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Realizing the Potential of Rapid-Cycling *Brassica* as a Model System for Use in Plant Biology Research

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Abstract

Rapid-cycling *Brassica* populations were initially developed as a model for probing the genetic basis of plant disease. Paul Williams and co-workers selected accessions of the six main species for short time to flower and rapid seed maturation. Over multiple generations of breeding and selection, rapid-cycling populations of each of the six species were developed. Because of their close relationship with economically important *Brassica* species, rapid-cycling *Brassica* populations, especially those of *B. rapa* (RCBr) and *B. oleracea,* have seen wide application in plant and crop physiology investigations. Adding to the popularity of these small, short-lived plants for research applications is their extensive use in K–12 education and outreach.

Key words: *Brassica rapa*; *Brassica oleracea*; Fast plants; Rapid-cycling; Model

Development of a Successful Model System for Research and Teaching

Introduction

The genus *Brassica* comprises a diverse group of plants with worldwide economic importance. Vegetable types of *Brassica* are best known in the United States (broccoli, Brussels sprouts, cabbage, cauliflower, collard, kale, mustard greens, turnip, rapini). Worldwide, especially in Canada, Northern Europe, China, and India, rapeseed (oil-seed *Brassica* spp.) is the predominant edible oil crop. Other uses of *Brassica* spp. include production of condiment mustard, animal feed, and fodder (Williams and Hill 1986).

Agricultural and horticultural selection has given rise to divergent growth forms within the genus (Figure 1). These economically important *Brassica* species include three diploid species and three naturally occurring allotetraploid species (Table 1). The ease with which diploid and tetraploid species may be intercrossed makes transfer of useful traits among *Brassica* spp. convenient. Before development of rapid-cycling populations, the relatively long (0.5–2 y) life cycles for most of the economically important brassicas was a negative element in this otherwise promising scenario for continuous plant improvement.

In search of sources of disease resistance, Williams and Hill (1986) began growing *Brassica* from a worldwide collection of 2,000 accessions. They noticed heterogeneity in days to flowering in a few plants from each species and set about to select

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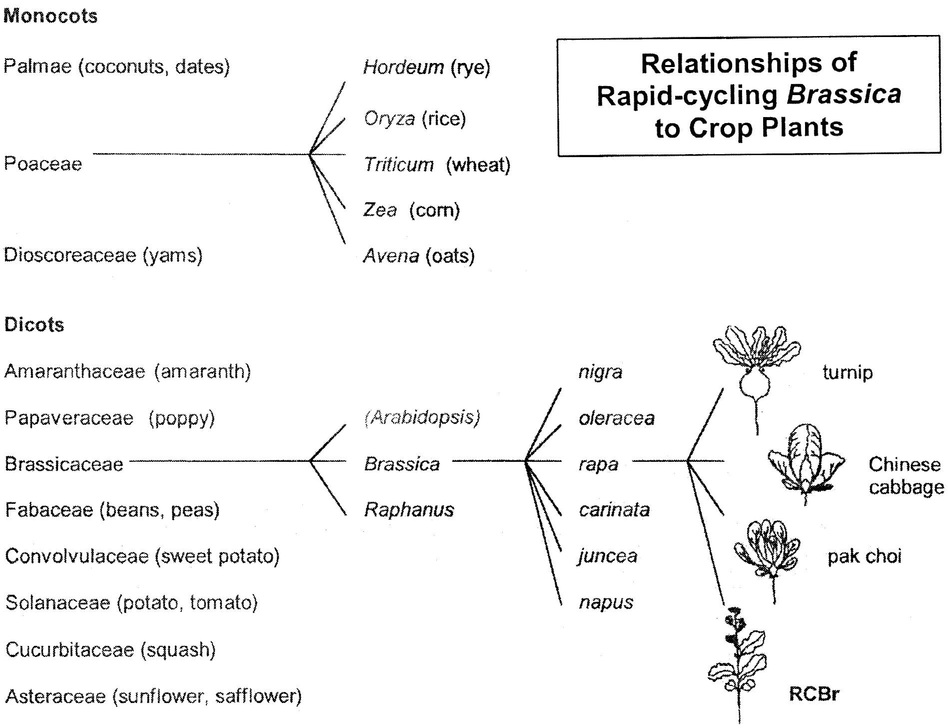

Figure 1. The species *B. rapa* contains a number of morphotypes, including turnip, Chinese cabbage, and RCBr. Similar diversity in morphotypes is found in the other *Brassica* species and spans the range from oil seed types in *B. napus* to the numerous vegetable and fodder types in *B. oleracea*. Taxonomically, the relationship between *Brassica* and the model plant *Arabidopsis* is analogous to the relationship between the world's major crop plants rice and wheat. RCBr is unique among model plants discussed in this volume because of its close genetic relationship to economically important plants.

populations with short reproductive times. Through recurrent selection and breeding under conditions of high plant density, small root zone, and continuous illumination from artificial light, populations were created by interpollinating the early flowering types in each species. In successive generations the following selection criteria were used:

