchgrass was selected in 1992 as the herbaceous model species to develop dedicated cellulosic bioenergy crops. Breeding and genetics studies began on switchgrass in the 1950s, focused on utilization in livestock agriculture. Recent developments have rapidly increased the rate of gain for biomass yield, largely by increasing the focus and intensity of selection and improving the choice of germplasm and selection methods. Modern genomics tools are rapidly being incorporated into switchgrass breeding programs to increase the rate of gain for important agronomic and bioenergy traits, as well as to create new variability that can be captured in commercial cultivars.

# 2.1 Introduction

Switchgrass is a highly versatile grass, used for soil and water conservation, livestock production, and biomass production for conversion to energy. The species is native to North America, east of the 100th meridian, ranging from southern Canada to northern Mexico. It was once one of the dominant species of the tallgrass prairie and associated ecosystems that included savanna, sand barrens, forest margins, and grassland–wetland transition zones. The most significant taxonomic division within switchgrass occurs at the ecotype level and is related to

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habitat. Upland and lowland ecotypes were named largely for an obvious phenotypic differentiation that was originally associated with habitat. Upland ecotypes were found on upland sites that were subject to occasional or frequent droughts, while lowland ecotypes were found on lowland sites that were prone to seasonally wet soils. The upland–lowland taxonomic division figures prominently in nearly all the cultivation and breeding history of this species.

#### 2.2 Biogeography

Switchgrass germplasm has been preserved in remnant prairies and associated ecosystems throughout its historic range (Fig. 2.1). While less than 1% of the tallgrass prairie and associated savanna ecosystems have been preserved, the species native to these habitats have been preserved in thousands of remnant sites [1]. Most of these sites have never been developed or plowed, but some represent abandoned farmlands, which have been allowed to return to their native habitat and species assemblage. Presumably the native species on these sites have been restored to these sites as a result of viable seed banks. In some cases, human intervention has been used to assist recovery, in the form of seeds introduced from other sites.

Prairie and savanna remnants in North America have been preserved under the leadership and custodianship of a wide range of organizations, including the U.S. Forest Service, many state agencies responsible for preservation and use of natural resources (e.g. State of Wisconsin Department of Natural Resources), non-governmental agencies such as The Nature Conservancy, and numerous private organizations that include railroad right-of-ways, rural cemeteries, and private conservationists. The size of these remnants ranges from tiny family cemeteries of several hundred square meters up to several national grasslands, some of which exceed 0.5 M ha in size (e.g. Cimarron, Comanche, Rita Blanca, and Black Kettle National Grasslands). These grasslands are highly variable in species composition, due to variations in climate and soil type, with switchgrass occupying a range of positions from the dominant species to rare or absent in many cases.

Switchgrass is a highly polymorphic species with considerable morphological and physiological variation that is closely related to climatic factors. It is highly photoperiodic along its north–south adaptation range. In their native habitat, northern accessions may flower as early as late June or early July after only 3-4 phytomers have been produced. Conversely, accessions from the extreme southern portion of the range may flower as late as mid-October after production of 7-10 phytomers. Photoperiodism and extreme flowering times have created a strong adaptation gradient associated with both photoperiod and temperature. Most recommendations are to move switchgrass germplasm no more than one hardiness zone (5°C increments) north or south of its origin to avoid stand loss. Northern germplasm is insufficiently heat tolerant and too early in flowering to be productive at southern locations, while southern germplasm may lack sufficient cold tolerance to survive at northern locations [2–4].

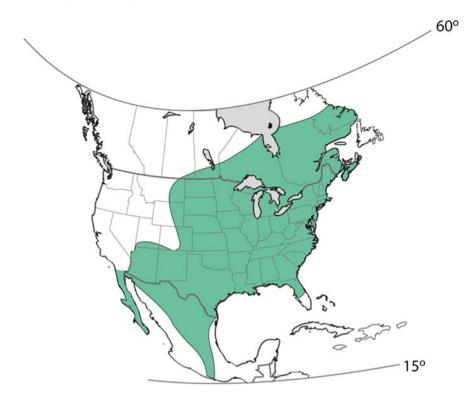


Fig. 2.1 Historical range of switchgrass in North America ([92], reprinted with permission)

Two ecotypes form the principal taxonomic division within switchgrass (Fig. 2.2). These two ecotypes were originally described based on observations of a distinct polymorphism associated with upland and lowland habitats, hence their names: upland and lowland ecotypes. Upland ecotypes are widely adapted north of 34°N latitude, extending into much of eastern Canada, but extremely rare at latitudes below 34°N. Lowland ecotypes are widely adapted up to approximately 42°N in the western portion of the range, but can be found as far north as 45°N in eastern North America due to climate-moderating oceanic effects. Upland and lowland ecotypes have generally been differentiated on the basis of plant phenotype: lowland plants are taller, have fewer and larger tillers, longer and wider leaves, thicker stems, and are later in flowering than upland plants (Table 2.1). Most lowland ecotypes also have a distinct blue coloring on stems and leaves, believed to be due to a waxy bloom on the epidermis. The blue hue is easily removed from some genotypes by touching or rubbing plant tissue between two fingers. As suggested by their names, upland ecotypes tend to be more drought tolerant than lowland ecotypes [5].

Upland and lowland switchgrass diverged on the evolutionary tree of life approximately 0.8–1.0 Mya [6–8]. Since that time, there have been approximately

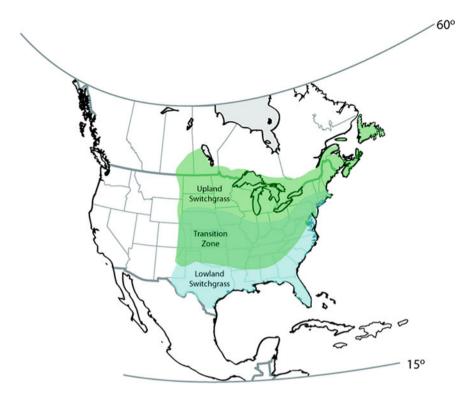


Fig. 2.2 Native ranges of upland and lowland switchgrass ecotypes in North America ([27], reprinted with permission)

**Table 2.1** Summary of the most common range of phenotypic values for upland and lowland switchgrass plants grown in direct-comparison experiments in Wisconsin and New Jersey (40–42°N latitude)<sup>a</sup>

Ecotype	Heading date <sup>b</sup> (doy)		Flag leaf length (cm)	Flag leaf width (mm)	Number of tillers (# plant <sup>-1</sup> )	diameter		CIE y-scale color <sup>b</sup>
Upland	180-195	0.9-1.7	32–48	9–11	150-300	3–5	x < 0.4	0.4 < y < 0.8
Lowland	205-220	1.9–2.2	50–58	12-14	40–90	5–7	x < 0.2	0.2 < y < 0.4

<sup>a</sup> Cortese et al. 2010 [18]; Casler et al. 2010, unpublished data

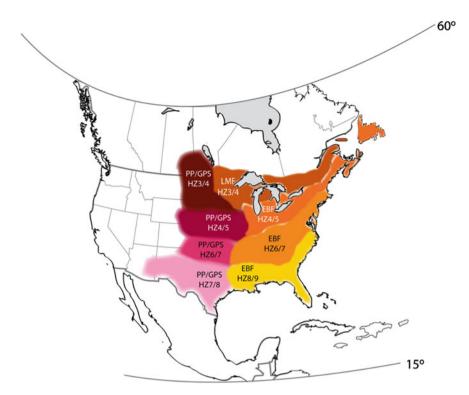
<sup>b</sup> Heading date = day of year. Color reference: McLaren [94]; http://www.colorbasics.com/ CIESystem/

12–15 major ice age cycles [9] that have compressed the native range of switchgrass into a relatively narrow band along the current coastline of the Gulf of Mexico [10]. Ice ages forced upland and lowland switchgrasses to occupy a relatively narrow region for tens of thousands of years, allowing upland and lowland ecotypes to occasionally mate with each other.

These matings probably occurred at a relatively low frequency, due to differential flowering time, but resulted in significant and measureable gene flow between the two ecotypes [8, 11]. As such, visual or morphometric assessments of plant phenotype are no longer reliable as a mechanism of classifying plants into the upland or lowland ecotype taxa [12]. The most reliable classification method is based on sequence-based marker analysis of plastid DNA, specifically chloroplast DNA, which evolves very slowly over time, much more accurately reflecting the ancient division between the two ecotypes [6–8].

Following each ice age, gradual and punctual warming of the earth's climate resulted in a slow, gradual, and highly discordant northward migration of species to repopulate their former habitats. Because plants are sessile organisms and former habitats became buried under many meters of glacial outwash and sediment, northward migrations of switchgrass and other species required assistance from birds and mammals. Deep sampling of pollen from lakebed sediments has shown that this process required thousands of years and followed a progression of tundra, taiga, boreal forest, deciduous or mixed forest, and grassland. Minor climatic shifts were often sufficient to cause local extinctions and short-term cyclic changes between dominant habitats during the early and mid-Holocene period [13–15]. Because switchgrass and other species were wholly dependent on animals for transport to new sites, it should not be surprising to observe multiple genetic lineages of switchgrass at any particular site; indeed, this is a common occurrence [6, 8, 11, 12].

McMillan [16] proposed that switchgrass was preserved in three refugia during the ice ages, corresponding largely to the eastern Gulf Coast, western Gulf Coast, and western montane (dryland) regions. His research was based on the most extensive collection and evaluation of switchgrass accessions ever conducted, representing nearly the entire range within the USA. Molecular marker analyses of both nuclear and plastid DNA have confirmed that Calvin McMillan was largely correct, but suggest that his "three" refugia might not have been isolated from each other to the extent that he hypothesized. During the Holocene period that followed the Pleistocene glaciation, tallgrass prairie and savanna establishment was largely completed by approximately 2-3,000 year BP. Since that time, climate and animals have continued to mold the genetic landscape of switchgrass, due largely to its highly outcrossing nature and the dominance of wind-aided pollination. Individual accessions of switchgrass, samples of plants collected from a single remnant site, typically possess between 65 and 80% of the genetic variability observable within the species, based on DNA markers [8, 11, 12, 17-22]. Genetic differentiation occurs on a fine-scale in some cases, e.g. changes in soil type or microclimate, but is largely associated with the photoperiod-temperature cline and an east-west cline of humidity and/or precipitation. McMillan hypothesized that the western montane refuge was largely the source of switchgrass genotypes that populated the western portion of its range, where dryland environments and drought tend to be more frequent than in eastern North America. More recent phenotypic studies across a range of sites have confirmed a tendency for eastern accessions to have relatively poor performance at western sites and vice



**Fig. 2.3** Proposed gene pools for deployment of regionally adapted switchgrass germplasm and cultivars for use in breeding programs or in conservation and restoration projects *PP* prairie parkland, *GPS* great plains steppe, *LMF* laurentian mixed forest, *EBF* eastern broadleaf forest [23]; *HZ* USDA hardiness zone [93] ([92], reprinted with permission)

versa [2]. Anecdotal observations have suggested that eastern accessions lack the drought tolerance to perform well in the western regions, while western accessions lack the disease resistance to perform well in the more humid eastern regions.

One net result of these studies has been the development of a concept of gene pools for switchgrass (Fig. 2.3). Each of the proposed gene pools spans a region that includes two neighboring hardiness zones, with a range in mean temperature of no more than  $10^{\circ}$ C. The east–west division approximately follows the Mississippi River Valley, splitting the range according to historic tallgrass prairie versus historic savanna ecosystems [23], Sanderson et al. [24] has estimated that cultivar recommendations that follow this regional gene pool concept are responsible for approximately a 20–25% increase in local biomass yields, simply associated with choosing appropriately adapted cultivars. There is currently at least one switchgrass breeder located in each of the eight regions shown in Fig. 2.3, creating opportunities to develop regionally adapted cultivars that take advantage of the significant genotype x environment interactions that are common to this species.

#### 2.3 Genetics and Cytology

Switchgrass has a basic chromosome number of x = 9, with a wide range of chromosome numbers for somatic cells ranging from n = 18 to 108 [25, 26]. Lowland ecotypes of switchgrass are largely tetraploid with 2n = 4x = 36 chromosomes, while octoploids with 2n = 8x = 72 chromosomes are very rare [11]. Conversely, upland ecotypes exist at both tetraploid and octoploid levels, with the octoploid form approximately two to three times more abundant than the tetraploid form. Tetraploids contain approximately 3.1 pg DNA per nucleus with a haploid genome size of ~1.5 Gb [27]. True hexaploids, 2n = 6x = 54, are extremely rare but have been observed within remnant sites that possess both tetraploid and octoploid plants, suggesting their potential role in interploidy gene flow [11]. Recent results based on genotype-by-sequencing suggest that hexaploids likely arise by union of a normal gamete and an unreduced (2n) gamete (Costich et al. 2011, unpublished data). Although 2n gametes are common in grasses [28], they have yet to be verified in switchgrass. Aneuploids appear to be common in switchgrass, particularly at the octoploid level, and largely characterized by chromosome loss from the normal euploid number [29].

Switchgrass is a disomic polyploid, or allopolyploid, with largely diploid inheritance at the tetraploid level [30]. Tetraploid switchgrass has 18 linkage groups arranged in two highly homologous sets that are highly conserved with other C<sub>4</sub> grasses, e.g. *Sorghum* and *Setaria* [30]. Meiosis of both tetraploid and octoploid individuals is largely characterized by normal bivalent pairing [31, 32]. A high frequency of DNA markers are characterized by segregation distortion and multi-locus interactions that could be caused by low frequencies of quadrivalent pairing including homeologous chromosomes [30], which could occur in a recent polyploid such as switchgrass.

Switchgrass is predominantly cross-pollinated with a gametophytic selfincompatibility system similar to the S-Z system found in many grasses [33]. Pollen is dispersed by wind and early reports suggested that the percentage of selfed seed was low in most genotypes, generally <1% [33, 34]. More recent reports suggest that some genotypes are capable of producing selfed seed in frequencies as high as 50% (Buckler et al. 2011, unpublished data; [35]).

Upland and lowland ecotypes of switchgrass can be easily crossed with each other at the tetraploid level, reflecting their relatively recent divergence on the evolutionary scale [33]. A single hybrid between a random upland and a random lowland plant produced a cross with an average of  $\sim 35\%$  high-parent heterosis, suggesting that the evolutionary divergence between the two ecotypes has been sufficient to create genetic divergence and allelic complementarity. A post-fertilization incompatibility system between ploidy levels minimizes the opportunity for interploidy crosses and gene flow. The existence of 2n gametes in switchgrass would be an effective mechanism to bridge this barrier. Vogel [36] suggested that tetraploid and octoploid plants that are sympatric within a single remnant prairie site are effectively members of different interbreeding populations.

# 2.4 Germplasm, Ecotypes, and Early Use

Early cultivars of switchgrass are exclusively represented by seed increases from source-identified remnant prairies (Table 2.2). These natural-track cultivars have generally undergone no direct selection for agronomic traits or been subjected to any plant improvement efforts. Most of these cultivars were given a name that reflects the geographic location of the original accession. Most of these cultivars were collected and evaluated by personnel of the plant materials centers (PMC) of the Soil Conservation Service (SCS), which later became the Natural Resource and Conservation Service (NRCS), an agency of the USDA. Many evaluations included common-garden agronomic evaluations of numerous accessions, allowing personnel to chose only one or two of the best populations for use in each region. There are 26 PMC locations within NRCS, 15 of which have been involved in collection and/or release of switchgrass cultivars using this approach. Because these populations represent little or no breeding history, they represent the natural genetic diversity within specific regions of the switchgrass range. Nevertheless, there are exceptions to this, in which different seed lots of a single cultivar have been shown to have diverged from each other, most likely due to seed increase under different environmental conditions [19].

Natural-track cultivars of switchgrass formed the basis for development of a switchgrass seed industry, shared between a small number of private companies and public organizations such as the Nebraska and South Dakota Crop Improvement Associations. The principal use of switchgrass since the 1940s has been for pasture and rangeland, largely in the Great Plains region of the USA, but only sparsely in the eastern portions of North America. In addition, switchgrass has been a component of seed mixtures for prairie and savanna restoration projects since the mid-twentieth century. Early agronomic trials of unimproved cultivars quickly established the presence of large amounts of ecotypic variation, suggesting that some sense of local adaptation should be used in developing recommendations for the geographic range of individual cultivars [36].

With the choice of switchgrass as a herbaceous model species for the U.S. Department of Energy (DOE) Biofuel Feedstock Development Program (BFDP) in 1992, public and commercial interest in switchgrass rapidly increased. Because the seed industry was largely based on unimproved cultivars that represented source-identified collections, these cultivars came under rapid and high demand as source material for agronomic field trials and demonstration plots for studying conversion of herbaceous biomass into biofuel. This interest led to an expansion of the switchgrass seed industry and the establishment of hundreds of field experiments across the native range of switchgrass in the USA and Canada. Many of these field experiments were highly valuable in helping to define the limits of adaptation of individual cultivars. Even though they were not centrally coordinated or conducted under uniform conditions, these experiments were responsible for identifying Cave-in-Rock and Alamo as cultivars with remarkably broad adaptation. Alamo, from central Texas, can be successfully grown throughout the southeastern USA and along the Atlantic Seaboard into southern New England [37]. Cave-in-Rock is broadly adapted in hardiness zones 4

Cultivar	PI number <sup>a</sup>	Ecotype	Ploidy	Year of release	Geographic origin	USDA hardiness zones <sup>b</sup>
Alamo	422006	Lowland	4x	1978	Southern Texas	6, 7, 8, 9
Kanlow	421521	Lowland	4x	1963	Northern Oklahoma	6, 7
Pangburn		Lowland	4x	$NA^d$	Arkansas	6, 7
Penn Center		Lowland	NA	2010	Coastal South Carolina	8
Stuart	422001	Lowland	4x	1996	Southern coastal Florida	9, 10
Timber		Lowland	4x	2009	Unknown mixture <sup>e</sup>	6, 7, 8
Miami	421901	Up/Low <sup>c</sup>	4x	1996	Southern Florida	9, 10
Wabasso	422000	Up/Low	4x	1996	Southern coastal Florida	9, 10
Dacotah	537588	Upland	4x	1989	Southern North Dakota	2, 3, 4
Falcon	642190	Upland	4x	1963	New Mexico	4, 5, 6
Grenville	414066	Upland	NA	1940	Northeastern New Mexico	4, 5, 6
High Tide		Upland	NA	2007	Northeastern Maryland	5, 6, 7
KY1625	431575	Upland	4x	1987	Southern West Virginia	5, 6, 7
Blackwell	421520	Upland	8x	1944	Northern Oklahoma	5, 6, 7
Caddo	476297	Upland	8x	1955	Central Oklahoma	6, 7
Carthage	421138	Upland	8x	2006	North Carolina	5, 6, 7
Cave-in-Rock	469228	Upland	8x	1973	Southern Illinois	4, 5, 6, 7
Central Iowa	657600	Upland	NA	2000	Central Iowa	4, 5
Forestburg	478001	Upland	8x	1987	Eastern South Dakota	3, 4
Nebraska 28	477003	Upland	8x	1949	Northeast Nebraska	3, 4
Shelter		Upland	8x	1986	Central West Virginia	4, 5, 6
Southlow	642395	Upland	NA	2003	Southern Michigan	4, 5, 6

 Table 2.2 Switchgrass cultivars and released germplasm populations representing various habitats in the central and eastern USA, largely representing local ecotypes with minimal or no selection for plant traits

<sup>a</sup> GRIN accession number (http://www.ars-grin.gov/). Empty cells indicate that an accession is not available through GRIN; <sup>b</sup> USDA Hardiness Zones are defined in approximately 5°C increments of mean annual minimum temperature (http://www.usna.usda.gov/Hardzone/ushzmap.html); <sup>c</sup> Upland cytoplasm, but lowland phenotype and nuclear DNA, suggesting an ancient hybrid origin [12]; <sup>d</sup> *NA* information not available; <sup>e</sup> DNA marker analyses suggest a mixture of germplasm from the southern Great Plains and the southeastern USA [8, 11]

through 7, covering much of the eastern USA and Canada north of 35°N latitude, but is poorly adapted to dryland regions [38]. Most other cultivars have significantly narrower ranges of adaptation, more aligned with the regional gene pools shown in Fig. 2.3.

Numerous collections of switchgrass have been generated throughout the species range. The official USDA collection of switchgrass accessions is located at Griffin, GA, part of the national plant germplasm system (NPGS) and germplasm resources information network (GRIN). At the time of this writing, the GRIN collection consists of 497 historical accessions, of which 174 are currently available for distribution. A small number of seeds are made available to anyone anywhere, upon request through the web link.<sup>1</sup>

<sup>&</sup>lt;sup>1</sup> http://www.ars-grin.gov/

There are thousands of additional accessions that are currently being stored in collections made by both public and private organizations involved in restoration, conservation, production, breeding, and genetics. Regardless of where they are housed, these collections are all essentially private, because they are not made broadly available to the public. Any accessions can be donated to GRIN, simply by contacting the switchgrass curator via the GRIN web link. Either seed or living tillers can be donated, but seed is preferred because it requires less urgency for care and handling. Source-identified accessions are preferred and basic passport or descriptive information about the collection site is highly desirable as a link between each accession and its natural environment. Donations can be made of switchgrass germplasm at any stage of development, ranging from wild populations to highly bred cultivars.

## 2.5 Genetic Improvement

#### 2.5.1 Breeding Objectives

Switchgrass breeding was initiated at the University of Nebraska in the 1950s, largely based on regional seed collections as the basis for breeding populations [39]. Early objectives were focused largely in improving forage quality for livestock production systems, as well as fundamental research to develop a more thorough understanding of reproductive biology and breeding behavior of switchgrass.

Development of a high-throughput in vitro dry matter digestibility (IVDMD) assay [40] was one of the major research breakthroughs that led to some of the most significant breeding gains in switchgrass. Three cycles of selection increased IVDMD by 5% over the original population mean. Simultaneous improvements in both IVDMD and forage yield are possible, but at a reduced rate of gain for both traits due to the reduced individual-trait selection pressure required for multi-trait selection [34, 41–43]. Several improved cultivars have been derived from these efforts to improve forage quality of switchgrass (Table 2.3).

Genetic improvements in forage quality traits such as IVDMD are remarkably stable across environmental conditions and management systems [44, 45]. This principle allowed the rapid cycling of high-IVDMD switchgrasses from breeding nurseries into small-plot trials of agronomic traits and large-plot grazing trials to measure livestock performance. Early grazing trials demonstrated that a 40 g kg<sup>-1</sup> increase in IVDMD was realized as an increase in daily live-weight gains 0.15 kg animal unit<sup>-1</sup>, an increase in beef cattle production of 67 kg ha<sup>-1</sup>, and an increase in profit of \$59 ha<sup>-1</sup>, measured in 1998 \$US [46]. Documentation of improved livestock production, directly associated with increases in IVDMD, was responsible for rapid adoption and success of these cultivars on many thousands of hectares in the years following release of these improved cultivars.

Cultivar	PI number <sup>a</sup>	Ecotype	Ploidy	Year of release	Principal traits selected during cultivar development <sup>b</sup>	USDA hardiness zones <sup>c</sup>
EG2101		Upland	8x	2009	Biomass yield, spring vigor, rust resistance	4, 5, 6
Pathfinder	642192	Upland	8x	1967	Biomass yield and vigor	4, 5
Shawnee	591824	Upland	8x	1996	IVDMD, biomass yield	5, 6, 7
Sunburst	598136	Upland	8x	1998	Large seed size and mass	3, 4, 5
Trailblazer	549094	Upland	8x	1984	IVDMD, biomass yield	4, 5
Summer	642191	Upland	4x	1963	Earliness, rust resistance	4, 5
BoMaster	645256	Lowland	4x	2006	IVDMD, biomass yield	6, 7, 8
Cimarron		Lowland	4x	2008	Biomass yield	6, 7, 8
Colony	658520	Lowland	4x	2009	IVDMD, biomass yield	6, 7, 8
EG1101		Lowland	4x	2009	Biomass yield, spring vigor, rust resistance	8, 9, 10
EG1102		Lowland	4x	2009	Biomass yield, spring vigor, rust resistance	6, 7, 8
Performer	644818	Lowland	4x	2006	IVDMD, biomass yield	6, 7, 8
TEM-LoDorm	636468	Lowland	4x	2007	Reduced post-harvest seed dormancy	6, 7, 8

 Table 2.3 Improved switchgrass cultivars and germplasm releases representing significant breeding and selection activities

<sup>a</sup> GRIN accession number (http://www.ars-grin.gov/). Empty cells indicate that a cultivar is not available through GRIN; <sup>b</sup> *IVDMD* in vitro dry matter digestibility; <sup>c</sup> USDA Hardiness Zones are defined in approximately 5°C increments of mean annual minimum temperature (http://www.usna.usda.gov/Hardzone/ushzmap.html)

Increases in IVDMD have been largely associated with reductions in lignin concentration [44] and reduced ratios of p-coumaric/ferulic acid [47]. Low-lignin switchgrass genotypes have also significantly reduced cortical fiber and secondary wall thickenings in stem tissues, compared to high-lignin genotypes [48]. These low-lignin genotypes have significant potential to improve conversion efficiency of switchgrass biomass to bioenergy in a fermentation system where lignin is one of the chief factors limiting ethanol production [49, 50].

Three different approaches have been used in efforts to improve establishment capacity of switchgrass. Selection for large seed size was highly effective in increasing mean seed mass by up to 50% compared to other cultivars, resulting in double the emergence rate and 6-week seedling height [51]. Selection for high seedling shoot mass was highly successful, but did not translate into consistent improvement of seedling vigor, root growth, or establishment capacity in field studies [52]. Finally, divergent selection for elevated versus reduced crown node position failed to affect seedling vigor or establishment capacity [53]. Given the persistent establishment problems associated with switchgrass, especially as a monoculture for bioenergy production systems, there has been surprisingly little research conducted on breeding approaches or directed breeding efforts to improve establishment capacity. Instead, research efforts have largely focused on establishment methods to reduce weed competition (see Chap. 4).

Seed dormancy is a significant problem in many natural populations of switchgrass, but every cycle of selection in a breeding program moves one step closer to reducing seed dormancy problems for improved cultivars, simply by the process of eliminating seeds that do not germinate. Gradual elimination of seed dormancy problems in switchgrass is currently the first of several wild traits to be eliminated on the road to domestication of this species.

A number of pathogens and herbivorous or boring insects utilize switchgrass as a host in various portions of its range [36, 37]. Breeding for resistance to many of these pests is a high priority for most switchgrass breeders, but is largely conducted as a secondary objective in field-based breeding programs. There are no reports of pest screens conducted under highly controlled or uniform conditions of artificial inoculation. Rather, breeders have tended to rely heavily on natural inoculum in field nurseries, which can often be highly variable, inconsistent, and unreliable. Susceptibility to pests is one of the principal sets of traits that are used to eliminate plants from breeding nurseries prior to measurement of traits related to productivity and quality. Genetic variation is known to exist for some pests (e.g. [54]) and disease epiphytotics and insect infestations will likely elevate pest resistances to higher priorities in the future. There are several recent reports of widespread insect pests that are capable of drastically reducing biomass yields and/ or seed yields of switchgrass [55, 56]. Smut, caused by Tilletia maclaganii, has been reported in several switchgrass fields in Iowa, USA, already causing up to 50% reduction in biomass yield with high infection rates [57].

The global emphasis on development of herbaceous energy crops has led to increased emphasis on biomass yield as the principal breeding objective for most switchgrass breeding programs. Economic studies have clearly indicated that biomass yield of on-farm production systems is the most important factor limiting economic viability of switchgrass as a bioenergy crop [58]. Significant improvements, directly associated with selection and breeding, have been demonstrated in a diverse array of environments and breeding populations [41, 42, 59–61]. Typical rates of gain have averaged about 1-2% year<sup>-1</sup> and some have been sustained for multiple cycles of selection. Realistically, these results suggest that switchgrass breeders have likely increased biomass yields by an average of 20–30% since the inception of the U.S. DOE BFDP in 1992. Many of these gains are yet to be realized or quantified in agronomic or field-scale demonstration trials due to the 8–10 year lag required to complete evaluations, cultivar releases, and seed multiplications.

While the use of locally adapted and representative germplasm is a cornerstone of restoration and conservation applications for switchgrass, breeders are bounded only by what germplasm will survive or can be modified to survive in their target environments. Switchgrass breeders are beginning to take advantage of southern germplasm, collecting and evaluating germplasm from as far south as possible in an effort to extend the effective growing season. Biomass yield of switchgrass peaks near anthesis [43], often leaving 6–8 weeks of unutilized growing season in northern climates. Lowland ecotypes, which can be 4–6 weeks later than upland ecotypes in flowering time, are currently being selected and bred for improved

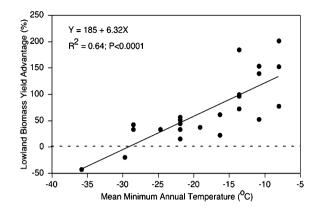


Fig. 2.4 Relationship between lowland-ecotype biomass-yield advantage and mean minimum annual temperature (hardiness zone definitions—[93]) for 23 cultivar-evaluation trials conducted under varying climatic conditions in the USA. Each point is represented by a mean of at least two upland and two lowland cultivars and the difference is expressed as a percentage of the upland mean. Data were collected from [3] (Arlington and Spooner, WI; Mead, NE; Manhattan, KS; Stillwater, OK), [95] (Hope, AR; College Station, Dallas, and Stephenville, TX), [96] (Princeton, KY; Raleigh, NC; Jackson and Knoxville, TN; Blacksburg and Orange, VA; Morgantown, WV), [97] (Chariton, IA); and [4] (Beeville, College Station, Dallas, Stephenville, and Temple, TX)

cold tolerance and biomass yield at northern latitudes. A meta-analysis of 23 field trials conducted in a wide range of climatic conditions shows a strong relationship between lowland-ecotype biomass yield advantage and mean minimum temperature (Fig. 2.4). Lowland cultivars are generally at least twice as productive as upland cultivars at the most southern locations, but this advantage gradually declines with increasing latitude. Lowland cultivars can be expected to have biomass yields 30–50% higher than upland cultivars in the transition zone where both are well adapted. At the most extreme northern locations, biomass yield of lowland cultivars is limited by their inability to survive multiple winters [3].

#### 2.5.2 Breeding Methods

By far the most common breeding method used on switchgrass is some form of phenotypic recurrent selection, mostly using one or more restrictions as proposed by Burton [62] and described in detail by Vogel and Pedersen [63] and Burson [64]. Breeding begins with the assembly of germplasm to be evaluated for inclusion in adapted populations. Nearly all switchgrass breeders conduct initial switchgrass germplasm screens as spaced-plant nurseries at a single location. Because most breeding programs are focused on regional adaptation (Fig. 2.3), a single representative location is typically sufficient to make gains. Spaced plantings are used to conduct efficient evaluations of individual genotypes over 2 or

3 years, allowing each genotype to express itself without competition. Many breeders use some form of mental ideotype in these nurseries, selecting for a range of traits that provide the morphological form desired for the region and the production system. For example, several studies of both upland and lowland ecotypes [65–67] have suggested that tiller density may be the most efficient indirect spaced-plant selection criterion for improving biomass yield.

Although spaced-plant nurseries are generally ineffective toward improving biomass yield of  $C_3$  forage crops [45], they have been highly useful for improving biomass yield of switchgrass. All of the gains cited in the previous section were based on selection in spaced-plant nurseries. Controlling spatial variation within spaced-planted nurseries is critical to ensure a moderate to high heritability for biomass yield. Missaoui et al. [60] accomplished this using a moving-mean or Papadakis-type analysis, similar to nearest-neighbor analysis. Casler [59] controlled spatial variation by conducting all selection within 10-plant blocks, forcing each block to be a mini-selection nursery. Rose et al. [61] evaluated both low- and high-yield environments for selection nurseries and observed greater genetic gain for biomass yield in the low-yield environments.

Spaced plantings are highly efficient for improving quality traits related to feeding value for ruminants or conversion to bioenergy. Traits such as IVDMD and lignin concentration have sufficiently high heritabilities for unreplicated spaced plants that gains can easily exceed 2% cycle<sup>-1</sup> [44, 68]. Quality traits also possess high genetic correlations between spaced-plant and sward-plot conditions, allowing gains made on spaced plantings to be quickly transferred to real-world pastures and hay fields where performance is measured in terms of livestock production [69, 70].

Duration of spaced plant nurseries can be a critical factor in the success of a breeding program. Reducing cycle time to speed up gains can result in insufficient time in the field to evaluate critical traits such as cold tolerance, heat tolerance, pest resistance, and tiller production. Three cycles of selection for high IVDMD were highly successful, but the third cycle resulted in catastrophic winter injury in the Cycle-4 nursery (<10% survival), largely attributed to selection based 3-year-old plants that had survived only two winters of cold stress [43]. Selection of survivors from this nursery, combined with an additional winter of observation prior to final selection decisions has been attributed to reversing this loss in plant fitness [43].

A typical spaced planting in a switchgrass nursery consists of 1,000–10,000 plants. Once selections are made from a spaced planting, plants are intercrossed to create seed to begin the next cycle of selection. Intercrossing can be conducted in situ leaving the unreplicated plants in place [59], by transplanting selections to an isolated crossing block [43], or by vegetatively propagating the genotypes and transplanting them into a replicated polycross block [63]. If the final data collection and selection decisions are made late in autumn, plants can be transplanted once they are dormant, or transplanting can take place very early in spring without compromising the ability of plants to intercross and produce seed. Some breeding programs intercross selections in the glasshouse, which can be effective following

selection and transplanting from the field at numerous times during the growing season. Switchgrass does not require vernalization to flower, so the most critical factor is to obtain clonal ramets that possess sufficient numbers of tillers or tiller buds to generate inflorescences. Photoperiod adjustment, including low-irradiance 24 h photoperiod, can be used to promote flowering in the glasshouse and to synchronize flowering among genotypes of widely different origins [71].

Family-based selection methods have become commonplace in switchgrass breeding programs. Half-sib families are the most common type of family, largely because they are simple and efficient to produce. Family-based selection methods strive to utilize as much genetic variability as possible by conducting selection among and within families. Both of the USDA-ARS breeding programs in Lincoln, NE and Madison, WI rely heavily on half-sib family selection, using family rows of spaced plants. Both programs attempt to create a competitive environment for individual plants using two different methods. In Lincoln, highly rhizomatous plants are spaced 1.2 m apart, but their spread is constrained to  $0.5 \times 0.5 \text{ m}^2$  by frequent tillage [72]. Plants are harvested and biomass yield is expressed on a unitarea basis. In Madison, family rows are created with a plant spacing of 0.3 m within rows and 0.9 m between rows, allowing plants to begin competing with each other in the second year [59]. If half-sib family seeds are produced in sufficient quantity in the field, half-sib family breeding methods can utilize drill-seeded plots to more accurately simulate a realistic agricultural production system [59].

Cycle time for switchgrass breeding programs ranges from 2–7 years, depending on the objectives and specific methods. Theoretically, each cycle could spin off a new and improved population that could move into candidate-cultivar status [63]. Most breeding programs establish new field trials of candidate cultivars every 2–4 years, depending on timing and resources. These field trials are more extensively replicated than selection nurseries, utilizing multiple locations throughout the target region of environments, replicated and randomized experimental designs, drill-pots that can provide accurate biomass yield assessments, and 2–4 years of agronomic-trait measurements. Because many breeders do not have access to a wide array of test sites, some switchgrass breeders collaborate by pooling resources and sharing test sites for candidate-cultivar field trials, particularly when some of the candidate cultivars may have expected adaptation to broader regions than the breeder's range of test sites.

Hybrid breeding is expected to be a significant activity in the future. The evolutionary divergence between upland and lowland ecotypes has been sufficient to create significant allelic differentiation for a wide range of DNA markers and sequences throughout the genome (previously discussed). This allelic diversity has created a certain level of complementarity between uplands and lowlands, such that F1 hybrids have significantly superior performance to their parents [72, 73]. This allelic complementation, manifested as hybrid vigor or heterosis, does not occur in F1 hybrids within either the upland or lowland ecotype. The observation of 30–35% heterosis, superiority of the F1 hybrid to the best of the two parents, lends great optimism to this approach, particularly since the parents were selected more-or-less at random, without any selection for specific combining ability.

Commercial production of F1 switchgrass hybrids is still many years from reality, due to two massive logistical problems. Simply crossing upland and lowland ecotypes with each other is extremely difficult, due to the differences in flowering time of 4–6 weeks (Table 2.1). Flowering time can be relatively easily manipulated in glasshouse or growth chamber environments using temperature, light intensity, and day length [37]. However, commercial hybrids will require field-scale seed production between two clonal genotypes or two inbred lines. Growth regulators, such as the families of compounds that are used to reduce or delay flowering in grasses [74], might be a viable mechanism to promote fieldscale interpollination between upland and lowland parents. Field-scale propagation of the parents poses a second problem. Current approaches to F1-hybrid development are based on heterozygous genotypes as parents, requiring some form of efficient and high-throughput vegetative propagation methodology, e.g. somatic embryogenesis or micropropagation [75]. Both parents would be propagated in the laboratory and transplanted in alternate rows to a hybrid-seed production field using existing transplanting technologies (Fig. 2.5). Commercial development will require parents to be screened for specific combining ability (genetic capacity for hybrid vigor in the progeny, complementing the other parent) and competency for large-scale vegetative propagation.

Ideally, and in the long term, such a system should strive to replace heterozygous clonal parents with inbred lines. However, inbred lines are many years from realization in switchgrass, due to self-incompatibility and inbreeding depression. Self-incompatibility is genetically controlled, so it can be modified by selection and breeding for selfing competence. Inbreeding depression can also be partially overcome by long-term selection for vigor and seed production during the inbreeding process, a proven phenomenon in maize breeding, allowing the development of inbred lines capable of seed propagation. This is an extremely long-term goal in switchgrass that is theoretically possible, based on the maize model, but has no precedent in perennial plants.

#### 2.5.3 Impact of Genomics on Breeding

Bi-parental linkage-mapping populations have provided the most definitive evidence that tetraploid switchgrass is characterized primarily by disomic inheritance, acting largely as a diploid organism with 18 pairs of chromosomes [30, 76]. These populations consist of two highly heterozygous genotypes from contrasting populations, crossed under controlled conditions to create a pseudo testcross population. Each parent serves as a tester for markers and quantitative-trait loci (QTL) that are heterozygous in the other parent. Second-generation linkage populations, which would allow detection of markers and QTL that are homozygous for different alleles in the two parents, have yet to be reported in switchgrass. The 18 linkage groups of switchgrass consist of two highly homologous sets of nine chromosomes that are highly collinear with *Sorghum* and *Setaria* [30].

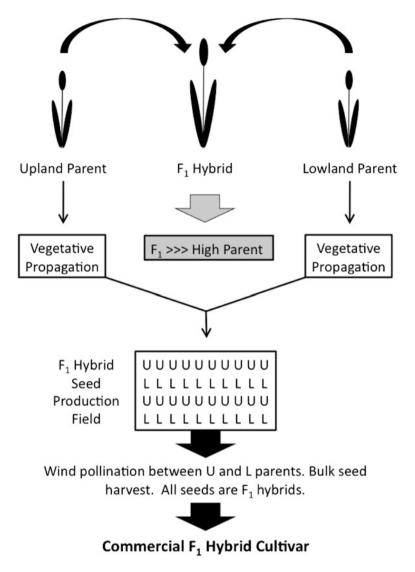


Fig. 2.5 Seed production scheme to develop  $F_1$  hybrids between vegetatively propagated genotypes of upland and lowland switchgrass ecotypes

Numerous DNA marker systems have been adapted for use in phylogenetic studies of switchgrass and development of DNA markers that can be used in switchgrass breeding [27]. Based on these marker systems, both wild switchgrass populations and bred cultivars contain levels of variability equivalent to 65–85% of that found across the range of the species. The remaining variability is associated with the two major taxa (upland and lowland), geographic differentiation

[8, 12], and fine-scale differentiation due to natural selection [6]. The consistent similarity of wild populations and bred cultivars indicates that the limited number of generations of selection in breeding current switchgrass cultivars has not created significant bottlenecks to impact the overall genetic diversity within the cultivars. Selection and breeding has likely impacted a relatively small number of genes scattered throughout the switchgrass genome, preserving genetic variability at the genomic level.

A reference genome sequence is not available for switchgrass, largely due to the complexity and expense involved in sequence assembly of such a complex polyploid. Current efforts by DOE Joint Genome Institute (JGI), Walnut Creek, CA are focused on deep sequencing the parents and 192 progeny from the AP13  $\times$  VS16 (Alamo  $\times$  Summer) bi-parental cross. An existing linkage map of this population will be used to localize genomic scaffolds to one of the 18 linkage groups, creating a reference map that can be used to order and localize future sequence data from other genomic resources of switchgrass, including bacterial artificial chromosomes (BAC, [77]), expressed sequence tags (EST, [78]), and exome sequences that are currently under development [27].

Marker-trait associations have yet to be specifically identified in switchgrass. The AP13  $\times$  VS16 bi-parental cross has undergone phenotypic evaluation for several morphological and agronomic traits in southern Oklahoma, but data have yet to be analyzed at the time of this writing. Two association panels of switch-grass were assembled and established in field studies. A northern panel, consisting largely of upland accessions, consists of 10 genotypes each of 60 populations and has been evaluated for single nucleotide polymorphic (SNP) markers and phenotypic traits at Ithaca, NY. A southern panel, consisting largely of lowland accessions, consists of 10 genotypes each of 48 populations and has been evaluated for phenotypic traits at Athens, GA and Ardmore, OK. Future plans will include genome-wide association studies (GWAS) of marker-trait associations within each panel and across the two panels [27].

Finally, genomic selection (GS) offers considerable potential to increase the rate of gain for important traits such as biomass yield [79]. Genomic selection offers two mechanisms to increase selection efficiency and rate of gain. Once a training population of genotypes and families is established, predictive equations are developed and validated to predict phenotype from genotype. This predictive equation can be applied to seedlings to apply indirect selection pressure to important agronomic traits prior to establishment of field-based nurseries, dramatically increasing the proportion of genetic variation utilized in selection [80]. Second, recurrent selection could be altered to eliminate the field-based evaluation in some cycles, relying on short-term maintenance of linkage disequilibrium in the population following only one or two recombination events. For example, Cycle 1 could consist of a field evaluation of biomass yield, equation development and validation, and within-family selection. Cycle 2 (and possibly Cycle 3) could proceed with simple phenotypic selection using the predicted breeding values based on seedling DNA marker analyses, prior to returning to the field for another cycle of field evaluation and recalibration of the predictive equations. Seedling selection offers huge time advantages because it can be accomplished within 1 year, as opposed to a minimum of 5 years for a typical field-based selection cycle. Validation and computer simulation studies will be critical in determining the number of seedling selection cycles, if any, that can be utilized to speed up the selection process.

Current efforts in Wisconsin USA are focused on development of three training populations: an upland population selected for biomass yield, a lowland population selected for winter survival, and a hybrid population. All populations are structured as half-sib families and the basic selection method will involve selection among families for field-based biomass yield in plots replicated across locations and years and selection of seedlings within families for breeding values predicted from DNA markers. If the accuracy of breeding-value prediction is >0.3, GS with 10% within-family selection intensity has >35% higher expected gains than any phenotype-based selection protocol [80]. If genotyping costs are sufficiently low to increase within-family selection intensity to 1%, expected gains for GS are 70% greater than phenotypic selection.

#### 2.5.4 Genetic Engineering and Risk Assessment

Switchgrass can be transformed with the addition of specific functional genes from other organisms using one of two methods. *Agrobacterium*-mediated transformation uses a biological vector whereas particle bombardment uses a non-biological vector to insert new genes into the switchgrass genome [81]. *Agrobacterium*-mediated transformation tends to result in lower copy number and fewer genomic rearrangements than particle bombardment [82–84]. While efficient, high-throughput genetic transformation systems have been developed for switchgrass, these systems are currently genotype-dependent. Certain genotypes are more responsive to both the tissue culture and transformation phases of this process (Fu and ZY Wang, 2011, personal communication), creating a need for more genotype-independent methodologies.

Genetic transformation of switchgrass to reduce recalcitrance of biomass for conversion to energy has received the greatest amount of attention. Manipulation of one gene is sufficient to create measurable and significant changes to cell-wall composition and structure, impacting sugar release and downstream processing of biomass to energy [85–87]. Reductions in lignin concentration or modifications to lignin structure can positively impact the availability of cell-wall carbohydrates in a fermentation system to produce liquid fuels and in a livestock-production system where switchgrass is a livestock feed [50]. Such changes not only increase energy that is available to microorganisms that conduct fermentation, but also create opportunities to significantly reduce input costs of production, allowing reduced pretreatment severity and enzyme requirements in the case of biofuel fermentation systems [85].

Genetically modified (GM) organisms have traditionally been highly regulated, requiring several years of extensive testing and risk assessment studies [88]. Scientific issues that have potential to impact the regulatory process include the effects of transgenes on plant fitness under a wide range of environmental conditions and their potential to be transmitted to non-transgenic switchgrass. In the case of switchgrass, transmission can occur via either pollen or seed. Pollen-flow studies have not been conducted on switchgrass, but pollen has been known to travel as far as 21 km in *Agrostis* [89]. Seed is literally unbounded in its ability to travel across the landscape, facilitated by birds, mammals, and humans [8]. Risk assessment is further complicated by the need for most assessments to be designed specifically for the gene in question (related to its function in the plant and both proposed and unintended potential functions in the environment), the biology of the species, the likelihood and frequency with which natural stands of compatible relatives exist within the agricultural range of the species, and the range of environmental conditions under which the species will be utilized [90].

Based on its broad natural range, the existence of thousands of native prairie and savanna remnants, and the range of management systems and environmental conditions under which switchgrass can be grown for biomass or forage, deregulation of any switchgrass transgene is guaranteed to result in its dissemination and introduction into natural populations. Switchgrass is a wild plant that still contains many traits common to wild species, including seed dormancy, seed dispersal by shattering, variability in flowering time within individual panicles and plants, and small seeds that can easily escape containment, each of which can contribute to switchgrass seed dispersal and viability over space and time. The scientific community is highly dichotomous over the potential implications of genetic improvement of switchgrass, the agricultural community generally in favor of speeding up domestication and incorporating useful traits (e.g. [85]) and the ecological community more cautious and concerned about limiting impacts outside of agricultural fields (e.g. [91]). In the USA, genetically modified switchgrass is regulated by the Animal and Plant Health Inspection Service (APHIS) of the USDA. APHIS is currently experiencing pressure from the U.S. DOE which has invested millions of dollars into development of new GM concepts and technologies, aimed at improving production and processing of switchgrass biomass for energy production.

#### 2.6 Conclusions

Genetic resources of switchgrass are vast and untapped. Modern cultivars represent no more than five or six cycles of selection removed from wild germplasm, insufficient to create significant genetic bottlenecks. Rates of gain have historically been modest, largely focused on one or two principal traits and likely based on relatively low numbers of genes. As such, switchgrass is still an undomesticated plant with vast potential for improvement of agronomic and biofuel traits. Huge stores of genetic variability exist for adaptive traits such as pest resistances, stress tolerances, biomass yield and quality traits, and phenological traits, providing a basis for utilizing genetic resources from a broad geographic area to generate highly targeted improvements within regions suitable for biomass production. Genomic resources are rapidly being developed to create opportunities for increasing selection efficiency and rate of gain. Development of a formal switchgrass research community, already underway in 2010, is expected to improve communications among researchers of diverse interests and disciplines and provide mechanisms for researchers to keep up with rapidly developing and changing technologies.

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# Chapter 3 Crop Physiology

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Abstract In this chapter, we review the physiology of switchgrass from seed dormancy till the effects of water and nutrients stress on grown plants. These characteristics are presented and discussed mainly at the canopy and whole-plant level with emphasis on the agro-physiology of the species in view of the possible contribution of crop physiology to agricultural development. Switchgrass is noted for the variable degrees of seed dormancy regulated by endogenous and exogenous factors that determine the successful seedling establishment. Plant growth rates are determined by temperature while the reproductive phase is controlled mainly by photoperiod. There is also evidence that some physiological attributes, such as photosynthesis, transpiration, and water use efficiency differ between tetraploid, hexaploid, and octoploid ecotypes. But despite these differences, in general switchgrass combines important attributes of efficient use of nutrients and water with high yields thanks to its ability to acquire resources from extended soil volumes, especially at deep layers. Moreover at canopy level, resources capture and conservation are determined by morpho-physiological characteristics

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( $C_4$  photosynthetic pathway, stomatal control of transpiration, high leaf area index, low light extinction coefficient) that enhance radiation use efficiency and reduce carbon losses. However, specific information on switchgrass physiology is still missing, in particular deeper understanding of physiological principles controlling the water and nutrients acquisition mechanisms and allocation under suboptimal growing conditions. The physiology of tillering and root respiration are also factors that need further investigation.

#### 3.1 Introduction

Switchgrass (*Panicum virgatum* L.) is a perennial grass of temperate zones that has evolved into a forage crop and more recently into an energy crop. The species originates from the central plains of North America as a component of the tall grass prairie [1]. Basically switchgrass is a warm season, deep-rooted, photosensitive, C<sub>4</sub>-type metabolism species with a high adaptability to a wide geographical range and soil types. Considering habitat preferences and other plant characteristics, switchgrass is classified into upland and lowland ecotypes associated with latitudinal origin (northern and southern ecotypes) [2, 3]. In general switchgrass presents a high interspecific variability in physiological characteristics, with its own response adaptations to photoperiod, temperature, water logging or drought, and other stresses. These traits influence the carbon fixation efficiency and therefore crop production potential.

Surprisingly, a comprehensive review on switchgrass physiology as a crop species is lacking. In most of the cases, switchgrass physiology is covered along that of other forage grasses. Sanderson et al. [4], for example, provide a general overview of the morphological and physiological response to stress of forage grasses, but specific information on switchgrass is limited. Moreover, in a 10-year research program designed by the US Department of Energy to evaluate and develop switchgrass as an energy crop and that involved a large network of research sites, universities, laboratories, and US Department of Agriculture facilities, only two institutions were listed as interested in switchgrass physiology [5]. Hence, most of the information available on switchgrass physiology comes from previous studies that focused on its forage end use. Topics were wide ranging from seed dormancy physiology to the crop growth determinants. Across the world there is an increasing interest in switchgrass as a multipurpose crop species, but the establishment of a permanent switchgrass physiology program as an important part of agricultural research is needed.

In this chapter, we review the physiology of seed dormancy, seedling establishment, above- and belowground biomass development, resource use efficiency, and the effects of water and nutrient stress. These characteristics are presented and discussed mainly at the canopy and whole-plant level with emphasis on the agrophysiology of the species in view of the possible contribution of crop physiology to agricultural development. Organ and cellular levels are not presented here.

#### 3.2 Seed Germination and Seedling Establishment

## 3.2.1 Physiology of Seed Dormancy

Switchgrass seeds, even within the same lot of seeds, have variable degrees of dormancy, which is an optimum strategy to survive in the wild and trough periods of environmental stress but at the same time is a major obstacle for its wide-spread cultivation as a forage or biomass crop [6, 7]. At harvest, more than 90% of the seeds of some cultivars could be dormant [6, 8]. Although dormancy declines naturally with time, the mechanisms of dormancy in switchgrass are not well understood. Several authors indicate that a combination of physical and physiological factors may be involved and, to a lesser extent, morphological factors too [9-13]. The seed coat is in part responsible for switchgrass dormancy. These outer layer coverings act as barriers for water and oxygen uptake, produce and encapsulate germination inhibitors, modify the light reaching to the embryos, and act as physical barriers that inhibit germination [10].

Since the embryos of switchgrass are fully developed at harvest time, it is suggested that after-ripening is not a major factor inducing dormancy break in switchgrass [9, 13]. The aforementioned authors reached such a conclusion based on their observations that after injuring, cutting, or completely removing the embryos, endosperms, and/or the seed coat, unspecified germination inhibitors were released, thus the germination percent increased significantly. Hence, primary dormancy in switchgrass may not be related to underdeveloped embryos but to dormancy mechanisms within the embryo and the required quiescence period of the seeds [6, 9, 13]. Under natural conditions this period could last months if not years [7, 13]. In order to accelerate the decay of dormancy, several artificial methods can be used. Mechanical and chemical scarification of switchgrass seeds, for example, resulted in 73% and 61% increased germination, respectively [10, 14]. Such a process may weaken the fiber tissue in the lemma, allowing more gas exchange and water uptake, and eliminate or weaken the physical barrier posed by the lemma and palea that impede the embryo expansion [10].

Impermeable membranes to oxygen but permeable to water in switchgrass seeds seem to be also responsible for dormancy. Under suboptimal temperatures, these membranes prevent the respiration of the stored energy within the seed and therefore delay or inhibit germination. However, by exposing seeds to cool temperatures and moisture (stratification), oxygen can be absorbed by the seeds and therefore dormancy reduced or broken. For example, Sanderson et al. [8] indicated that naturally (e.g., cool and wet conditions prevail in early springtime) and artificially stratified seeds germinated well and provided good stands and yields.

Moreover, Shen et al. [6] showed evidence that germination of Cave-in-Rock switchgrass seeds could be increased up to 80% within a 14-day stratification period at 5°C. However, dormancy break by stratification is not straightforward and unidirectional process, and it depends on undetermined factors within the seed and the surrounding environment. Shen et al. [6] indicated that stratified

switchgrass seeds could become dormant again after the seeds are dried for mechanical planting. This tendency toward secondary dormancy was termed as reversibility. The authors suggested that this reversibility was linked to a physiological continuum that the seeds enter when the right environmental conditions occur for germination. In case such conditions do not occur or stop, the seed goes dormant again, which is called by some authors residual dormancy [12]. However, reversibility depends on the degree on which dormancy was removed. Shen et al. [6] suggested that a 42-day period of stratification would be enough to completely remove dormancy, but the length of such a period will depend on how well the seeds were after-ripened or aged, as stratification and after-ripening have additive effects. Moreover, residual dormancy is responsive to the modification of endogenous levels of nitric oxide (NO) and/or reactive oxygen species (ROS) [12]. In general, environmental stresses (e.g., drought, temperature, light, etc.) can cause mutations in the genes responsible for germination. Such mutations can be associated with the degree of seed maturation and with the biosynthesis of dormancyregulator hormones [15, 16].

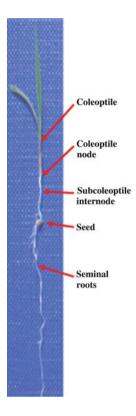
Toward seed maturity, the concentration of germination inhibitors, such as abscisic acids (ABA) in switchgrass, as in other dormant grass species, remains high in comparison to growth promoters, such as gibberellic acid (GA); therefore, dormancy prevails [17, 18]. The balance between ABA and GA seems to be affected by endogenous and micro-environmental factors; however, the mechanisms of such shifts are not fully understood [12]. These authors, however, indicated that NO and ROS are important reactive pathways to perceive ABA. Then these receptors and cognate proteins drive signaling cascades resulting in biological outputs [17, 18]. For example, exogenously applied ROS, as  $H_2O_2$ , inhibited the effects of ABA probably by overriding the ABA-dependent signals, resulting in enhanced switchgrass germination [17, 18]. Even though the exogenously applied NO was not able to overcome the ABA-dependent signals, probably because of a NO scavenger, Sarath et al. [11, 17, 18] indicated that high levels of endogenous NO are required for germination. Exogenously applied H<sub>2</sub>O<sub>2</sub> may stimulate the production of endogenous NO in the aleurone layer, the main site for NO synthesis in switchgrass [17, 18], and therefore overcome the ABA inhibition of germination.

#### 3.2.2 Seedling Establishment

Seedling establishment comprises the germination and emergence phase, and the adventitious root development phase [19]. A seed is considered germinated and emerged when the radicle protrudes from the seed coat and when the coleoptiles become visible; radicle extension precedes the coleoptile emergence [11, 12]. A rapid initial development of roots enables seedlings to acquire the necessary water and nutrients for growth. When the coleoptile emerges from the soil surface, subcoleoptile internode elongation stops, the coleoptile opens, and shoot growth

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Fig. 3.1 Switchgrass seedling showing the placement of the crown, subcoleoptile elongation zone, and seminal roots. Photo by Andrea Monti



and chlorophyll synthesis begins ([20]; Fig. 3.1). The speed and rate of germination and emergence are affected by environmental factors such as water, temperature, and light. In general, it is indicated that the base temperature for germination is between 8.1 and 10.3°C, and optimum is between 25 and 30°C, with maximum germination occurring after 72 h of imbibition [21, 22]. Maximum temperature for germination may be as high as 45°C [22], but all of these conditions seem to be cultivar dependent. Germination rate is affected by the degree of dormancy, imbibition rate, and respiration, which are temperature-dependent factors, while maximum seed germination and emergence is mainly affected by the degree of water uptake [22, 23].

Since the seeds of switchgrass are very small (Table 3.1), the amount of water required for germination is also very small, especially at the hydration phase. The water requirements will increase as the seedling develops and its juvenile root system (composed of the primary root, seminal roots, and subcoleoptile internode roots) starts growing and functioning [24]. Radicle protrusion coincides with radicle emergence, which is characterized by short duration. Some authors consider it to be a negligible part of a switchgrass seedling [25]. However, it plays a fundamental role in the early establishment phase of the young seedling, a role that has not yet been clearly defined [24]. Proper soil moisture is essential at this stage

Cultivar	Ecotype	Ploidy level	Origin of germplasm (latitude)	Maturity	Seed weight (mg 100 seeds <sup>-1</sup> )	References
Alamo	Lowland	Tetraploid	Texas (28°)	Very late	94	[35, 36, 119]
Blackwell	Upland	Octoploid	Oklahoma (37°)	Mid/late	142	[35, 36, 119]
Caddo	Upland	Octoploid	Southern Great Plains (35°)	Late	159	[35, 36, 119]
Carthage	Upland	Octoploid	North Carolina (35°)	Late	148	[100, 119]
Cave-in- Rock	Upland	Octoploid	Illinois (38°)	Mid/late	166	[35, 36, 119]
Dacotah	Upland	Tetraploid	North Dakota (46°)	Very early	148	[35, 119]
Expresso	Lowland	Tetraploid	Missisippi	?	?	[22]
Forestburg	Upland	Octoploid	South Dakota (44°)	Early	146	[35, 119]
Kanlow	Lowland	Tetraploid	Oklahoma (35°)	Very late	85	[35, 36, 119]
Nebraska 28	Upland	Octoploid	Nebraska (28°)	Early/mid	162	[36, 119]
Pangburn	Lowland	Tetraploid	Arkansas (34°)	?	96	[36]
Pathfinder	Upland	Octoploid	Kansas (40°)	Mid/late	187	[35, 36, 119]
Shelter	Upland	Octoploid	Virginia (40°)	Mid	179	[35, 36, 119]
Stuart	Lowland	Tetraploid	Florida (29°)	Late	?	[36]
Summer	Upland	Tetraploid	Nebraska (41°)	Late/mid	114	[35, 36, 119]
Sunburst	Upland	Octoploid	Dakota (44°)	Mid	198	[35, 36]
Trailblazer	Upland	Octoploid	Nebraska (40°)	Mid	185	[35, 36]
Tusca	Lowland	Tetraploid	Mississippi	?	?	[22]
Wabasso	Intermediate	Tetraploid	Florida (27°)	Very late	?	[36]
NL 93-1 <sup>a</sup>	Lowland	Tetraploid	?	?	121	[42]
NU 94-2 <sup>a</sup>	Upland	Octoploid	?	?	173	[42]
SL 93-2 <sup>a</sup>	Lowland	Tetraploid	?		87	[42]
SL 93-3 <sup>a</sup>	Lowland	Tetraploid	?	?	140	[42]
SL 94-1 <sup>a</sup>	Lowland	Tetraploid	?	?	142	[42]
SU 94-1 <sup>a</sup>	Upland	Octoploid	Oklahoma	?	183	[42]

Table 3.1 Ploidy levels, origin and average seed weight of the principal cultivars of switchgrass

<sup>a</sup> Cross from different genotypes of diverse origin.

Source Alderson and Sharp [119]; Gunter et al. [35]; Hopkins et al. [36]; Stout et al. [100]; Seepaul et al. [22]; Taliaferro and Hopkins [42]

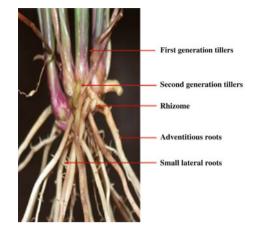
as the establishment of a viable seedling is not yet ensured. However, information on switchgrass water requirements for germination and seedling establishment is scarce. The few data available indicate that under drought stress (-0.3 MPa) only 2.5% of all sowed seeds emerged, representing 5% survival of the germinable seeds [26]. Hence, these results suggest that the lower limit of moisture availability for switchgrass germination might be around -0.3 MPa, regardless of the sowing depth and soil texture.

Adequate soil surface moisture is also essential for the formation of adventitious roots. The long-term survival of the seedling will be determined by the development of robust adventitious roots as they will become the major root system of the seedlings ([1, 19]; Fig. 3.2). In any case, Smart and Moser [27] suggested that few long adventitious roots that reach moist subsurface soil layers are enough for the successful early establishment of seedlings. Moreover, Newman and Moser [19] showed that in switchgrass the number of adventitious roots increased rapidly between 4 and 8 weeks after planting following 4 or more days of consecutive rain. However, during the first 4 weeks after planting. there were few adventitious roots even under adequate soil surface moisture conditions. In fact, switchgrass starts to develop adventitious roots by the thirdleaf stage [24]. After this period, water flow may be preferential to the growing shoot, as it is suggested to happen in blue grama [28]. Xu et al. [29] found that switchgrass seedlings (fifth- to sixth-leaf stage) exposed to continuous soil dehydration increase by 11% their allocation of carbohydrates to the roots. Such a change in carbon partitioning may be a useful strategy for the seedling survival.

Adventitious roots develop in clusters from the coleoptilar node or seedling crown ([19]; Fig. 3.2). Since the crown node is pushed to the soil surface by the elongating subcoleoptile internode until a certain light level is sensed, the location of the seedling crown and that of the adventitious roots will be close to the soil surface regardless the sowing depth [19, 20]. At greater soil depths, moisture and temperature conditions are more favorable for the successful development and functioning of the adventitious roots, which in turn will secure the seedling establishment and survival. On the other hand, if the seedling's crown is at or close to the soil surface, its exposition to faster soil desiccation may be abortive or limiting for the development of adventitious roots, especially in drier environments [1, 19]. In general, switchgrass has excessive crown node elevation, which makes its successful establishment difficult. However, Elbersen et al. [20] demonstrated that populations with low crown placement can be selected and that this trait is heritable. Such genotypes have shorter subcoleoptile internodes which facilitate water flow toward the coleoptile and transpiring leaves and therefore accelerate emergence and establishment. Thus, these genotypes are better able to withstand drought conditions before adventitious roots are developed [30]. In fact, in field trials with alternating wet and dry periods, it was shown that the selected genotype for low crown placement had greater seedling germination and emergence rates [30].

Selection for seed size can also improve switchgrass seedling establishment. Several authors indicated that larger seeds accelerated the germination, emergence, growth rates, and development of adventitious roots, but all these advantages associated with seed size were no longer evident at later growth stages, even when soil moisture was suboptimal [1, 27, 31, 32].

Fig. 3.2 The origin of firstand second-generation tillers, and formation point of adventitious roots and rhizomes. Photo by Andrea Monti



## 3.3 Canopy and Root Development

## 3.3.1 Phenological Stages

Based on habitat, morphological characteristics, and ploidy level, switchgrass has been classified into two ecotypes: lowland (mainly grown in lower and wetter environments) and upland (mainly grown in mesic environments). Each ecotype is further subdivided according to its geographical origin into southern and northern ecotypes ([33, 34]; Table 3.1). Casler et al. [2] indicated that latitude is the largest determinant of switchgrass productivity and survival. Lowland ecotypes are usually tetraploid (2n = 4x = 36 chromosomes), while the upland types range from tetraploid to hexaploid (2n = 6x = 54 chromosomes) to octoploid (2n = 8x = 72)chromosomes) [35-38]. This large genetic diversity result in morphologically and physiologically different plants. In general, the lowland ecotypes are taller, with thicker stems, longer bluish-green leaves, have larger panicles, and produce higher yields but have lower initial growth rates than upland ecotypes [1, 39]. When lowland/southern ecotypes are moved to northern latitudes, they produce higher biomass yields because they remain vegetative for a longer period of time with a longer photosynthetically active period, but they may not reach maturity and form seeds. In fact, in order to survive the winter, switchgrass should acquire adequate dormancy and translocate storage carbohydrates to the roots, but due to the extended photoperiod, southern ecotypes may start this process too late when moved to northern latitudes, and their survival may be compromised. Moreover, the harvested biomass from immature plants can have high moisture and ash contents due to the partial translocation of nutrients to the rhizomes, which is unfavorable for energy purposes. On the other hand, upland/northern cultivars moved southward have lower biomass yields and are more susceptible to diseases [2, 33, 40, 41] because they are exposed to shorter-than-normal days during the

summer, which leads to early flowering. So far, hybridization attempts between these two ecotypes have been unsuccessful [42], and therefore at the moment, the functional advantages of one ecotype cannot be introduced into the other. In order to study the growth and development of switchgrass as function of environmental and management variables, descriptive indices of its phenological stages have been developed (Table 3.2). The general method used for the study of perennial grasses proposed by Moore et al. [43] and specifically adapted by Sanderson [34] for switchgrass indicates five main phenological stages: (a) emergence, (b) vegetative/leaf development (c) stem elongation, (d) reproductive/floral development, and (e) seed development and ripening. Each stage is sub-divided into sub-stages, which are identified by numerical codes that go from 0.5 to 35. These codes provide a descriptive attribute of the physiological and ecological status of the plant and enable researchers to quantitatively assess the successive growth stages and statistically determine deviations from the normal growth. Under natural prairie conditions, seed shattering and complete dormancy could also be considered as additional growth stages during the life cycle of switchgrass [40]. In switchgrass as in other perennial grasses, the vegetative growth stages are discrete, so leaf growth continues even when the stem elongation stage has started [44]. Moreover, the floral development starts when there are still some leaf primordia and unemerged leaves on the apex [45].

#### 3.3.2 Growth Analysis

The physiological development of switchgrass is typical of that of perennial grasses and follows the general growth sequence indicated above (Table 3.2). Al-though the duration of each stage is cultivar dependent, the vegetative growth of switchgrass (including leaf development and internode elongation) is strongly influenced by temperature [46]. Sanderson and Wolf [44], for example, indicated that when Cave-in-Rock (northern/upland) and Alamo (southern/lowland) cultivars that are grown close to their place of origin require 200 and 430 growing degree-days (GDD) above a base temperature of 10°C to complete the leaf elongation stage and 378 and 1,020 GDD for the stem elongation phase. On the other hand, the reproductive phase of switchgrass is controlled mainly by the day length (photoperiod), regardless of temperature and moisture availability [41], suggesting a facultative short-day response, though the influence of other factors cannot be excluded completely. The effect of photoperiod extension was studied by Van Esbroeck et al. [41] on two switchgrass cultivars; when Cave-in-Rock was subjected to long days (16 hr), panicle emergence was delayed by 18 days and the time for panicle exsertion was increased by 243% as compared to the control treatment (12-hr photoperiod). Such delay was associated with an increase in the phyllochron (the time between the appearance of two successive leaves) and in leaf size, while the number of leaves was not altered. In the case of the Alamo cultivar, the number of leaves and their size decreased. In both cases, however, the

Growth stage <sup>1</sup> Index G0 0.0				
G0 0.0	x	Description	Index	Description
		Dry seed		
G1 0.1		Imbibition		
G2 0.3		Radicle emergence		
G3 0.5		Coleoptile emergence		
G4 0.7		Mesocotyl and/or coleoptile elongation		
G5 0.9		Coleoptile emergence from soil	0.5	Emergence
V0 1.0		Emergence of first leaf	1 - 10	Leaf development
V1 (1/N) +	) + 0.9	First leaf collared		
V2 (2/N) +	) + 0.9	Second leaf collared		
Vn (n/N) +	) + 0.9	Nth leaf collared		
E0 2.0		Onset of stem elongation	11–19	Stem elongation (n. internode $> 1$ cm Es 14 = 4 internode $> 1$ cm)
E1 (1/N) +	) + 1.9	First node palpable/visible		
E2 (2/N) +	) + 1.9	Second node palpable/visible		
En (n/N) +	) + 1.9	Nth node palpable/visible		
R0 3.0		Boot stage	20	Boot stage
R1 3.1		Inflorescence emergence/1st spikelet visible		
R2 3.3		Spikelets fully emerged/peduncle not emerged	21–29	Percent of inflorescence visible
R3 3.5		Inflorescence emerged/peduncle fully elongated	30	Spikelets visible with peduncle
R4 3.7		Anther emergence/anthesis	31	Beginning of anthesis
R5 3.9		Post-anthesis/fertilization	32	End of anthesis
S0 4.0		Caryopsis visible		
S1 4.1		Milk	33	Milk/dough stage
S2 4.3		Soft dough		
S3 4.5		Hard dough		
S4 4.7		Endosperm hard/physiological maturity	34	Physiological maturity
S5 4.9		Endosperm dry/seed ripe	35	Seed shattering

duration of the panicle exsertion was extended. Then, an increase in the duration of the panicle development could maximize seed production, but its effects on biomass accumulation remain unclear. On the other hand, early flowering results in fewer leaves, reduced photosynthetic capacity, and lower yields.

In switchgrass, the leaf appearance rate (LAR) is somehow also related to photoperiod. The LAR decreases when days are long and increases when days are short. The faster LAR is associated with a short period between floral initiation and floral emergence [41, 45], thus reducing the vegetative period and potential biomass yield. In early maturing cultivars from northern latitudes, the phyllochron was almost double than that of southern late cultivars, suggesting an important role of latitude in controlling maturity time [45]. In general the lamina extension rate (LER) range from 0.20 to 0.30 cm  $\text{GDD}^{-1}$ , with the longest leaves located near the middle of the canopy up to the seventh leaf. Even though leaf growth continues until the leaf collar has emerged [47], when panicle development begins (around 1,000–1,200 GDD) LERs decline and shorter leaves are formed on the top [45], probably due to the increased sink force of the emerging panicles. Among several switchgrass cultivars, the final number of leaves on spring-emerged tillers range from 9 to 11, while from summer-emerged tillers the range was from 6 to 8 [45]. The same authors indicated that leaf formation in spring tillers, and thus biomass accumulation, continues until environmental conditions induce floral development. Flowering in switchgrass is induced by decreases in day length following the summer solstice. However, the photoperiod requirements of the diverse cultivars change depending on the latitude of origin of each ecotype.

Beaty et al. [48] indicated that switchgrass tillers behave as true biennale tillers; that is, the first-year tillers remain as rhizomes buds. Then in the coming spring, when temperatures are adequate, a flush of tillers emerges (Fig. 3.2). The physiological mechanisms responsible for tillering initiation in switchgrass have not been fully studied. Perhaps, as is suggested for other perennial grasses, the antagonistic actions of hormones such as auxin produced in the apical meristems, and cytokinin and strigolactones produced in the roots, together with resource availability (e.g., nutrients, water) and photosensitivity to red and far red light play an important and decisive role in the growth of axillary meristems [49], but specific information on such mechanisms is still lacking. Lower internodes begin to elongate after some leaves have been produced and continue until the inflorescence has emerged [48, 50], at which stage the carbohydrate reserves in the stem bases are the lowest [51]. Elongation rates can range from 1.4 to 2.8 cm  $d^{-1}$ depending on the cultivar and environmental conditions, with the more southernorigin ecotypes having greater growth rates [2, 46]. Upland ecotypes can reach 1.5-2 m in height, while lowland ecotypes are 3-4 m tall [52]. In general, tiller density during the vegetative growth stage is high but declines with advancement towards the following growth stages [53]. The final tiller density is, however, highly variable in number and physiological stage, depending on cultivar and environmental conditions. For example, 3-year-old stands of Cave-in-Rock and Dacotah cultivars, grown between 43° N (Arlington, WI) and 44° N (Brookings, SD) in the USA had tiller densities of 677 and 1,355 tillers  $m^{-2}$ , respectively.

while the reproductive tiller fraction, averaged across cultivars, was 81 and 8% at Arlington and Brookings [54].

The tillering capacity is an important component of switchgrass plasticity and its ability to respond to environmental stimuli in time and space. The general issues concerning the physiology of tillering in crop plants may apply to switchgrass, but specific information is not available. For example, in dense grass canopies, tiller elongation rate is stimulated and apical dominance is enhanced due to the low red–far red light ratio and low blue light perceived by the phytochromes [55]. However, information on this subject is still lacking in the case of switchgrass.

In addition to the genotype, photoperiod, and temperature, other factors such as plant density, irradiance, water, and nutrition may influence tiller initiation. Muir et al. [56] indicated that nitrogen fertilization and row spacing have a direct effect on the number of productive tillers, which play an important role in increased productivity. However, the same authors indicated increased tiller mass rather than an increased number of tillers is the main mechanism by which biomass yield is increased. Moreover, as in other grasses, new switchgrass tillers may obtain water from the mother plant until functional adventitious roots are developed; otherwise, the new tiller may die [57].

#### 3.3.3 Root Growth and Function

Initial root growth of switchgrass is very rapid, especially during the first 3 weeks after sowing, and then slows down gradually but remains the major carbohydrate sink at least for the first 15 weeks after sowing [58]. The root-to-shoot ratio of 3-week-old seedlings was 5.5, while at 15 weeks of age the ratio was reduced to 2.0 [58]. Such a large allocation of carbohydrates and rapid initial root growth rate are fundamental to the successful establishment of switchgrass. The C<sub>4</sub> physiology of switchgrass may allow a large and well-structured root system to develop that would ensure more active and efficient acquisition of soil resources and increase the nutrient storage capacity. Unfortunately there is no information on the root growth patterns and carbon partitioning of older plants, especially at what growth stage the tillers would become the major carbon sink. Actually most of the information available is for plants that have already reached maturity (4 or more years old), but in general mature plants follow a similar pattern to the one described above. The whole function of the plant is then determined by the canopy architecture and carbohydrate allocation [5]. For example, Garten et al. [59] indicated that the root-to-shoot ratio of a 4-year-old switchgrass stand averaged over four cultivars changed from 5.8 at the beginning of the growing season (April) to 0.76 at mid season (July) and to 0.77 at the end of the season (October). In contrast to the initial growth stages of switchgrass seedlings where most of the seed reserves are allocated to the development of a vigorous root system, in the case of mature plants a well-developed root system is already present where most of the nutrient reserves, mainly N and nonstructural carbohydrates, are accumulated and therefore able to sustain a rapid growth of the aboveground parts of the plant in the following season. In mature plants, starch is the primary and most dynamic nonstructural carbohydrate stored in the roots. Sucrose is secondary in importance [60].

The dynamic root growth throughout a growing season is heavily influenced by the annual harvest/clipping practices of the aboveground biomass, but typically the root system of switchgrass continues to grow even until advanced autumn, probably due to the continuous production of rhizomes. In fact, the highest root biomass production occurs at the end of the growing season (from midsummer to autumn; [59]), while aboveground biomass production is maximized from spring to midsummer. In central Iowa, the largest mass of fine (0–2 mm diameter) and probably small roots too (2–5 mm diameter) were found between August and October at 6- and 7-year-old plantations, while the lowest root mass was found in May [61]. Similarly, Xu et al. [62] found uninterrupted growth of root mass throughout the season (from April to November) and throughout the whole soil profile (from 0 to 1.50 m depth) in a 5-year-old switchgrass plantation in Northwest China. These observations suggest that an increased root system of switchgrass is not limited to shallower layers and that root growth terminates well beyond the flowering stage.

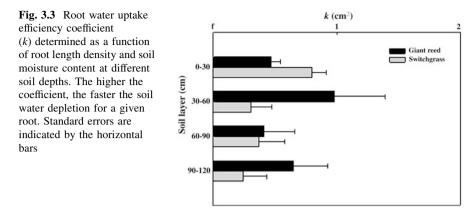
Roots of mature switchgrass plants can reach more than 3 m in depth [63], but the bulk of the roots is commonly found in the upper 1 m of the soil profile [61, 63, 64]. In a few of the studies available that measured the root length density (RLD) of switchgrass, Monti and Zatta [64] reported a RLD of 311 cm cm<sup>-3</sup> and that only 35% of the root mass was located in the top 0.35 m of the soil, while at lower layers, up to 1.2 m, roots were more uniformly distributed. In the study of Ma et al. [63], however, more than 68% of the roots were found in the top 0.15 m of the soil. Similarly, Bolinder et al. [65] indicated that at least 78% of the roots were located in the top 0.15 m of the soil at the peak standing crop growth stage. Garten and Wullschleger [66] indicated that up to 94% of coarse root mass (>2 mm diameter) was located in the upper 0.4 m of soil. These results differ from those of Monti and Zatta [64] possibly due to the different soil types and cultivars used in each study.

Soil respiration at a switchgrass plantation, as with any other species, can be related to live roots that directly contribute to soil respiration and dead roots and exudates that provide energy and nutrients for microbial respiration. However, there is limited information on switchgrass root respiration and turnover rates. Tufekcioglu et al. [67] and Frank et al. [68] reported similar soil respiration patterns throughout the growing season of several switchgrass cultivars; they concluded that soil respiration increased rapidly from winter to midsummer and then decreased rapidly toward the autumn. Such seasonal changes were highly related to temperature changes and, to a lower degree, to soil moisture changes. Moreover, Tufekcioglu et al. [67] indicated that the annual soil respiration rates were strongly related to the production fine roots (<2 mm) and soil organic carbon, suggesting that the large resources allocated to produce an extensive fine root system not only increase the resource capture capacity of the plant but also the soil

carbon inputs through root turnover. Frank et al. [68] indicated that about half of the carbohydrates captured in plant biomass during a growing season is lost through soil respiration. Garten and Wullschleger [69] estimated the range of turnover to be between 2.4 and 4.3 years for particulate organic matter and between 26 and 40 years for more recalcitrant mineral-associated organic matter.

Water uptake capacity and efficiency of switchgrass roots seem to be directly related with RLD but independent of root distribution along the soil profile ([64]: Fig. 3.3). Thus water may passively move into switchgrass roots in response to water potential gradients, rather than actively pumping solutes in order to create an osmotic gradient, in the cell-to-cell pathway as the wetting front moves downwards. A general feature of drought-resistant crops is a deep root system which facilitates access to deep-moist soil layers. Eggemeyer et al. [70], using the stable isotopes of hydrogen and oxygen, determined the water sources of switchgrass in the Sandhills grasslands of Nebraska. In agreement with the results shown in Fig. 3.3, Eggemeyer et al. [70] found that throughout the growing season switchgrass mainly extracts water from the upper 0.5 m of the soil profile. During the dry period (August), they found that water uptake increased at deeper layers but the amount acquired was insignificant in relation to the total amount of water used by the crop. The reason for the limited contribution was not discussed, but reduced hydraulic conductivity due to lignification of deep roots may be excluded since Garten et al. [59] indicated that lignin concentration in deep coarse and fine live switchgrass roots was lower than that in shallower layers. Hence, the amount of available water at deep layers might be the limiting factor.

In general, switchgrass has low nutrient amendment requirements compared to annual cereals, mainly because a pool of nutrients is recycled/conserved annually. The long-lived rhizomes (up to 10 years) may act as sites of nitrogen and carbon storage [71]. Several authors indicated that nutrients and nonstructural carbohydrates are translocated from the canopy to the crown/root system at the end of each growing season (after anthesis but before killing frost) and vice versa during resprouting [60, 72-74]. This is corroborated by Reynolds et al. [75], who found a higher nitrogen concentration in aboveground biomass of diverse switchgrass genotypes when harvested in mid season than when harvested during the fall. Moreover, in the fall harvest more nitrogen was present in the aboveground biomass in a double harvest system than in a single harvest system, probably because in the double harvest system the re-grown tillers were at a younger stage, and translocation of nutrients to roots was interrupted because they did not reach full senescence [75]. In a single harvest system, Garten et al. [59] indicated that nitrogen reserves in the root system declined to  $1.4 \text{ g N m}^{-2}$  due to acropetal translocation during the period of fast growth of the canopy. On the other hand, about 50% of the nitrogen fixed in the aboveground biomass was translocated to the roots by the time the plants had become dormant [59]. Lemus et al. [72] estimated that the total amount of nitrogen remobilized from roots to shoots and vice versa may range from 40 to 100 kg N ha<sup>-1</sup>. Griffin and Jung [76] reported that phosphorus levels in switchgrass and big bluestem decreased with maturity. They found that stem tissue phosphorus content declined from an average of



0.24–0.14% with increasing maturity. Smith and Greenfield [77] reported the highest phosphorus accumulation in the inflorescence. Radiotis et al. [78] reported 0.12% phosphorus in switchgrass tissues at the reproductive stage, and it declined to 0.04% when it was left to overwinter. Seasonal recycling of phosphorus, potassium, and other nutrients follow more or less a similar pattern to nitrogen [5, 73], but instead of translocation, the main recycling mechanisms may be related to leaching from senesced/dead leaves within the switchgrass extensive rooting zone. In either case, nutrients are returned to the soil during the winter, helping to maintaining soil fertility and reducing fertilizer requirements.

## 3.3.4 Crop Modeling

Field trials for the herbaceous energy crop switchgrass are beginning to provide valuable insights into the climatic, genetic, soil, and management practices that govern the production of biomass for this species [79–81]. Bioenergy crop models are a useful tool for summarizing information gained through field studies and for understanding the potential supply, resource utilization, and environmental impacts associated with the large-scale expansion of bioenergy crops [82].

Bioenergy models for many first- and second-generation energy crops, including switchgrass, can be broadly classified into empirical and mechanistic models. Empirical models are developed using statistical methods that establish relationships between biomass yield and biophysical and agronomic variables. Wullschleger et al. [83] developed an empirical biomass yield model for switch-grass using 39 field trials conducted across the United States. A nonlinear parametric model was used to determine the relationship between biomass yields, with physical, climatic, and management variables such as precipitation, temperature, nitrogen fertilization, and ecotype (lowland and upland cultivars). Results showed that lowland cultivars produced 1.5 times more biomass than did upland cultivars.

Temperature, precipitation, and nitrogen application during the season showed a significant and positive effect on biomass yield. Jager et al. [80] further explored the determinants of biomass yield in switchgrass cultivars by developing an ecotype-specific empirical model based on a slightly expanded dataset from that used by Wullschleger et al. [83]. The results showed that the responses to several biophysical and management variables were different for lowland and upland ecotypes. The impact of growing season temperature and precipitation on the production of biomass was greater for lowland than for upland ecotypes. The minimum winter temperature showed a positive response on biomass yield of both ecotypes but was highly significant only for biomass yield of upland ecotypes. Applied nitrogen also showed a positive response to soil moisture was found for upland but not for lowland ecotypes.

In contrast to empirical models, mechanistic models provide details of underlying physiological and morphological processes and their interactions on crop vield. Two models in particular are widely used in switchgrass, ALMANAC and EPIC. ALMANAC was developed to understand crop growth and yield across varied environments by accounting for competition from other crops/weeds and abiotic stresses [84], while EPIC was developed to account for the environmental impact of production practices along with biomass yield estimation [85, 86]. EPIC, which simulates switchgrass growth and development, is a process-based model capable of simulating a wide array of ecosystem processes including plant growth, crop yield, water and nutrient balances and soil erosion. ALMANAC is related to EPIC in many ways; ALMANAC uses biophysical subroutines and process descriptions from EPIC with additional details for plant growth processes and is capable of simulating several crops including switchgrass [87]. Inputs for both models are consistent with field measurements gathered for field crops including leaf area index, radiation use efficiency, carbon gain and allocation, and phenological stages of development. Thus, crop models for switchgrass are relatively easy to parameterize and interpret. Biomass produced under a range of scenarios can be derived using EPIC or ALMANAC at individual sites [88, 89] or larger spatial scales more suitable for regional analyses [82, 90].

Although mechanistic models require more extensive parameterization, they are widely used to forecast yields at spatial scales from local to regional level and, in some instances, to relate the production of biomass to other environmental consequences including soil erosion and water quality. This information is essential for developing a technically feasible, environmentally sound, and economically viable supply of bioenergy. Models that allow users to obtain reliable estimates of bioenergy crop production provide an essential framework to understand site-specific information that in turn can facilitate pragmatic decisions regarding the suitability of certain regions where bioenergy crops can be sustainably produced and any environmental benefits or consequences associated with that production.

#### **3.4 Resource Use Efficiency**

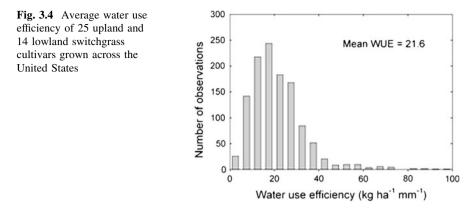
#### 3.4.1 Radiation Use Efficiency

Radiation use efficiency (RUE) can be defined as the biomass production per unit light interception. Monteith [91] was the first to provide a strong and convincing theoretical foundation for this parameter by demonstrating experimentally a robust relationship between light interception and stress-free biomass production for several agricultural crops. Since then, RUE has been considered a crop-specific parameter and a widely used efficiency measure for comparing plant productivity across different crops and management practices.

Field experiment-based RUE measurements for switchgrass are very limited, and any available data covers only North America. In general, RUE is higher for switchgrass as compared to other traditional cultivated crops. A mean RUE value of 4.7 g  $MJ^{-1}$  intercepted photosynthetically active radiation (IPAR) was reported for Alamo switchgrass and 3.7 g  $MJ^{-1}$  IPAR was reported for maize in Texas [92]. This is not surprising because switchgrass possesses ideal qualities that support high RUE, such as C<sub>4</sub> photosynthesis, a high leaf area index, and a low light extinction coefficient [93]. In general octoploid switchgrass cultivars have higher leaf gas exchange rates than tetraploid ones (Table 3.3), and this is attributed to the greater activity of RuBP carboxylase, PEP carboxylase, and NADmalic enzymes and concentration of biochemical constituents and smaller cell size [94]. However, Wullschleger et al. [38] indicated that, more than ploidy level, photosynthetic rates in either type are determined by seasonal changes and/or water stress.

RUE measurements vary widely across switchgrass cultivars, growing locations, growing seasons, and management practices. Mean RUE value for Alamo switchgrass ranged from 3.04 g MJ<sup>-1</sup> IPAR for the high plains of Texas to 5.05 g  $MJ^{-1}$  for Missouri [93]. The same switchgrass cultivar had different RUE values for two different growth periods: 3.2 g MJ<sup>-1</sup> for 1995–1997 and 4.4 g  $MJ^{-1}$  IPAR for 2008–2010 [93, 95]. Madakadze et al. [96] reported that RUE values vary across different upland switchgrass cultivars in Canada: 1.98 g  $MJ^{-1}$  IPAR was reported for the cultivar Sunburst to 2.38 g  $MJ^{-1}$  IPAR for the cultivar Cave-in-Rock. Heaton et al. [97] showed that under North America conditions (IL), RUE for Cave-in-Rock can be as low as 1.2 g MJ<sup>-1</sup> IPAR. Kiniry et al. [93] conducted a comparative study of different cultivars for RUE measurements in Missouri. Results of this study revealed that mean RUE for the cultivar Cave-in-Rock was 3.17 g MJ<sup>-1</sup> IPAR, which is below the mean for the lowland cultivar Alamo (4.3 g  $MJ^{-1}$  IPAR) but noticeably higher than the earlier report for Cave-in-Rock from IL by Heaton et al. [79]. Management practices also play a major role for different RUE values for the same switchgrass cultivar. Under irrigated conditions, Alamo exhibited higher RUE as compared to the water deficit condition, and the relative reduction in RUE under a water deficit environment was greatest for fields with higher plant density than lower plant density [93, 95].

Table 3.3 Some	physiological character	istics of switchgrass det	ermined across the United	Table 3.3 Some physiological characteristics of switchgrass determined across the United States <sup>a</sup> and on the Loess Plateau of China <sup>b</sup>	China <sup>b</sup>
Cultivar	Photosynthesis (µmol Transpiration (mmol Stomatal conductance $m^{-2} s^{-1}$ ) $m^{-2} s^{-1}$ ) (mol $m^{-2} s^{-1}$ )	Transpiration (mmol $m^{-2} s^{-1}$ )	Stomatal conductance $(mol m^{-2} s^{-1})$	Instantaneous water use efficiency Dark respiration ( $\mu$ mol ( $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> ) m <sup>-2</sup> s <sup>-1</sup> )	Dark respiration ( $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> )
Range of 25 cultivars <sup>[a]</sup>	17.5–30.8	6.2–13.0	0.16-0.30	2.08–3.77	1.76-2.24
Alamo <sup>[a]</sup>	27.90	8.2	0.23	3.60	1.98
Cave-in-rock <sup>[b]</sup>	10.60	3.1	1	4.01	I
Blackwell <sup>[b]</sup>	10.79	3.0	1	4.43	I
Dakota <sup>[b]</sup>	8.48	2.2	1	3.91	I
Forestberg <sup>[b]</sup>	9.93	2.5	1	4.38	I
Nebraska 28 <sup>[b]</sup>	10.35	1.8	1	5.74	I
Pathfinder <sup>[b]</sup>	9.71	2.2	I	4.53	I
Sunburst <sup>[b]</sup>	8.88	2.4	Ι	3.87	I
<i>Source</i> <sup>a</sup> Wullsch <sup>b</sup> Ma et al. [106]	<i>Source</i> <sup>a</sup> Wullschleger et al. [38, 134] <sup>b</sup> Ma et al. [106]				



Modeling based on RUE is considered to be the most preferred approach for crop growth modeling because of the sheer simplicity in factors needed and its straightforward implementation [98]. Two commonly applied crop simulation models for switchgrass such as EPIC and ALMANAC are based on a RUE approach for biomass simulation. In ALMANAC, a RUE value of 4.7 g MJ<sup>-1</sup> IPAR was used for simulating switchgrass biomass at several sites across the United States [87–89].

## 3.4.2 Water Use Efficiency

Switchgrass is often described as a drought-tolerant, warm season perennial due to its deep roots and C<sub>4</sub> metabolism. Physiologically, C<sub>4</sub> plants are known to efficiently use water through stomatal control of transpiration. As indicated before, root systems of perennials, especially C<sub>4</sub> grasses, can access water stored deep within the soil profile and thus extract more water at depth than annual crops [99]. Although this rooting characteristic could translate to higher rates of water use for switchgrass, few studies report estimates of either water use or water use efficiency (WUE) for this emerging bioenergy feedstock. Early investigations by Stout et al. [100] and Stout [101] measured WUE for switchgrass cultivated in the northeastern United States. In their studies, WUE for the cultivar Cave-in-Rock was 25.0 kg ha<sup>-1</sup> mm<sup>-1</sup> for summer-growth switchgrass. More recently, Hickman et al. [102] compared rates of evapotranspiration, water use, and WUE for switchgrass, maize, and miscanthus grown in Illinois. Rates of water use exceeded 750 mm whereas WUE was 9.7 kg ha<sup>-1</sup> mm<sup>-1</sup> for the cultivar Cave-in-Rock grown in Illinois.

While information on water use and WUE is sparse, the database compiled by Wullschleger et al. [83] can be used to derive estimates of WUE for a large number of lowland and upland cultivars of switchgrass. Here annual biomass production can be analyzed along with growing season precipitation to obtain a reasonable estimate of WUE. Across the entire database, which includes almost 1,200 observations of biomass and precipitation for 25 upland and 14 lowland cultivars, WUE averaged 21.6 with a range from 2.3 to 103.1 kg ha<sup>-1</sup> mm<sup>-1</sup> (Fig. 3.4). Sixty-eight percent of the observations fell within the range of 10 to

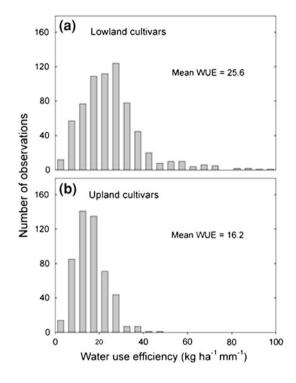


Fig. 3.5 Water use efficiency of upland and lowland switchgrass cultivars

30 kg ha<sup>-1</sup> mm<sup>-1</sup> interval. Separation of the data revealed that WUE was, on average, higher for lowland than for upland cultivars (Fig. 3.5). WUE for lowland cultivars averaged 25.6 (Fig. 3.5a), whereas WUE for upland cultivars averaged 16.2 kg ha<sup>-1</sup> mm<sup>-1</sup> (Fig. 3.5b).

## 3.4.3 Nutrient Use Efficiency

Nitrogen use efficiency (NUE) is dependent on many factors including soil nitrogen availability, uptake and assimilation, and carbon–nitrogen flux and is one of the major limiting factors in increasing crop productivity.

Although NUE can be calculated in a number of ways [103], a simple yet useful measure is biomass yield per unit of nitrogen applied to the soil. More specifically, NUE can be calculated from a series of nitrogen addition plots where annual biomass yield is determined for a range of soil nitrogen additions, including a control where no supplemental soil nitrogen is applied: NUE = (yield of N<sub>x</sub>-yield at N<sub>0</sub>)/kg of nitrogen applied; where N<sub>x</sub> = N rate > 0, and N<sub>0</sub> = no N applied. Although the definition of NUE is simple, and although the response of biomass yield to applied nitrogen has been repeatedly studied for switchgrass [1], NUE for

bioenergy crops including switchgrass has not been well quantified (Table 3.4). While annuals depend more on acquired nutrients for growth, perennial crops, as indicated before, may derive benefits through traits such as remobilization of carbon and nitrogen reserves in the spring that can then support growth from overwintering rhizomes or roots. Thus, perennial plants have a higher NUE than annual crops [104]. In addition, switchgrass has a higher NUE than traditional annual crops in part due to differences in harvest time and management, which allow higher rates of translocation of nitrogen to storage organs like stems and roots. Based on field trials conducted at various locations, Staley et al. [105] and Lemus et al. [72] have reported the most thorough analysis of NUE for switchgrass to date. In those studies, these authors examined the NUE, nitrogen concentration, total nitrogen uptake, and apparent nitrogen recovery for switchgrass fertilized with 0, 90, 180, or 270 kg nitrogen per hectare. Field data collected over the years revealed a diminishing return or inefficiency in NUE with higher rates of nitrogen (Table 3.4). Averaged across all treatments in the study of Lemus et al. [72], there was a yield advantage with nitrogen fertilization of about 9 kg of biomass per kg of applied nitrogen per year. These findings suggest that applying <90 kg nitrogen per hectare per year would provide good yields for switchgrass produced with two cuttings. In a subsequent study by these same investigators [73], it was shown that nitrogen removal exceeded the amounts of nitrogen applied in both one- and twocut management, suggesting that nitrogen was being supplied via mineralization or other processes. Others have obtained similar results, leading Parrish and Fike [1] to conclude that switchgrass is quite efficient and inherently thrifty in its use of applied nitrogen with a capacity to obtain nitrogen from sources that other crops cannot tap. Studies are just now beginning to examine the potential shifts in microbial community composition beneath bioenergy crops and the potential exists for unknown associations of microbes that facilitate nitrogen acquisition and uptake for energy crops like switchgrass and miscanthus [106].

The high NUE of switchgrass is also in part attributed to its deep root system and its symbiotic associations with mycorrhiza. Huang et al. [107] indicated that about 22% of the total nitrogen required by the crop could be supplied by deep roots (deeper than 1.2 m). Moreover, the capacity of the root system to recover/use deep nitrogen sources changes with the season, with the maximum recovery occurring just before/during anthesis; afterwards a significant reduction was registered due to shoot senescence. In general switchgrass can uptake between 1.49 and 2.63 kg nitrogen ha<sup>-1</sup> d<sup>-1</sup>, depending on soil nitrogen levels and nitrogen fertilization [108].

#### 3.5 Yield Gap

Evaluations of existing commercial varieties and new cultivars have served to identify the most productive ones and some management practices that would improve productivity. Yield improvements of up to 50% were already obtained

Table 3.4 Svdifferent ratesrespectively <sup>a</sup>	Switchgrass (cv. tes and locations $q^{a}$	. NJ-50	)) yield, water he	nitrog olding	en conc capacitie	entration, niti es at Klinnes	<b>Table 3.4</b> Switchgrass (cv. NJ-50) yield, nitrogen concentration, nitrogen use efficiency, total uptake, and percentage derived from nitrogen fertilizer at different rates and locations. The water holding capacities at Klinnesville, Calvin, and Leck Kill (Northern Appalachian ridge) were 4.9, 14.4, and 25.3, respectively <sup>a</sup>	total uptak sck Kill (No	e, and perc orthern App	entage deriv alachian rid	ed from nitrog ge) were 4.9,	gen fertilizer at 14.4, and 25.3,
Site	Nitrogen rate	Yield	Yield (Mg ha <sup>-1</sup> )	$\mathfrak{1}^{-1}$		Nitrogen coi	Nitrogen concentration (g kg <sup>-1</sup> ) Total nitrogen uptake (kg ha <sup>-1</sup> ) Nitrogen from fertilizer ( $\%$ )	Total nitr	ogen uptake	: (kg ha <sup>-1</sup> )	Nitrogen fron	n fertilizer (%)
		Cut 1	Cut 2 Tot		NUE <sup>b</sup>	Cut 1	Cut 2	Cut 1	Cut 2	Tot	Cut 1	Cut 2
Klinnesville 0	e 0	3.47	0.63	3.98	I	9.3	8.5	31.6	5.2	35.0	0.0	0.0
	90	6.63	1.03	7.31	37.0	11.3	9.1	71.7	8.7	77.5	35.0	19.8
	180	7.80	1.55	8.83	26.9	13.6	12.0	104	17.1	115	39.3	27.4
Calvin	0	5.52	0.99	6.18	I	8.9	9.7	48.7	9.5	55.0	0.0	0.0
	90	8.86	1.41	9.80	40.2	11.1	9.4	98.5	13.3	107	27.8	18.2
	180	9.76	1.81	11.0	26.8	13.0	11.5	127	20.2	140	33.2	30.4
Leck Kill	0	5.68	1.25	6.52	I	9.0	9.6	49.0	12.2	57.2	0.0	0.0
	90	8.66	1.73	9.82	36.7	11.7	9.6	104	16.8	115	24.1	13.5
	180	I	I	I	I	I	I	I	I	I	I	I
Average	0	4.89	0.96	5.56		9.07	9.27	43.10	8.97	49.07	0.00	0.00
	90	8.05	1.39	8.98	38.0	11.37	9.37	91.40	12.93	99.83	28.97	17.17
	180	8.78	1.68	9.92	24.2	13.30	11.75	115.50	18.65	127.50	36.25	28.90
Source: <sup>a</sup> S	Source: <sup>a</sup> Staley et al. [105]	_										

<sup>b</sup> Nitrogen use efficiency (NUE), NUE = (yield at  $N_x$ -yield at  $N_0$ )/(kg N applied), where  $N_x = N$  rate >0, and  $N_0 = no N$  applied

#### 3 Crop Physiology

with the identification of the most suitable varieties to determined agroecological zones [90]. However, within a production system, switchgrass is usually thought of as being suitable for or allocated to marginal areas where soil resources such as water, nutrients, etc., are available at minimum levels. Such stresses reduce the crop's growth and potential yield; therefore, increased scientific knowledge on how stresses limit the productivity of switchgrass is needed in order to move forward and further improve the productivity and sustainability of switchgrass.

#### 3.5.1 Limitation of Productivity by Water and Salinity

Under drought conditions, switchgrass yield losses can be limited by a combination of drought avoidance and tolerance mechanisms, as with most perennial grasses [4]. Such mechanisms include the development of deep roots, a high leaf area index, pubescent and waxy leaves, changes in leaf orientation, leaf senescence, and osmotic adjustment. However, in upland and lowland ecotypes, some acting mechanisms may not be the same. For example, an interesting difference that might contribute to the reduction of transpiration losses in upland cultivars and upland x lowland switchgrass reciprocal hybrids is the presence of different amounts of pubescent hairs on the leaf blades [3, 109]. Leaf blades in lowland cultivars are more bluish and with waxy covers [95]. Adaxial leaf rolling is another defense mechanism in both ecotypes that reduces the leaf surface area (stomata) exposed to sunlight and therefore reduces the radiation load on the leaves [110, 111]. This may allow a reduction in the water stress level while remaining photosynthetically active.

Barney et al. [26] indicated that biomass production, plant height, number of tillers, leaf area, and specific leaf area were significantly reduced (up to 80%) by severe drought and extreme drought (-4.0 and -11.0 MPa) in both upland and lowland ecotypes. In general, upland ecotypes are considered to be more drought tolerant [112], but more conclusive evidence is needed as Stroup et al. [113] did not find any significant reduction in biomass production of both ecotypes when the drought stress was less severe (-1 MPa). However, upland ecotypes were somewhat less affected than lowland ecotypes under such stress level, indicating the generic capacity of switchgrass to tolerate drought. In response to drought, both ecotypes increase the proportion of leaves with respect to the total dry matter produced [113], probably due to shorter tillers. On the other hand, even though lowland ecotypes outperformed upland ecotypes under waterlogged conditions, the reduced tiller numbers and length, leaf area, and biomass of both ecotypes were not that large, suggesting the wetland facultative properties of switchgrass [26, 114].

Several studies indicate that photosynthetic rates and leaf water potential of switchgrass are reduced to varying degrees depending on the water stress level. For example, Sanderson and Reed [115] indicated that at a soil water tension lower than -45 kPa, photosynthesis and xylem water potential are reduced by 10% and 48%, respectively. However, transpiration efficiency of diverse switchgrass cultivars was not affected by drought probably because the leaf components, mainly proteins, and

enhanced transpiration efficiency [116]. Knapp [117] indicated that the photosynthetic activity of switchgrass was virtually stopped during the driest period of the season, but after a substantial rainfall, photosynthetic rates recovered to about predrought period values. The decreased photosynthesis in switchgrass is accompanied with a decrease in stomatal conductance and significant osmotic adjustment. The capacity of switchgrass to adjust osmotically reflects its capacity to recover from drought. Apart from that, drought-induced reductions in photosynthesis are associated with shoot nitrogen retranslocation to the roots as a probable mechanism to ensure the availability of resources for growth and survival after drought [118]. Even though variations in photosynthetic rates and ploidy levels were identified, it is not yet clear how these differences affect productivity [38].

In switchgrass, physiological growth stages are delayed by drought stress at the primary and regrowth stages [113]. Depending on the stand age and growth stage of the drought occurrence, yields would be variably affected. Sanderson and Reed [115] suggested that switchgrass is more sensitive to water stress at the seeding year than when the plants are already fully established. Moreover, in a dry year, the typical 30-37% of the total biomass concentrated in the roots can be increased up to 60-73% [95] as a response mechanism to limited water availability. Then the increased root growth may ensure a better plant water status and improved nutrient acquisition.

In general, salt stress reduces seed germination, stand establishment, and yield of perennial grasses, such as switchgrass, to varying degrees [4]. Information on the salt tolerance of switchgrass ecotypes, however, is almost nonexistent. Most of the limited available information is focused on germination and seedlings establishment but not on mature plants, except for a study (as far as we know) that found lowland Alamo to have moderate tolerance to salinity [119]. Aboveground biomass yield of switchgrass was only 29% of the control when NaCl was applied in a 2.65 M solution for 5 weeks in pots [120]. Dkhili and Anderson [121] tested the effects of soil salinity (1.1, 6.5, 9.8, and 14.9 dS/m) in combination with different amounts saline irrigation water (0, 4, and 8 dS/m) on pathfinder switchgrass seedlings growth. Their results showed that switchgrass seedlings cannot survive soil salinity levels of 14.9 dS/m or irrigation water with an electric conductivity of 8 dS/m. Moreover, even slight soil salinity levels (6.5 dS/m) delayed emergence, decreased percentage of emergence, reduced seedling height, and reduced dry matter production of aboveground and belowground organs. However, the interactions between saline irrigation water and soil salinity decreased the salt effects as the amount of irrigation increased. Similarly, Kim et al. [122] indicated that the growth and development of Cave-in Rock switchgrass started to show the effects of salinity and ion imbalances in plant tissues at around an electric conductivity of 5 dS/m.

Although switchgrass increases the size of its stomata and develops salt glands to excrete salt excess, these response mechanisms to salinity seem to not function well as large amounts of sodium accumulate in the roots and shoots, even when exposed to moderate salinity levels [122].

## 3.5.2 Limitation of Productivity by Nutrients

Plant nutrients are essential for the growth and development of the different plant parts and for their correct functioning. Nitrogen is mostly involved in enzymatic processes and in proteins. Biomass productivity of upland and lowland switchgrass ecotypes is mainly determined by nitrogen availability rather than by water [113]. The same authors indicated that at low levels of nitrogen (10 kg ha<sup>-1</sup>) the plants were so small that their water requirement never reached stressful levels even though their water supply was limited. Similarly, Sanderson and Reed [115] indicated that well-watered nitrogen-deficient plants developed a higher soil water tension than droughted high-nitrogen-fertilized plants, probably because the smaller transpiring canopy of nitrogen-deficient plants. Other studies, however, indicate that shortage of water may be the most important limiting factor for switchgrass growth in semiarid regions [123, 124] probably because nitrogen mobility decreases substantially as soil moisture decrease. In any case, Stout et al. [123] showed evidence that when precipitation was evenly distributed, nitrogen level accounted for 80% of the variation in yield and water use efficiency.

Nitrogen-deficient plants are chlorotic, with lower photosynthetic rates, lower growth rates, and lower aboveground and belowground resources acquisition capacity [4, 115, 113]. Suplick et al. [125] reported that the LAR and LER in switchgrass respond to increasing nitrogen fertilization in a quadratic fashion, with the highest rates around 164 kg nitrogen ha<sup>-1</sup>. The lower LAR and LER rates at lower nitrogen levels than the aforementioned threshold could be attributed to reduced cell division rather than reduced cell elongation [126]. Because nitrogen deposition in the growing zone (cell division zone) of elongating leaves is reduced at low nitrogen levels, LER and therefore yield are reduced due to lower number of cells produced [127]. Moreover, Suplick et al. [125] found that LER was highly correlated with the total dry biomass production. However, Stroup et al. [113] suggested that the reduced partitioning of carbohydrates to stems and sheaths, commonly associated with nitrogen limitation, is the main reason for reduced yields at low levels of nitrogen. On the other hand, high nitrogen fertilization rates result in increased weight and number of tillers [113].

Apart from storing nitrogen in the roots for regrowth the following spring, perennial grasses such as switchgrass remobilize nitrogen to the roots when availability of external sources decline [128]. However, this nitrogen reserve could be completely depleted if additional nitrogen sources are not made available. Although no signs of deficiency were reported, Lemus et al. [72] demonstrated that in the course of 3 years the internal root-stored nitrogen reserves accumulated during the first year decreased from 1.05 to 0.50% in the following years in the absence of fertilization following crop establishment. They also speculated that 0.50% may be the lower limit before inadequate nitrogen levels start to affect productivity.

Currently there is not much information on the effects of phosphorus deficiency on switchgrass productivity. Brejda [129] and Muir et al. [56], among others, reported little or no yield response of switchgrass to phosphorus fertilization, while Parrish

and Fike [1] indicated that switchgrass is inherently thrifty in the use of applied phosphorus. Phosphorus provides plants with, among other things, a means of using the energy harnessed by photosynthesis to drive its metabolism [130]. According to Mills and Jones [131], concentrations between 0.8 and 1.7 g kg<sup>-1</sup> of phosphorus are sufficient for optimal switchgrass growth. In general, phosphorus is a relatively immobile element in the soil, and the majority of the phosphorus absorbed is via diffusion; therefore, roots have to grow toward where the pool of phosphorus is located or other factors, such as mycorrhizae and exudation of hydroxyl ions and organic acids [132], have to intervene to make it available for the plant. Research indicates that switchgrass phosphorus uptake increased by 37 times when mycorrhizae were present [133]. The higher phosphorus uptake may be related to the enlarged root surface absorption by the symbiotic association with mycorrhizae.

Understanding the basic processes of crop resource capture (nutrients, water, etc.) and allocation have been invaluable tools for designing and evaluating agronomic management practices to improve crop resistance to stress. However, there are still many unrevealed agro-physiological characteristics at canopy and root level that may contribute to improve switchgrass resource use efficiency and to enrich its agronomic outcome. The establishment of a permanent switchgrass physiology program as an important part of agricultural research is urgently needed in ordered to incorporate the already acquired knowledge and further develop the scientific understanding of physiological mechanisms underpinning the control of growth and plant resources use.

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# Chapter 4 Crop Management of Switchgrass

Matt A. Sanderson, Marty Schmer, Vance Owens, Pat Keyser and Wolter Elbersen

**Abstract** Management of switchgrass for bioenergy and forage share some commonalities, of particular interest in bioenergy crop production is: (1) rapid establishment of switchgrass to generate harvestable biomass in the seeding year, (2) highly efficient management of soil and fertilizer N to minimize external energy inputs, and (3) harvest management to maximize yields of lignocellulose. Bioenergy cropping may entail management for multiple services in addition to biomass yield including soil C sequestration, wildlife habitat, landscape management, and water quality protection. Management is a critical factor especially as land classified as marginal or idle land will be emphasized for bioenergy

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production to reduce conflicts with food production. Marginal land may also be more risky. To date, there has been no long-term commercial production of switchgrass on a large scale and there is little in the way of hands-on, practical farm experience with switchgrass managed as a bioenergy crop. In this chapter, we lay out the key best management practices for switchgrass as a bioenergy crop including establishment, soil fertility, and pest management.

### 4.1 Introduction

Switchgrass management as a bioenergy crop is relatively new. Early in the development of switchgrass as a bio-energy crop it was assumed that management for bioenergy would be similar to forage management [1]. For example, it was assumed that establishment methods and weed management guidelines for switchgrass as a forage crop would work as well for its use as a bioenergy crop. Although management for bioenergy and forage share some commonalities, of particular interest in bioenergy crop production is: (1) rapid establishment of switchgrass to generate harvestable biomass in the seeding year (2) highly efficient management of soil and fertilizer N to minimize external energy inputs, and (3) harvest management to maximize yields of lignocellulose. Harvest management may differ the most between bioenergy and forage cropping because the goal is to maximize yields of lignocellulose in bioenergy rather than to optimize forage yield and forage quality. However, extension material for establishing and managing switchgrass for bioenergy has been developed based on the best-available information [2].

Bioenergy cropping may entail management for multiple services in addition to biomass yield including soil C sequestration, wildlife habitat, landscape management, and water quality protection. Management is a critical factor especially as land classified as marginal or idle land will be emphasized for bioenergy production to reduce conflicts with food production. Marginal land may also be more risky.

Switchgrass has been grown for grazing, hay, and conservation uses for decades and a sizable body of scientific and practical information has been accumulated [3–5]. To date, there has been no long-term commercial production of switchgrass on a large scale and there is little in the way of hands-on, practical farm experience with switchgrass managed as a bioenergy crop. Most of the technical information on the management of switchgrass as a bioenergy crop has come from small-plot research studies, pilot-scale demonstration projects of a few acres, and a handful of field- and farm-scale demonstration projects, such as the Chariton River Valley project in southern Iowa and the Tennessee Biofuels Initiative,<sup>1</sup> which includes 6,000 acres (2,400 ha) of switchgrass in eastern Tennessee.

<sup>&</sup>lt;sup>1</sup> http://www.iowaswitchgrass.com/; http://renewablecarbon.tennessee.edu/Partners.html

In this chapter, we lay out the key best management practices for switchgrass as a bioenergy crop including establishment, soil fertility, and pest management. Where management protocols translate well from and are firmly founded in forage management, we briefly summarize and direct the reader to the appropriate authoritative source.

#### 4.2 Strategic Planning for Bioenergy Crop Management

As mentioned at the outset, there is no large-scale production of switchgrass for bioenergy. When bioenergy cropping matures, however, farmers will need to do some strategic planning regarding production systems. For example, the first planning decision the farmer may face is whether or not to grow switchgrass for bioenergy. Economic considerations (e.g., price paid for biomass, costs of production) will certainly drive that decision but other considerations, such as flexibility to grow other crops in rotation or to manage switchgrass as a forage crop to take advantage of other markets, will affect decisions as well. Knowledge of switchgrass production will also influence decision making. A survey of Tennessee farmers in 2005 indicated that farmers were unfamiliar with switchgrass; however, nearly a third of those surveyed would grow switchgrass if it were profitable. Some of their concerns included the need for technical assistance to grow and manage switchgrass and the lack of developed markets [6]. In a survey of Illinois farmers, most respondents indicated that growing perennial grasses for bioenergy may be worthwhile, however, they had limited knowledge of the grasses and how to grow or manage them [7]. Some of their concerns regarding production included lack of a local market for the biomass and a hesitancy to replace annual row crops with perennial grasses. Farmers who rented land were reluctant to consider perennial grasses because their landlord may not approve of the cropping system change. Most respondents stated that perennial grasses would fit best on their marginal cropland.

The option to use switchgrass both as cattle forage and biomass for energy could offer an attractive incentive for farmers wherever livestock production is an important enterprise. Flexibility in its use could also provide an incentive to adopt switchgrass production as a new enterprise [8].

Strategic planning also includes considerations for site selection for growing switchgrass. Site selection criteria may include suitability of soils for switchgrass production among other factors. Planning for establishment should begin at least one or more years in advance so that soil deficiencies (e.g. low pH) can be corrected or to control weed populations through appropriate crop rotation along with herbicide use.

Other important considerations in the planning process are to consider if the proposed crop rotation has the flexibility to respond to markets or climate with alternative crop choices. Producers should also consider risk assessment and the probability of success and to be completely aware of potential to realize all outcomes. Risk management practices such as participation in government incentive programs [e.g., the biomass crop assistance program (BCAP)], and the availability of crop insurance should also be considered. In the USA, the BCAP program provides eligible producers reimbursement of up to 75% of the cost of establishing a perennial bioenergy crop along with assistance (up to \$45 per ton) for harvest, transport, and storage of bioenergy crops. Risk management also applies to harvest decisions. Harvesting switchgrass early to obtain quality forage for livestock and using regrowth for biomass may provide flexibility for the producer but can risk stand longevity and future productivity. Managing for a single autumn harvest can maximize yield, whereas allowing the stand to remain in the field over winter to reduce mineral and water levels for efficient harvest and storage risks yield loss.

Proper management is needed to realize the best yields possible for a given land area so as not to replace (or reduce) or compete with land for food production [9]. The importance of proper management was illustrated by Lemus et al. [10] who showed that simply improving agronomic management by controlling weeds and implementing a standard harvest schedule (without N fertilizer) increased yields from 2.3 to 5 Mg ha<sup>-1</sup>.

Implementing appropriate management practices can be an important part of reducing production risk. Appropriate establishment techniques protect against risk of stand failure. Use of best management practices positions the farmer for the best yield response in favorable growing conditions. Similarly, implementing best practices can protect against losses from pests, diseases, and abiotic stresses.

#### 4.3 Switchgrass Establishment

#### 4.3.1 Site Selection and Preparation

Like most perennial species, switchgrass establishes best on well-drained, fertile soils but it also can establish and persist under highly variable soil conditions. Switchgrass used in bioenergy is likely to be grown on land that is unsuitable for crop production either based on erosion potential or low crop productivity. Switchgrass establishment practices for bioenergy plantings will require rapid establishment and effective weed control.

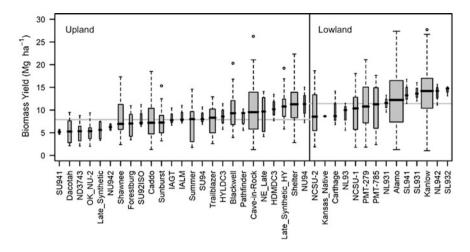
Proper planning is important for establishment success. Key factors to consider for successful establishment are measuring soil fertility status, proper field preparation, proper cultivar selection, and using high quality seed. Soil sampling before planting is recommended to determine soil nutrient status with the number of soil samples required being dependent on the soil heterogeneity of the land to be converted. At a minimum, soil should be tested for P, K, and pH before planting with subsequent soil sampling every three to five years thereafter. Optimal seed germination occurs between pH of 6–8 [11] but switchgrass seedlings can tolerate pH values between 3.7 and 7.6 under field conditions [5, 12, 13]. The overall effectiveness of liming before switchgrass establishment has been mixed but liming is likely beneficial on soils with pH values below 5.0 [13].

Planting switchgrass into existing cropland, pastureland, or conservation land requires land management preparation one to two years in advance. For example, scouting for and controlling perennial weeds within fields before switchgrass establishment will minimize stand failures. Allelopathic effects from previous crops on switchgrass establishment have not been well documented but certain crops can increase or decrease the likelihood of successful establishment depending on weed suppression, herbicide carryover, and residue amount. Herbicide-resistant crops in general provide good weed suppression the following year for effective establishment. For example, soybean (Glycine max L. Merr.) provides a firm seed bed, minimal residue, and good weed control which are important attributes for successful establishment of switchgrass. High-residue crops, such as maize (Zea mays L.) may require heavy duty drills to plant switchgrass under no-till conditions or tillage practices for effective seed-to-soil placement and uniform seeding depth. Establishing switchgrass in former pastureland or conservation land is more challenging and requires multiple management tools such as non-selective herbicides, tillage, and burning.

## 4.3.2 Cultivar Selection

Selecting the proper cultivar is critical for both establishment and persistence. Switchgrass cultivars are morphologically divided into upland and lowland ecotypes (see Chap. 1). Lowland ecotypes are taller, have longer more bluish-green leaves, have longer ligules, are higher yielding, grow like a bunchgrass, are more rust (*Puccinia emaculata* Schwein.; *Puccinia graminis* Pers.; *Uromyces gramincola* Burrill) resistant, and have a coarser stem than upland ecotypes. Upland ecotypes are shorter growing, have a finer stem, and are more tolerant of dry climatic conditions than lowland ecotypes [4]. Within ecotypes there are northern upland, southern upland, northern lowland, and southern lowland strains based on responses to latitudinal effects [14]. Viable switchgrass seed can be produced when lowland ecotypes are crossed with the same chromosome number [15]. Development of F1 hybrids derived from upland and lowland ecotypes has shown increased biomass yields compared with the parental lines [16].

Estimates of economic yields of switchgrass biomass vary, but McLaughlin et al. [17] indicated that 9 Mg ha<sup>-1</sup> average annual yield across all production areas was economic in the USA. In a meta-analysis of switchgrass biomass yields from 39 sites in 17 states of the USA, lowland ecotypes of switchgrass averaged  $12.0 \pm 5.9$  Mg ha<sup>-1</sup> and upland ecotypes averaged  $8.7 \pm 4.2$  Mg ha<sup>-1</sup> (Fig. 4.1; [18]). Based on their empirical model of switchgrass yield, greatest biomass yields were predicted to occur in a region from the mid-Atlantic region to eastern Kansas and Oklahoma.

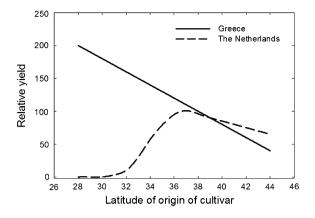


**Fig. 4.1** Variation in biomass yields of upland and lowland switchgrasses at several locations in the USA (from Wullschleger et al. [18], with permission, copyright American Society of Agronomy)

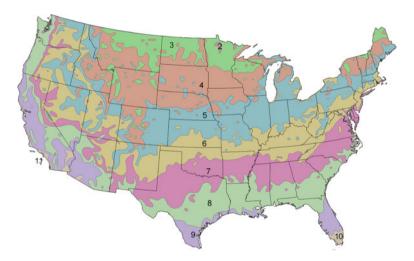
European research also indicates that lowland ecotypes yielded more than upland ecotypes. Averaged over four years and two systems of cutting in Italy, lowland ecotypes averaged 14.9 Mg ha<sup>-1</sup>, whereas upland ecotypes averaged 11.7 Mg ha<sup>-1</sup> [19]. Yields for several switchgrass varieties ranged from 7.1 to 21.3 Mg ha<sup>-1</sup> during four years in Greece and 0.9–26.1 Mg ha<sup>-1</sup> in Italy [20].

Switchgrass is photoperiod sensitive, requiring short days to induce flowering [21]; however, there is variation among switchgrass cultivars in photoperiod response [22, 23]. When switchgrass is grown north of the original adaptation area, it is exposed to longer photoperiods resulting in a longer vegetative stage and more biomass is produced than existing populations originating within that latitudinal environment. Switchgrass populations moved south of their original adaptation area produce less biomass than in their original adaptation area because floral initiation begins earlier. Switchgrass populations moved too far north in a temperate climate do not survive the winter because the plants do not cold harden before the onset of freezing temperatures. Switchgrass populations that originated in high latitudes or low latitudes within the United States have the most defined plant responses to latitude [14]. Generally, switchgrass cultivars should not be planted more than one hardiness zone (Fig. 4.2) north of their area of origin [4, 24]. Longitudinal response by switchgrass populations is less defined than latitudinal responses and is variety specific [24].

The optimal latitude for growing a specific switchgrass variety will also differ between Europe and North America. For example, the cultivar Cave-in-Rock, which originates in southern Illinois USA (38.30° North), is probably best adapted to northwest Europe (Netherlands and United Kingdom  $\sim 52^{\circ}$  North) [25]. When cultivars are grown too far north, they may not survive winter or have reduced



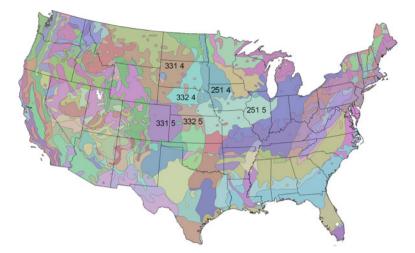
**Fig. 4.2** Expected relative yields of varieties from low and high latitude of origin when grown at a Northern location ( $52^{\circ}$  north, the Netherlands) and at a southern location ( $38^{\circ}$ , Greece) (from Elbersen, with permission)



**Fig. 4.3** USDA plant hardiness zones for the 48 contiguous states in the USA (http:// www.usna.usda.gov/Hardzone/ushzmap.html). Switchgrass cultivars should not be planted more than one hardiness zone north of their area of origin. The average minimum annual temperature (°C) ranges by zone: zone 1, <-45.6; zone 2, -45.5 to -40; zone 3, -39.9 to -34.5; zone 4, -34.5 to -28.9; zone 5, -28.8 to -23.4; zone 6, -23.3 to -17.8; zone 7, -17.7 to -12.3; zone 8, -12.2 to -6.7; zone 9, -6.6 to -1.2; zone 10, -1.1 to 4.4; zone 11, >4.5

stand life. A generalized relationship between latitude of origin of a cultivar and the yield at a southern and northern site in Europe is illustrated in Fig. 4.3.

Cultivars originating from the semi-arid Great Plains may be more susceptible to disease when established in more humid locations, whereas cultivars originating in humid climates may be less adapted to the drought conditions of semi-arid



**Fig. 4.4** Plant adaptation region map for the 48 contiguous states of the USA (from [26]). Plant adaptation regions are derived from combining ecoregion and plant hardiness zone classification systems. Example plant adaptation regions labeled include the Great Plains Steppe hardiness zones 4 and 5 (331-4, 331-5, 331-5, and 332-5) and the Prairie Parkland Temperate hardiness zones 4 and 5 (251-4 and 251-5)

climates. Plant adaptation regions define suitable environments for leading switchgrass populations (Fig. 4.4, [26]).

The majority of released switchgrass cultivars were developed for conservation or traits desirable for pasture grazing. Current switchgrass varieties tend to be from (1) seed increases from random plants originating from a remnant prairie or (2) improved cultivars based on selection of defined traits such as yield, digestibility, seed dormancy, or disease resistance [27], see Chap. 2]. Most improved switchgrass cultivars have been developed in the Central Plains of the USA. Breeding for specific bioenergy traits (e.g. low lignin, high cellulose) will be dependent on the conversion process used within a given region [1].

## 4.3.3 Seed Quality

Seed quality attributes include viability, purity, cleanliness, and vigor [28]. Seed vigor relates to the ability of a seed to germinate and establish a viable seedling under field conditions (see Chap. 3). Growing conditions, harvest timing, seed drying, cleaning and processing procedures, storage conditions, field sanitation, diseases, and insects influence switchgrass seed quality [28]. Agronomic practices that improve seed quality include N fertilization, use of row cultivation, spring burning, and control of smut (*Tilletia maclaganii* (Berk.) G.P. Clinton) and rust. Selection for increased seed size can increase viability and vigor. Average seed

weight for switchgrass is 850 seeds  $g^{-1}$  but variation exists among and within cultivars for seed size [5]. High germination rates and greater emergence have been reported for switchgrass seed with higher than average seed weights [29, 30]. Under field conditions, switchgrass seedlings from heavy seed had greater germination and earlier growth than lighter seed but no growth differences were detected 8–10 week after emergence [31].

#### 4.3.3.1 Seed Quality Tests

Standard germination and purity tests have limited utility in the direct calculation of planting rates for switchgrass. Pure live seed (PLS) is used in expressing seed quality and determining recommended field seeding rates. Pure live seed is calculated as: PLS (%) = [seed purity (%) × seed germination (%)]/100. Switchgrass germination testing protocols from the Association of Official Seed Analysts include a 14 day cold stratification (5°C) period before germination testing, which reduces the amount of primary dormancy found in switchgrass. The germination percentage found on seed tags tends to overestimate the percent of viable seeds, which complicates determining proper field seeding rates [13]. Germination trials without cold stratification are recommended if seed dormancy is expected [5]. Seed vigor tests that evaluate emergence from depth or accelerated aging tests have been recommended as alternative methods in determining field seeding rates [32, 33]. Switchgrass seed lots with the same germination percentage but different seedling vigor have resulted in emergence differences of more than 40% [28].

#### 4.3.3.2 Dormancy

Seed dormancy reduces seedling vigor and establishment. Dormant seed can be defined as seed that is unable to germinate even when subjected to suitable conditions [34]. The mechanisms for seed dormancy of switchgrass are complex but the expression of seed dormancy is caused by structures that surround the embryo and mechanisms within the embryo [35]. Dormancy is likely caused by genetics and environmental effects during seed production, harvesting, and processing. Genetic selection for low dormancy seed has been shown to lower overall primary dormancy in lowland ecotypes [36]. Primary dormancy of switchgrass seed can generally be broken by an after-ripening period or by cold stratification [4]. After-ripened switchgrass seed is generally one-year-old or more. Switchgrass seed that is stored for three or more years at room temperature may have poor seedling vigor and reduced establishment [28]. Secondary or latent dormancy occurs when viable seed becomes dormant after unfavorable environmental conditions [35]. Environmental or chemical methods can be used to break secondary dormancy. Seeds that have undergone secondary dormancy-breaking techniques but still demonstrate low germination have residual dormancy [37]. Residual dormancy can be reduced in switchgrass when endogenous levels of nitric oxide (NO) and reactive oxygen species (ROS) are altered [38]. High abscisic acid (ABA) levels can increase dormancy in monocot seeds [39]. Exogenous ABA and diphenyleneiodonium levels in switchgrass seeds are believed to block germination by restricting ROS and NO activity [38]. Gibberellic acid, sodium nitroprusside, potassium ferrocyanide, potassium nitrate, polyethylene glycol, and hydrogen peroxide have been used to reduce residual dormancy in switchgrass but overall success of each treatment is cultivar-specific [40, 41].

#### 4.3.3.3 Seed Treatments

Several seed treatments have been investigated for their ability to increase switchgrass germination and establishment. Seed priming, an osmotic process where seed is hydrated to a level where metabolic activity begins but radicle emergence does not occur, may enhance switchgrass germination [42, 43]. Seed water uptake is regulated when priming media such as synthetic calcium silicate is used as a water source until equilibrium between the seed and media is reached. Hydrogen peroxide treatment of non-dormant switchgrass seeds increased seed germination and emergence along with more uniform development of seedlings [38]. Sodium nitroprusside, a NO donor, promoted germination of the lowland ecotype Kanlow [37]. Field conditions at planting influenced overall effectiveness of primed seed [42]. Blending primed and non-primed seed may reduce overall seeding costs and increase stand establishment under variable field conditions [44]. Karrikinolide [3-methyl-2H-furo[2,3-c]pyran-2-one], a compound from smoke that has been found to promote germination and seedling establishment in several native species, did not increase switchgrass germination or seedling vigor [45]. Switchgrass seeds coated with fungicides have been used in humid climates to increase seedling emergence; however, it is unclear if fungicide application limits the symbiotic relationship between switchgrass and arbuscular mycorrhizal fungi. Insecticides have also been shown to improve establishment [46] and can be used as a seed coating applied before planting.

#### 4.3.4 Seedbed Preparation

Switchgrass can grow under variable soil conditions ranging from sand to clay loam [5]. Switchgrass has been successfully established under various tillage practices but side-by-side tillage studies and preceding crop comparisons have been limited [13]. Switchgrass is mainly established through direct seeding using a culti-packer seeder, grain drill, or no-till drill. Seed-drill calibration is necessary to ensure proper seeding rates. Broadcast seeding also has been used in conservation plantings but lack of stand uniformity may limit its potential use for bioenergy plantings. Seedbed preparation likely will be predicated on equipment accessibility, soil erosion concerns, preceding crop, and initial soil moisture conditions. A firm seed bed is recommended for proper seed placement regardless of planting method since switchgrass is planted at a shallow depth. Planting switchgrass using conventional tillage methods is a common practice for effective establishment. A grain drill with a small-seed box and small-seed tube attachment or a cultipacker seeder is effective in establishing switchgrass under conventional tillage practices. Conventional tillage can control or reduce cool-season weed populations before seeding as well as reduce residue from previous cropping systems that may interfere with proper seed placement. Soil temperatures will be higher under conventional tillage than no-tillage practices during a spring seeding. Following a tillage practice, soil clods need to be reduced or eliminated by successive tillage, packing, or firming the soil to provide good seed-to-soil contact at time of planting. Soil firming before seeding has been more effective in switchgrass establishment than soil firming after seeding [47]. Conventional tillage practices are not recommended on fields with steep slopes because of the risk of soil erosion. Soil carbon loss via  $CO_2$  emissions from tilled soil is also a concern, especially on land that was previously in set-aside programs or other perennial grassland systems which tend to maintain high levels of soil carbon [48]. No-tillage seeding of switchgrass has also been effective under variable climate conditions and previous cropping systems. No-tillage practices have been successful in establishing switchgrass in existing grasslands and are highly effective on soybean stubble [49]. A no-till drill is recommended when planting in sod or heavy residue because the drill has coulters to remove residue before seed placement and it is heavier than a conventional grain drill. No-tillage seeding provides greater water conservation benefits than conventional tillage especially near the soil surface. For bioenergy purposes, both pre-emergent and post-emergent herbicides are critical under no-tillage practices to control or reduce weed populations during the establishment year.

### 4.3.5 Fertilization

Nitrogen fertilizer is not recommended during the planting year because N will encourage weed growth, increase competition for establishing seedlings, increase establishment cost, and increase economic risk associated with establishment if stands should fail [49]. Sanderson and Reed [50] found that there was no biomass yield response to N at rates of 22 or 112 kg ha<sup>-1</sup> during the establishment year of switchgrass and indicated that switchgrass was able to use the available N found in the soil during the establishment year. Starter fertilizer (9 kg N ha<sup>-1</sup> and 27 kg P ha<sup>-1</sup>) applied at planting did not improve switchgrass establishment or initial yields [51]. As stated earlier, soil tests (P, K, pH) are recommended before planting. Phosphorus levels (Bray & Kurtz #1 method) should exceed 25 mg kg<sup>-1</sup> when establishing warm-season grasses [52, 53]. Phosphorus and K levels are generally adequate for switchgrass growth in most agricultural fields.

#### 4.3.6 When to Plant

Successful establishment of switchgrass seedlings is determined by seeding depth, soil texture, soil moisture, and soil temperature [13]. Soil temperatures above 20°C are required for switchgrass germination and seedling growth [54, 55]. Optimal germination of several switchgrass cultivars was found to be between 27 and 30°C [56] but germination tolerance to temperature varies by cultivar [57]. Switchgrass has a panicoid type of seedling root development (see Chap. 3) which places the crown node (from which adventitious roots develop) near the soil surface by elongation of the subcoleptile internode or mesocotyl [58]. The subcoleoptile internode has a relatively small xylem cross-sectional area in most warm-season grasses. This limits the amount of water and nutrients that can be transported from the primary root system to developing leaf area and contributes to seed establishment failure in warm-season grasses before adventitious roots develop [59]. Selection for low crown node placement (i.e., a shorter subcoleoptile internode) increased switchgrass seedling survival in the southern Great Plains [60]. Greater planting depths reduce warm-season seedling emergence [61]. Recommended planting depths for switch grass are 0.5-2 cm [4] but seeding depths of 3 cm have been recommended on coarse textured soils [13].

The most important factor for switchgrass to develop adventitious roots is adequate soil surface moisture over a period of time [62]. Rainfall frequencies between 7 and 10 days were found to be crucial for seedling survival under south central US climate conditions [63]. Under favorable environmental conditions, warm-season grasses initiate adventitious roots at 2–4 weeks after emergence [59]. Switchgrass will typically have one adventitious root by the three-leaf emergence stage [62]. Adventitious roots have a larger xylem cross-sectional area than the subcoleoptile internode resulting in higher water uptake and nutrient absorption than the primary root system [59].

Seeding dates for warm-season grasses are recommended in early spring when soil moisture is not limiting and soil temperatures are low enough to cold-stratify seeds. For the Northeast United States, switchgrass planting is recommended 3 weeks before and 3 weeks after corn planting [64]. In Iowa, switchgrass planted in mid-April to early May had higher dry matter production than when planted later in the season [65]. Planting during late April to mid-May was optimum for warm-season grasses in mid-latitude areas of the United States [54, 55]. In Nebraska, planting in March resulted in higher standing crop biomass than April or May plantings [66]. Dormant planting (late autumn) or frost seeding (late winter) may be used to establish switchgrass for conservation purposes.

#### 4.3.7 Seeding Rate

Recommended seeding rates for switchgrass are 200–400 pure live seeds (PLS) m<sup>-2</sup>. With excellent weed control, however, a seeding rate of 107 PLS m<sup>-2</sup> gave adequate stands for conservation plantings [67]. Seeding rates of 4.48–11.20 kg ha<sup>-1</sup> resulted

in similar biomass yields during post-establishment years in the southeast US [68]. Lower seeding rates are recommended for regions with limited rainfall but this recommendation is based on conservation plantings and may not be appropriate for bioenergy-specific plantings. Switchgrass swards appear to regulate plant density depending on the climate conditions and initial seedling density with low seedling density tending to increase with sward age and high seedling density tending to decrease until equilibrium has been reached for a particular site.

### 4.3.8 Mixed Species Planting

Mixed species plantings are common for conservation and forage purposes and may be useful for bioenergy purposes as well. The use of low-input high-diversity mixtures has been proposed as a sustainable way to produce bioenergy on degraded land [69]. Species and cultivar selection is important for long-term success and should have similar growth characteristics, seed vigor, forage quality characteristics, maturity dates, and tolerance to selected herbicides. Warm-season grass species of big bluestem (Andropogon gerardii Vitman), indiangrass [Sorghastrum nutans (L.) Nash], switchgrass and little bluestem [Schizachyrium scoparium (Michx.) Nash] were the dominant species of tallgrass prairie in North America. These species are common in mixtures for set-aside plantings within the central US. A positive relationship has been found between soil heterogeneity and plant diversity for grassland ecosystems. Planting mixed species would likely increase establishment success on areas with variable soil types or topography by allowing certain species to grow based on broad or narrow niche requirements. When switch grass is planted as a mixture with other perennial grasses, it is recommended that no more than 20% of the seeds should be switchgrass [5].

In a multilocation study in Minnesota, biomass yield increased by 28% when species richness of mixtures increased from 1 to 8 species; however, there was no further yield increase at species richness levels of 8–24 species [70]. In most instances, only a few species produced most of the biomass yield. A comparison of warm-season grass monocultures with polycultures of 4–16 species of grasses and forbs demonstrated that monocultures produced more biomass more economically than polycultures [71]. Observational research on conservation grasslands in the northeastern USA indicated a negative relationship between plant species diversity and biomass production [72].

Companion crops have been used successfully to establish perennial grasses especially when herbicide options are limited. Advantages to companion crops include reduced erosion potential under conventional tillage along with a potential cash crop that can be harvested, which may reduce overall establishment costs especially if a switchgrass stand failure occurs. Companion crops also reduce weed populations during switchgrass establishment but management practices are important to ensure proper establishment. A disadvantage to using companion crops is that in certain regions, an establishment year switchgrass harvest is not possible because of limited biomass. Switchgrass has been successfully established under maize and sorghum-sudangrass (*Sorghum bicolor*  $\times$  *sudanense*) [73, 74]. Winter wheat (*Triticum aestivum* L.), spring oat (*Avena sativa* L.) and other annual cool-season grasses managed as a hay crop have been successfully used for establishing perennial grasses like switchgrass. Soybean planted in wide rows (76 cm) can be used in cooler regions where row canopy closure is delayed until late-summer. Seeding densities for the companion crop should be reduced from optimal monoculture plantings to allow more light to reach the switchgrass seedling.

Establishing switchgrass on land formerly in set-aside programs or pastures has been done using either conventional tillage or no-tillage methods [13]. Management practices for effective establishment may include autumn or spring hay harvest, prescribed burning in autumn or spring, pre- and post-herbicide treatments, tillage, and the use of companion crops. Planting set-aside land or pastures with herbicide-resistant soybeans or spring-planted cover crop mixtures one to two years before switchgrass establishment may also be an effective management tool for seedbed preparation.

## 4.3.9 Weed and Pest Control During the Establishment Year

Weed competition is a major reason for switchgrass stand failure during establishment. Acceptable switchgrass production can be delayed by one or more years by weed competition and poor stand establishment [75]. The most common weeds in establishing warm-season grasses are annual grasses such as crabgrass [*Digitaria sanguinalis* (L.) Scop.], green foxtail [*Setaria viridis* (L.) Beauv.], yellow foxtail [*Setaria glauca* (L.) Beauv.], autumn *panicum (Pancicum dichotomiflorum* Michx.), and barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.] [76]. The recommended practice of controlling weeds in fields planted with switchgrass is with the use of pre-emergent herbicides particularly for annual grass control. Non-selective herbicides, such as glyphosate [N-(phosphonomethyl) glycine] are effective in weed control before switchgrass emergence especially under no-till plantings. *It is important to follow all herbicide regulations, label directions, and safety precautions. Extension staff or professional advisors should be consulted regarding proper use and application of all herbicides.* 

Atrazine [2-chloro-N-ethyl-N'-(1-methylethyl)-1, 3, 5-triazine-2, 4-diamine] has been an effective herbicide during switchgrass establishment controlling mainly cool-season annual grasses and broadleaf weeds [65, 77]. Switchgrass biomass yields were higher with atrazine application than without [52, 77]. Quinclorac (3, 7-dichloro-8-quinolinecarboxylic acid) is another effective herbicide in establishing switchgrass [2, 78]. Quinclorac controls warm-season annual grasses such as giant foxtail [*Setaria faberi* (L.) Beauv.], yellow foxtail, green foxtail, and barnyardgrass along with a limited number of broadleaf species [79]. Switchgrass treated with a pre-emergent combination of quinclorac and atrazine

had greater biomass yields and comparable switchgrass stand frequencies compared with switchgrass treated with atrazine or quinclorac alone and both herbicides were equally effective on upland and lowland ecotypes [80]. Post-emergent application of quinclorac reduced switchgrass yields compared with atrazine but was highly effective in controlling annual grasses [81]. Pre-emergent applications of imazeth-{2-[4,5-dihydro-4-methyl-4-(1-methlethyl)-5-oxo-1H-imidazol-2-yl]-5-ethylapyr 3-pyridine-carboxylic acid} has been effective in switchgrass establishment [82]. Post-emergent sulfosulfuron [1-(4,6-dimethoxypyrimidin-2-yl)-3-(2-ethylsulfonylimidazo[1,2-a]pyridin-3-yl)sulfonylurea] applications on switchgrass are more effective in controlling smooth pigweed (Amaranthus hybridus L.) than quinclorac but are less effective on annual grasses [78]. The use of 2,4-D (2,4-dichlorophenoxyacteic acid) is cost effective for broadleaf weed control when applied post-emergence at the 4- or 5-leaf stage. Broadleaf weed control using a mechanical treatment (mowing) can be successful when broadleaf weeds are taller than switchgrass and the mowing application can be done to minimize switchgrass leaf loss [83]. After successful establishment, only limited herbicide use should be necessary.

Research on pest and disease control on switchgrass grown for bioenergy has been limited. Establishment year insecticide applications like carbofuran (2,3-dihydro-2,2-dimethly-7-bensofuranyl) have had variable success on switch-grass yield and stands [13, 84]. Insect injury on switchgrass seedlings is dependent on climatic conditions, seeding dates, and weed populations. Outbreaks of rust and smut can occur during the establishment year but are generally more likely to occur post-establishment.

#### 4.3.10 How to Evaluate Establishment Success

A grass seedling is considered fully established when adventitious roots are formed, able to overwinter, and survive the following growing season [85, 86]. Switchgrass seedlings must develop two or more tillers to survive winter [87]. At the field-scale, switchgrass stands are evaluated based on morphological development, weed control, and seedling density. Integrating a global position system receiver and the use of geographic information systems (GIS) with ground-based scouting tools can assist in quantifying stand densities and weed populations across fields. Maps can be generated using GIS-derived data to identify areas within fields for possible reseeding or weed control measures. Switchgrass will typically not reach full yield potential until 1–2 years after the establishment year. Under normal weather conditions and proper agronomic management, switchgrass can achieve  $\geq$ 50% of full yield potential during the establishment year [75]. Harvesting switchgrass in the establishment year reduces farm-gate production costs and improves economic returns [88].

The definition of an acceptable stand density is dependent on the desired management goal. Stand densities of  $\geq 10$  seedlings m<sup>-2</sup> are acceptable for conservation purposes [89–91], whereas switchgrass managed for grazing or bioenergy purposes will require higher initial stand densities. Establishment year stands of >20 switchgrass seedlings m<sup>-2</sup> with minimal weed pressure are considered to be fully successful, whereas stands between 10 and 20 seedlings m<sup>-2</sup> are considered adequate, and stands below 10 seedlings m<sup>-2</sup> may require reseeding. Switchgrass has the ability to produce similar biomass yield under different seedling densities as a result of compensatory responses to tiller number and sizes [50]. When comparing recommended seeding rates (200–400 PLS m<sup>-2</sup>) and desired seedling densities, planted seed to established seedling success rate is generally <20%.

Stand frequency measurements have been used to quantify seeding establishment and assess grassland improvements [92, 93]. Frequency is determined by the number of samples in which a species occurs, within a given area, and is expressed as a percentage of the total. Vogel and Masters [93] developed a frequency grid tool that can be used to assess plant populations at numerous sampling areas within a field and provide a conservative estimate of stand density. In a study conducted in the northern Great Plains USA on 10 farms, switchgrass fields with stand frequency of 40% or greater provided a successful establishment year stand threshold for subsequent post planting year biomass yields [75]. The frequency grid tool was designed for monitoring plantings under culti-pack drop seeders or conventional seed drills. Switchgrass planted into wider drill rows (>38 cm) may require alternative methods to quantify establishment success, such as a frequency rod or line transects [77, 94].

# 4.4 Soil Fertility and Crop Fertilization in the Established Stand

Fertilizer requirements of switchgrass managed for bioenergy depend on yield potential, soil productivity, management practices, and weather. Most switchgrass fertility research has focused on N because it is often the most limiting nutrient [95–97], and before 1990 the primary goal was to better understand the effect of fertility on switchgrass grown for forage rather than bioenergy [98]. This is an important consideration because a biomass harvest, especially in northern environments, is often done around a killing frost in the autumn when some nutrients will have been translocated to underground tissue [99].

#### 4.4.1 Nitrogen

Optimum N rates for switchgrass managed for bioenergy vary by geographic region, environmental and weather conditions (specifically precipitation),

N-supplying capability of the soil, and harvest frequency [97, 100-103]. Maximum yields of 'Kanlow' switchgrass were achieved with 448 kg N ha<sup>-1</sup> applied in April in a 3-cut system in Oklahoma [97]. Multiple harvests each year and high N application rates, however, had a greater negative effect on average annual biomass production than a single harvest system over a four-year period at one of two study sites and was not economically feasible. Aravindhakshan et al. [100] concluded that harvesting once annually and applying only 69 kg N ha<sup>-1</sup> was most economical in a similar environment. In a comparison of a 1- or 2-cut system with added N (50 kg  $ha^{-1}$ with one cut or 100 kg  $ha^{-1}$  with two cuts), Lemus et al. [102] noted that N removal was significantly greater in the 2-cut system because of the high concentration of N in the mid-summer harvest. Although mean switchgrass yields were slightly higher in the 2-cut, 100 kg N ha<sup>-1</sup> system than in a 1-cut, 50 kg N ha<sup>-1</sup> system, they concluded that the cost of additional inputs and greater N removal rates would not be worth the small yield increase. Applying 56 kg N ha<sup>-1</sup> increased switchgrass biomass production in several locations in South Dakota, USA, but application rates above this amount resulted in no further yield advantage and increased weed pressure [103]. Switchgrass response to N levels up to 220 kg ha<sup>-1</sup> was linear or quadratic in Iowa, USA [104].

Applying 10–12 kg N ha<sup>-1</sup> for each Mg ha<sup>-1</sup> biomass removed under a single late-summer or autumn harvest is recommended in the Midwest USA [99]. This allows for replacement of N removed in biomass because switchgrass typically contains 60–120 kg N Mg<sup>-1</sup>. Regardless of amount applied, N is typically applied after green-up in the spring to minimize weed encroachment, particularly from invasive cool-season grasses.

Nitrogen response of switchgrass may depend on harvest management. For example, in western Europe, switchgrass generally is harvested after senescence in winter (i.e., delayed harvest) and yield response to N is minimal, even if the crop is grown for many years with no fertilizer N [105, 106]. The low N removal (because of N recycling within the plant) combined with high rates of atmospheric N deposition and generally high soil N levels probably contribute to the lack of a N response in delayed harvest switchgrass in Europe.

# 4.4.2 Phosphorus and Potassium

Less research has been done relative to P and K fertilization of switchgrass for biomass or forage. Recommendations for P and K application are based on soil test levels and soil characteristics, [101]. There was no response of switchgrass to P application at two locations in Texas, USA over a 3 or 7 year period [96]. Switchgrass production increased when P and N or P, K, and N were applied together with lime compared to N alone on five different soils in Louisiana, USA [107]; however, the authors speculated that response to P fertilization would be limited without N.

Switchgrass grown on acid soils (pH < 5) may be subjected to P deficiencies. Switchgrass performs better on acid soils when grown in symbioses with arbuscular mycorrhizal fungi [108, 109]. Phosphorus may be more available to plants with this association because arbuscular mycorrhizal fungi are better able to access more soil P.

# 4.4.3 Lime

Switchgrass has limited response to lime. In a greenhouse pot experiment with five acidic soils, yield did not increase when soil pH was brought to 6.5 with lime [107]. A yield response was noted, however, when N and P or N, P, and K were co-applied with lime. On a strongly acid (pH 4.3–4.9) soil in Pennsylvania, USA, untreated switchgrass yielded approximately 50% of that receiving the high lime and fertilizer rate [110].

# 4.4.4 Manure

Cattle manure may be used as a source of nutrients on switchgrass. Lee et al. [111] compared three equivalent N rates (0, 112, and 224 kg ha<sup>-1</sup>) of cattle manure or ammonium nitrate and found that switchgrass yields increased with the medium application rate of either N source. However, ammonium nitrate had a greater deleterious effect on switchgrass stand persistence and weed encroachment than the equivalent rate of manure. They speculated that this may have been due to the slow release of N from manure compared to the rapid availability of N from ammonium nitrate. Switchgrass biomass yields increased linearly with dairy manure applied at rates of 0–600 kg N ha<sup>-1</sup> [112]. Switchgrass filter strips effectively reduced concentrations of nutrients and pollutants in runoff water from the dairy manure-treated plots.

## 4.5 Pest and Weed Management in the Established Stand

#### 4.5.1 Diseases

A number of diseases have been reported in the literature for switchgrass. Disease pressure will likely increase if large scale production of switchgrass for bioenergy is realized [13]. Rust (*Puccinia emaculata*) has been found on switchgrass in central and eastern South Dakota. Rust symptoms have been more severe on cultivars of northern origin; however, heritability exists for rust resistance [113]. Other diseases reported in a review by Vogel [5] include anthracnose

[*Colletotrichum graminicola* (CES) G.W. Wils; now suggested to be caused by *Colletotricum navitas* J.A. Crouch [114]], smuts [*T. maclaganii* (Berk.) G.P. Clinton], Phoma leaf spot (*Phoma spp.*), and *Fursarium* root rot (*Fusarium spp.*). Trailblazer switchgrass seemed to be more susceptible to anthracnose than Cave-in-Rock in Pennsylvania [115]. Reduced switchgrass biomass and seed yields were attributed to smut in several production fields in Iowa, USA in the late 1990s [116]. Smut was found in 15 of 17 fields surveyed, and the authors estimated that 50–82% of the area in switchgrass production was infested with *T. maclaganii*. Fields with smut incidence >50% yielded less than half of the expected biomass and some seed production fields were a total loss in 1999 [116]. Thomsen et al. [117] estimated yield losses from *T. maclaganii* of 2–40% in switchgrass fields sampled in Iowa, USA, and noted the critical need for research on disease management approaches if switchgrass is to be a successful biomass crop.

#### 4.5.2 Insects

Few insects have been identified as potential pests of switchgrass; however, damage from insects may increase if or when switchgrass monocultures are grown on large production fields. Distribution and symptoms of a stem-boring caterpillar (*Blastobasis repartella* Dietz.) were described by Prasifka et al. [118]. In this survey *B. repartella* was consistently found in cultivated and natural switchgrass stands in eight northern states. In the four northern states (Illinois, Nebraska, South Dakota, and Wisconsin), sampling indicated that 1–7% of tillers were damaged by *B. repartella* [118]. A new species of gall midge [*Chilophaga virgate* Gagne (Diptera: *Cecidomyiidae*)] was recently discovered in South Dakota, USA [119]. Proportion of tillers infested with the gall midge in 10 switchgrass genotypes ranged from 7 to 22%, mass of infested tillers was 35% lower than normal tillers, and infested tillers produced no appreciable seed. Grasshoppers (*Orthoptera*) are known to feed on switchgrass, but the extent of the damage has not been quantified [13].

## 4.5.3 Weeds

In established stands of switchgrass, weed pressure during the second growing season is often worse than in subsequent years if there was poor site occupancy by switchgrass seedlings during the seeding year. In that scenario, a number of weeds can become established during or after the first summer. The problem is often compounded because where site occupancy may not have been high during the seeding year, stand vigor still may be reduced in the second year and, therefore, vulnerability to weed competition may be increased. With adequate weed control during the first two years of a stand, subsequent problems with competition can be limited in switchgrass managed as a biofuel feedstock.

Cool-season annual weeds usually are not a concern in switchgrass unless the infestation is severe. In such cases, use of either a broadleaf formulation (2,4-D, dicamba {3,6-dichloro-2-methoxybenzoic acid}, picloram {4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid}, metsulfuron {2-[[[[(4-methoxy-6-methyl-1,3,5triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoic acid}, sulfosulfuron, or aminopyraild {4-amino-3,6-dichloro-2-pyridinecarboxylic acid} active ingredients) or a broad-spectrum herbicide, such as glyphosate, will provide effective control; where grasses are the concern, the latter is the proper choice. A burn-down chemical, such as paraquat {1,1'-dimethyl-4,4'-bipyridinium}, is also an option in that instance. It is important to apply non-selective herbicides before the switchgrass breaks dormancy to avoid crop injury.

It is important to follow all herbicide regulations, label directions, and safety precautions. Extension staff or professional advisors should be consulted regarding proper use and application of all herbicides.

Cool-season perennial weeds are relatively easy to control in switchgrass because their growing season differs from that of switchgrass. Dormant-season applications of glyphosate can be used to control cool-season grasses. Switchgrass has modest tolerance to glyphosate early in the growing season [120], which allows some flexibility in terms of timing spring treatments. Regardless, it is best to use glyphosate before spring dormancy break or in autumn after switchgrass is fully dormant.

Summer annual weeds are usually not a problem unless the switchgrass stand density is low. Aggressive competitors such as crabgrass or seedling johnsongrass [Sorghum halapense L. (Pers)] usually will not establish and compete after switchgrass has developed a full canopy and is able to overtop these species. However, where grass weeds do persist imazethapyr [for crabgrass and signalgrass (Brachiaria platyphylla (Munro ex C. Wright) Nash)], quinclorac [for foxtails (Setaria species) and (Echinochloa crus-galli (L.) P. Beauv.)], or sulfosulfuron (for johnsongrass) can be useful, but generally must be applied when weeds are small (6–20 cm, depending on the weed species). Warm-season broadleaf weeds may be controlled with the same herbicides used during the cool-season, except that broad-spectrum chemicals should not be used once switchgrass is growing actively.

Low-growing summer perennial weeds often are not able to compete with wellestablished stands of switchgrass. Johnsongrass, on the other hand, can persist in switchgrass stands because of its tall growth habit. Lowland varieties of switchgrass typically will outcompete johnsongrass. Where that does not occur, and control is still necessary, sulfosulfuron can be effective. Perennial warm-season broadleaf weeds can be controlled with the same herbicides that are used to control cool- or warm-season annuals. With perennials, attention must be paid to stage of plant development because it affects application timing and rates.

As with any use of herbicides, attention should be paid to crops that may be planted on the site in the next 12 months because they may be sensitive to some herbicides. Also, there are some scenarios in which producers may want to use some part of the switchgrass crop otherwise intended for biofuels for livestock forage. In such cases, it is important to consider animal feeding restrictions stated on the herbicide label in deciding which product to use for weed control.

Early-season prescribed fire can also be effective for weed control [76]. Switchgrass should not be burned after mid-April, for the southerly and middle latitudes of the USA, so as not to reduce biomass yield [120]. There must be adequate fuel to carry a fire. In some cases, dense stands of early spring weeds, and the large amount of green vegetation they may produce, can hamper burning. Burning earlier (early to mid-March) may be effective before the weed cover becomes too dense. Clipping or mowing may be useful but will reduce yields if it occurs beyond the very early portion of the growing season. If there is a risk of a particularly undesirable weed going to seed or weeds otherwise reducing stand vigor or quality, clipping may be the desirable choice despite potential short-term reduction in yield.

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# Chapter 5 Switchgrass Harvest and Storage

**Rob Mitchell and Marty Schmer** 

**Abstract** The feedstock characteristics of the conversion platform will influence the optimal harvest and post harvest management practices for switchgrass. However, many of the harvest management practices are tied to plant phenology and will be similar across platforms. Proper harvest and storage of switchgrass will help provide a consistent and high-quality feedstock to the biorefinery. Bioenergyspecific switchgrass strains are high-yielding and in most cases can be harvested and baled with commercially available having equipment. Many options are available for packaging switchgrass for storage and transportation, but large round bales or large rectangular bales are the most readily available and are in use on farms. Large round bales tend to have less storage losses than large rectangular bales when stored outside, but rectangular bales tend to be easier to handle and load a truck for transport without road width restrictions. Although there is limited large-scale experience with harvesting and storing switchgrass for bioenergy, extensive research, as well as a history of harvesting hay crops for livestock in many agroecoregions, makes harvesting and preserving switchgrass for bioenergy feasible at the landscape scale.

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# 5.1 Introduction

Switchgrass is not a new crop and switchgrass research is not a new phenomenon. The USDA location in Lincoln, Nebraska, USA has been conducting switchgrass research continually since 1936. Although the first 50 years of research focused on switchgrass for livestock and conservation, the research since 1990 within USDA-ARS, numerous universities, and more recently private industry, has emphasized bioenergy [1, 2]; see also Chap. 1). Although there is limited large-scale experience with harvesting and storing switchgrass for bioenergy, more than 20 years of bioenergy research from small plots to on-farm trials provides experience and critical insights.

Switchgrass is native to the North American tallgrass prairie and is broadly adapted to habitats east of the Rocky Mountains and south of 55°N latitude [3]. Switchgrass plants are generally caespitose or with short rhizomes and reproduce both sexually and asexually. Switchgrass has two primary ecotypes (upland and lowland) and two primary ploidy levels (tetraploid and octoploid) [2]. Switchgrass genotypes are largely self-incompatible and seed production results from cross-pollination by wind [1]. Switchgrass generally grows 1–3 m tall depending on location and genetic background and can develop extensive root systems to a depth of 3 m [1]. The aboveground growth and root structure makes switchgrass well-suited for dual use as a biomass crop and vegetative filter strips which have removed 47–76% of the total reactive P in surface runoff water in areas treated with manure [4].

Morphology and phenological development are important to understand when managing switchgrass for bioenergy. The growth form of both the caespitose and rhizomatous plants is erect with leaf blade length ranging from 10 to 60 cm depending on genotype, environment, and location within the plant [2]. Switchgrass plants tend to be less prone to lodging than other warm-season grasses. Switchgrass is photoperiod sensitive and requires shortening day length for floral induction, which helps explain why switchgrass morphology is strongly correlated to day of the year (DOY) and growing degree days (GDD) [5]. Switchgrass has a determinate growth habit where most vegetative growth terminates with inflorescence development [5, 6], which has implications for regrowth following harvest. Following floral induction, tillers advance to the seed ripening stages, growth stops, and tiller senescence occurs. In switchgrass swards in eastern Nebraska, there were no vegetative tillers present by DOY 196 and 100% of the tillers had elevated apical meristems [7]. Any regrowth following a harvest at or after this stage will occur only from retillering. In eastern Nebraska, sufficient regrowth to warrant a second harvest after a killing frost occurs about one year out of four [8]. For a more complete review of the morphology and tiller dynamics of warm-season grass swards, see Mitchell and Moser [9].

Canopy architecture affects the physiology of growing plants and compositional characteristics of harvested biomass [10] and breeding for increased biomass and digestibility changed the canopy architecture of switchgrass [11]. Canopy

architectural traits such as tiller density, phenology, and leaf area index (LAI) are in a continual state of flux and functions of tiller morphology and the growth stage distribution of tillers within the tiller population [11, 12]. In Trailblazer switchgrass, there was an inverse relationship between advancing phenology and tiller density, with tiller density declining by an average of 9.4 tillers  $m^{-2} d^{-1}$  and an average tiller density of 1,525 tillers  $m^{-2}$  during the 2 year study [7]. Quantifying the phenology of tiller populations provides information for understanding these architectural changes in the grass sward. For example, switchgrass phenology advanced linearly with DOY and GDD across six environments in Nebraska and Kansas [5]. The predictability of switchgrass development in response to DOY and GDD indicates switchgrass management recommendations for adapted cultivars may be made based on DOY within a region [5]. Switchgrass LAI increased as phenology advanced and varied across years with maximum LAI ranging from 4.9 to 7.7, with at least 95% of the variation in LAI explained by DOY [7]. If the selected conversion platform targets feedstock material harvested after senescence, there will be less variability in the phenologic stage of the swards at harvest and may provide a more uniform product to the biorefinery. However, the morphological status during the growing season will have implications for other management decisions.

#### 5.2 Harvest Management

The bioenergy conversion platform likely will determine the optimal harvest and post harvest management practices for switchgrass [2]. However, many of the harvest management practices will be similar for all conversion platforms. Many agroecoregions in the US have a history of harvesting and preserving hay for livestock, so making adjustments to harvesting for bioenergy production will be an easy transition. Due to the extensive research conducted on switchgrass, best management practices and extension guidelines have been developed for many regions [8, 13, 14]. High-yielding switchgrass fields (>12 Mg ha<sup>-1</sup>) can be harvested and baled with commercially available having equipment, but some important items must be considered [8]. For example, self-propelled swathers with rotary heads (disc mowers) will be required to optimize efficiency and handle the volume of material harvested from switchgrass bioenergy production fields [8]. Cutting height is easily adjusted and in most cases will be 10–15 cm, which keeps the windrows elevated above the soil surface to facilitate air movement and more rapid drying to less than 20% moisture content prior to baling [2]. After harvest, switchgrass can be packaged for storage and transportation in large round bales or large rectangular bales [2, 8]. Large round bales tend to have less storage losses than large rectangular bales when stored outside, but rectangular bales tend to be easier to handle and load a truck for transport without road width restrictions [8]. These technologies are in use on farms to harvest and package forages for livestock and are discussed in more detail in later sections.

#### 5.2.1 Timing and Frequency

Maximum biomass yield with high lignocellulose content is the primary objective of most herbaceous bioenergy feedstock harvests [8, 15]. Depending on ecoregion, switchgrass biomass can be maximized with a one-cut or multi-harvest system [13, 16–18]. Most research supports a single annual harvest for optimizing biomass and energy inputs, as well as maintaining stands. For example, Sanderson et al. [17] concluded a single harvest near DOY 260 maximized biomass yield in the southcentral USA. In most rainfed environments of the Great Plains and Midwest USA, maximum first-cut yields and long-term stand maintenance can be achieved by harvesting switchgrass once during the growing season to a 10-cm stubble height when panicles are fully emerged to the post-anthesis stage, near DOY 215 [8, 18, 19]. However, harvesting after frost minimizes nutrient removal, especially N [13]. With upland ecotypes, plant material senesces rapidly and is completely dormant within 7 days of killing frosts. However, lowland ecotypes grown in northern latitudes senesce and enter dormancy slowly after exposure to killing frost. This difference in response by ecotype is illustrated by upland and lowland plants harvested 27 days after the first killing frost and exposed to low temperatures of less than  $0^{\circ}$ C on 17 of the 27 days. The completely dormant material is Shawnee, whereas the material with green stem bases is a lowland strain selected from Kanlow (Fig. 5.1).

This delay in entering dormancy may be one explanation for the winter injury susceptibility of lowland ecotypes. However, harvest strategies for upland and lowland ecotypes have not been compared in agro-ecoregions where both ecotypes occur, so harvest strategies may vary [8]. Proper harvest timing, cutting height and maintaining adequate N fertility are important management practices required to maximize yield and ensure persistent switchgrass stands [2, 8]. As previously mentioned, time of harvest research generally indicates a single harvest at postanthesis maximizes yield, but harvesting after a killing frost ensures stand persistence and productivity, especially during drought [2, 8]. For example, Vogel et al. [18] reported switchgrass biomass increases up to anthesis, then decreases by 10–20% until killed by frost. This fits well with recommendations by Mitchell et al. [8] who reported switchgrass should not be harvested within 6 weeks of the first killing frost or below a 10-cm stubble height to ensure carbohydrate translocation to the plant crowns for setting new tiller buds and maintaining stand productivity.

Wullschleger et al. [20] compiled a database comprised of switchgrass biomass production studies conducted at 39 field sites in 17 states which supported the single harvest for bioenergy. They reported the switchgrass mean biomass yield across all locations was  $8.7 \pm 4.2$  Mg ha<sup>-1</sup> for upland cultivars and  $12.9 \pm 5.9$  Mg ha<sup>-1</sup> for lowland cultivars and the yield difference between ecotypes was significant. Additionally, they reported that there was no evidence that small plots biased switchgrass yield when compared to field-scale sites and stressed the importance of single harvest systems for biomass energy.

Several studies throughout the Great Plains and Midwest have evaluated switchgrass harvest management. Phenologic stage at first harvest did not affect



Fig. 5.1 Upland and lowland ecotypes of switchgrass enter dormancy at different rates when grown in the same environment. Both photographs were taken following field-scale switchgrass harvest in eastern Nebraska on 15 November, 2011. Notice that the upland cultivar **a** 'Shawnee' is completely dormant, whereas the lowland experimental strain **b** still has *green* stem bases (photos by Rob Mitchell)

switchgrass persistence, but regrowth potential decreased as first harvest was delayed to later stages of development and later DOY [21]. Harvesting switchgrass two or three times each year resulted in the greatest stand reductions [22]. Switchgrass harvested once at anthesis in Nebraska and Iowa had greater biomass than areas harvested twice [18]. Biomass was maximized with a single harvest during anthesis and yields ranged from 10.5 to 12.6 Mg ha<sup>-1</sup> yr<sup>-1</sup> with no stand reduction [18]. In Texas, Sanderson et al. [17] harvested several switchgrass strains once or twice per growing season from multiple environments and concluded that a single harvest in autumn maximized biomass and maintained stands.

In general, delaying harvest until after a killing frost reduces yield, but ensures stand productivity and persistence, especially during drought, and reduces N fertilizer requirements for the following year by about 30% [2, 8]. Post-frost harvests allow N and other nutrients to be mobilized into roots for storage during winter and use for new growth the following spring, but will reduce the amount of snow captured during winter and will limit winter wildlife habitat value [8]. Harvesting after a killing frost is a logical management decision for thermo-chemical conversion platforms and biopower because N, Ca, and other plant nutrients that function as contaminants in the thermo-chemical process are minimized in the plant tissue. Another alternative harvest time is to leave switchgrass standing in the field over winter and harvest the following spring [23]. Delaying harvest until spring reduced yield by 20–40% compared with harvesting in autumn after a killing frost, but had no effect on gasification energy yield [23]. Yield losses associated with delaying harvest until spring may be acceptable if wildlife cover during winter is critical [23].

These studies from a broad geographic range in the USA support a single annual harvest will maximize biomass and maintain stand persistence, but harvest timing needs to be considered for optimizing biofuel production. Additionally, the conversion process is an important consideration when determining the optimum harvest date. With good harvest and fertility management, productive stands can be maintained indefinitely and certainly for more than 10 years [8].

#### 5.2.2 Nutrient Removal

Harvesting biomass, whether crop residue or dedicated herbaceous perennial feedstocks such as switchgrass, removes large quantities of nutrients from the system [19]. In most agro-ecoregions, nitrogen (N) is the most limiting nutrient for switchgrass production and is the most expensive annual production input. Consequently, reducing N removal from the switchgrass production system has a positive effect on the economic and environmental sustainability of the system. For example, harvesting 10 Mg ha<sup>-1</sup> of switchgrass DM with whole-plant N concentration of 1% will remove 100 kg of N ha<sup>-1</sup>, whereas if harvest is delayed until after senescence, N concentration can decline to 0.6%, resulting in the removal of only 60 kg of N ha<sup>-1</sup>. Depending on conversion platform and a predictable harvest window in autumn or winter, this 40 kg of N ha<sup>-1</sup> reduction in N removal may be an acceptable trade-off for the yield losses associated with delaying harvest.

Collins et al. [24] reported the average yield for three switchgrass cultivars irrigated in the Pacific Northwest ranged from 14.5 to 20.4 Mg dry matter ha<sup>-1</sup> yr<sup>-1</sup>. They reported each kg of N produced 83 kg of biomass and the macronutrient export averaged 214 kg N ha<sup>-1</sup>, 40 kg P ha<sup>-1</sup>, 350 kg K ha<sup>-1</sup>, 15 kg S ha<sup>-1</sup>, 60 kg Ca ha<sup>-1</sup>, 38 kg Mg ha<sup>-1</sup>, and 6 kg Fe ha<sup>-1</sup>. Averaged across cultivars, switchgrass removed less than 1 kg ha<sup>-1</sup> of B, Mn, Cu, and Zn. Additionally, delaying harvest until spring reduced ash content and leached nutrients from the vegetation [23]. Although management of all nutrients in the system is important, N is the most expensive, has the greatest potential for environmental contamination, and has the greatest influence on life cycle assessment.

Nitrogen removal in switchgrass production systems is a function of biomass yield and N concentration, with biomass N concentration increasing as N fertilization rates increase [18]. In a multi-environment study evaluating numerous N rates and harvest dates, biomass was optimized when switchgrass was harvested at the boot to post-anthesis stage and fertilized with 120 kg N ha<sup>-1</sup> [18]. At this harvest date and fertility level, the amount of N removed at harvest was similar to the amount of N applied, and soil NO<sub>3</sub>-N did not increase throughout the study [18]. Consequently, it is important to consider the interaction of N rate and harvest date to only replace the N needed for the production system to prevent overfertilization and soil N accumulation.

#### 5.2.3 Soil Carbon

As mentioned previously, switchgrass has an extensive perennial root system which protects soil from erosion and sequesters carbon (C) in the soil profile [25]. Soil organic carbon (SOC) typically increases rapidly when annual cropland is converted to switchgrass [26, 27]. The amount of C sequestered depends on the climate, soil type, original soil C content, time, and placement depth of C [28, 29].

For example, switchgrass grown and managed for bioenergy on three marginally productive cropland sites in Nebraska resulted in an average SOC increase of 2.9 Mg C ha<sup>-1</sup> yr<sup>-1</sup> in the top 1.2 m of soil in just 5 years [30]. In South Dakota, switchgrass grown in former cropland enrolled in CRP stored SOC at a rate of 2.4–4.0 Mg ha<sup>-1</sup> yr<sup>-1</sup> at the 0–90 cm depth [31]. McLaughlin et al. [32] reported an average of 1.7 Mg C ha<sup>-1</sup> yr<sup>-1</sup> sequestered in the Southeast U.S. on switchgrass experimental plots. Soil carbon levels on low-input switchgrass fields have been shown to increase over time, across soil depths, and are higher than adjacent cropland fields in the Northern Plains [25]. A similar result was found between switchgrass and a corn (Zea mays L.)-soybean (Glycine max (L.) Merr.)-alfalfa (Medicago sativa L.) rotational system in Iowa [33]. Switchgrass managed for bioenergy on multiple soil types in the Northern Plains was carbon-negative, sequestering 4.42 Mg C ha<sup>-1</sup> yr<sup>-1</sup> into the soil profile [34]. In the Southeast U.S.A., an estimated 0.17–0.21 Mg C ha<sup>-1</sup> yr<sup>-1</sup> was sequestered on switchgrass plots, managed as a bioenergy crop, based on SOC that was near steady state [35]. Nitrogen applications on switchgrass plots did not alter root C storage when compared with non-fertilized plots in a 2 year study [36]. However, fertilization of grasslands increased the amount of C sequestered by  $0.30 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  on 42 studies throughout the world [28]. Microbial biomass carbon increased after establishment of switchgrass and carbon mineralization increased by 112 and 254% at depths of 0-0.15 m and 0.15-0.30 m, respectively [36]. Soil organic C increased at rates ranging from 1.7 to 10.1 Mg C ha<sup>-1</sup> yr<sup>-1</sup> after switchgrass establishment throughout North America [31, 34, 35, 37].

### 5.3 Storage Management

Substantial amounts of switchgrass biomass will need to be safely stored on a year-around basis to supply a cellulosic biorefinery. Cellulosic biorefineries in the U.S. are expected to keep only a 72 h feedstock inventory with the remaining feedstock inventory at the edge of field or at satellite storage facilities [38]. At present, there is uncertainty on the overall capacity of cellulosic biorefineries but techno-economic models have evaluated refinery sizes ranging from 535 to 8,000 dry Mg feedstock per day [39–41]. Offsite storage management will be critical to maintain desirable composition characteristics and to ensure feedstock access under variable weather conditions. Storage infrastructure requirements will need to be cost effective, maintain desirable quality characteristics depending on conversion technology, provide an aerobically stable environment, and have flexible delivery schedules depending on regional weather factors [42].

Storage requirements and management will be dependent on how switchgrass is harvested. In the near-term switchgrass will be harvested and baled using commercial hay equipment. Self-propelled mower/conditioners (swather) with rotary heads are effective in harvesting high-yielding (>12 Mg ha<sup>-1</sup>) switchgrass fields (U.S. [43]). The conditioner component on a swather accelerates switchgrass

drying by crushing plant stems but not altering plant structure and consolidates the switchgrass into a windrow [38]. After harvest, the baling step bundles switchgrass into a more condensed form to ease handling, transport and storage. Variable chamber round balers or rectangular balers will likely be used to consolidate and bundle switchgrass. Round balers will typically make a bale that is 1.2–1.8 m in diameter and 1.2–1.8 m in length. Large rectangular bale size ranges from 0.9 to 1.2 m in height and width and 1.8–2.4 m in length. Round balers and large rectangular balers require switchgrass moisture levels to be  $\leq 18$  and  $\leq 16\%$ , respectively, at time of baling to reduce storage losses. Bale moisture content in excess of these respective values may result in composition degradation or spontaneous combustion. Field drying prior to baling is required to meet safe moisture levels for baling which may be hindered depending on the region and harvest date. Balers can be modified to spray preservatives (e.g. propionic acid) onto hay limiting microbial growth and removing excess moisture for hay with 20–25% moisture content [44].

The density of a round bale or a large rectangular bale will vary depending on harvest period with anthesis harvest bales having a greater density than post-killing frost harvest bales. There are advantages and disadvantages for the round baling or large rectangular baling methods but both are capable of processing switchgrass and are commercially available to producers. The round baler is one-fourth to one-third the capital cost as a large rectangular baler [45] but the field capacity of a round baler is lower because the baler needs to be stopped to wrap and release the bale. Large rectangular balers continuously bales without the need for stopping and is estimated to cost less per unit of harvested area [46]. Smaller bioenergy producers may opt for the round baler is custom harvesting enterprises that are equipped with large rectangular balers. Rectangular bales need to be removed from the field soon after baling and protected from precipitation events because the flat surface of the bale does not shed water and resultant DM losses can be large [44].

Commercially available self-propelled or pull-type round or rectangular bale stacking equipment collect bales within the field and are able to place bales at the edge of field for short-term or long-term storage until feedstock delivery to a biorefinery. These stacking systems significantly lower energy use and increase field capacity efficiency when compared with a single bale loader system. Switchgrass round bales have less storage losses than large rectangular bales when stored outside as they are less prone to water penetration especially when net wrapped [38]. Net wrapped round bales had 60–70% lower DM losses when compared with round bales tied with plastic twine [47]. Rectangular bales tend to be easier to handle and load a truck for transport without road width restrictions. The time required to load bales onto semi trailers is double for round bales than it is for rectangular bales [38]. Unless cellulosic biorefineries stipulate a certain baling method or alternative harvest method, both baling methods will likely occur for a given region.

Consolidation methods other than baling may be implemented in regions where weather conditions or existing infrastructure enterprises allow for alternative harvesting scenarios [19, 48–51]. Wet storage methods have been proposed for switchgrass in regions where drying conditions for baling operations are not possible because of high relative humidity and increased chance of a precipitation event after harvest [50]. Switchgrass harvested using wet storage methods include either a swather harvest and then chopped using a self-propelled forage harvester with a windrow pickup or directly cut with a self-propelled forage harvester with an attached rotary head that blows the material into adjacent semi bulk trailers.

Moisture content for switchgrass at time of pickup under wet storage methods are >40%. Advantages to wet storage methods include reduced harvest costs, lower DM losses during storage, improved switchgrass cell wall recovery during enzymatic hydrolysis and lower potential risk of fire during storage [50]. Disadvantages for the wet storage method include higher equipment and storage structure costs than a conventional baling system [44]. The wet storage method was found to be more expensive than other collection and storage methods for cellulosic refinery sizes greater than 1,500 Mg switchgrass per day because of the high cost of the ensiling pit and transportation of wet material by truck [51].

Regions where silage harvesting is common would likely have increased participation in storing switchgrass under wet conditions. Field chopping using a forage harvester can be done at moisture levels similar to baling in less humid regions. Field chopping has an added advantage to baling in that particle size is much smaller which may eliminate a preprocessing step at the biorefinery [19]. Estimated delivery costs for chopped switchgrass biomass are less than for a conventional baling system [51]. Chopped biomass requires specific storage areas either at farm site or at a satellite storage facility. Chopped biomass has the lowest bulk density and densification may be an issue for long-term storing and transporting the material [19]. Southeastern U.S. researchers have proposed increased densification of chopped switchgrass by using modulizing technology developed for the cotton industry [48, 49].

A loafer stacker system has also been proposed as a cost effective method to collect switchgrass for biomass production [52]. The loafing system is similar to the field chopping system (dry storage) with the exception that instead of blowing switchgrass material into a semi trailer the loaf stacker picks up switchgrass from the windrow and makes a biomass stack approximately 2.4 m wide, 6 m long, and 3.6 m high [51]. The roof of the loafer stacker has a dome shape which creates a biomass stack that resembles a bread loaf and is designed to shed water. Field capacity of a loafer stacker is lower than either conventional baling system or a forage chopper. Once the loafer stacker is full, the operator needs to immediately transport the biomass stack after harvest. Biomass stacks are also susceptible to large biomass loss in regions with significant wind velocities if placed perpendicular to prominent wind direction.

The U.S. Department of Energy has proposed a uniform-stacking feedstock supply design that can pre-process switchgrass and other cellulosic materials regardless of collection method for use in a large-scale cellulosic biorefinery ( $\geq$ 4,535 Mg d<sup>-1</sup>) which would increase regional and producer flexibility to harvest and collect switchgrass [38].

#### 5.3.1 Desirable Storage Characteristics

The ideal storage management procedures are to preserve switchgrass so that it enters and leaves the storage phase in an unaltered state [53]. Key factors in minimizing storage loss for bales are to ensure low moisture levels prior to storage and protection from moisture during the storage phase. Low relative humidity and low ambient temperatures during storage also reduce DM loss and composition degradation. Maintaining low biological activity during storage to reduce microbial growth and subsequent storage loss is also important.

Switchgrass with higher levels of N or with increased soluble sugars have increased potential for microbial growth and degradation during bale storage [38]. Harvest dates determine overall N and soluble sugar content in switchgrass [54]. Storage conditions that reduce the potential for spreading crop diseases, low rodent populations, and mold spore formation are also desirable [38].

# 5.3.2 Storage Platforms

Although there is limited research on switchgrass storage platforms for specific bioenergy purposes, there is significant storage research on forages that offer insights into the advantages and disadvantage of different storage options. Near-term storage strategies include placing bales outside on well-drained surfaces (i.e. gravel, crushed rock), tarping, bale wrapping in plastic and indoor placement. Optimal storage platforms are dependent on expected bale storage losses and projected storage costs to offset these losses. For example, enclosed buildings are the most expensive storage platform but also ensure the greatest switchgrass value and lowest storage loss [55]. Proper storage of wet material or ensiling has been well documented for a number of feedstocks including switchgrass [38, 44, 56]. Pre-processing steps such as pelletizing or briquetting switchgrass provides decreased storage losses and decreased transportation costs [57]. Estimated capital costs for a pellet mill or briquettor, however, potentially offset any near-term savings in storage or transportation costs [41].

#### 5.3.3 Storage Losses

Limited research has been conducted on DM losses during switchgrass storage with most research evaluating storage loss using the baling method. In Texas, DM losses for large, round bales ranged from 1 to 5%, with larger losses occurring with drier material [58]. Switchgrass round bales stored for 6–12 months inside had 0-2% DM losses, whereas bales stored outside lost 5–13% of the original bale weight [58]. In southeastern U.S., round bales with higher initial moisture content

and longer storage times caused increased DM loss when stored outside [59]. In Indiana, switchgrass round bales wrapped in twine had 13% DM loss on sod but bales stored on crushed rock had 5% DM loss after six months [60]. Switchgrass round bales stored outside on either sod or gravel showed similar DM losses 12 months after baling in Texas [58]. Estimated DM storage losses in excess of 16% are required to cover the initial cost of storage sites using crushed rock for improved drainage [38]. In southern Europe, switchgrass round and rectangular bales showed minimal storage loss and no visible microbial activity when stored under a sheltered roof [61]. Storage loss was found to be greater for tarped large rectangular bales than for tarped round bales and that delivery costs increased with larger storage times due to increased storage losses [62]. Tarped and untarped large rectangular bales had DM losses of 7% and up to 25%, respectively, six months after harvest in Nebraska [8]. Water and temperature together determines microbial damage for storage systems with regions having high relative humidity and having temperatures results in increased storage degradation on portions of biomass in direct contact with air [38]. In general, biomass stored dry should be kept at moisture levels below 15% to prevent biomass degradation by filamentous fungi and bacteria [63]. Additional physical factors that cause storage loses include wind erosion or handling losses, moisture partitioning, bulk settling, and dust accumulation [38].

# 5.3.4 Changes in Composition During Storage

Composition changes during storage will likely be more unfavorable for biochemical conversion than either thermo-chemical conversion technology or direct combustion for electrical generation. Switchgrass round bales stored unprotected outside lost up to 11% of ethanol extractables, which could significantly reduce conversion to ethanol [64]. Biomass quality heterogeneity will occur within bales with portions of the bale showing no signs of degradation while other portions having significant spoilage or composition degradation. Round bales can be segmented to four portions based on the potential for deterioration [65].

Approximately 33% of a round bale circumference contacts the ground after settling which can absorb moisture and result in spoilage [38]. The first round bale portion is where the round bale contacts the ground up to 15 cm. A transitional area above this portion (15–30 cm) can also be degraded depending on moisture conditions and length of storage. Sanderson et al. [58] noted that switchgrass round bales stored on sod had a large, black layer where the bale was in contact with sod whereas round bales stored on gravel did not have this layer of spoilage indicating outside storage method will influence composition heterogeneity of bales. The third portion of the round bale is the outer 15 cm of the round bale not in contact with the ground. This portion can also have compositional changes depending on weather factors, length of storage, and wrapping methods (i.e. plastic twine, plastic net-wrap). The final portion of the round bale is the core which is the least likely to



Fig. 5.2 Large rectangular bales are susceptible to spoiling on the *top* and *bottom* of the bale if not stored properly. This bale was cut in half to expose the spoilage on the bale interior (photo by Rob Mitchell)

have compositional changes during storage. Biomass degradation from weather and microbial activity can be as high as 42% by volume for a round bale [65]. For large rectangular bales, moisture can penetrate at the top of the bale or can be absorbed at the bottom of the bale. A large rectangular bale is comprised of a number of layers of switchgrass compressed together. Water channeling can occur within these layers causing heterogeneous spoilage ([38]; Fig. 5.2).

Large rectangular bales are typically stacked so water channels between layers can cause biomass degradation to adjacent bales as well. Chopped switchgrass in a dry form will have the most compositional changes around the outer surface layer (0–0.8 m) with the interior portions unaltered. The amount of compositional changes by volume from dry chopped switchgrass piles is a result of the overall stack size. Sulfuric acid pretreatment on switchgrass stored under wet conditions inhibited microbial activity and resulted in 7% higher ethanol conversion efficiency than untreated switchgrass [50].

## 5.4 Conclusions

This brief overview has scratched the surface of switchgrass harvest and storage management. Proper harvest and storage management is paramount to providing a consistent and high-quality feedstock to the biorefinery. Although the bioenergy conversion platform will guide the switchgrass harvest and post harvest management practices, proper handling will ensure optimum biofuel recovery. Continued research on the effects of harvest and storage management on feedstock characteristics is critical as landscape scale deployment of switchgrass for bioenergy moves forward. Important areas for continued research include the effects of compositional changes during storage on biofuel production; harvest timing effects on ecosystem services, especially SOC sequestration, wildlife, and

pollinator habitat, and GHG emissions and mitigation; and long-term research evaluating harvesting effects on macronutrient and micronutrient removal, as well as developing strategies for maintaining soil nutrient status.

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# **Chapter 6 Environmental Impacts of Switchgrass Management for Bioenergy Production**

R. Howard Skinner, Walter Zegada-Lizarazu and John P. Schmidt

**Abstract** In this chapter, we review major environmental impacts of growing switchgrass as a bioenergy crop, including effects on carbon sequestration, greenhouse gas emissions, soil erosion, nutrient leaching, and runoff. Information from life cycle analyses, including the effects of indirect land use change (iLUC), is examined to quantify the full impact of migration to bioenergy cropping systems on both managed and natural ecosystems. Information on the environmental impacts of switchgrass cultivation is scarce and there exists a critical need for additional research. What limited information there is suggests that switchgrass provides multiple environmental benefits compared to annual crop cultivation. However, benefits generally appear to be similar to other perennial crops.

# 6.1 Introduction

An evaluation of the environmental impacts of switchgrass (*Panicum virgatum* L.) depends on contrasts to alternative crop species or cropping systems that switchgrass will potentially displace or to which switchgrass might be preferred.

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Pioneer Hi-Bred International, Inc., A Dupont Business, Champaign Research Center, 985 County Road 300 E, Ivesdale, IL 61851, USA e-mail: john.schmidt@pioneer.com In the Midwestern United States, an area dominated by maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] production, an important contrast will be based on impacts relative to these crops. Alternative perennial crops could be various cool-season ( $C_3$ ) grasses, forage legumes, or another  $C_4$  grass, *Miscanthus x giganteus* Greef & Deuter ex Hodkinson & Renvoize (hereafter referred to as *miscanthus*). The most relevant contrasts are those that represent realistic alternatives. If switchgrass is grown as a bioenergy crop, it will likely compete for a place on the landscape with maize, soybean, cool-season perennials, and *miscanthus*.

Switchgrass was selected as a model bioenergy crop in the U.S. because it is a native plant, produces substantial above-ground biomass (20 Mg  $ha^{-1} yr^{-1}$ ), and has an extensive areal range in North America. The development of this selection is described by Wright and Turhollow [1]. Another bioenergy crop, miscanthus, offers a particularly interesting alternative to switchgrass. *Miscanthus* has been the focus of bioenergy research in Europe because it produces as much as 40 Mg ha<sup>-1</sup> yr<sup>-1</sup> above-ground biomass with similar or fewer N fertilizer inputs as switchgrass [2]. Both *miscanthus* and switchgrass are desirable bioenergy crops because: (1) biomass yield is high; (2) they are perennial rhizomatous plants that cycle nutrients seasonally between the above- and below-ground portions of the plant, thus minimizing fertilizer requirements and corresponding environmental impacts; (3) they provide a clean burning fuel when they are harvested after senescence; (4) planting is required only once, so minimal fuel costs associated with tillage and planting are incurred; and (5) they are both  $C_4$  plants, which are photosynthetically more efficient than  $C_3$  species [2]. A potential advantage of switchgrass over *miscanthus* is that it is reproduced by seeds, whereas *miscanthus* reproduction is vegetative with accompanying higher establishment costs and need for specialized equipment.

In this chapter, we review major environmental impacts of growing switchgrass as a bioenergy crop including effects on carbon sequestration, greenhouse gas emissions, soil erosion, nutrient leaching, and runoff. Where available, information from life cycle analyses, including the effects of indirect land use change (iLUC), will be examined to quantify the full impact of bioenergy crops on both managed and natural ecosystems.

#### 6.2 Climate Change

# 6.2.1 Carbon Sequestration

One important impact of growing switchgrass or other bioenergy crops will be the potential for C sequestration or loss of C from the soil. The number of studies looking at C sequestration in switchgrass stands is limited, but those that exist have shown that replacing annual crops with perennials such as switchgrass increases C sequestration. In an analysis of published estimates of soil organic carbon (SOC)

changes following conversion of natural or agricultural lands to biofuel crops, Anderson et al. [3] found that removing maize grain and residue as a bioenergy feedstock led to rapid loss of SOC at rates up to 4.2 Mg ha<sup>-1</sup> yr<sup>-1</sup> compared with management for grain removal only. They calculated that 10 years of maize biomass removal resulted in a loss of about 3 Mg ha<sup>-1</sup> yr<sup>-1</sup> at 25% residue removal and about 8 Mg ha<sup>-1</sup> yr<sup>-1</sup> at 100% removal. In contrast, SOC accumulation under switchgrass ranged from about 0.4 to 0.7 Mg ha<sup>-1</sup> yr<sup>-1</sup> depending on the statistical model used to estimate changes.

Anderson et al. [3] reviewed the existing literature and identified three general principles governing the C balance of biofuel crops. First, conversion of uncultivated soil to biofuel crops initially entails a loss of SOC; second, crops differ in the ability to sequester C with perennial crops outperforming annuals such as maize; and third, a tradeoff exists between biomass removal and C sequestration. In another review of the literature, Blanco-Canqui [4] also concluded that residue removal reduced SOC concentration, whereas, planting warm-season grasses such as switchgrass increased C sequestration.

In a paired comparison of 120 cm deep soil samples from 42 switchgrass/ cropland sites, Liebig et al. [5] found that SOC was greater in switchgrass stands at soil depths of 0–5, 30–60, and 60–90 cm and that the differences in SOC were especially pronounced at deeper soil depths. They attributed the difference at depth to the greater switchgrass root biomass below 30 cm compared with cropland sites. In a modeling study, simulations with the DAYCENT model [6] predicted an increase in SOC of 45–300% after 15 years of switchgrass growth compared to cotton production. However, another modeling exercise using DAYCENT suggested that maize had slightly greater SOC than switchgrass [7] and that little change in SOC was predicted to occur over a 10 year period for either cropping system.

In contrast to the studies cited above, Tolbert et al. [8] found that both no-till maize and switchgrass had accumulated SOC after three growing seasons, with no significant difference in accumulation rate between the two. However, SOC was numerically greater under switchgrass even though differences were not significant. In a Canadian study [9] switchgrass increased SOC by 3 Mg ha<sup>-1</sup> yr<sup>-1</sup> compared with maize at a higher fertility site, whereas, there was no significant difference after 4 years between switchgrass and maize on a rocky shallow-soil.

Because SOC content changes relatively slowly against a large background pool, long-term studies are often needed before differences in accumulation rates are translated into significant differences in the magnitude of SOC pools. Most of the switchgrass studies cited here, and reviewed by Anderson et al. [3] lasted less than 5 years, highlighting the critical need for more long-term studies.

Comparisons between switchgrass C sequestration and other perennial systems generally revealed little difference between systems, or even lower C sequestration potential for switchgrass sites. Non-significant differences in SOC or C sequestration rates were observed when switchgrass was compared with forests or cool-season grasses [10], smooth bromegrass (*Bromus inermis* Leyss.) [11], or short-rotation poplar plantations [12]. When Chamberlain et al. [6] simulated land use conversion

from unmanaged grasses to switchgrass, SOC decreased if the switchgrass was not fertilized, was unchanged when 45 kg N ha<sup>-1</sup> was applied, and increased when 90 and 135 kg N ha<sup>-1</sup> were applied. Omonode and Vyn [13] observed little difference in surface SOC content between switchgrass and mixed native warm-season grasses but when SOC mass was calculated to a depth of 1.0 m, SOC was 8% higher under switchgrass than under the native mixture.

Two modeling studies have suggested that C sequestration would be less for switchgrass compared with other perennial species. Growing willow (*Salix alba x glatfelteri* L.) was calculated to increase SOC by 9.0–9.5 Mg ha<sup>-1</sup> yr<sup>-1</sup> compared with 3.0–3.5 Mg ha<sup>-1</sup> yr<sup>-1</sup> for switchgrass [9]. Davis et al. [7] estimated that SOC under switchgrass would be similar to native prairie but that both would be significantly less than *miscanthus*. While switchgrass appears to have greater C sequestration potential than annual crops, including maize grown as a biofuel, there is no indication that it has any greater C sequestration potential than other perennial systems.

The potential to sequester C depends on a number of factors including initial soil C content, prevailing soils and climate, and management practices. Sequestration is generally greater when existing SOC pools have been depleted, in cool compared with warm climates, in fine-textured compared with course-textured soils, and where soil fertility is high [14]. Perhaps the most widely studied variable is the effect of N fertility. In a switchgrass study in the southern USA, Ma et al. [15] found no difference in SOC among N application rates of 0, 112, and 224 kg N ha<sup>-1</sup>. They attributed the lack of N effect to the short, 3 year, duration of the study. However, in a study of similar duration in the northern Great Plains, Lee et al. [16, 17] applied 112 or 224 kg N ha<sup>-1</sup> as either ammonium nitrate or as manure to mature switchgrass stands. Applying N as manure increased SOC accumulation rate to a depth of 90 cm by 33-125% compared with mineral fertilizer, probably because of the additional C input from the manure. All fertilizer rates and sources increased C sequestration compared with no fertilization but there was no difference between the 112 and 224 kg N ha<sup>-1</sup> application rates. Averaged across N rates, C sequestration was 2.4 Mg  $ha^{-1}$  yr<sup>-1</sup> for plots receiving mineral fertilizer compared with 4.0 Mg  $ha^{-1}$  yr<sup>-1</sup> when manure was applied.

In the simplest terms, C sequestration represents the net balance between C inputs into the system, mainly from photosynthesis but potentially from application of organic sources such as manure or crop residues, and C outputs, mostly from soil and plant respiration but also from removal of harvested material and potentially from runoff or leaching. An assessment of CO<sub>2</sub> fluxes during the first 4 years of switch-grass establishment by Skinner and Adler [18] found that photosynthetic inputs varied little from year to year, ranging from 9.2 to 9.4 Mg-C ha<sup>-1</sup> yr<sup>-1</sup>. In contrast, ecosystem respiration ranged from 6.8 to 9.1 Mg-C ha<sup>-1</sup> yr<sup>-1</sup>, and harvested biomass removal ranged from 0 to 2.4 Mg-C ha<sup>-1</sup> yr<sup>-1</sup>. Mean C sequestration over the 4 years was 0.4 Mg-C ha<sup>-1</sup> yr<sup>-1</sup>, and clearly depended more on processes affecting C loss than on C uptake. Similar dependence of sequestration on C loss rather than uptake were observed for cool-season pastures in the northeastern U.S. [19] and for forests along a north–south transect in Europe [20].

Several factors have been found to affect the C dynamics of switchgrass systems. Stepwise regression analysis by Lee et al. [16, 17] found that soil temperature was highly correlated with soil  $CO_2$  flux, whereas, soil moisture was not. Garten and Wullschleger [21] also observed slower decomposition rates in cooler climates. In contrast to the results from Lee et al. [16, 17], Frank et al. [22] measured lower soil  $CO_2$  flux during a drought year compared to  $CO_2$  fluxes during 2 years of above average precipitation.

Lee et al. [16, 17] also found that manure application increased soil respiration but ammonium nitrate application did not. The manure effects were due to increased soil microbial biomass C and potentially mineralizable C. Soil texture may also exert some control over dynamic soil C fractions such as microbial biomass C and thus affect soil respiration. In turn, microbial biomass C will be affected by C input from roots coupled with the influences of soil moisture and temperature [23]. Ma et al. [23] also found that harvest frequency affected soil respiration and attributed the results to the effect of harvest frequency on root lifespan.

Establishment of any new crop, including switchgrass, usually entails an initial loss of soil C, incurring a "carbon debt" that must be repaid before net C sequestration can occur. Corre et al. [10] reported that conversion from cool-season grass to switchgrass initially resulted in a loss of SOC, and that it took 16–18 years after planting for SOC under switchgrass to approach that under the undisturbed cool-season grass. It has been suggested that growing perennial grasses on former cropland soils might result in little or no carbon debt, whereas, replacing uncultivated land could incur a debt that might require decades or even centuries to repay [3, 24]. Whatever the magnitude of the debt, it is important that initial C losses be considered when evaluating the C sequestration potential when replacing existing vegetation with switchgrass or other bioenergy crops.

#### 6.2.2 Nitrous Oxide

Among carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O), the three primary greenhouse gases associated with agricultural production systems, the latter has the greatest global warming potential. Although N<sub>2</sub>O is found at lower atmospheric concentrations, its global warming potential can be as high as 296 times that of CO<sub>2</sub> over a 100 year period [25]. In nature, soil and oceans are sources of N<sub>2</sub>O, where it is produced by microbial processes of nitrification and denitrification. Nitrification is the aerobic oxidation of ammonium to nitrate, and denitrification is the anaerobic reduction of nitrate to nitrogen gas. N<sub>2</sub>O is a gaseous intermediate in the reaction sequence of denitrification and a minor by-product of nitrification [26].

Almost all agricultural systems are a significant source of direct (from agricultural lands) and indirect (from volatilization/deposition and leaching/runoff)  $N_2O$  emissions through the application of N fertilizers and animal manures.

In general annual crops produce about three times more emissions than unmanaged successional lands and perennial crops such as poplar [27]. According to these authors, the major determinant of  $N_2O$  emissions is the amount of nitrogen available in the soil. However, Stehfest and Bouwman [28] indicate that nitrogen fertilization rate, crop type, fertilizer type, soil organic content, soil pH, and texture also play an important role in controlling the activity of nitrifiers and denitrifiers and thus the  $N_2O$  emissions from agricultural fields. Therefore, cropping systems and crops such as switchgrass with lower nutrient demands or more efficient utilization of fertilizer inputs are likely to have a greater potential to reduce  $N_2O$  emissions and be more profitable to the farmer.

In general, the well developed root systems of grasses like switchgrass have a great capacity for N uptake and large amounts of inorganic N seldom accumulate in soils where they are grown [29]. Moreover, Bransby et al. [30] indicated that switchgrass has the ability to recover about 66% of applied N, which is about 16% higher than an established standard of wheat (*Triticum aestivum* L.) or maize, confirming its high potential to reduce GHG emissions compared to annual crops. Moreover, a comprehensive review of switchgrass and *miscanthus* agronomy indicated that switchgrass has a stronger response to N fertilization than *miscanthus* [2]. This higher response to fertilization could be one of the reasons for the 75% lower N<sub>2</sub>O emission from switchgrass than *miscanthus* as reported by Zeri et al. [31] in one of the few side-by-side comparisons of N<sub>2</sub>O fluxes from these grasses.

The fertilization rates for switchgrass vary widely; several authors indicated that economically and energetically viable yields can be obtained with 0–100 kg ha<sup>-1</sup> of N fertilization, depending on site-specific soil conditions, water availability, and crop management [32–34]. Moreover, for optimum biomass yields, Vogel [35] indicated that switchgrass requires between 10 and 12 kg N ha<sup>-1</sup> for each Mg ha<sup>-1</sup> of biomass produced.

Since Bransby et al. [30] showed evidence that the N recovery capacity of switchgrass does not change with varieties or harvesting times but only with the yield levels, it is assumed that N<sub>2</sub>O emission could be decreased by increasing switchgrass productivity. However, due to the sometimes quadratic [36] dependency between increased fertilization and yields, the effective potential to reduce GHG emission can be counteracted by N<sub>2</sub>O emissions that may or may not be proportional with the amount of nitrogen fertilization. For example, Heggenstaller et al. [36] indicated that total plant N content with fertilization rates of 220 kg N ha<sup>-1</sup> was less than with 140 kg N ha<sup>-1</sup>, indicating that at higher fertilization rates more N remained in the soil with a greater potential for N loss from the system. The N loss was probably by a combination of volatilization, denitrification, and/or leaching. However, because information is lacking on the relative contribution of each N loss pathway, an exact determination of the greater N<sub>2</sub>O emission potential at the higher fertilization rate is difficult.

Actual quantification of  $N_2O$  emissions from switchgrass fields is almost nonexistent, not only because installing the measuring chambers is costly, but also because of the high spatial and temporal variability in  $N_2O$  fluxes [25]. Some studies have shown that the highest N<sub>2</sub>O fluxes occur just after N fertilizer application and/or after large rainfall events [37, 38], making it difficult for spot measurements with small chambers to be representative of total GHG emissions. Therefore, most of the emission values reported in the literature are estimated based on emission factors and calculation guidelines developed by the IPCC [26] and life cycle analysis (LCA) studies such as Qin et al. [39], Adler et al. [40], Crutzen et al. [41], among others. Discrepancies and uncertainties between reported emissions depend on how they were calculated and expressed in the respective LCAs.

Adoption of fertilizer best management practices is one strategy that could reduce  $N_2O$  emissions by 30–40% [42]. The appropriate amount, timing, and placement of fertilizers are examples of best management practices [30, 37, 42, 43], but the particular response of switchgrass to such practices depend on climatic, management, and mycorrhizal symbiotic relations [35].

Other agronomic practices such as intercropping with legumes contribute to reduce emissions, although the decomposition of organic residues may contribute to postharvest  $N_2O$  emissions. In any case, the limited available results suggest that switchgrass, when compared to other crops, is particularly good at mitigating the soil  $N_2O$  emissions associated with N fertilizer applications. However, more studies based on actual measurements of  $N_2O$  fluxes are needed to confirm or provide more precise emission factors to be used in LCA and other studies because the general figure that 70% of GHG emission from agricultural activities comes from  $N_2O$  emissions seems to be an underestimation [41, 44]. If  $N_2O$  emissions are higher than the IPCC estimations, its mitigation will become a priority or at least of equal importance as C sequestration [45].

#### 6.2.3 Methane

CH<sub>4</sub> is a greenhouse gas with global warming potential equivalent to 21 times that of CO<sub>2</sub> [46]. Lately, its atmospheric concentration has increased significantly mainly due to agricultural activities and the use of fossil fuels [44]. Soils can act as sources or sinks for CH<sub>4</sub>, depending on land use and climatic conditions [25, 46, 47]. Soil temperature, moisture, pH, and soil N status are factors affecting the capacity of a soil to act as a CH<sub>4</sub> sink [48]. Moreover, forest soils and grasslands are net consumers of CH<sub>4</sub> and have a greater sink potential than cultivated soils, as agronomic and fertilization practices reduce the sink potential of the soil [46–50]. For example, Mosier et al. [51] indicated that annual fertilization increases N<sub>2</sub>O fluxes and at the same time decreases CH<sub>4</sub> uptake in the soil by 41%.

In mid and late unmanaged successional forests,  $N_2O$  emissions were almost completely offset by  $CH_4$  oxidation [27]. Moreover, in unfertilized and undisturbed grasslands  $CH_4$  uptake was 1.4 and 2 times higher than that in fallow lands and cultivated wheat fields [51]. Since switchgrass is a typical perennial grass with low fertilization and tillage requirements,  $CH_4$  emissions from this crop may be

Source of emission by activity	Emissions (kg $ha^{-1}$ )	
	N <sub>2</sub> O	CH <sub>4</sub>
Land preparation	2.22E-4	1.23E-2
Crop growth	1.11E-3	5.93E-2
Crop harvest	2.72E-3	1.23E-1
Transport harvested material	2.72E - 2	1.41E-0
Production and use of fertilizers and atrazine	5.01E-0	1.6E-0
Use of lime	2.47E-4	1.23E-2
Biomass degradation losses	0	6.10E+1
Combustion in boilers	2.22E - 0	3.46E-0
Post combustion activities	4.20E-5	2.07E-3

Table 6.1  $\rm N_2O$  and  $\rm CH_4$  emission factors from switchgrass feedstock production in the power generation chain

*Data source* Qin et al. [39], assumed switchgrass biomass yield 25 Mg ha<sup>-1</sup>, stand life 10 years, transport distance 40 km

close to zero, or there may be significant CH<sub>4</sub> uptake. For example, Adler et al. [40] indicated that CH<sub>4</sub> uptake by switchgrass was 1.41 g CO<sub>2</sub>-eq m<sup>-2</sup> yr<sup>-1</sup>. However, another study estimated that during the agronomic practices to establish switchgrass the total CH<sub>4</sub> emission were 23 g CO<sub>2</sub>-eq m<sup>-2</sup> and that during the harvesting operations (mowing, bailing, etc.) emissions were 17.4 g CO<sub>2</sub>-eq m<sup>-2</sup> [52].

Currently, however, limited information is available on  $CH_4$  flux contributions to net GHG emission from switchgrass. Qin et al. [39] in a LCA study estimated that the largest  $CH_4$  emissions are produced during the processing/combustion phase of switchgrass (Table 6.1), but even then they remained of low significance. As far as we know actual  $CH_4$  flux measurements in a switchgrass stand are nonexistent, probably because most studies do not consider it relevant to include these measurements because of the assumed small effect on GHG emissions. Therefore,  $CH_4$  flux based on field measurements are urgently needed to precisely determine the most impacting phases (cultivation, transformation, etc.) of switchgrass when used as a feedstock for diverse purposes.

# 6.2.4 Life Cycle Assessment

In theory, LCA is an all-inclusive account of the inputs and outputs of a productions cycle [53]. Inputs and outputs can include energy requirement and yield, economic cost and benefit, and environmental impacts whether positive or negative. However, the meaning of 'all-inclusive' can be somewhat nebulous, and a clear definition of comparable system boundaries, both for alternative and traditional fuel sources, is necessary but potentially difficult to achieve when conducting a LCA. The purpose of this review is not to evaluate the appropriateness of various LCAs, but analysis boundaries must be kept in mind when evaluating LCA results.

Because the use of biofuels was prompted by the recognitions of human impacts on global warming and the need to reduce GHG emissions, an appropriate starting point for LCA would be to examine total GHG emissions from various bioenergy systems. In an early LCA comparing switchgrass with other bioenergy crops, Adler et al. [40] found producing ethanol and biodiesel from switchgrass and hybrid poplar reduced GHG emissions by 115% compared with gasoline and diesel. In comparison, maize rotations reduced GHG emissions by 40% and reed canary grass by 85%. They found that displaced fossil fuels were the largest GHG sink, followed by soil C sequestration. They also concluded that GHG reductions resulting from biomass gasification for electricity generation were greater than for biomass conversion to ethanol.

Other studies have found smaller GHG savings from ethanol production from switchgrass. Thus, Cherubini and Jungmeier [43] calculated that the use of switchgrass in a biorefinery reduced GHG emissions by 79% with soil C sequestration responsible for a large part of the GHG benefit. Bai et al. [54] found a 65% reduction in GHG emissions with switchgrass ethanol fuels, and Hsu et al. [55] suggested a 43–57% reduction compared with cars operating on conventional gasoline.

The LCA by Adler et al. [40] suggested that  $N_2O$  emissions were the largest GHG source. According to Qin et al. [39] the largest source of  $N_2O$  emissions during the crop production phase are: the production and use of fertilizer and other chemicals, the transport, harvest, and growth stages, in that order of importance (Table 6.1). When comparing a biorefinery fed with switchgrass biomass with a traditional fossil fuel refinery, Cherubini and Jungmeier [43] indicated that during the first 20 years of operation of the biorefinery the use of switchgrass had a net reduction in GHG emissions, but that the emissions of  $N_2O$  were about 10 times higher than in the fossil fuel refinery. This was due to  $N_2O$  emissions from the N fertilizer (112 kg N ha<sup>-1</sup>) applied to the soil and possibly because of the decomposition of the soil organic matter and dead roots but it seems that this point is not taken into account by the authors. According to their computations, the production phase of switchgrass was responsible for 80% of the GHG emissions and from that 40% were  $N_2O$  emissions.

Several other studies also indicated that the crop production phase is the main source of  $N_2O$  emissions [40, 56, 57]. Therefore, one of the best options to reduce the large impact of fertilization in GHG emissions would be to minimize the use N fertilizers, or to use and develop more efficient N-use strategies. This would also be the case when manures are the fertilizer source because losses of ammonia to the atmosphere and nitrate to groundwater are larger with manures than from synthetic inorganic fertilizers [45].

It is important to also consider other environmental costs and benefits when evaluating bioenergy production systems. In one such analysis, Harto et al. [58] investigated the life cycle water use of biofuel and other low-carbon transport systems. They found that adoption of electric vehicles and some algae-based and

switchgrass systems could contribute to the decarbonization of transportation systems with little additional water consumption. However, use of irrigated biofuel crops could have a significant potential impact on water resources. Whereas, non-irrigated cellulosic ethanol production would require less than 10 gallons of water per gallon of fuel produced, irrigated maize or cellulosic ethanol would consume more than 400 gallons of water per gallon of fuel. They concluded that using irrigated switchgrass to provide 10% of transportation fuel demand in the US would require 7% of total consumptive water demand. Supplying 50% would require 37% of total national annual consumption. Non-irrigated switchgrass, on the other hand, would only consume 1.4% of annual water use to supply 50% of the national fuel demand.

In addition to global warming potential, Bai et al. [54] conducted LCAs for bioenergy production effects on abiotic depletion, eutrophication, photochemical oxidation, ozone layer depletion, human toxicity, eco-toxicity, and acidification. Results were mixed, with bioenergy production reducing global warming, abiotic depletion, and ozone layer depletion, but increasing human toxicity, eco-toxicity, acidification, photochemical oxidation, and eutrophication. Reduced emissions from crude oil production caused the reduction in ozone layer depletion, whereas, abiotic resource depletion decreased due to reduced use of crude oil. They attributed the higher eutrophication score to nitrate leaching from N fertilizer application, whereas, human and eco-toxicity increased due to the use of agrochemicals. Increased acidification resulted from ammonia emissions from agriculture. Cherubini and Jungmeier [43] also concluded that biofuel production had greater impacts on acidification and eutrophication.

These studies suggest that additional environmental impacts should not be disregarded when evaluating the impact of bioenergy production systems on GHG emissions, but no recommendations were made concerning how to rank the importance of competing environmental impacts, or on how to compute an overall "environmental score" for bioenergy production. Such information will be crucial for evaluating the advisability of using bioenergy sources to replace fossil fuels.

### 6.2.5 Indirect Land Use Change

The production of switchgrass for energy purposes at an industrial level requires, as with any other crop, large expanses of agricultural croplands. Since projections indicate that the global population will continue to increase, as will their food, feed, and energy demands [59, 60], it is foreseen that the croplands dedicated to produce energy feedstocks such as switchgrass will most probably come from the displacement of existing crops or from the conversion of grasslands and forests. In either case, the new uses of the land will lead to direct and indirect changes in the carbon balance within and outside the boundaries of the newly introduced system with the consequent effects on global climate, food and feed supplies, and ecosystem services [61].

In general, when a natural forest or pasture is replaced by an annual crop the soil and biomass carbon emissions increase significantly [59]. On the other hand, when a cropland cultivated with annual crops or an abandoned cropland is converted to a perennial grass such as switchgrass, large amounts of carbon could be sequestered in the soil and therefore a net reduction in the atmosphere could be expected. However, this process is not always straightforward because many variables are involved [30, 59, 62, 63]. The degree of impact will be a function of the type of crop replaced, root mass, soil depth, soil bulk density, climatic conditions, and crop management and intensity, among others.

In general, it has been estimated that when perennial grasses are introduced into croplands the carbon stocks in the soil increase at a rate between 1.1 and 1.2 Mg C ha<sup>-1</sup> yr<sup>-1</sup> [64–66]. In agreement with such general estimations, Garten and Wullscheleger [67] predicted that during a 10–30 year conversion period of a cropland to switchgrass the SOC sequestration rate would be 0.78 and 0.53 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively, probably because the large amount of assimilates accumulated in its extended root system. On the other hand converting native grasslands, such as those in U.S., to maize resulted in 5.6 Mg C ha<sup>-1</sup> yr<sup>-1</sup> emissions [24].

Land use change (LUC) is not a new topic as the expansion of the agricultural frontier led to, and will continue to lead to significant  $CO_2$  emissions, especially when tropical forest (rich in above-and below-ground carbon) are converted into agricultural lands [68, 69]. Beyond the environmental effects, LUC could have short-term effects on crop prices, stocks, farm incomes, and demand of agricultural products. However, only recently have the direct and iLUC effects been taken into account in the estimations of GHG emission and LCA studies of biofuel and bioenergy production systems.

However, the estimation process has a number of limitations and there is not a conventionally accepted estimation procedure. Since there are not reliable data on LUC effects, the estimations are highly variable and susceptible to economical, political, social, and environmental influences. For example, Fargione et al. [24] and Searchinger et al. [70] indicated that including the effect of LUC and iLUC in the analysis could result in GHG emissions being even higher for bioenergy crops than that of current fossil fuels. On the other hand, other estimates indicate that the positive benefits of biofuels could be seen under certain circumstances and type of crops such as sugarcane or other perennial grasses [60]. Switchgrass, being a perennial grass with low input requirements and high carbon sequestration capacity, may be part of the group of crops with positive environmental effects. But the available information is not sufficient to determine, with an acceptable degree of approximation, its indirect and direct effects.

Changing the preexisting vegetation to bioenergy crops causes removal or sequestration of  $CO_2$ , but such changes could be negated or enhanced elsewhere because of the spatial and temporal nature of the replacement effects [71]. Therefore, the iLUC effects are non-local and not specific to a feedstock, so they cannot be quantified directly but only through modeling [71]. Since the dynamics of iLUC are dominated by international trading trends, food and feed prices,

agricultural policies, climatic conditions, among others, its global nature makes it very difficult to model. In fact, the validity of the current available methods is hotly debated. But in general two approaches are widely used. In the economic approach, linkages between complex macro- and micro-economic models with biophysical models are used to estimate GHG emissions associated with iLUCs. While in the deterministic approach, the iLUC analysis is based on the export/ import trends of agricultural commodities in the most relevant countries.

Examples of the most common economic and deterministic models used to estimate iLUC effects can be found in Searchinger et al. [70] and Fritsche et al. [61]. In both cases, however, the results and predictions remain vague and variable, mainly because of insufficient analysis of market distortions, complexity of the factors considered, and insufficient analysis of trading levels. A recent study [60], in which seven agro-economic models were compared, indicated a wide range (from 10 to 80 g  $CO_2$  MJ<sup>-1</sup> of biofuel produced) of overall emissions from iLUC. The large variability mainly depended on the assumptions used in each model. However, in the case of switchgrass none of these models may be applicable as none of them considered the iLUC effects of second generation feed-stocks, showing the urgent need to develop estimation procedures that take into account perennial grasses. In the case of the deterministic model, it was shown that adding iLUC plus LUC emissions in LCAs could almost double GHG emissions per unit energy [71].

Some authors consider model simulation approaches to not be sufficiently accurate, therefore they use the risk-adder method, which estimates the average LUC area per additional hectare of bioenergy production [60, 70, 72], to determine the maximum possible effects of iLUC. Based on that approach, Searchinger et al. [70] indicated that even when U.S. maize fields are converted to switchgrass, GHG emissions still increase by 50% over a period of 30 years. Such results raise great concerns about the potential of switchgrass to reduced GHG emissions associated with iLUC. However, this seems to be an overestimation mainly because of the arbitrary assumptions and non-replicable parameters used in the study. But it is clear from this and other studies that the approach of eliminating any iLUC risk provides very rough estimates, which in turn seem insufficient for generalization and rulemaking. Therefore, further studies are needed to define more precise evaluation methods and specific criteria to quantify consistent iLUC values in order to opportunely include them in GHG emission balances.

In any case, it is clear that the production of biofuel and bioenergy leads to GHG emissions associated with iLUC, and controlling them could be an important factor for mitigating the global warming process. Several authors suggest that optimizing the use of byproducts as biofuels feedstocks, maximizing the use of crop residues as biofuels feedstocks, and cultivation of feedstocks on abandoned croplands are measures that to some extent could reduce the iLUC effects on GHG emissions [45, 60]. In addition, technological developments along the supply chain, improved feedstocks, crop management, and improved conversion efficiencies (e.g. bioelectricity instead of biofuels from lignocellulosic crops such as switchgrass) will reduce the impact of the bioenergy feedstock on the GHG

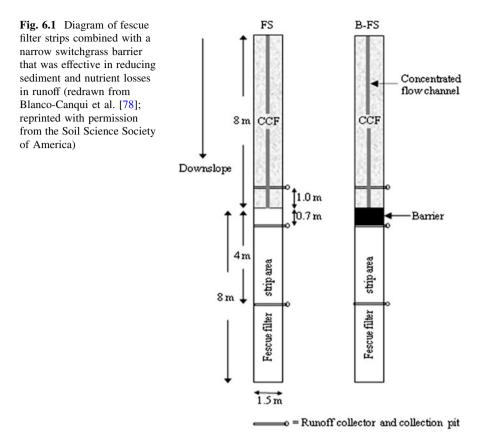
balance [60, 73]. Global climate policies with emissions caps would also help to control iLUC effects. In fact, any measure that reduces the land requirements for feedstocks will contribute to mitigate the effects of direct and iLUCs. As for switchgrass, the research window remains completely open as information on the aforementioned aspects is almost nonexistent.

### 6.3 Impact on Water Quality

### 6.3.1 Runoff, Nutrient, and Sediment Losses

Before switchgrass became the focus of research as a bioenergy crop, its environmental impact on surface water runoff and quality were considered from the perspective of using this grass species in vegetative buffer strips. Switchgrass has upright and stiff stems and a rhizomatous growth habit, characteristics that make it desirable for intercepting sediment from surface runoff and allowing it to regrow through sediment that has been deposited on the soil surface. The USDA Natural Resource Conservation Service identifies these criteria for selecting desirable species in vegetative buffers (Code 601; [74]). In one study in which several grass species were evaluated as a narrow hedge (0.75-1.0 m) grown along the contour to impede surface water runoff, Dabney et al. [75] determined that the primary explanation for the sediment trapping efficacy of the hedge was the result of flow constriction and water backing up above the hedge. The backed up water slowed runoff and the sediment load was deposited, thus the filtering efficacy of the hedge. In a field study in which the effectiveness of a switchgrass hedge was determined for runoff and sediment loss from maize plots, the hedge reduced runoff (41%) and sediment (63%) losses [76]. While species of grass in the hedge seemed unimportant in these studies as long as there was the physical constraint of backing up the surface runoff, cool-season grasses may not withstand the sediment deposition as well as non-bunch, rhizomatous warm-season grasses like switchgrass.

Rainfall simulations were used in a plot  $(1.5 \times 16 \text{ m})$  study to contrast the effectiveness of filter strips of different grass species, including: tall fescue [*Lolium arundinaceum* (Schreb.) S.J. Darbyshire], tall fescue with a switchgrass barrier (0.7 m wide), and a mixture of grasses and forbs native to the Midwestern United States with a switchgrass barrier [77]. Compared to a tilled plot, tall fescue improved surface water quality on this Mexico silt loam soil by reducing organic N (55%), NO<sub>3</sub>–N (27%), NH<sub>4</sub>–N (19%), particulate P (36%), and PO<sub>4</sub>–P (376%). The addition of the switchgrass barrier (similar to a narrow hedge) provided greater effectiveness, reducing organic N by 67% compared to the tilled plot, and also reducing NO<sub>3</sub>–N (68%), NH<sub>4</sub>–N (50%), particulate P (53%), and PO<sub>4</sub>–P (54%). Expanding this experiment to consider concentrated flow paths for the same fescue strips with switchgrass barriers (Fig. 6.1), Blanco-Canqui et al. [78] determined that dormant or actively growing switchgrass were similarly effective



in reducing runoff and sediment losses. As with the previous study, the switchgrass barrier was more effective than only tall fescue in reducing sediment and nutrient losses.

In a direct comparison between cool-season grasses [smooth bromegrass, timothy (*Phleum pretense* L.), and tall fescue] and switchgrass, switchgrass was more effective than the cool-season grasses in removing sediment, N, and P from surface runoff [79]. This comparison was based on 3 or 6 m wide filter strips. The 6 m wide strips were generally 50% more effective than the 3 m wide strips in removing sediment and nutrients. While the switchgrass was usually significantly better than the cool-season grasses at filtering runoff sediment and nutrients, differences were generally less than 10% (Table 6.2).

Switchgrass hedges have also been effective in reducing nutrient runoff losses from plots receiving manure or fertilizer [80]. In no-till plots receiving manure or fertilizer, the adjacent (and downslope) hedge reduced runoff concentrations of dissolved P (47%), bio-available P (48%), particulate P (38%), total P (40%), and NH<sub>4</sub>–N (60%). In the disked plots, the reduction in runoff concentration was not as great for dissolved P (21%), but was generally comparable for the other nutrients.

Strip							
Width (m)	Area ratio	Grass	Sediment (%)	Total N (%)	NO <sub>3</sub> –N (%)	Total P (%)	PO <sub>4</sub> –P (%)
6	20:1	Switchgrass	78.2 a <sup>*</sup>	51.2 a	46.9 a	55.2 a	46.0 a
6	20:1	Cool-season	74.8 b	41.1 b	37.5 b	49.4 b	39.4 b
Overall a	average		76.5	46.2	42.2	52.3	42.7
3	40:1	Switchgrass	69.0 c	31.7 c	28.1 c	39.5 c	38.1 b
3	40:1	Cool-season	62.0 d	23.5 d	22.3 d	35.2 c	29.8 c
Overall a	average		65.5	27.6	25.2	37.4	34.0

 Table 6.2 Efficacy of switchgrass and cool-season grasses in reducing sediment and nutrient runoff (redrawn from [79])

 active

 $^*$  Percent within a column for reduction followed by a different letter are significantly different (P < 0.05)

Water quality research related to switchgrass hedges has mostly focused on the reduction of runoff losses and improvement in associated water quality characteristics, attributing these reductions to water backing up above the hedge. Improving infiltration and/or hydraulic conductivity of the soil surface within a switchgrass stand would contribute to additional water quality improvements by filtering fine sediment particles and other soluble nutrients. Measuring field-saturated hydraulic conductivity  $(K_{fs})$  was the focus of a study in Iowa on a Monona silt loam soil [81]. In this study  $K_{fs}$  was measured at three different locations on the landscape: (1) 7 m upslope from the switchgrass hedge in a maize or soybean field, (2) 0.5 m upslope from the hedge in the sediment depositional area, and (3) within the grass hedge. These hedges had been in place for 10 years. The K<sub>fs</sub> within the grass hedge (107 and 154 mm  $h^{-1}$ ) was more than seven times greater than the K<sub>fs</sub> in the row crop field (13.5 and 22.5 mm  $h^{-1}$ ) and more than 24 times greater than the depositional area (1.4 and 9.4 mm  $h^{-1}$ ). Infiltration was measured under conditions of increasing soil water tension. As tension increased to 50 and 100 mm, infiltration within the hedge was still greater than in the row crop field; but as tension was increased to 150 mm, the infiltration within the hedge and row crop field became similar. A reduction in sediment losses due to a switchgrass hedge can probably be attributed mostly to water backing up above the hedge, but the reduction in nutrient loss is likely attributable to the greater infiltration within the hedge.

The efficacy of a 7.1 m buffer of switchgrass was compared to the switchgrass buffer with an additional 9.2 m length of switchgrass and woody species mix [82]. In a 2 h rainfall simulation (22 mm h<sup>-1</sup>), the switchgrass buffer trapped 70% of the incoming sediment and 64, 61, 72, and 44% of incoming total N, NO<sub>3</sub>–N, total P, and PO<sub>4</sub>–P, respectively. The additional length of switchgrass-woody species buffer improved these numbers to 92% of the sediment and 80, 92, 93, and 85% of the respective nutrients. The woody species in the additional buffer provided greater infiltration, plus the additional length of buffer contributed to the overall greater effectiveness of the buffer in the latter scenario.

When compared to row crops in small-plot studies, switchgrass is very effective at reducing sediment and nutrient loads in surface runoff. The examples provided here were often side-by-side comparisons for standing crops. Because switchgrass is a perennial crop that will not require additional tillage or re-establishment of the crop, an annual (and long-term) comparison of water quality between a row crops and switchgrass should be even more favorable for switchgrass. When switchgrass was compared to cool-season grasses, such as tall fescue, improvements in the sediment, N, and P from surface runoff were slightly better with switchgrass, but the differences were generally less than 10%. Switchgrass makes an effective ground cover for improving water quality.

### 6.3.2 Expanding the Spatial and Temporal Scale of Switchgrass Impacts

Numerous land use studies considering changes to switchgrass have evaluated the environmental impact at larger scales, from the small watershed to the Mississippi River basin. Using the Soil and Water Assessment Tool (SWAT), hillslope processes were modeled and the environmental impacts were considered if planting switchgrass to 10, 20, 30, and 50% of the Walnut Creek watershed (51.3 km<sup>2</sup>) near Ames, IA [83]. Filter strips of switchgrass representing 10–50% of the sub-basin could lead to a 55–90% reduction in NO<sub>3</sub>–N load during an average rainfall year. In the larger Delaware River basin of NE Kansas, SWAT was used to consider sediment yield, surface runoff, NO<sub>3</sub> in surface runoff, and edge-of-field erosion [84]. If the cultivated cropland (119,400 of 300,000 total ha) were converted to switchgrass, sediment loss would be reduced by 99%, surface runoff by 55%, NO<sub>3</sub> loss by 34%, and edge-of-field erosion by 98%. Evaluating a shift to switchgrass in the large, agriculturally dominated Raccoon River watershed of central Iowa (9,364 km<sup>2</sup>), results from SWAT indicated that lower water yield will correspond with less NO<sub>3</sub>, less P, and less sediment loss [85]. These scientists suggested that even though a shift in land use (i.e. toward more switchgrass) might resemble a pre-1940s land use and land cover, the extensive tile drainage network would prevent the hydrology from ever resembling pre-1940s condition. Tile drainage is meant to move water quickly away from agricultural fields with the inadvertent consequence of carrying its nutrient load with it. Nevertheless, growing maize results in lower annual evapotranspiration and therefore greater runoff and drainage than a perennial cropping system, such as switchgrass; resulting in a shift toward fewer environmental impacts when more switchgrass is grown.

Expanding the spatial scale even further to an area encompassing much of Missouri, Iowa, Nebraska, and Kansas in the Midwestern U.S. (15,100 km<sup>2</sup>), Brown et al. [86] used the Erosion Productivity Impact Calculator (EPIC) to consider future environmental impacts of growing more switchgrass in this region. Their model scenarios also extended the temporal scale as well by considering

crop yields and sediment loss under increased atmospheric CO<sub>2</sub> (560  $\mu$ g g<sup>-1</sup>). Alternative climate conditions were considered using the general circulation model (GCM) from CSIRO. With increased temperature, switchgrass yield increased by 5 Mg ha yr<sup>-1</sup>, whereas other crop yields decreased (Mg ha<sup>-1</sup> yr<sup>-1</sup>): maize, 1.5; sorghum, 1.0; soybean, 0.8; and wheat, 0.5. With additional CO<sub>2</sub> under this otherwise similar future scenario, all crops responded with greater yield compared to the future scenario with only increased temperature. Greater rainfall predicted with climate change corresponded with greater runoff and generally increased sediment loss, except with switchgrass for which sediment loss generally decreased. A stochastic model was used in another study to evaluate the impact of producing cellulosic ethanol (i.e. growing switchgrass) compared to maize-derived ethanol in the Mississippi River basin [87]. They concluded that cellulosic ethanol production would result in a 20% decrease in NO<sub>3</sub> delivered to the Gulf of Mexico.

Growing switchgrass compared to growing a row crop will increase water use and increase infiltration, both of which will have the net effect of reducing runoff. These impacts described and measured at the small plot scale translate to reduced nutrient losses at the larger watershed and basin scales. Including switchgrass on the landscape will have a favorable impact on improving water quality.

### 6.3.3 Seasonal Nitrogen Dynamics: Implications for Management and Environmental Impacts

Perhaps some of the more interesting questions about growing switchgrass as a bioenergy crop are intertwined in the N dynamics of physiological characteristics, production management, and their corresponding impacts on N in the environment. For example, when is N taken up by switchgrass? When will switchgrass be harvested? What is the biomass N content at harvest? How much N fertilizer will be applied? How do these physiological characteristics impact NO<sub>3</sub> water quality and N<sub>2</sub>O emissions? Some of these questions have already been addressed with recent research, though some remain unanswered.

Cropland in the Midwestern U.S. is extensively tile-drained, designed to move water quickly from the field to improve soil conditions for maize and soybean production. This conduit for water also effectively moves  $NO_3$  from agricultural soils to streams and rivers [88, 89], significantly contributing to the increase in  $NO_3$  flux in the lower Mississippi River [89]. Compared to continuous maize or maize–soybean crop rotations, perennial crops will reduce  $NO_3$  leaching in this tile-drained landscape from more than 60 kg N ha<sup>-1</sup> yr<sup>-1</sup> to less than 5 kg N ha<sup>-1</sup> yr<sup>-1</sup> [90]. This reduction in  $NO_3$  loss from perennial crops can be attributed to both lower  $NO_3$  concentration in the tile water and reduced drainage as a result of greater evapotranspiration.

In a recent study [91], the impact of *miscanthus* and switchgrass managed as bioenergy crops on hydrology and NO<sub>3</sub> leaching was evaluated in the tile-drained landscape of the Midwestern U.S. The soil profile water content was consistently

less with *miscanthus* than with either switchgrass or a maize-soybean rotation, especially later in the growing season. Nitrate leaching was much greater in the maize-soybean rotation  $(34-45 \text{ kg N ha}^{-1} \text{ yr}^{-1})$  than either switchgrass  $(0.3-3.9 \text{ kg N ha}^{-1} \text{ yr}^{-1})$  or *miscanthus*  $(1.6-6.6 \text{ kg N ha}^{-1} \text{ yr}^{-1})$ . Although N fertilizer was not applied to switchgrass in this study, N applications will likely be a component of switchgrass management as a bioenergy crop; but this study illustrates that a perennial switchgrass crop will contribute much less N to ground water than a crop rotation including maize and soybean.

In a 2 year study in Illinois [92], the biomass and N content of *miscanthus* and switchgrass were evaluated in a side-by-side trial at three locations spanning a 5° latitudinal range (37-42° N). Biomass was harvested on five dates between June and February. As much as 60 Mg ha<sup>-1</sup> of biomass was harvested for *miscanthus* and more than 20 Mg ha<sup>-1</sup> for switchgrass. Nitrogen concentrations of the biomass for both species decreased from 1.5 to 2.5% in June to <0.5% in December. Nitrogen concentrations in February were similar to concentrations in December, so there was not an incentive to postpone harvest until February. Harvest in December corresponded with greater biomass than in February and similar N concentrations. When harvested in June, switchgrass removed as much as 187 kg N ha<sup>-1</sup> and *miscanthus* as much as 379 kg N ha<sup>-1</sup>, whereas N removal in February corresponded to as little as 5 and <17 kg N ha<sup>-1</sup>, respectively, because N within the plant was translocated to the rhizomes during winter dormancy. The December harvest, corresponding to low N concentrations and high biomass yield, was the most suitable harvest date for providing a large amount of desirable biofuel feedstock and represents an efficient N recycling cropping systemmeeting economic and environmental objectives.

A 5 year study in Knoxville, TN contrasted a two-cut (summer and fall) harvest plan to a one-cut (fall) plan for switchgrass [93]. Biomass yield was similar for the two approaches (17.4–18.7 Mg ha<sup>-1</sup>), but much less N was removed with the one-cut plan (48 kg N ha<sup>-1</sup>) than with the two-cut plan (116 kg N ha<sup>-1</sup>). One other study provided similar results, concluding that less N can be applied and less N removed in the biomass when switchgrass is harvested late in the fall [94]. If less N is removed (but remains in the rhizomes), less N fertilizer will be required; thus reducing loss risks associated with NO<sub>3</sub> leaching and N<sub>2</sub>O emissions after N fertilizer applications.

Root distributions and dynamics and total soil respiration were evaluated for an edge-of-field and riparian area that included zones of poplar (*Populus* x *euro-americana*' Eugenei), switchgrass, cool-season grasses (smooth brome, timothy, and tall fescue), and soybean or maize [95]. The fine root biomass for switchgrass increased from 7 to 10 Mg ha<sup>-1</sup> between May and November, remained relatively constant for poplar and cool-season grasses (6-8 Mg ha<sup>-1</sup>) during this same period. Fine root biomass was always less than 2 Mg ha<sup>-1</sup> for maize and soybean. Small root biomass (2–5 mm) was significantly greater in switchgrass (2 Mg ha<sup>-1</sup>) than for any of the other species (<0.65 Mg ha<sup>-1</sup>), with no differences across sampling dates. Coarse root biomass (>5 mm) was only observed under poplar (3.8 Mg ha<sup>-1</sup>), soybean (1.1 Mg ha<sup>-1</sup>), and maize (0.3 Mg ha<sup>-1</sup>).

Root density was greater in the poplar, switchgrass, and cool-season grasses than the maize and soybean, for the 0–50, 50–100, and 100–125 cm depths. Soil respiration was greatest in the poplar and cool-season grasses. Soil respiration under switchgrass was less than for poplar or cool-season grasses; but greater than with maize or soybean. The implications from this research is that the additional roots provided by poplar, switchgrass, or cool-season grasses provide a carbon source that is greater and to greater depths than provided by maize or soybean. The additional C contributes to the riparian zone denitrification potential and the presence of growing roots has implication for additional NO<sub>3</sub> removal; consequently, roots deeper in the soil profile represents two effective means of  $NO_3$ removal from ground water.

Switchgrass is a perennial warm-season grass that is native to North America. It grows to a height of about 2 m, has a deep and fibrous root system, and will produce between 5 and 20 Mg ha<sup>-1</sup> yr<sup>-1</sup> of above-ground biomass [96]. A stand of switchgrass may maintain this productivity for 15-20 year with much less fertilizer and chemical inputs than usually applied to crops such as maize and soybean. Some of the physiological characteristics that suggest that switchgrass should have a favorable impact on the environment compared to most other agriculture cropping systems include: (1) after crop establishment the soil will remain undisturbed for many years, reducing soil erosion and energy inputs (as fuel); (2) low N fertilizer inputs translates into low energy demand for growing the crop and reduced risk of N<sub>2</sub>O emissions and NO<sub>3</sub> leaching that might occur with almost any N fertilizer application; (3) low energy inputs relative to harvested biomass; and (4) a perennial crop with a fibrous root system translates to reduced water and nutrient losses through leaching as well as an effective surface runoff filter. If and when land use currently in traditional cropping systems is converted to switchgrass, environmental impacts should be favorable.

#### 6.4 Conclusions

The number of studies looking at the environmental impacts of switchgrass cultivation is extremely limited, especially when switchgrass was managed for bioenergy production. In particular, additional studies at multiple locations are needed to identify the climatic and edaphic drivers of soil C sequestration. Similar research is needed for N<sub>2</sub>O emissions, but in addition, continuous flux measurements throughout the year are needed to indentify the contribution of periodic high-emission events to total annual emission rates. Additional research on how N fertilization rates affect N<sub>2</sub>O emissions and NO<sub>3</sub> leaching and on interactions between environmental effects of N fertilization and biomass production is also warranted. What limited information there is suggests that switchgrass provides multiple environmental benefits compared to annual crop cultivation. However, benefits generally appear to be similar to other perennial crops.

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# Chapter 7 Biochemical and Thermochemical Conversion of Switchgrass to Biofuels

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Abstract With dwindling oil reserves and growing environmental concerns, researchers are looking at producing sustainable biofuels and chemicals from renewable resources like switchgrass. Biofuels and biochemicals will be produced in the near future from switchgrass in biorefineries using both biochemical and thermochemical platforms. We have summarized recent literature pertaining to different processing steps within the biochemical platform (pretreatment, enzyme hydrolysis, microbial fermentation, protein extraction) and thermochemical platform (pyrolysis, bio-oil, gasification, combustion, hydrothermal process) in this chapter. Though we have improved our fundamental understanding on the different processing steps to produce biofuels, several challenges still have to be overcome to create a bioeconomy and produce fuels and chemicals from biomass in an economic and sustainable manner.

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### 7.1 Introduction

Like a petroleum refinery which uses crude oil as a feedstock to produce different products, the biorefinery combines different process technologies to convert biomass residues generated from agriculture and forest industries to fuels and chemicals. Lignocellulosic feedstocks offer several advantages, such as availability in great abundance, being a renewable resource and non-edible and does not interfere with food industries. It is comprised of a complex network of 60–70% carbohydrates (cellulose, hemicellulose) and 15–20% lignin as major constituents. The remaining 10–25% constitutes minor components such as protein, ash, oil/ waxes and others. There are two distinct platforms for producing fuels and chemicals (Fig. 7.1).

The first platform is a biochemical route where the biomass residues are chemically pretreated using different process technologies followed by enzymatically hydrolyzing the pretreated biomass to fermentable sugars. Since sugars are the building blocks for most microorganisms; they are fermented to produce different fuels and chemicals.

The second platform is using a thermochemical route where the biomass residues are subjected to hydro-thermolysis, pyrolysis, thermolysis or burning to produce bio-oil/bio-char/syngas followed by catalytically converting them into fuels and chemicals. Both platforms cogenerate heat and power to meet their internal energy demand. There are some hybrid biorefinery concepts where the hydrolysed sugars or the fermented products are catalytically converted to produce intermediates and final products.

Green biorefineries is another concept in which green plants are processed to produce a fiber-rich press cake and nutrient-rich green juice. The fiber is used for making biofuels via either the biochemical or thermochemical route, while the green juice which contains proteins, minerals and nutrients can be used as an animal feed supplement [1, 2].

### 7.2 Biochemical Platform

The processing steps involved in this platform include: biomass harvest and transport [3], milling, pretreatment [4], solid liquid separation (optional), high solid loading enzymatic hydrolysis [5], microbial fermentation [6], product recovery and burning un-hydrolyzed biomass to generate heat/electricity [7] (Fig. 7.2). The number of processing steps can increase or decrease depending on the choice of pretreatment. For example, lignin (e.g., with organosolv pretreatment) or hemicellulose (e.g., with acid pretreatment) can be removed which can be used as a starting material for making other products [8]. In some pretreatments the biomass composition is unaltered [e.g., Ammonia Fiber Extraction (AFEX<sup>TM</sup>)] and hence there is no need for solid–liquid separation. There are different methods

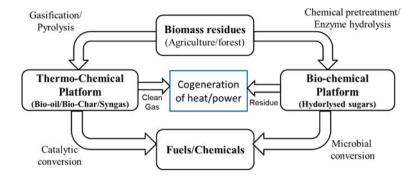


Fig. 7.1 Different biorefinery platform for making fuels and chemicals

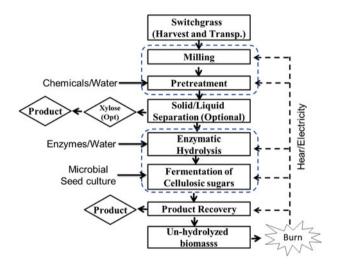


Fig. 7.2 Different processing steps involved in biochemical platform

of converting pretreated biomass to biofuels, including separate hydrolysis and fermentation (SHF) or simultaneous saccharification and co-fermentation (SSCF). In both cases, commercial enzymes and microbial seed cultures are needed for the process. Alternatively consolidated bioprocessing (CBP) can be performed using a microbe which produces enzymes and also ferments the sugars to fuels and chemicals [6]. The fermentation broth is further processed to recover products from un-hydrolysed solids and insoluble lignin is burned to generate enough heat/ electricity to carry out the other processing steps. In order to make the biorefinery process environmentally sustainable and economically feasible compared to a petroleum refinery, the amount of water and energy used in different processing steps should be minimized, the catalyst used in the process should be recovered and recycled, enzyme cost should be minimized [9] and the microbe used should

be highly efficient. In addition, several co-products (e.g., lignin, proteins) can be generated which will further reduce the processing cost.

### 7.2.1 Thermochemical Pretreatments

Pretreatments are necessary to reduce the native recalcitrance of lignocellulosic biomass to biological conversion. The choice of pretreatment technology (thermal, thermo mechanical, thermo chemical or biological) would also have pervasive impacts on all aspects of the biorefinery operation ranging from choice of feedstock selection to product recovery and waste-water treatment [4]. However, in order to correctly estimate the impact of different pretreatments on biomass conversion it is necessary to carry out these studies using a standard basis. The Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) was established as a partnership among several university and federal labs that were leaders in biomass pretreatment and hydrolysis in late 1999 in Dallas and early 2000 in Chicago to establish a common standard to assess pretreatments [10]. Three CAFI projects have been carried out over the last decade on three different feedstocks: corn stover, poplar and switchgrass [10-12]. Three different varieties of switchgrass, supplied by Ceres Corporation (Thousand Oaks, CA), were used in the last CAFI project: Alamo (lowland), Dacotah (upland), and Shawnee (upland) with variable planting and harvesting time periods (spring vs. late-fall). The variable composition (glucan and lignin, in particular) seemed to depend strongly on harvest time than on variety or ecotype.

Six different pretreatments were investigated in this project; AFEX<sup>TM</sup>, dilute acid (DA), liquid hot water (LHW), lime, sulfur-dioxide impregnated steam explosion (SO<sub>2</sub>), and soaking in aqueous ammonia (SAA). The optimal pretreatment conditions that were determined for Dacotah switchgrass variety are highlighted in Table 7.1. All chemical pretreatments are known to modify plant cell walls through various physicochemical modifications depending on pretreatment chemistry (acid vs. base) and type of biomass that eventually helps enhance enzymatic digestibility via improving enzyme accessibility to embedded polysaccharides.

The CAFI pretreatments can be split into two main categories; acidic (DA, LHW, SO<sub>2</sub>) or basic (AFEX<sup>TM</sup>, Lime, SAA), that vary in thermochemical severity. In recent years there have been several advances highlighting the physicochemical mechanism of acidic and ammonia based pretreatments for monocots and dicots [13–18]. Cleavage of lignin-carbohydrate complex (LCC) linkages between lignin and hemicellulose helps improve accessibility of cellulose. While in acidic pre-treatment the glycosidic bonds are cleaved to result in the formation of lower molecular gluco-/xylo-oligomers and monomers; in the case of alkaline pretreatments only oligomers are released. Lignin-based ether linkages are prone to cleavage only during acidic (or oxidative) pretreatments further coupled with the re-condensation and deposition of lignin-based globular structures (10–10,000 nm

Pretreatment category	Temperature (°C)	Reaction (min)	Pretreatment chemical	Catalyst loading (g/g BM)	Water loading (g/g BM)
AFEX <sup>TM</sup>	140	30	NH <sub>3</sub>	1.5	2
DA	140	40-45	$H_2SO_4$	0.01	10
LHW	200	10-20	None	-	6.7
Lime	120	240	Ca(OH) <sub>2</sub> , O <sub>2</sub>	1	15
Steam expl.	180	10-15	None or SO <sub>2</sub>	0.05	10
SAA	160	60–80	NH <sub>4</sub> OH	1.4	7.7

Table 7.1 Optimal pretreatment conditions for Dacotah switchgrass (adapted from [11])

in cross-section diameter) on outer wall surfaces. On the contrary, the lignin ether linkages are intact during AFEX<sup>TM</sup> with deposition of heterogeneously-shaped lignin rich extractives (10-500 nm) on outer wall surfaces only under certain pretreatment conditions [19]. High resolution analytical microscopy imaging techniques on untreated and variously pretreated switchgrass samples have shown that the relative extent of cell wall delamination, lignin re-localization, porosity and cell wall morphological disruption is closely dependent on pretreatment type [20] and [19]. Both DA and AFEX<sup>TM</sup> pretreatment were found to significantly alter the ultrastructure of the compound middle lamella and the outer secondary cell walls for corn stover and switchgrass suggesting mass transfer is a major limitation for effective cell wall pretreatment. Cellulose degree of polymerization has been shown to decrease for acidic pretreatments with no major decrease in crystallinity index [21]. Though pretreatment of cellulose with anhydrous liquid ammonia has been shown to produce a novel allomorph named cellulose IIII (an allomorph that has up to 5 fold higher rate of saccharification than native cellulose  $I_{\beta}$ ), without producing significant amounts of amorphous cellulose, no major alteration in cellulose crystal structure (to a more readily digestible form) is seen during conventional AFEX<sup>TM</sup> or other aqueous pretreatments [15, 19].

Acidic pretreatments have similar chemistries but vary in thermochemical severity. The acid is either added externally ( $H_2SO_4$ ,  $SO_2$ ) or formed during pretreatment (e.g., degradation of polysaccharides and lignin to short-chain aliphatic acids and phenolic acids, respectively). Water is a strong acid at high temperatures above 200°C as well [22]. The extent of hemicellulose (88–93% removal) and lignin (13–19% removal) removal depends on several factors (pH, temperature, residence time and liquid to solid loading) as highlighted in Table 7.2. In alkaline pretreatments, the pretreatment catalysts varies considerably such as in AFEX<sup>TM</sup> (43% anhydrous ammonia in water solution), SAA (15% anhydrous ammonia in water solution), and oxidative lime (7% calcium hydroxide solution with dissolved oxygen).

The low liquid-to-solid loading employed during AFEX<sup>TM</sup> is responsible for the low mass loss seen after pretreatment in contrast to SAA and lime pretreatment where a significant fraction of lignin (55–60%) and hemicellulose (38–40%) is solubilized. One critique of the acetyl mass balance for AFEX<sup>TM</sup> treated switchgrass is that conventional chromatography methods used are unable to detect acetamide that is only formed during AFEX<sup>TM</sup> [16], hence underestimating the

Pretreatment category	Recovery (% solids)	Cellulose (% residual)	Xylan	Arabinan	Acetyl	Lignin	Others
	(70 solids)	· /					
Untreated	-	35.6	22.6	3.1	3.6	21.1	13.9
AFEX <sup>TM</sup>	100	35.9	22.5	3.4	2.4	24.4	11.4
DA	60	50.3	4.5	0.5	0.3	29.4	15.0
LHW	60	50.1	2.5	0	0.3	30.6	16.6
Lime	65	53.0	21.5	1.7	0	14.6	9.2
Steam expl.	62	53.9	2.7	0.7	0.5	27.6	14.6
SAA	62	55.6	21.9	2.4	1.5	13.9	4.7

**Table 7.2** Relative extent of solids recovery and individual component mass balance for various CAFI pretreatments conducted on Dacotah switchgrass (adapted from [97])

true extent of acetyl removal. Previous work on AFEX<sup>TM</sup> and DA pretreatments for corn stover has shown that more than 75 cell wall decomposition products are formed (e.g., amides, acids, furans, imidazoles, phenolics, aldehydes) due to degradation of carbohydrates and lignin [16]. A similar study needs to be carried out for switchgrass since varying thermochemical severity would have significant impact on formation of degradation products that can differentially impact enzyme and microbial action.

### 7.2.2 Effect of Enzyme Combination on Hydrolysis of Pretreated Switchgrass

#### 7.2.2.1 Effects of Cellulases

Cellulase enzymes are needed to break down the sugar polymers to monomeric sugars [23]. The effect of CAFI pretreatment followed by enzymatic hydrolysis on total monomeric and oligomeric sugar release (glucose and xylose only) is highlighted in Table 7.3. Hydrolysis experiments were done using commercial enzymes. Spezyme CP (Genencor Division of Danisco US, Inc, NY, USA), with a protein content of 82 mg/mL and specific activity of 50 FPU mL<sup>-1</sup> was loaded at 15 FPU  $g^{-1}$  glucan in untreated biomass.  $\beta$ -Glucosidase (Novozyme 188, Novozymes Corp.) with a protein content of 67 mg mL<sup>-1</sup> and specific activity of 600 CBU mL<sup>-1</sup> was loaded at 30 CBU g<sup>-1</sup> glucan in untreated biomass. The sugar yields are reported based on maximum possible total glucose contribution of 60.6% and xylose contribution of 39.4% from the Dacotah switchgrass at 1% glucan loading during hydrolysis. Acidic pretreatments solubilized higher levels of glucose in stage 1 (during pretreatment) than alkaline pretreatments. All pretreatments except lime and  $SO_2$  were effective in releasing near-theoretical yields of glucose and xylose after enzymatic hydrolysis (Stage 2). Most xylose was released during acidic pretreatments in stage 1; however, the relative extent of monomeric and oligomeric sugar yield depended on severity of pretreatment. Nearly one third of total available xylose sugars, as oligomers, are released during

Pretreatment category	% Glucan conv.	conv.	Total Gluc.	% Xylan conv	nv.	Total Xyl.	Combined Sugars	MESP (\$/gal EtOH)
	Stage 1	Stage 2		Stage 1	Stage 2			
Untreated	I	8.4	8.4	I	1.9	1.9	10.3	I
Theor. max			60.6			39.4	100	I
$AFEX^{TM}$	0.8/0.8	47.1	47.9/0.8	11.1/11.1	25.6/3.0	36.7/14.1	84.6/14.9	2.50
DA	4.3/0.5	42.2	46.5/0.5	29.3/1.7	3.4	32.6/1.7	79.2/2.2	2.59
LHW	4.1/3.8	47.3	51.4/3.8	25.9/17.2	5.30/1.1	31.3/18.3	82.6/22.1	2.32
Lime	0.9/0.8	54.0/3.0	54.9/3.8	13.6/13.6	22.4/0.8	36.0/14.3	90.9/18.2	2.63
Steam expl.	3.0/1.5	48.3	51.4/1.5	28.7/1.5	3.2	31.9/1.5	83.2/3.0	2.73
SAA	0.2/0.2	39.8/1.2	40.0/1.4	9.5/8.7	17.8/6.9	27.3/15.5	67.3/17.0	2.93
Stage 1 refers to pretreatment and Stage 2 refers to the enzymatic digestion of the solids produced during pretreatment. First and second value reported each column corresponds to total monomeric/oligomeric sugars and oligomers only released, respectively. A single value indicates release of only monome	ttment and St s to total mor	tage 2 refers to nomeric/oligou	o the enzymatic meric sugars and	digestion of th l oligomers onl	le solids prod y released, re	luced during pr spectively. A si	etreatment. First and se ingle value indicates rel	nent and Stage 2 refers to the enzymatic digestion of the solids produced during pretreatment. First and second value reported in o total monomeric/oligomeric sugars and oligomers only released, respectively. A single value indicates release of only monomers
(adapted from [11])								

Table 7.3 Sugar yields from pretreatment (Stage 1) and enzymatic hydrolysis at a fixed cellulase loading of 30 mg/g glucan (Stage 2) for prewashed and

a post-wash of AFEX<sup>TM</sup> pretreated switchgrass (only to allow fair comparison with other pretreatments since during conventional AFEX<sup>TM</sup> no water washing of pretreated biomass is necessary following AFEX<sup>TM</sup> treatment).

This suggests that a significant proportion of hemicelluloses are solubilized and re-deposited on outer cell walls during AFEX<sup>TM</sup> pretreatment, however, these oligomers can be easily removed as shown elsewhere as well [15, 19]. Unlike the previous work with corn stover, CAFI pretreatments show distinct effectiveness on late-spring harvest Dacotah switchgrass. A detailed techno-economic analysis was carried out for all CAFI pretreatments based on the results reported for Dacotah switchgrass [24]. The minimum ethanol selling price (MESP) per gallon fuel for an integrated, commercial-scale lignocellulosic ethanol process based on each pretreatment has been provided in Table 7.3 (inclusive of oligomers credits) that highlights LHW and AFEX<sup>TM</sup> as the less expensive options.

For industrial processing, a high solid loading (>18%) is preferred since it gives higher product concentrations resulting thereby lowering energy and water input. It is important to note that as the solid loading increases from 3 to 18% or higher, the sugar conversion drops significantly [5]. The possible reason for such inhibition is due to enzyme inhibition at higher sugar concentration (particularly cellobiose and oligomeric sugars), higher concentration of pretreatment degradation products and lesser adsorption of enzymes to substrates. Several studies are under way to overcome this problem for economically producing biofuels through biorefinery process.

#### 7.2.2.2 Effect of Hemicellulases

By looking at the composition of the pretreated switchgrass residues (Table 7.2), it is clear that hemicellulose is either partially or completely retained. Therefore, supplementing cellulases with accessory enzymes like hemicellulases may be important to further improve both glucose and xylose yields for some pretreatments. About 5–10% increase in glucan conversion and about 5–20% improvement in xylan conversion is usually observed depending on the enzyme loading for different alkaline pretreated biomass [25]. The dilute acidic, sulfur dioxide both produced solids with very low xylan contents of 4.5 and 7.3%, respectively. Supplementing at low levels, xylanase had negligible effect on xylan hydrolysis yields.

## 7.2.3 Co-Production of Protein and Biofuel from Switchgrass

With the increasing cost of food and feed products, alternative feeds such as leaf protein concentrate (LPC) are gaining more attention. LPC is protein from herbaceous biomass such as grasses or alfalfa that has been removed from the cell wall carbohydrates, and is generally 50–80% protein by weight [26]. This concept

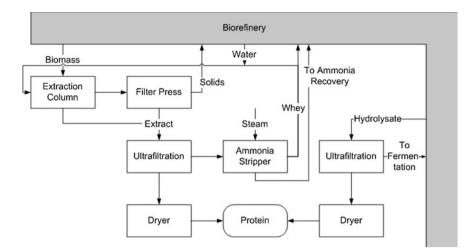


Fig. 7.3 Schematic representation of leaf protein processing of switchgrass integrated with a cellulosic biofuel production facility ('biorefinery') using an ammonia based pretreatment [34]

of a green biorefinery has been studied for decades and has been scaled up and commercialized using alfalfa as a feedstock [26, 27]. In the basic process, the fresh, wet feedstock is first macerated and then mechanically pressed to produce a protein-rich juice. This juice is rapidly heated via steam injection to precipitate the protein, which is then dried and sold. The de-proteinated juice can be evaporated and the stillage added to the fiber to be sold as an animal feed or, alternatively, as a feedstock for biofuel production [27, 28] Alternatively, dried biomass can be added to a dilute alkaline solvent to extract the protein, and ultrafiltration can be used as an alternative to heat for coagulation for protein recovered during the pressing/extraction step and 50–60% of those proteins recovered during the coagulation/filtration step.

Most of the research on LPC has focused on using alfalfa as a feedstock, but switchgrass has also been considered as a protein source. Prior to biofuel research, switchgrass was considered forage for animals, noting that protein in excess of 12% of the total weight of the plant was available if harvested in early summer [31]. Protein extraction of a late May harvest of Alamo switchgrass were reported [32]. Only 35% of the protein was extracted in most cases. However, the remaining protein could be solubilized during cellulose hydrolysis and is also potentially recoverable. A later study showed that the protein was only partially recoverable, but that the process when integrated with biofuel production (Fig. 7.3) would produce a net profit of \$34 Mg<sup>-1</sup> biomass if moderate improvements in extractability and recovery were possible [33]. Proteins have been extracted from fresh and stored switchgrass harvested in the summer and autumn [35]. Protein content within the biomass, extraction yields, and concentration yields were lower than obtained from orchardgrass,

suggesting that this may not be a viable co-product for biofuel production from switchgrass.

### 7.2.4 Impact of Harvest Dates and Phenotype Variety on Biofuel Yields

While numerous studies exist on pretreatment and enzymatic hydrolysis of switchgrass, the massive variation in switchgrass harvest locations, harvest dates, and phenotype variety make it difficult to directly compare these studies. There are many varieties of switchgrass broadly categorized in two types; lowland switch-grass such as Alamo tends to have greater biomass yields while upland switchgrass such as Cave in Rock are more suitable to cooler climates [35]. As a perennial grass, it can be harvested multiple times throughout the growing season. It is known that forages have significant structural changes over the course of the growing season, with cellulose and lignin content increasing over time and protein and soluble sugars decreasing. The stem to leaf ratio also increases, and leaves will drop off as senescence occurs if the plant is left standing over winter.

Little research has been performed on differences in biofuel yields of different harvest dates. Sugar yields from three different stages of switchgrass growth pretreated with DA were reported [36]. While total sugar yields released were similar among the three stages, the later stages had greater cellulose content. Thus, the percentage yield was lower for the later stages, with a steeper decline for glucose than non-glucose sugars. The CAFI project also compared multiple varieties harvested in early winter with a Dacotah switchgrass harvested after allowing it to stand all winter [37]. The Dacotah harvest had significantly poorer glucose yields among all pretreatments tested, with the largest decreases in AFEX<sup>TM</sup>, DA, and SAA (Fig. 7.4).

The xylose trend was less clear, with significant decrease in yields seen only for the two ammonia pretreatments. Two different switchgrass varieties harvested in July and October: one planted in Michigan and one in Alabama were investigated [38]. Differences in fiber digestibility were seen in the Michigan harvest but not in Alabama. This is likely due to a shorter growing season in Michigan, causing the July harvest to be more immature than an equivalent July harvest in Alabama as well as the fact that the October harvest in Alabama was not yet at full senescence. In general, however, a clear trend of decreasing cellulose digestibility with a slight decrease in hemicellulose digestibility as the growing season continues is present in all studies.

In contrast, different strains of switchgrass can also have different digestibility, although there is currently very little research in this field. Shawnee and Alamo switchgrass, both planted in Oklahoma and harvested in December did not show much difference for any pretreatment between sugar yields [38]. A separate study considered ethanol yields from pretreated switchgrass varieties specifically bred

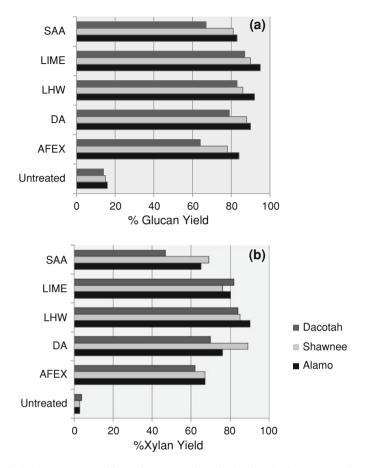


Fig. 7.4 Switchgrass pretreated by various methods and hydrolysed using commercial enzymes. **a** % glucan yield, **b** % xylan yield. The experiments were done at 3% solid loadings using commercial enzymes (15 FPU cellulase in Spezyme CP plus 30 CBU  $\beta$ -glucosidase in Novozyme 188) per g glucan of untreated raw switchgrass (equivalent to total 27 mg protein/g glucan in untreated biomass). The hydrolysis was carried out at 50°C and an agitation rate of 150 rpm. Pretreatment conditions are given in Table 7.1. Sugar yield is based on glucan or xylan in pretreated/hot washed solids for all pretreatments except for AFEX<sup>TM</sup> (adapted from [37])

for either high digestibility or high biomass yield. The highly digestible sample did not significantly increase ethanol yields over one of the lower digestibility breed, but did increase yields over the other breed [39]. In contrast, Sarath et al. [40] found significant differences in varieties specifically bred for changes in dry matter digestibility, and noted that most of the change in ethanol yield after DA pretreatment came from variations in stem lignin content [40]. Other important factors were present, however, including the amount of hemicellulose that could be solubilized during pretreatment. Besides the total lignin produced, the syringyl/guaiacyl ratio within lignin can be important, and genetically engineered strains that improved these ratios have been developed and shown to greatly improve biofuel potential [41].

Several studies have also considered different strains for fiber digestibility in sheep and cattle, which has been shown to correlate strongly with biofuel yields [42]. Pathfinder and a variety bred specifically for feed were tested for high fiber digestibility, and noted a slight but not significant difference in fiber digestibility when fed to lambs [43]. Likewise, Trailblazer and Pathfinder switchgrass were compared and also no differences were found in fiber digestibility [44]. More recently, three varieties across three separate years were compared, and found that the differences among years were greater than the differences between strains [45]. A later study did show that Trailblazer had significantly higher cellulose digestibility than Cave in Rock and Shawnee, but not hemicellulose digestibility [46]. The authors note that the difference is likely due to a different leaf/stem ratio as opposed to differences in cell wall structure.

### 7.2.5 Biofuel Production

Biochemical conversion of lignocellulosic biomass to biofuel consists of four biological events: saccharolytic enzymes production, enzymatic hydrolysis, hexose fermentation and pentose fermentation [6]. Depending on how the four biological steps are integrated, the process configuration can be separate hydrolysis and fermentation/co-fermentation (SHF/SHcF), simultaneous saccharification and (co)fermentation (SSF/SSCF), or CBP [6]. SHF/SHcF performs the four biological steps separately (co-fermentation performs hexose and pentose fermentation together). SSF combines enzymatic hydrolysis and hexose fermentation together. SSCF further integrates hexose fermentation and pentose fermentation. CBP performs the four biological steps in a single bioreactor. The major advantage of SHF/SHcF is that enzymatic hydrolysis and fermentation can be carried out at optimal conditions [47]. Nevertheless, the enzymes are easily inhibited by the endproducts (sugars) and thus affecting final sugar conversions. SSF/SSCF removes sugars by fermentation and thereby reduces the sugar inhibition [47]. SSF/SSCF also has other advantages such as lower cost, shorter process time, and lower contamination risk. CBP is the ultimate low-cost industrial configuration to produce biofuel [48, 49]. However, currently there's no perfect microbe or microbial consortium capable of degrading lignocelluloses, utilizing all the carbohydrates and at the same time producing ethanol at a high yield as CBP requires [48].

Biofuel (such as ethanol) production from switchgrass is affected by many factors such as switchgrass type, harvest time, pretreatment, glucan loading, enzyme loading, fermentation strain, and process configuration (Table 7.4).

Switchgrass type and harvest time affect the sugar compositions in the biomass and hence affect the biofuel yield [33]. The two factors might also affect other chemicals or nutrients in the switchgrass, which in turn affect fermentation

Table 7.4 E	Table 7.4 Ethanol production from switchgrass	in from switchgr	ass							
SG type (harvested time)	Pretreatment	Process configuration	Strain(s)	Glucan loading (%)	Cellulase loading (FPU/g glucan)	Nutrients suppl.	EH + ferm. Time (h)	Ethanol titer (g/L)	Ethanol yield (g/g biomass)	Ref.
Unspecified	Lime	SSF	S. cerevisiae D5A	3	25	$\mathrm{YEP}^\mathrm{b}$	168	14	$0.16^{a}$	[98]
Unspecified	Dilute acid	SSF	S. cerevisiae D5A	3	25	YEP	168	14	$0.15^{a}$	[66]
Kanlow	Hydrotherm.	SSF	K. marxianus IMB3	4.2	15	YE & amm. Sulf.	96	19	0.15 <sup>a</sup>	[100]
Kanlow	Hydrotherm.	SSF	S. cerevisiae D5A	4.2	15	YE & amm. Sulf.	168	22	$0.18^{a}$	[100]
Kanlow	Hydrotherm.	SSF	K. marxianus IMB4	4.1	15	YE & amm. Sulf.	72	17	$0.15^{a}$	[62, 101]
CIR	Aq. ammonia	SSF	S. cerevisiae D5A	9	38.5	YEP	96	22	$0.15^{a}$	[102]
CIR	Aq. ammonia	$SSF^{c}$	S. cerevisiae D5A	2	LL	YEP	72	10	$0.16^{a}$	[103]
CIR (Oct)	AFEX <sup>TM</sup>	SHcF	S. cerevisiae 424A (LNH-ST)	9	15	None	96 + 96	35	0.18	[63]
CIR (Oct)	AFEX <sup>TM</sup>	SSCF	S. cerevisiae 424A (LNH-ST)	9	15	None	8 + 192	32	0.17	[63]
CIR (Oct)	AFEX <sup>TM</sup>	SSCF	S. cerevisiae 424A (LNH-ST)	9	15	YEP	8 + 192	36	0.19	[63]
CIR (Jul)	AFEX <sup>TM</sup>	SHcF	S. cerevisiae 424A (LNH-ST)	6.1	10	None	72 + 96	34	0.16	[33, 42]
CIR (Oct)	AFEX <sup>TM</sup>	SHcF	S. cerevisiae 424A (LNH-ST)	6.7	12.2	None	72 + 96	30	0.14	[33, 42]
Alamo (Jul) AFEX <sup>TM</sup>	AFEX <sup>TM</sup>	SHcF	S. cerevisiae 424A (LNH-ST)	6.5	9.8	None	72 + 96	30	0.14	[33, 42]
									c)	(continued)

Table 7.4 (continued)	continued)									
SG type	Pretreatment	Process	Strain(s)	Glucan	Cellulase	Nutrients	EH + ferm.	Ethanol	Ethanol	Ref.
(harvested		configuration		loading	loading	suppl.	Time (h)	titer	yield	
time)				$(0'_{0})$	(FPU/g		(FPU/g (g/L)	(g/L)	g/g	
					glucan)				biomass)	
Alamo	$AFEX^{TM}$	SHcF	S. cerevisiae 424A	3.3	9.7	None	72 + 96 21		0.2	[33, 42]
(Oct)			(LNH-ST)							
Unspecified	Inspecified Dilute acid	SSF	14 &	$4.4^{a}$	26	YEP	$168 \sim 192$	unspecified 0.16 <sup>a</sup>	$0.16^{a}$	[61]
			S. cerevisiae D5A							
St6-3F	Dilute acid	SHF	S. cerevisiae	$1.1^{a}$	$127^{a}$	None	72 + 48	unspecified 0.08	0.08	[39,
			ATCC 24859							104]
CIR Cave in rock	rock									

CIR Cave in rock <sup>a</sup> Calculated or estimated <sup>b</sup> *YEP medium* yeast extract and peptone medium; *YE* yeast extract <sup>c</sup> Pilot scale

performances [33]. For instance, more inhibitory effects were observed during the fermentation of the biomass harvested in July when compared to October [33]. Pretreatment largely determines the substrate properties for biofuel production. Degradation products generated during pretreatment reactions also affect fermentation performance [50, 51]. Typically, DA pretreatment destroys the nutrients present in the biomass and hence nutrient supplementation is required during fermentation. However, AFEX<sup>TM</sup> pretreatment conserves nutrients, which renders nutrients supplementation unnecessary and reduces cost [51, 52]. Glucan loading determines the final biofuel concentration. Higher glucan loading typically leads to higher biofuel concentration and reduces the cost of biofuel recovery/distillation as well as water use. However, higher glucan loading normally results in lower sugar conversions and hence lower biofuel yield [5]. Commercial enzyme supplementation is a large cost in the biofuel production process. High enzyme loading can enhance the biofuel yield while also increase the production cost.

The oldest ethanol fermentation strain, Saccharomyces cerevisiae, cannot natively ferment pentose. During the past decades, a great deal of effort has been made to genetically engineer microorganisms, such as Escherichia coli [53], Zymomonas mobilis [54], and Schefferosomyces stipitis [55] in order to efficiently ferment xylose into ethanol. However, the robustness of E. coli and Z. mobilis during fermentations of cellulosic hydrolysates might not be ideal [56]. Dissolved oxygen control is the key for ethanol production using *P. stipitis* [57]. Genetically modified S. cerevisiae strains were also widely utilized for glucose and xylose co-fermentation [58-60]. Depending on the biofuel production strains capacity, the process can convert glucose or both glucose and xvlose to biofuel. Typically, higher biofuel yield can be obtained when xylose was also converted. For instance, Yang et al. [39] reached a yield of 0.08 g ethanol per gram switchgrass using a SHF process configuration, while Bals et al. [33] achieved higher than 0.14 g ethanol per gram switchgrass even at a higher solids loading and lower enzyme loading. Comparing to SHF/SHcF, SSF/SSCF has a potential to reach higher yields [61]. The ethanol yields from switchgrass using SSF were around 0.14-0.18 (Table 7.4). One large concern of SSF is the discrepancy of the optimal temperature for enzymes, which is around 50°C, and for fermentation strains, which is around 30-37°C. Typically, SSF is performed at fermentation temperatures as a compromise resulting in lower enzyme activities. High temperature tolerance strain such as *Kluyveromyces* marxianus was also investigated for SSF of switchgrass [62]. However, lower ethanol yield was obtained probably due to the ethanol fermentation was not as good as S. cerevisiae.

Those SSF studies mentioned above were all performed at a relatively low glucan loading, which resulted in low ethanol concentrations that did not reach the industrial titer threshold of 40 g  $L^{-1}$ . Jin et al. [63] tried 6% glucan loading using AFEX<sup>TM</sup> treated switchgrass aiming to produce an industrial relevant ethanol titer in both SHcF and SSCF configurations; 35 and 32 g  $L^{-1}$  ethanol was produced, respectively. It turned out that the solid switchgrass biomass showed severe

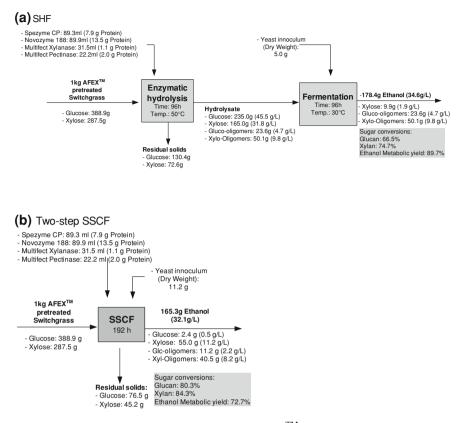


Fig. 7.5 a Mass balance for SHcF, b SSCF (b) of AFEX<sup>TM</sup> pretreated switchgrass (Cave-in-rock, harvested in October). Data were collected from 6% (w/w) glucan loading experiments (adapted from [63])

inhibition on the yeast fermentation, which rendered the SSCF results were not as good as the SHcF ones. However, with washing and supplementation of YEP medium, actions typically performed with other pretreatments, SSCF yielded 36 g L<sup>-1</sup> ethanol (Table 7.4). The mass balances for SHcF and SSCF of AFEX<sup>TM</sup> treated switchgrass were shown in Fig. 7.5. Low sugar conversions were observed for SHcF process. SSCF enhanced the sugar conversions while the ethanol metabolic yield was reduced by solids. It should be noted that there was a considerable amount of sugar loss in the oligomeric forms. Improved enzymes or engineered yeasts able to consume oligomers would help resolve this problem.

CBP of dilute-acid pretreated switchgrass using *Clostridium thermocellum* was also tried with a 0.32 g L<sup>-1</sup> ethanol yield [64]. ABE (Acetone butanol ethanol) fermentation was also tested on dilute-acid pretreated switchgrass and yielded 14.6 g L<sup>-1</sup> [65]. It is promising to produce biofuels from switchgrass, while some improvements are still required such as fermentation strains and saccharolytic enzymes.

### 7.3 Thermochemical Conversion Processes for Switchgrass to Biofuels

Switchgrass is an oxygenated, low energy density feedstock when compared to conventional fossil fuels including crude oil and coals. About 38–42% of the dry matter of switchgrass is oxygen [66]. A useful means of comparing biomass and fossil fuels is in terms of their oxygen to carbon (O:C) and hydrogen to carbon (H:C) ratios. The lower the respective ratios the greater the energy content of the material [67]. Therefore, removal of oxygen from switchgrass is the major objective for upgrading its energy density. The deoxygenation can be achieved most readily by dehydration, which removes oxygen in the form of water, and by decarboxylation, which removes oxygen in the form dioxide [68, 105]. For an efficient carbon conversion to fuels, it is desirable to keep decarboxylation as low as possible.

Thermochemical conversion processes involve the use of temperature and/or catalysts to convert biomass to liquid or gaseous intermediate products which is further upgraded to transportation fuels or chemicals. The choice of an appropriate conversion technology depends on several factors such as biomass composition, availability, logistics, processing characteristics and types of biofuels desired. The robustness of the technology, production of fungible fuels, ability to process diverse feedstocks, and almost complete utilization of biomass components are some of the major advantages of the thermochemical pathways. Pyrolysis, gasification, and combustion that are conducted at a temperature of several hundred degrees Celsius are the most promising technologies under thermochemical pathways to convert switchgrass to liquid fuels and/electricity (Fig. 7.6). These three major technologies can be compared based on the basis of oxygen supply during the respective conversion processes (Fig. 7.6b) using equivalence ratio ( $\Phi$ ) which is defined as the actual air fuel ratio/the air fuel ratio for complete combustion.

The proximate and ultimate analysis, ash/inorganic contents, moisture content, heating value, and bulk density are some of the important properties which play a very important role in thermochemical conversion processes. The elemental composition of switchgrass is a basic property that is required to determine the carbon conversion efficiency during different thermochemical processes and potential of switchgrass for biofuels applications. Literature average value of proximate analysis of switchgrass is 81.4% volatile matters, 3-6% ash, and 15% fixed carbon with a higher heating value in the range of 18.5-18.7 MJ kg<sup>-1</sup>. High volatile matter of biomass makes it more readily devolatilized than coal leaving less fixed carbon. The elemental compositions of switchgrass are comparable with other potential biofuels feedstock such as hybrid poplar and corn stover. Recently the chemical properties of lowland and upland varieties of switchgrass (Table 7.5) which are relevant to biofuel production were reviewed [69]. Ash is an integral part of the plant structure which consists of a wide range of mineral matter such as salts of calcium, potassium, sodium, silica, and magnesium. Ash content depends

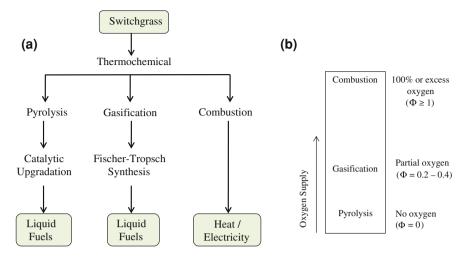


Fig. 7.6 a A simplified schematics of thermochemical routes for switchgrass, b comparison of pyrolysis, gasification, and combustion with respect to equivalence ratio

Variety	Ultim basis)		lysis (w	t% dry	Ino	rganic	constitue	ents (m	g/kg, dı	ry bas	sis)	
	С	Н	Ν	0	Al	Cl	Κ	Si	Р	S	Ca	Ash%
CIR	47.5	6.8	0.51	42.5	74	1,624	9,148	8,623	3,577	820	3,572	6.0
Alamo	47.3	5.3	0.51	41.6	_	_	_	-	_	-	_	5.2
Trailblazer	45.9	6.0	0.96	_	75	1,500	8,674	9,420	4,176	920	3,712	6.4
Blackwell	46.3	6.0	1.10	_	82	1,514	9,323	9,904	3,662	881	3,792	6.2
Kanlow	48.0	5.4	0.41	41.4	76	1,596	10,894	8,767	3,844	865	3,512	5.4

Table 7.5 The ultimate analysis and inorganic constituents of different switchgrass species [69]

on the type of the plant and the soil contamination in which the plant grows. The time and frequency of harvesting of switchgrass is also important and these conditions affect the ash content along with the total dry matter yield. As an example, Monti et al. [70] reported that harvesting twice per year may have advantages from time-management point of view, but biomass quality was significantly affected leading to higher ash content in biomass. The biomass productivity and plant vigour generally decreased after initial two years in twice-cut system. Therefore, the additional harvesting costs due to low re-growth biomass yield potentially offsets the benefits [71].

The chemical composition, ash content, and mineral composition (C, N, Al, Ca, Cl, Fe, K, Mg, Na, P, S, Si) of switchgrass varies along the stems, leaves, and flower head. Generally, leaves have higher mineral concentration when compared with other parts of the plant. Monti et al. [70] compared mineral compositions and ash contents of six major energy crops (four perennial and two annual crops). Switchgrass and miscanthus showed the overall better biomass quality with respect

Process	Time (s)	Bio-oil	Gas (%)	Biochar
Fast pyrolysis	1–2	75	13	12
Slow pyrolysis	30-1,800	30	35	35

Table 7.6 Products of biomass pyrolysis

to ash and mineral contents. It has been demonstrated that ash contents also negatively affects the heating value of biomass. The ash content of perennial grasses (e.g. switchgrass, giant reed, miscanthus) are generally higher than wood probably due to the soil contamination. The time of harvesting also affects the biomass yield and quality. Switchgrass yield is generally decreased due to delayed harvesting, but delayed harvesting has been reported to have less ash and moisture content in switchgrass thereby increasing the overall heating value of the biomass [72].

Although the ash content of switchgrass is lower than coal, the ash chemistry is different when subjected to the thermochemical conversion processes. In general, the ash present in biomass has low ash fusion temperature (750–1,000°C) which becomes a concern due to the propensity of agglomeration, fouling, deposits, slagging and corrosion in the reactor at high temperature. Further, ash needs to be properly disposed after the conversion process to minimize its impact on process equipment. The melting and slagging of ash in biomass is dependent on the concentrations of K, Cl, S, Al and Si in ash [73]. The problems arising from the high ash content of energy crops during thermochemical conversion may be addressed by separating the problematic elements through leaching or by adding additives such as lime to increase the melting point of slag. Another possibility is to blend the perennial crops with wood which has comparatively lower ash content and also higher ash fusion temperature [74].

### 7.3.1 Pyrolysis

Pyrolysis, in the form of wood distillation, has been used since the historical times. Pyrolysis is the thermal degradation of biomass in the temperature range of 400–550°C in the absence of oxygen. Overall, biomass pyrolysis is an endothermic process, which means that energy needs to be supplied to drive the process. The process yields gaseous (non-condensable gases e.g., CO, CO<sub>2</sub>, CH<sub>4</sub>, and H<sub>2</sub>), liquid (bio-oil and water) and solid (bio-char) products with varying yields (Table 7.6) depending upon the pyrolysis conditions such as feedstock composition (lignin, ash, and carbohydrates), pyrolysis temperature, vapor residence time, heating rate, and particle size. Primarily following three basic approaches (fast, medium, and slow pyrolysis) are used based on the product requirement:

• Fast pyrolysis where technologies are dedicated to bio-oil production and so biochar is an undesirable by-product;

- Medium pyrolysis where technologies are dedicated for co-production of biochar and useful by-products (liquid fuels, syngas, chemicals, heat, and electricity);
- Slow pyrolysis where technologies are dedicated to bio-char production, without or with minimum production of useful by-products (gases and liquids).

To produce transportation fuels, the process is generally optimized for the production of bio-oil (fast pyrolysis) that can be catalytically upgraded to transportation fuels, chemicals, adhesives, and many other applications. Fast pyrolysis typically requires dry (<10% moisture) and finely ground biomass (2–6 mm). The thermal degradation of lignocellulosic feedstock follows a complex mechanism, which can be influenced by heating and cooling rates. The rapid heating and rapid quenching can produce intermediate bio-oil, which condenses before further decomposing into gaseous products.

Bio-char which is the non-liquefied carbonaceous solid product resulting from the thermal decomposition of biomass has historically been used in soil to enhance plant growth [75]. Switchgrass bio-char has also been reported to be a potential adsorbant for both organic pollutants and heavy metals [76, 77].

#### 7.3.2 Bio-oil

Bio-oil is a dark brown, free-flowing organic liquid that is comprised of highly oxygenated compounds with a density of about 1.2 g mL<sup>-1</sup>. The synonyms for bio-oil include pyrolysis oil, pyrolysis liquids, bio-crude, wood liquids, wood oil, liquid smoke, wood distillates, pyroligneous acid, and liquid wood. Bio-oil yield from fast pyrolysis can be as high as 70-80% based on the starting dry biomass weight [78]. It is important to note that bio-oil produced via fast pyrolysis is not stable and its composition tends to change toward thermodynamic equilibrium during storage [79]. The oxygen content of bio-oils is usually 35–40 wt% and the single most abundant component of bio-oil is water. It contains major groups of compounds, including acids, alcohols, aldehydes, esters, ketones, sugars, phenols, guaiacols, syringols, furans, lignin derived phenols and extractible terpene with multi-functional groups. Bio-oil has water content of typically 15-30 wt%, which cannot be easily removed by conventional methods. The presence of aldehydes and ketones make bio-oils especially hydrophilic and highly hydrated, which leads to the water being difficult to eliminate [79]. Oxygen containing components present in the oil, especially the carbonyl compounds and low pH value are responsible for bio-oil's instability [80]. In fact, bio-oil is usually not miscible and chemically very different than the petroleum due to the presence of water, high oxygen content, higher viscosity, and low pH. The higher heating value of bio-oil is about 15–22 MJ kg<sup>-1</sup>, which is much lower than that of liquid fuels due to presence of oxygenated compounds.

Table 7.7 Physical           properties of switchgrass bio-           cil produced at three	Property	450 (°C)	500 (°C)	550 (°C)
oil produced at three pyrolysis temperatures [82]	Density at $15^{\circ}$ C ( $10^3$ kg m <sup>-3</sup> )	1.17	1.13	1.11
pyrorysis temperatures [62]	pH value	2.74	3.29	2.96
	Water content (wt%)	20.1	24.7	27.5
	HHV (MJ kg <sup>-1</sup> )	18.7	17.4	17.1
	Viscosity at 40°C (centistokes)	28.2	20.9	17.0
	Solid content in bio-oil (wt%)	1.62	1.44	1.50
	Ash content (wt%)	0.42	0.40	0.43

#### 7.3.2.1 Switchgrass Bio-Oil

There have been several studies which reports the fast pyrolysis of switchgrass to produce bio-oils and discusses the bio-oil properties [69, 81-83]. The bio-oil vield varied from 43 to 77% depending upon the pyrolysis conditions and types of switchgrass [69]. It has been reported that the switchgrass bio-oils had much higher levoglucosan levels, less aromatic compounds, and also less nitrogen containing compounds than alfalfa stems bio-oils [69]. Alkali metal, especially potassium present is switchgrass has been reported to have a strong catalytic effect on pyrolysis [84]. Table 7.7 shows the important properties of bio-oil produced from the fast pyrolysis of switchgrass. He et al. [82] studied fast pyrolysis of switchgrass in a pilot fluidized-bed pyrolysis reactor at three different temperatures (450, 500, and 550°C) and moisture (5, 10, and 15%). Switchgrass was collected in Iowa (USA) from mature stands of the Cave-in-Rock cultivar while dormant (early spring). The study concluded that moisture content and pyrolysis temperature caused large variations in product yield, chemical composition, and most of the measured physicochemical properties of bio-oil. Solid content in bio-oil are generally fine char particle entrained with vapors.

The inconsistent physicochemical properties of bio-oil possess a significant barrier to the commercialization. However, the volumetric energy density of bio-oil is 5–20 times higher than the original biomass [80]. This makes the concept of developing small scale fast pyrolyzer available near to the feedstock where bio-mass can be converted to bio-oil and subsequently, the liquid product can be transported to a central refining location for upgrading it to transportation fuels. The deleterious properties of bio-oil such as high viscosity, thermal instability and corrosiveness can be addressed by different upgrading processes. Some of the recent bio-oil up gradation techniques include hydro-de-oxygenation, catalytic cracking of pyrolysis vapors, emulsification, steam reforming, and chemicals extraction techniques [79].

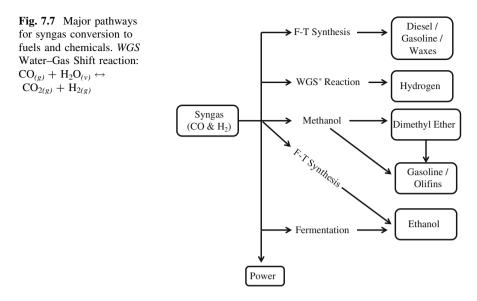
# 7.3.3 Gasification

Gasification is another transformation pathway for production of liquid fuels from biomass. Biomass gasification is an endothermic process where biomass is converted into combustible gas under high temperature and limited supply of oxygen (partial oxidation). Biomass gasification processes vary considerably, and typical operation is conducted at >700°C and 1-5 atmosphere pressure under limited supply of air/oxygen/steam using relatively dry feedstock (moisture <10 wt%) [85, 86]. Biomass inside a gasifier mainly goes through four conversion stages: (1) drying (>150°C); (2) pyrolysis or devolatilization (150–700°C); (3) combustion (700-1,500°C); and (4) reduction (800-1,100°C). The stages 1, 2, and 4 absorb heat (endothermic) whereas step 3 releases (exothermic) heat. The gasifiers are generally classified according to the relative movement of the fuel and the gasifying medium as either fixed beds (up-draft, down-draft and cross-draft) or fluidized beds (bubbling, circulating, spouted and swirling). The key step in the gasification process includes feedstock conditioning, gasification, heat recovery, gas cleaning and conditioning, and gas utilization. The product gas from a gasifier is mainly composed of CO, CO<sub>2</sub>, H<sub>2</sub>O, H<sub>2</sub>, CH<sub>4</sub>, other gaseous hydrocarbons (CH<sub>s</sub>), tars, char, inorganic components, and ash. The gas composition can greatly vary depending upon the gasification process, the gasifying agent, and the biomass composition. A generalized reaction scheme can be written as:

$$\begin{array}{rll} CH_xO_y+\ O_2\rightarrow CO\ +\ CO_2+\ H_2O\ +\ H_2+\ CH_4+\ other\ (CH_s)\ +\ tar \\ +\ char\ +\ ash \end{array}$$

The product gas is either a medium-energy content gas referred synthesis gas (syngas) or a low-energy content producer gas. Syngas consists primarily of carbon monoxide and hydrogen. Higher quality syngas can be produced by using pure oxygen as the oxidizing agent. Producer gas results if air is used as the oxidizing agent, which dilutes the combustible components of the gas with nitrogen. Generally, producer gas is adequate for power generation and avoids the energy use associated with oxygen production. Syngas is required for chemicals and liquid fuels production. Gasification technology has been widely used to produce commercial fuels and chemicals. Technological advances particular to biomass gasification have been successfully demonstrated and being developed at commercial scales. The main advantage of technology is its ability to produce a reliable, high-quality syngas product from organic matters that can be used for energy production or as a building block for chemical manufacturing processes (Fig. 7.7).

In fact, the easiest way to convert biomass into liquid transportation fuels is via routes involving C<sub>1</sub>-building blocks (i.e. synthesis gas) [68]. In 1923, German scientists Franz Fischer and Hans Tropsch first studied conversion of coal-derived syngas to liquid hydrocarbons over metal catalysts through a series of chemical reactions. The process is known as Fischer-Tropsch (FT) synthesis.



\* WGS: Water-Gas Shift Reaction:  $CO_{(g)} + H_2O_{(v)} \leftrightarrow CO_{2(g)} + H_{2(g)}$ 

FT synthesis is typically carried out in the temperature range of 150–300°C and at high pressures (15–40 bars) described by the following equation [87]:

$$nCO + (2n + 1) H_2 \rightarrow C_n H_{2n+2} + nH_2O$$

where n is the number of carbon atoms in the hydrocarbon produced.

All reactions (for varying value of n) are exothermic ( $\Delta H = -165$  kJ mol-CO<sup>-1</sup>) and the product is a mixture of different hydrocarbons mainly consisting of paraffins and olefins [87]. The relative distribution of the products depends on the catalyst and the process conditions (temperature, pressure, and residence time). The ratio of H<sub>2</sub> and CO in syngas is an important parameter and ideally, a good mole ratio is 2:1 to form alkanes (-CH<sub>2</sub>-) [88]. The synthesis gas from carbohydrates and other biomass normally has 1:1 molar ratio (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>  $\rightarrow$  6CO + 6H<sub>2</sub>). However, H<sub>2</sub> to CO ratio can be adjusted by the use of water–gas shift (WGS) reaction (CO + H<sub>2</sub>O  $\rightarrow$  CO<sub>2</sub> + H<sub>2</sub>).

The gasification process has several benefits for near-term commercial application due to the inherent advantages over combustion including more flexibility in terms of energy applications, higher economical and thermodynamic efficiency at smaller scales, and potentially lower environmental impact when combined with gas cleaning and refining technologies [88]. The directly evolved synthesis gas from a gasifier contains undesired impurities derived from biomass feedstock including tar, hydrogen sulfide, carbonyl sulfide, ammonia, hydrogen cyanide, alkali, and dust particles. It is important to clean synthesis gas to avoid FT catalyst poisoning. Impurities from synthesis gas can be removed through several routes

Compound	Switchgrass	Corn	Wheat straw
		stover	
H <sub>2</sub>	23.5	26.9	24.4
CO	33.2	24.7	27.5
$CO_2$	19.4	23.7	22.0
CH <sub>4</sub>	17.0	15.3	16.3
$C_2H_4$	5.1	4.2	4.3
$C_2H_2$	0.34	0.45	0.31
C <sub>3</sub> H <sub>8</sub>	0.82	0.40	0.81
C <sub>3</sub> H <sub>6</sub>	0.10	0.12	0.10
%Mass closure	101.1	97.5	0.08
H <sub>2</sub> /CO	0.71	1.09	0.92
Gas yield (kg/kg feed)	0.62	0.54	0.54

**Table 7.8** Average gascomposition [90]

including physical separation, thermal cracking, and catalytic hot gas cleanup [89]. The significant problem with FT synthesis is the cost of clean-up and tar reforming. The presence of tars in gas causes coking on catalyst surfaces which must be removed to sustain an effective catalyst.

## 7.3.3.1 Switchgrass Gasification

There have been few studies on gasification of switchgrass. One of the earlier studies in fluidized bed gasifier used air (10  $\text{Nm}^3 \text{ h}^{-1}$ ) as oxidizing medium. The gas production over the experiment was reasonably constant at 650°C, with 5% H<sub>2</sub>, 3% CH<sub>4</sub> and 11% CO as main combustible components. Lower heating value of the gas was 4 MJ/Nm<sup>3</sup>. Carpenter et al. [90] performed a detailed parametric study of the gasification of switchgrass, corn stover, and wheat straw on an experimental, fluidized bed pilot-scale (0.5 Mg  $d^{-1}$ ) gasification facility. The study compared the performance of the gasifier as a function of feedstock, in terms of the syngas production and composition. Biomass was continuously fed into gasifier and the product gases were further cracked in a tubular flow reactor. The resulting synthesis gas was scrubbed and analyzed. Biomass of particle size of less than 2.3 mm was fed continuously in fluidized-bed reactor. Table 7.8 provides the comparison of average gas compositions (vol% on a dry nitrogen-free basis) during a gasification experiments conducted at steam-to-biomass ratio of 1.0, a fluidized-bed reactor temperature of 650°C, a thermal-cracker temperature of 875°C and biomass feed rate of 20 kg  $h^{-1}$ .

The residence time in the fluidized-bed reactor was 6.7 s and the residence time in the thermal cracker was 0.63 s. The study concluded that there was no significant variation (<10%) in the total tar formation between the feedstock and the gasification results were in agreement with the literature.

# 7.3.4 Combustion

Biomass is one of the earliest sources of energy produced by combustion especially in rural areas where it is often the only accessible and affordable source of energy [91]. Direct combustion and co-firing with coal for electricity production from biomass has been found to be a promising method in the near future. Combustion is the burning of biomass in the presence of air to convert the chemical energy stored in biomass into heat, mechanical power, or electricity using stoves, furnaces, boilers, steam turbines, turbo-generators, etc. It is an exothermic process and can be used for any types of biomass. Fluidized bed combustion is the best technology used to burn a fuel such as biomass with low quality, high ash content and low calorific value. Co-firing (direct co-firing, indirect co-firing and parallel co-firing) of biomass in coal-fired power plants is an especially attractive option because of the high conversion efficiency of these plants.

Biomass is very different from coal with respect to physical properties and organic, inorganic, and energy contents. In comparison to coal, biomass has less carbon, more oxygen, higher hydrogen content, higher volatile matters, lower ash fusion temperature, more silica and potassium, and lower heating value [91]. Biomass offers important advantages as a combustion feedstock because of the high volatile matters and the high reactivity of both the fuel and the resulting char. The relative volatility which is defined as ratio of volatile matter (V) to fixed carbon (FC) is generally around five (V/FC) which is significantly higher than that of coal (V/FC < 1). Besides the advantages of biomass being a renewable fuel and important feedstock for the energy security, it is useful in the mitigation of hazardous emissions from combustion such as CO<sub>2</sub>, NO<sub>x</sub>, CH<sub>4</sub>, SO<sub>x</sub> and CO from power plants. It can be used for electricity generation in power plants which can provide a viable alternative for reducing greenhouse gases (GHG) and also for the combined heat and power (CHP) in the process industries.

#### 7.3.4.1 Combustion Properties of Switchgrass

The suitability of switchgrass for bioenergy through combustion is measured by several indices that reflect energy content, density, and ease of recovery. The study conducted by McLaughlin et al. [92] indicated that switchgrass should be a versatile bioenergy feedstock for combustion and have some important advantages over wood with respect to combustion properties. The heating value of switchgrass (17.4 MJ kg<sup>-1</sup>) is comparable to that of poplar (18.6 MJ kg<sup>-1</sup>) with significantly lower initial moisture content. Switchgrass is baled at moisture contents of 13–15%, while poplar typically contains 45–50% moisture at harvest. Moisture content at harvest influences the cost of drying, transportation and handling. Ash content and chemistry is important in the combustion process because it can

Emission factor	CO <sub>2</sub>	N <sub>2</sub> O	CH <sub>4</sub>	SO <sub>2</sub>	СО
Switchgrass	1,525	0.090	0.140	0.10	4.12
Coal	2,085	0.031	0.022	17.16	0.25

Table 7.9 Emission factor (g/kg fuel) from switchgrass and coal [91]

contribute to slagging problem. McLaughlin et al. [92] reported the average ash content of switchgrass across a wide variety of locations, varieties and treatments as 4.5% (range 2.8–7.6%). The ash fusion temperature of switchgrass is 1,016°C which is lower than poplar (1,350°C) and coal (1,287°C) [92]. Coulson et al. [93] measured the initial deformation temperature (IDT) of switchgrass ash as 1,035–1,160°C in oxidizing atmosphere (air) and 1,055°C in nitrogen atmosphere. The IDT is one of the most important indicators to anticipate the problems from slag melting during combustion. It is recommended to run thermochemical operations well below the temperature at which ash starts to soften. The GHG emissions (CO<sub>2</sub>, NO<sub>x</sub> and SO<sub>x</sub>) are considerably lower than that of coal (Table 7.9) which makes switchgrass a potential fuel to mix with coal to reduce the environmental impacts.

The lower heating value, low bulk density, issues with storage, collections, and handling of switchgrass possess a significant challenge in commercialization of cofiring with coal. The direct injections of switchgrass with coal in existing coal-fired power plants possess significant challenges due to its difference in physical properties with coal. However, the environmental benefits from reduced emission of  $NO_x$ ,  $SO_2$ , fossil  $CO_2$ , and trace metal emissions such as mercury makes a favorable case to employ switchgrass or other biomass as a renewable energy source in electricity generating stations.

# 7.3.5 Hydrothermal Processes

Hydrothermal processes refers to processing of biomass in sub- and super-critical water (critical point: 374°C, 221 bar) medium. One of the major challenges in utilization of biomass is its high moisture content and the variable feedstock composition. The conventional thermochemical processes require dry biomass for the efficient production of biofuels. Hydrothermal processes, which can utilize wet biomass, capitalizes on the extraordinary solvent properties of water at elevated temperature for converting biomass to high energy density fuels and functional carbonaceous materials. Here, water acts as reactant as well as reaction medium which help in performing hydrolysis, de-polymerization, dehydration, decarboxylation, and many other chemical reactions. The technology has attracted much attention as a non-toxic, environmentally benign, inexpensive and tunable reaction medium for conducting ionic/free radical reactions. The process can be applied to produce solid (bio-char), liquid (bioethanol, bio-crude/bio-oil), and gaseous (methane, hydrogen) fuels from switchgrass depending on the processing temperature and pressure.

#### 7.3.5.1 Conversion of Switchgrass in Hydrothermal Medium

Kumar et al. [76, 77] used hydrothermal pretreatment of switchgrass to enhance and optimize its enzymatic digestibility during bioethanol production. More than 80% of glucan digestibility was achieved by pretreatment at 190°C. The nearcritical water (250–380°C) is used for the liquefaction of biomass to produce biocrude. Bio-crude can be upgraded to liquid fuel, hydrogen gas or chemicals by aqueous phase reforming process in hydrothermal medium. Switchgrass was effectively liquefied to produce bio-crude in subcritical water at a comparatively low temperature (235°C) in a flow-through reactor. More than 50 wt% of the organic carbon available in switchgrass was converted to bio-crude using subcritical water in the presence of 0.15 wt% of  $K_2CO_3$  [106].

Supercritical water has liquid-like density and gas-like transport properties. The lower density favors free-radical reactions, which is favorable for supercritical water gasification of biomass. Methane gas is a main product from biomass gasification in near-critical or supercritical water (350-400°C). At higher temperature in supercritical water, biomass is converted to hydrogen rich gas without catalyst or with non-metal catalysts. Adam et al. studied the hydrogen production by gasification of switchgrass bio-crude in supercritical water at 600°C. Nickel, cobalt, and ruthenium catalysts were tested on titania, zirconia, and magnesium aluminum spinel supports. Ni/ZrO2 gave 0.98 mol H2/mol C, the highest hydrogen yield of all tested catalysts; however, over time, increase in pressure drop lead to reactor plugging with all zirconia supported catalysts [94]. Ramsurn et al. [95] gasified switchgrass bio-char in hydrothermal medium at 400-650°C to produce syngas. The carbon gasification efficiency in hydrothermal medium was reported to be much better than that in the thermal medium [95]. Kumar [96] studied the hydrothermal carbonization (HTC) of switchgrass to produce the high energy density bio-char at 280°C. The results demonstrated that the HTC process can provide a promising route for high energy density (28 MJ kg<sup>-1</sup>) solid fuel and carbonaceous functional materials for use in a variety of applications.

# 7.4 Conclusions

We have given an outline for two different routes to processing switchgrass. For the biochemical platform, sugar conversion and products yield are summarized using the reported literature data. A different scenario of producing fuels and chemicals using the thermochemical route has also been presented. With the help of this review one can understand what has been accomplished so far in this field and identify other barriers which can be overcome in the near future for commercially producing biofuels from switchgrass in a cellulosic biorefinery.

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# Chapter 8 Estimating Region Specific Costs to Produce and Deliver Switchgrass

Anthony Turhollow and Francis Epplin

**Abstract** Feedstock costs are expected to be a major component of the cost of producing bio-based products from lignocellulosic biomass. The economic viability of using switchgrass to produce feedstock will depend on its cost relative to alternatives. The purpose of this chapter is to present estimates of the cost to produce, harvest, store, and transport switchgrass biomass. Enterprise budgets and sensitivity analysis are used to produce cost estimates. Delivered switchgrass costs can vary widely, depending on yields, input prices (seed, fertilizer and lime, and diesel fuel), input quantities (fertilizer and lime, herbicides), and land costs. Establishment costs amortized over 10 years range from \$38 to \$112 ha<sup>-1</sup> and reseeding costs (25% of land in second year) (amortized over 9 years) range from \$10 to \$18 ha<sup>-1</sup>. Baler productivity is important, and can impact costs up to a \$16 dry Mg<sup>-1</sup>. Costs of switchgrass delivered range from as low as \$42 to well over \$100 dry Mg<sup>-1</sup> if yields are low. The ultimate challenge is to formulate a profitable switchgrass production, storage, and delivery system simultaneously with profitable conversion to bio-based products.

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# 8.1 Introduction

Numerous cost estimates have been made for switchgrass. These estimates vary widely and depend greatly on the assumptions made about yield, land rents, fertilizer application rates, and machine costs (Table 8.1). In this chapter we provide a set of crop budgets for switchgrass that cover a range of possible scenarios. We use a discount rate of 6.5%, a labor cost of \$17.00 implement  $hr^{-1}$ , and a diesel fuel price of \$0.93 L<sup>-1</sup>.

# 8.2 Land Procurement

If switchgrass (or other purpose-grown crops) are to contribute significant amounts of biomass, many hectares of land are required. The vast majority of land in the United States capable of producing switchgrass and other biomass crops is privately owned. There is precedence in the United States for removing large quantities of cropland from production, most recently via long-term Conservation Reserve Program (CRP) leases. Historically, in the United States, the challenge facing the farm sector has been excess production capacity. Various federal government programs have attempted to reduce this by idling cropland from production of commodity crops [1].

Perlack and Stokes [2] estimate how much biomass crops (including perennial grasses such as switchgrass) could be produced at differing farm-gate prices (Table 8.2). In 2007 U.S. farmers planted 38, 24, and  $26 \times 10^6$  ha of corn (*Zea mays*), wheat (*Triticum aestivum*), and soybeans (*Glycine max*), respectively. To obtain between 9 and 21 million ha of land to grow switchgrass, switchgrass must compete economically with these and other crops and would impact cropland rental rates and land values.

An economically efficient switchgrass processing facility would be expected to be located near a concentration of feedstock production. Wright and Brown [3] estimated optimal biorefinery sizes and to achieve economies of scale annual feedstock requirements ranged from  $1 \times 10^6$  Mg for a fast pyrolysis-to-bio-oil facility to  $7 \times 10^6$  Mg for a gasification to mixed alcohol facility. Depending on yield, area required could range from 50,000 ha at 20 dry Mg ha<sup>-1</sup> to 644,000 ha at 11 dry Mg ha<sup>-1</sup>.

In the absence of spot markets (as in the case of corn for corn-to-ethanol facilities), obtaining a reliable flow of feedstock could involve: (1) contracts with individual growers, (2) contracts with a group of owners through a cooperative, (3) long-term leases similar to the CRP, and/or (4) land purchases. U.S. landowners have experience with long-term (10–15 year) CRP contracts, with more than  $13 \times 10^6$  ha having been under contract. CRP annual payments have been in the range of cropland rental rates and vary widely by state. Such contracts may provide a blueprint for biorefineries that need to ensure a reliable flow of feedstock

Year	Location	Nitrogen (kg ha $^{-1}$ )		Mature yield (dry Mg ha <sup>-1</sup> )	Farm gate cost (\$ Mg <sup>-1</sup> )	Source
2008	Indiana	90	173	11.2	50	Brechbill and Tyner [15]
2007	Iowa	112	198	9.0	90	Duffy [16]
1996	Oklahoma	56	74	9.0	25	Epplin [17]
2007	Oklahoma	90	148	8.4-14.6	41–58	Epplin et al. [18]
2008	Illinois	112	193	6.4	90	Khanna et al. [19]
2008	N. Dakota, S. Dakota, Nebraska	75	148	5.0	60	Perrin et al. [20]
2009		N/A	153	13.8	49	USEPA [21, 22]
2008	Tennessee	67	N/A	14.4	68 + land	UT Extension [23]
2008	Wisconsin	140	198	10.8	58	Vadas et al. [24]

Table 8.1 Estimates of switchgrass farm gate costs

**Table 8.2** Estimates of U.S. land converted to perennial grasses at three farm gate prices with a 4% annual growth rate in crop yield

Year	$44 \ Mg^{-1}$	$55 Mg^{-1}$	$66 {\rm Mg}^{-1}$
		ha (10 <sup>6</sup> )	
2017	2.9	8.5	12.1
2022	5.3	14.2	17.8
2030	9.3	18.6	21.4
		Percent of land base <sup>a</sup>	
2017	1.6	4.7	6.7
2022	2.9	7.8	9.8
2030	5.1	10.2	11.8
		Biomass produced (10 <sup>6</sup> dry Mg)	
2017	32	96	140
2022	91	245	307
2030	183	368	419

<sup>a</sup> Land base available in the POLYSYS model used in Perlack and Stokes [2] includes 183 million ha for the eight major commodity crops (corn, wheat, soybeans, cotton, barley, oats, sorghum, and rice), hay, cropland pasture, and non-irrigated permanent pasture

and for landowners that desire a reliable rent and reduced risk. However, the risk of default would be higher with a private biorefinery than with the U.S. government.

Given the required investment in harvest equipment and the need to provide a continuous flow of a consistent quality of biomass throughout the year and size economies in a biorefinery, due diligence would dictate a business plan that includes a well-designed and coordinated feedstock production, harvest, storage, and delivery system. Production characteristics and harvest cost economies could result in a structure for switchgrass production that more closely resembles

integrated timber production and processing. For a low-cost perennial feedstock with a long stand life and wide harvest window, such as switchgrass, market forces may drive the industry toward vertical integration. A mature switchgrass to bio-based products industry may more resemble a vertically integrated timber harvest, production, and processing industry than the atomistic corn grain system.

For a price, the history with the CRP indicates that a substantial quantity of U.S. farmland could be bid away from current uses and put into switchgrass for biomass production.

# 8.3 Machinery Costs

We cost machinery used in the establishment, maintenance, and harvest operations following the methodology set forth in AAEA [4], ASABE [5, 6], and Turhollow et al. [7]. Included are costs for depreciation and interest; repairs; insurance, housing, and taxes; fuel; and labor. Turhollow et al. [7] included operating interest for machinery in their methodology, but in this chapter operating interest is accounted for in the crop budgets. Table 8.3 summarizes the costs for machinery on a per hr and per ha basis. Hours of use for the costs in Table 8.3 are based on a 10 year machine life. In Table 8.4 we show how hours of annual operation affect costs. Note that there are two baler productivities, one from Larson and English [8] and the other from Shinners et al. [9], which are significantly different and affect baler costs.

The conclusion one can make from Table 8.4 is that if not enough hours of annual machine use occur, then machine costs are very expensive. A producer would be better off hiring custom operators or renting the equipment than owning if annual hours of use are limited. Harvest companies could develop in a manner similar to custom grain harvesting firms that harvest a substantial quantity of the grain produced in the U.S. Great Plains. Custom grain harvest firms take advantage of the economics of size associated with ownership and operation of machines used to harvest grain.

# 8.4 Establishment

Costs are incurred in the first year, and possibly second year for reseeding, to establish a stand of switchgrass. These include preparation of the soil, seeding, weed control, and possibly fertilization. Griffith et al. [10] have two conventional and two no-till establishment scenarios for (1) cropland harvested in fall or pastureland and (2) winter wheat grazed out or harvested for hay in April (Table 8.5). These were done for Oklahoma and are based on custom rates. Griffith et al. use a lower cost for seed and fertilizer (diammonium phosphate). We have updated the

Implement	Size (m)	Tracto (kW)		yr <sup>-1</sup> )		lement ha <sup>-1</sup> )	(\$ hr <sup>-1</sup>		$r^{-1}$	Total (\$ hr <sup>-1</sup> )	Total (\$ ha <sup>-1</sup> )
Chisel plow	5.49	97	200		0.26	6	19.84	61.6	3	81.46	21.70
Offset disk	3.66	97	200		0.35	i4	15.28	61.6	3	76.90	27.21
Fertilizer & lime spreader	12.2	45	120		0.10	)4	23.28	34.1	4	57.43	5.97
Boom sprayer	15.2	45	150		0.09	6	26.94	34.1	4	61.08	5.89
Press wheel drill	6.10	97	150		0.29	1	31.67	61.6	3	93.30	27.16
Mower-condit.	4.57	97	250		0.13	8	29.56	61.6	3	91.18	30.97
Side-delivery rake	2.44-3.66	45	250		0.19	1	5.90	34.1	4	40.05	18.89
			(kW)	(hr y	$(r^{-1})$	(dry Mg hr <sup>-</sup>	× ·	r <sup>-1</sup> ) (	\$ hr <sup>_</sup>	<sup>1</sup> ) (\$ hr <sup>-</sup>	<sup>1</sup> ) ( $\$ dry Mg <sup>-1</sup> )
Round bale mo	ver 1	4 bales	97	300		23.10	23.8	0 6	1.63	85.42	4.08
Rectangular bal	e mover 1	6 bales	97	300		18.33	34.8	3 6	1.97	96.45	5.80
Baler productiv	ity from La	rson and	l Englis	sh [ <mark>8</mark> ]							
Round baler <sup>a</sup>			97	150		5.44	59.7	2 6	1.63	121.34	22.29
Rect. baler <sup>b</sup>			119	300		10.89	82.4	8 7	5.60	158.08	14.52
Baler productiv	ity from Sh	inners e	t al. [ <mark>9</mark> ]								
Round baler <sup>a</sup>			97	150		19.00	59.7	2 6	1.63	121.34	6.39
Rect. baler <sup>b</sup>			119	300		25.20	82.4	8 7	5.60	158.08	6.27

Table 8.3 Machinery costs

 $^{\rm a}$  1.83 m dia, 1.52 m wide;  $^{\rm b}$  0.91  $\times$  1.22  $\times$  2.44 m

seed cost to a current quote of  $33.07 \text{ kg}^{-1}$  and for all fertilizers use a 4 year average (2008–2011) because fertilizer prices have been so volatile in recent years.

For conventional tillage, total costs are \$589 and \$508 for land that was previously in a fall harvested crop and winter wheat that was either grazed or hayed, respectively (Table 8.5). Amortized over 10 years, costs are \$82 and \$71 ha<sup>-1</sup>. If lime (assume 2.24 Mg ha<sup>-1</sup> at \$34.94 Mg<sup>-1</sup> and an additional application) and potassium (assume 90 kg ha<sup>-1</sup> as  $K_2O$  at \$0.697 kg<sup>-1</sup> as  $K_2O$ ) are needed, costs increase by \$147 ha<sup>-1</sup> and amortized costs increase by \$20 ha<sup>-1</sup>.

If no-till is used, overall costs are lower, with no plowing, disking, or cultipacking, but herbicide costs are higher (42 and 32 ha<sup>-1</sup> for land that was previously in a fall harvested crop and winter wheat that was either grazed or hayed, respectively). Total costs for no-till are 506 and 482 ha<sup>-1</sup> for land that was previously in a fall harvested crop and winter wheat that was either grazed or hayed, respectively (Table 8.5). If seed costs are only 13.23 kg<sup>-1</sup> (as they were at the time Griffith et al. did their calculation), then with minimal herbicides and no mowing, no-till establishment costs can be as low as 276 ha<sup>-1</sup> and amortized costs 338 ha<sup>-1</sup>.

Equipment	Baseline	ha of ar	nnual use				
	hours	50	100	200	400	800	1,600
Chisel plow-hr	200	13	27	53	107	213	
Chisel plow-\$ hr <sup>-1</sup>	32.68	163.96	89.91	55.33	39.94	32.13	
Offset disk-hr	200	18	35	71	142	283	
Offset disk - $\$ hr <sup>-1</sup>	15.28	68.64	37.61	23.65	17.29	13.64	
Fertilizer and lime spreader-hr	120	5	10	21	42	83	166
Fertilizer and lime spreader-\$ hr <sup>-1</sup>	23.28	189.40	99.38	55.35	35.48	26.37	21.02
Press wheel drill-hr	150	15	29	58	116	233	
Press wheel drill-\$ hr <sup>-1</sup>	31.67	125.73	70.89	46.33	34.60	27.53	
Boom sprayer-hr	150	5	10	19	39	77	154
Boom sprayer-\$ hr <sup>-1</sup>	26.94	305.31	157.59	84.20	49.80	34.58	26.68
Mower-conditioner-hr	250	17	34	68	136	272	
Mower-conditioner-\$ hr <sup>-1</sup>	29.56	156.06	84.45	51.11	36.35	28.86	
Side delivery rake-hr	250	24	47	94	189	377	
Side delivery rake-\$ hr <sup>-1</sup>	5.90	25.25	13.96	8.91	6.55	5.11	
	Hours of	annual us	e not bas	ed on a	rea, but	on crop	
	produc	ction					
Round baler-hr	150	100	200	400	800	1,500	
Round baler-\$ hr <sup>-1</sup>	59.72	67.71	55.19	46.80	41.04	37.51	
Rectangular baler-hr	300	100	200	400	800	1,500	3,000
Rectangular baler-\$ hr <sup>-1</sup>	82.48	127.91	94.35	75.76	63.29	55.40	49.49
Round bale mover-hr	300	100	200	400	800	1,500	
Round bale mover- $hr^{-1}$	23.80	37.03	27.34	21.74	17.80	15.23	
Rectangular bale mover-hr	300	100	200	400	800	1,500	
Rectangular bale mover-\$ hr <sup>-1</sup>	34.83	54.18	40.01	31.82	26.05	22.29	

Table 8.4 The effect of annual hours of use on machine costs

For herbicides, Mitchell et al. [11] suggest using a combination of quinclorac (0.56 kg ha<sup>-1</sup>) and atrazine (1.12 kg ha<sup>-1</sup>) to control grassy and broadleaf weeds for establishment and 2,4-D (1.06–2.13 kg ha<sup>-1</sup>) to control broadleaf weeds during the establishment year. This combination of herbicides is more expensive (65-76 ha<sup>-1</sup>) than those used by Griffith et al. [10] (21-42 ha<sup>-1</sup>).

We estimated costs for machinery using the values in Table 8.3 and used the herbicide costs based on Mitchell et al. [11] and include a potassium application of 90 kg ha<sup>-1</sup> (as K<sub>2</sub>O) at a cost of \$0.697 kg<sup>-1</sup> (as K<sub>2</sub>O) and a lime application of 2.24 Mg ha<sup>-1</sup> at a cost of \$34.94 Mg<sup>-1</sup>. Total establishment costs are \$721 and \$692 ha<sup>-1</sup> and amortized over 10 years are \$100 and \$96 ha<sup>-1</sup> for conventional and no-till, respectively.

Establishment costs will vary depending on land prices and the need for fertilizer and lime. Depending on the soil pH, lime could be applied at from 0 to  $2.24 \text{ Mg ha}^{-1}$ . Depending on soil levels of phosphorus and potassium, application may or may not be needed. There are large areas in the United States

Item	Units	$\$ unit <sup>-1</sup>	Previous crop				
			Croplan harvest pasture		Winter grazed harveste hay in S	or ed for	
			Conven	tional til	lage		
			Units ha <sup>-1</sup>	\$ ha <sup>-1</sup>	Units ha <sup>-1</sup>	\$ ha <sup>-1</sup>	
Chisel plow	ha	27.17	1	27.17	0	0	
Fertilizer (diammonium phosphate)	kg	0.744	48.2	35.83	48.2	35.83	
Apply fertilizer	ha	10.23	1	10.23	1	10.23	
Disk	ha	24.70	3	74.10	1	24.7	
Cultipack	ha	22.23	1	22.23	1	22.23	
Seed	kg	33.07	5.6	185.19	5.6	185.19	
Seeding	ha	33.10	1	33.10	1	33.10	
Rotary mow	ha	8.65	1	8.65	1	8.65	
Herbicide							
Glyphosate	kg	8.11	1.26	10.23	1.26	8.11	
Broadleaf, post emergent	ha	11.12	1	11.12	1	11.12	
Apply herbicide	ha	12.20	2	24.40	2	24.40	
Land	ha	111.15	1	111.15	1	111.15	
Interest on operating costs	\$	0.065	553.38	35.97	476.81	30.99	
Total	ha			589.35		507.81	
Amortize over 10 years	ha	0.1391		81.98		70.64	
Total without fertilizer, rotary mower, and glyphosate	ha			507.21		425.66	
Amortize over 10 years	ha	0.1391		70.55 No-till		59.21	
Total for no-till	ha			505.60		481.71	
Amortize over 10 years	ha	0.1391		70.33		67.01	
Total for no-till without fertilizer, rotary mower, and Glyphosate	ha			418.11		394.23	
Amortize over 10 years	ha	0.1391		58.16		54.84	

**Table 8.5** Establishment costs for conventional and no-till scenarios based on Griffith et al. [10], with seed at  $33.07 \text{ kg}^{-1}$ 

where limited or no potassium and lime are applied, especially in the western half of the United States. Land prices have a wide variance. For example in 2010, Iowa cropland and pastureland rented for \$435 and \$99 ha<sup>-1</sup>, respectively, and Texas non-irrigated cropland rented for \$64 ha<sup>-1</sup> [12]. With land cost at \$111 ha<sup>-1</sup>, based on Griffith et al. (Table 8.5) costs can range from \$394 ha<sup>-1</sup> (\$31.57 ha<sup>-1</sup> amortized over 10 years) for no-till with no potassium fertilization, no lime and minimal herbicides (\$276 and \$38 ha<sup>-1</sup> amortized if seed costs are \$13.23 kg<sup>-1</sup>) to, based on our calculation, \$721 ha<sup>-1</sup> (\$100.26 ha<sup>-1</sup> amortized over 10 years) for conventional tillage with fertilization/lime and a full spectrum of herbicides.

		Based on Griffith et al. [10]			Our calculations		
Item	Unit	$\$ unit <sup>-1</sup>	Quantity	$ha^{-1}$	\$ unit <sup>-1</sup>	Quantity	$ha^{-1}$
Seed	kg	33.07	1.4	46.30	33.07	1.4	46.30
Seeding	ha	33.10	0.5	16.55	13.23	0.5	13.58
Herbicide							
Glyphosate	kg	8.11	0.32	2.56			
Broadleaf, post emergent	ha	11.12	0.25	2.78			
Atrazine					9.53	0.14	1.33
Quinclorac					77.16	0.40	30.86
2,4-D					10.44	0.75	7.83
Herbicide application	ha	12.20	2	24.40	5.89	3	17.68
Interest on operating costs	\$	0.0325	92.58	3.01	0.0325	117.58	3.82
Total	ha			95.59			121.40
Amortize over 9 years	ha	0.1502		14.36	0.1502		18.24
Total with seed at \$13.23 kg <sup><math>-1</math></sup>				66.91			84.64
Amortize over 9 years	ha	0.1502		10.05	0.1502		12.72

 Table 8.6
 Reseeding costs (25% of land)

If land cost is \$198 ha<sup>-1</sup>, then establishment cost is  $813 \text{ ha}^{-1}$  (\$113.12 ha<sup>-1</sup> amortized over 10 years).

Some land planted to switchgrass may not successfully establish in the first year, so reseeding may be necessary. Based on Griffith et al. [10], if 25% of switchgrass needs reseeding, we estimate a cost of \$96 ha<sup>-1</sup> and amortized over 9 years' costs are \$14.36 ha<sup>-1</sup> (Table 8.6). Based on our calculations, including the herbicides suggested by Mitchell et al. [11], reseeding costs are \$121 ha<sup>-1</sup> and amortized over 9 years' costs are \$18.24 ha<sup>-1</sup>. If seed costs are \$13.23 kg<sup>-1</sup>, then reseeding costs are \$67 and \$85 ha<sup>-1</sup> and amortized over 9 years are \$10.05 and \$12.72 ha<sup>-1</sup>, based on Griffith et al. and based on our calculations, respectively. Depending on the assumed values for inputs, amortized reseeding costs can range between \$10.05 and \$18.24 ha<sup>-1</sup>.

The longevity of the stand and the interest (discount) rate affect the amortized cost of establishing switchgrass (Table 8.7). In Table 8.7 the establishment costs for no-till establishment on fall-harvested cropland or pasture from Table 8.5 ( $$505.60 \text{ ha}^{-1}$ ) amortized over the number of years in the first column plus the re-seeding cost from Table 8.6 ( $$92.58 \text{ ha}^{-1}$ ) amortized over the number or years in the first column minus one, are shown as affected by discount rate and years of stand life.

# 8.5 Maintenance

Once established, switchgrass should require limited attention until harvest. Fertilizer is assumed to be applied each year. Based on Griffith et al. [10], using up-dated fertilizer prices, maintenance costs are  $119 \text{ ha}^{-1}$ , including amortized

Years of stand life	Interest (disco	unt) rate	
	0.04	0.065	0.10
5	140	150	164
6	118	127	141
7	102	112	126
8	91	100	114
9	82	92	106
10	75	85	99
11	70	79	93
12	65	74	89
13	61	71	85
14	57	67	82
15	55	64	79
20	44	55	71

**Table 8.7** Effect of interest (discount) rate and years of stand life on amortized establishment and reseeding cost ( $ha^{-1}$  yr<sup>-1</sup>), based on no-till establishment on fall-harvested cropland or pasture

 Table 8.8
 Maintenance costs for years after establishment, excluding harvest, based on Griffith et al. [10]

Item	Unit	unit <sup>-1</sup>	Quantity	$ha^{-1}$
Urea	kg	0.554	103	57.13
Diammonium phosphate	kg	0.744	48	35.83
Fertilizer application	ha	10.23	1	10.23
Land	ha	111.15	1	111.15
Establishment+ reseeding cost amortized	ha			81.98
Interest on operating capital		0.0325	296.32	9.63
Total				305.95

establishment, reseeding and land (Table 8.8). In the Great Plains and Midwest, for each dry Mg of anticipated harvest, Mitchell et al. [11] recommend 10 kg N dry  $Mg^{-1}$  if harvested before a killing frost and 6–7 kg N if the previous year is harvested after a killing frost. We assume harvest takes place after a killing frost, and N is applied at a rate 6.5 kg N dry  $Mg^{-1}$ . Phosphorus and potassium may need to be applied, depending on the soil levels. In this analysis, we cost the application of 44 and 88 kg ha<sup>-1</sup> of phosphorus (as P<sub>2</sub>O<sub>5</sub>) and potassium (as K<sub>2</sub>O), respectively, as well as no potassium application (Table 8.9).

A well-established stand should typically require only herbicides to control broadleaf weeds only once or twice every 10 years, using 2-4,D at  $1.06-2.13 \text{ kg ha}^{-1}$ . One application of 2-4,D costs \$11-\$22 ha^{-1} and application cost is \$11.50 ha^{-1}.

		Yield (d	lry Mg ha	L <sup>-1</sup> )		
		4	8	12	16	20
Fertilizer	\$ kg <sup>-1</sup>	kg ha <sup>-1</sup>				
Nitrogen	1.23	26.0	52.0	78.0	104.0	130.0
Phosphorous (as P <sub>2</sub> O <sub>5</sub> )	1.18	44.8	44.8	44.8	44.8	44.8
Potassium (as K <sub>2</sub> O)	1.14	89.6	89.6	80.6	89.6	89.6
		$ha^{-1}$				
Nitrogen		32.04	64.08	96.12	128.17	160.21
Phosphorous (as P <sub>2</sub> O <sub>5</sub> )		53.04	53.04	53.04	53.04	53.04
Potassium (as K <sub>2</sub> O)		102.32	102.32	102.32	102.32	102.32
Application cost		5.97	5.97	5.97	5.97	5.97
Total ( $\ ha^{-1}$ )		193.37	225.41	257.45	289.50	321.54
Total (\$ dry Mg <sup>-1</sup> )		48.34	28.18	21.45	18.09	16.08
Total without potassium (\$ ha <sup>-1</sup> )		91.05	123.09	155.13	187.17	219.22
Total without potassium ( $\ dry Mg^{-1}$ )		22.76	15.39	12.93	11.70	10.96

Table 8.9 Fertilizer maintenance costs with and without potassium application as a function of yield

# 8.6 Harvest

Harvest is the single most expensive operation. The standard for herbaceous biomass to be harvested is as bales of hay. Costs for both large round (1.83 m diameter  $\times$  1.52 m wide) and large rectangular (square) bales (0.91  $\times$  1.22  $\times$  2.44 m) are estimated. The operations assumed for the harvest stage are: mow-condition, rake, bale, and move bales to field edge. Mowing-conditioning and raking are assumed to be independent of yield and are a function of area. Baling and bale moving are a function of how much biomass is handled. Based on work by Larson and English [8] on switchgrass, the throughput capacity for large round and large rectangular balers are 5.44 and 10.89 dry Mg hr<sup>-1</sup>, respectively. The throughput for the large rectangular baler is consistent with Kemmerer and Liu [13] with a throughput of 11.37 dry Mg hr<sup>-1</sup>, based on a 0.875 dry matter fraction. Large round bales are 156 kg m<sup>-3</sup> and 624 kg, while large rectangular bales use twine.

Shinners et al. [9] found similar bale densities, averaging 163 and 175 kg m<sup>-3</sup> for reed canarygrass and switchgrass, but much higher baler productivities, averaging 19.0 and 25.2 dry Mg hr<sup>-1</sup> for switchgrass for a round baler with net wrap and a large rectangular baler, respectively.

A mower-conditioner is used to cut the switchgrass and condition it so it will dry faster, and a rake puts it into a windrow for the baler. We assume that the costs of these two operations are independent of the yield and are based on the area covered. Including the tractor, the mower-conditioner costs  $30.97 \text{ ha}^{-1}$  and the rake costs  $18.89 \text{ ha}^{-1}$  (Table 8.10). So the cost of these two operations decreases as yield increases.

Equipment	\$ ha <sup>-1</sup>	
Mower-conditioner	30.97	
Rake	18.89	
	Round bales	Rectangular bales
	$dry Mg^{-1}$	
Baler (Larson and English [8] productivity)	22.29	14.52
Baler (Shinners et al. [9] productivity)	6.39	6.27
Twine/wrap	3.31	2.30
Bale mover (to field edge)	4.08	5.80
Transport	8.02	7.50

Table 8.10 Harvest (including tractor) and transport costs

Based on the baler productivities in Larson and English [8], baling costs (including wrap/twine) are \$26 and \$17  $Mg^{-1}$  for round and rectangular bales, respectively (Table 8.10). Based on the baler productivities in Shinners et al. [9], baling costs (including wrap/twine) are \$9.70 and \$8.60  $Mg^{-1}$  for round and rectangular bales, respectively, which are significantly lower (Table 8.10). Griffith et al. [10] use a cost (based on custom harvest) of \$24.50  $Mg^{-1}$  for rectangular bales.

Mooney and English [14] found that when harvest and transport costs and storage losses are considered, the use of a mixture of large round and large rectangular bales are optimal. The rectangular bales would be harvested and immediately taken to the biorefinery, whereas the round bales would be stored for later use. Using the baler productivities from Larson and English [8], harvest and trans-port costs for rectangular bales are slightly lower than round bales. Using the baler productivities from Shinners et al. [9], harvest and transport costs are lower for round bales.

# 8.7 Storage

Storage costs consist of out-of-pocket expenses as well as losses of dry matter. Dry matter losses from three studies were summarized by Turhollow et al. [7]. Shinners et al. [9] studied switchgrass losses (Table 8.11). If storage structures are only going to be used one time per year, then storage tends to be expensive. Turhollow et al. [7] present costs for a number of storage structures, plastic wrap on large rectangular bales, and a tarp (Table 8.12).

Turhollow et al. [7] estimated that the cost (out-of-pocket expenses for land rent at \$210 ha<sup>-1</sup>, structure or tarp, labor, and insurance and repair) for storing large rectangular bales four high on (1) the ground, (2) a gravel pad with a tarp, and (3) in a pole frame structure with one side open is \$1.12, \$10.87, and \$25.20 dry  $Mg^{-1}$ , respectively. If losses are for (1), (2), and (3) are 25, 6, and 2%, respectively, and switchgrass is valued at \$55.12 dry  $Mg^{-1}$ , then total costs of storage

Turhollow et al. [7]	% dry matter loss
Enclosed shed	2–5
Open-sided pole structure	3-10
Reusable tarp on ground	5-10
Plastic wrap on ground	4–7
Uncovered on gravel pad	13–17
Shinners et al. [9], switchgrass stored 293 days, large round bales	
Indoor	4.9
Plastic film, outdoor on grass	5.7
Breathable film, outdoor on grass	5.4
Net wrap, outdoor on grass	9.0
Plastic twine, outdoor on grass	9.3
Sisal twine, outdoor on grass	15.4

Table 8.11 Storage losses

 Table 8.12
 Initial costs for storage structures, a tarp, plastic wrap on large rectangular bales [7]

	Initial cost (\$	Useful life
	$m^{-2}$ )	(years)
Pole frame structure, all sides open	71.37	20
Pole frame structure, one side open	99.35	20
Pole frame structure, enclosed	111.62	20
Enclosed shed with concrete floor and foundation	169.43	20
Gravel storage pad	11.75	10
Asphalt storage pad	30.68	10
Hay tarp (including labor to place and remove)	2.91	5
Plastic wrap (2 large rectangular bales; equipment, labor, materials)	$6.71 \text{ dry } \text{Mg}^{-1}$	1

(including losses) are \$14.90, 14.18, and 26.30 dry  $Mg^{-1}$ , respectively. If losses for the plastic wrap option are 6%, then total storage costs for this option are \$11.34 dry  $Mg^{-1}$  (\$6.71 dry  $Mg^{-1}$  for the plastic wrap and \$0.084 dry  $Mg^{-1}$  for the land then increased by 6% to account for the losses, and \$3.31 for the lost dry matter). For outdoor storage if losses are kept to 9% [based on Shinners et al. [9] (Table 8.11)], then storage cost for net-wrapped large round bales is \$5.88 dry  $Mg^{-1}$  (including \$0.92 dry  $Mg^{-1}$  for land and 4.96 dry  $Mg^{-1}$  for lost biomass). If biomass can be directly taken from the field to a conversion facility then the storage costs (including losses) can be avoided. Land was valued at \$210 ha^{-1} in Turhollow et al. and bales were assumed to be stacked four high. For land valued at \$111 ha^{-1} and bales stacked one (two) high, the land cost is \$2.37 (\$1.19) dry  $Mg^{-1}$  (in Turhollow et al. the large rectangular bales are 463 kg, which is approximately the same as the large rectangular bales which are assumed to be 476 kg in this chapter).

For estimating storage costs in this chapter, it is assumed that round bales are not stacked and rectangular bales are plastic wrapped at a cost of  $6.71 \text{ dry Mg}^{-1}$ . Land area for storage allows space for equipment to maneuver around the piles and

is assumed to be 0.21 and 0.13 ha for 100 dry Mg of bales (before storage losses), for round and rectangular bales, respectively. Using a range of land costs of \$111–\$198 ha<sup>-1</sup>, land costs for storage are \$0.23–\$0.41 and \$0.14–0.26 dry Mg<sup>-1</sup> for round and rectangular bales, respectively. For simplicity, a cost of \$0.30 dry Mg<sup>-1</sup> is assumed for the land cost of storage. For storage, losses are assumed to be 9% for net-wrapped round bales and 6% for plastic-wrapped rectangular bales. The cost estimated for the plastic wrap in Turhollow et al. [7] was for 1.22 × 1.22 × 2.44 m bales, while in this chapter the bales are 0.91 × 1.22 × 2.44 m. The amount of wrap is reduced by 25%, so the cost is 75% of \$6.71 dry Mg<sup>-1</sup>, or 5.03 dry Mg<sup>-1</sup>.

## 8.8 Transport

We use a very simple model for transport costs, assuming a truck with a flatbed trailer costs \$75 h<sup>-1</sup>. Also assumed is that a round trip takes 2 h, so each load costs \$150. A load consists of 30 round bales (18.7 dry Mg) or 42 rectangular bales (20.0 dry Mg). Transport costs are \$8.02 and \$7.50 dry Mg<sup>-1</sup>. Griffith et al. [10] assume a transport cost for rectangular bales of \$4.50 per 680 kg bale (wet), or \$7.56 dry Mg<sup>-1</sup> assuming 12.5% moisture. In the low-cost scenario in the summary, we assume only 1 h is needed for a round trip, which cuts the transport cost into half.

# 8.9 Summary

Costs for the entire operation from growing to delivering the switchgrass are summarized in Table 8.13.<sup>1</sup> Low-cost and high-cost scenarios are presented to give an idea of the possible range of costs, which is significant. In the low-cost scenario, establishment costs are low because seed costs are low, no potassium and lime are applied, weed control (herbicide) requirements are low, and a lower land price is used; reseeding also uses a low seed cost; baling uses the lower cost harvest choice (round versus rectangular bales),<sup>2</sup> there is no storage (costs and losses), and only one hour (instead of two) is required for a round trip to transport bales to the biorefinery. Baler productivity makes a huge difference, as evidenced by the difference in harvest costs between those based on the productivities of Larson and English [8] and those of Shinners et al. [9], particularly the round baler

<sup>&</sup>lt;sup>1</sup> Note that storage costs in Table 8.13 consist of land rent plus the cost of plastic wrap for the rectangular bales. Storage losses, in the high-cost scenario, are accounted for as the difference between Total and Total after losses, and Total after loss is calculated as: Total/(1-loss), where loss is 9% for round bales in net wrap and 6% for rectangular bales in plastic wrap.

<sup>&</sup>lt;sup>2</sup> Rectangular bales for Larson and English [8] and round bales for Shinners et al. [9].

	<b>1</b> .	12 155.13 367.89 11.96 379.85	16 187.17	20	4	8	12	16	20
ishment ding zer sr-conditioner tal	<b>1</b> .	155.13 367.89 111.96 379.85	187.17						)
lishment ding zer r-conditioner tal		155.13 367.89 11.96 379.85	187.17						
ding zer r-conditioner tal		155.13 367.89 11.96 379.85	187.17		113.12				
zer er-conditioner tal		155.13 367.89 11.96 379.85	187.17		18.24				
r-conditioner tal		367.89 11.96 379.85		219.22	193.37	225.41	257.45	289.50	321.54
r-conditioner tal		367.89 11.96 379.85			197.60				
tal		367.89 11.96 379.85			30.97				
		367.89 11.96 379.85			18.89				
_		11.96 379.85	399.94	431.98	572.19	604.23	636.27	668.32	700.36
Interest on operating capital 9.67		379.85	13.00	14.04	18.60	19.64	20.68	21.72	22.76
Subtotal (\$ ha <sup>-1</sup> ) 313.68	68 346.77	22222	412.93	446.02	590.79	623.87	656.95	690.04	722.12
$[g^{-1})$	2 43.35	31.65	25.81	22.30	147.70	77.98	54.75	43.13	36.16
Baler	r productivity b	Baler productivity based on Larson and English [8]	nd English [8]						
\$ dry	$$ dry Mg^{-1}$								
Recti	Rectangular bales				Round bales	ales			
Baler 14.52	2				22.29				
Twine/wrap 2.30					3.31				
Bale carrier 5.80					4.08				
Telescopic handler 1.92					1.46				
Transport 3.75					8.02				
Storage					0.30				
Subtotal 28.28	8				39.46				
Interest on operating capital 0.92					1.28				
Subtotal 29.20	0				40.74				
Total 107.62	62 72.55	60.86	55.01	51.50	188.43	118.72	95.48	83.87	76.89
Total after losses					207.07	130.46	104.93	92.16	84.50

200

Table 8.13 (continued)											
	Low cost	st					High cost				
Yield (dry Mg $ha^{-1}$ )	4	8	12	16		20	4	8	12	16	20
	Baler pı	Baler productivity from Shinners et al. [9]	m Shinners	et al. [9]							
	Round bales	ales					Rectangular bales	ar bales			
Baler	6.39						6.27				
Wrap/twine	3.31						2.30				
Bale carrier	4.08						5.80				
Telescopic handler	1.46						1.92				
Transport	4.01						7.50				
Storage							5.33				
Subtotal	19.24						29.12				
Interest on operating capital	0.63						0.95				
Subtotal	19.87						30.06				
Total	98.29	63.21		51.52	45.68	42.17	177.48	107.91	84.72	73.12	66.16
Total after losses							186.29	112.27	87.60	75.27	67.87

productivities of 5.44 versus 19.0 dry Mg hr<sup>-1</sup>, respectively. In recent years the prices of fertilizers and lime have increased, giving regions that do not require potassium and/or lime a competitive advantage. Not having to apply potassium in the maintenance years lowers costs by  $102 \text{ ha}^{-1}$ . The balers in the Shinners et al. study were limited by their capacity to process the volume of switchgrass. Shinners et al. indicate that modifications to the baler pickup and throat might be needed to handle both the high tonnage and physical volume of biomass. Not having to store switchgrass lowers costs (from both out-of-pocket storage expense and avoiding loss of dry matter) by \$3-\$19 and \$8-\$17 for round and rectangular bales, respectively.

Yield has a large impact on the cost of producing switchgrass. In the high-cost scenario, the cost of switchgrass is above \$100 dry  $Mg^{-1}$  at either 4 or 8 dry Mg ha<sup>-1</sup>. In the low-cost scenario, at a yield of 8 dry Mg ha<sup>-1</sup> or greater with the baler productivity from Shinners et al. and a yield of 12 dry Mg ha<sup>-1</sup> or greater with the baler productivity from Larson and English, the cost of switchgrass is below \$61 dry Mg<sup>-1</sup>.

In the right circumstances, switchgrass using baling can provide biomass delivered to a biorefinery at a cost of less than \$60 dry Mg<sup>-1</sup>. These circumstances include a combination of some, but not necessarily all of: a reasonable land cost, limited need for fertilizers and herbicides, baling technology with the throughput from Shinners et al. [9] (19-25 dry Mg hr<sup>-1</sup>). In the high-cost scenario, using round baling and the throughput based on Larson and English [8] (5.44 dry Mg  $hr^{-1}$ ) the cost of switchgrass delivered to a biorefinery after accounting for a 9% loss of dry matter is \$84 dry Mg<sup>-1</sup> at a yield of 20 dry Mg ha<sup>-1</sup> (before losses). If the round baler can achieve a throughput of 19.0 dry Mg hr-1 (as in Shinners et al.) and transport cost is \$4.01 dry Mg<sup>-1</sup>, then delivered cost is \$62 dry Mg<sup>-1</sup>. If in addition the land price is at \$111 ha<sup>-1</sup> instead of \$198 ha<sup>-1</sup> and no potassium is needed, then at 12 dry Mg ha<sup>-1</sup> (before losses) delivered cost (after accounting for losses of 9%) is \$64 dry Mg<sup>-1</sup>. If land prices are high, then a high yield is needed to offset this cost. In the low-cost scenario, which represents geographic locations such as Oklahoma and Kansas, costs are below \$61 dry Mg<sup>-1</sup> for yields above 12 dry Mg ha<sup>-1</sup>. In the right circumstances it is possible to supply switchgrass at prices below \$61 dry  $Mg^{-1}$ .

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