- Minimum time from sowing to flowering (fastest 10%)
- Rapid seed maturation
- Absence of seed dormancy
- High female fertility

Repeated cycles of mass pollination within selected types led to stable populations, with 50% of individuals flowering within a 2- to 3-day period and created rapid-cycling base populations of six *Brassica* species (Table 1). These species were to serve as models for plant pathogen interactions with the idea that new sources of resistance could be efficiently screened in the rapid-cycling background and then readily transferred to the horticultural type. Because of the genetic heterogeneity of the base population, the reservoir of genes conferring differential response to disease-causing organisms was manifest in plant-to-plant variation. The great general utility of

Table 1. Characteristics of the Rapid-cycling Base Populations of *Brassica*

Species	Common Name	Days to First Flower	Days to Seed Maturity	Number of Chromosomes	Nuclear Genome
Diploid stocks					
B. rapa	Turnip group	16	36	20	aa
B. nigra	Black mustard	20	40	16	bb
B. oleracea	Cole crops	30	60	18	cc
Tetraploid stocks					
B. juncea	Mustards	19	39	36	aabb
B. carinata	Ethiopian mustard	26	56	34	bbcc
B. napus	Oilseed rape	25	55	38	aacc

Adapted from Williams 1982.

the rapid-cycling lines quickly became apparent, and attention focused on identifying interesting types within the fastest rapid-cycling brassica populations, RC *B. rapa* and *B. oleracea.*

Use in Education

After initial testing of rapid-cycling *B. rapa* in university genetics and plant breeding courses, Williams and co-workers received funding from the Educational Materials Development Program of the National Science Foundation to refine the genetic material and create the curriculum infrastructure needed to move the plants into the K–college classrooms. Included in the concept was development of a self-supporting plant culture system amenable to the realities of the classroom setting. A self-watering wickpot reservoir system that uses slow-release fertilizer, coupled with a compact growth area provided by lighting from banks of closely space fluorescent bulbs, made the growth of these Wisconsin Fast Plants™ (WFP) feasible for teachers.

Curricular materials developed and/or tested by teachers participating in the program addressed the educational goals of:

- Teaching basic concepts of biology
- Stimulating inquiry and problem solving
- Increasing the impact of genetics teaching
- Infusing the excitement of hands-on learning into the classroom

A master teacher system encouraged the rapid adoption of Fast Plants by schools across the country. For each master teacher receiving training at an NSF-sponsored Fast Plants Workshop, dozens more were trained when the master teacher conducted workshops in the local school district. These teachers then used the curriculum with hundreds of students in their classrooms. The development of a newsletter and subsequently the popular website (http://www.fastplants.org), updating teachers on new activities, was a key element in the success of the program (Williams 1990).

Initially the distribution of WFP seeds, growing kits, and curricular materials was shouldered exclusively by the WFP Program at the University of Wisconsin in Madison. Subsequently, the kits and manuals were commercialized by Carolina Biological Supply. An impressive collection of publications exists that are compendia of hands-on activities with WFP (AgriScience Institute and Outreach Program 1994; Williams 1993; Wisconsin Alumni Research Foundation 1989). Because of the classroom-ready tradition of the program, these publications often include all of the ancillary information and presentation materials necessary for the teacher to implement the project with students. This philosophy has been key to the widespread adoption of Fast Plants into all stages of science curricula.

Strengths of the System for Research Applications

Oil Seed Model

A chief value of the RCBr model is in the possibility of combining genetic research into the investigation and improvement of seed storage reserves in *Brassica.* Worldwide, rapeseed is the source of the fourth most commonly traded vegetable oil and contains 40% oil, which is pressed from the seed, leaving behind a high-protein seed meal used for animal feed and fertilizer (Williams and Hill 1986). Some rapeseed cultivars are important sources of erucic acid, a 22-carbon unsaturated fatty acid that cannot be metabolized by mammals but is used industrially for resins, lubrication oil, and in steel manufacturing. Erucic acid levels are controlled by a series of allelic genes (Robbelen and Thies 1980).

The timing of seed storage protein deposition during seed development has been examined relative to carbohydrate accumulation in RC *B. rapa* (Kuang and others 2000b; Sinniah and others 1998a). The ratio of oligosaccharides to total sugars correlated positively with the acquisition of desiccation tolerance (Sinniah and others 1998a). Water status of the mother plant greatly affected seed quality development (Sinniah and others 1998b). The onset of normal germination ability occurred as early as 12 days after pollination, when seeds were half filled, whereas tolerance to rapid desiccation began 16 days after pollination (Sinniah and others 1998b). This model has proven useful for the study of desiccation tolerance, because three types of stress proteins accumulate in RCBr seeds (HSP, a dehydrin, and a group 3 LEA protein) (Bettey and others 1998). Furthermore, Porterfield and colleagues (1999) used RC *B. rapa* as a model for studying the oxygen-depleted zones surrounding developing seeds inside siliques in the Brassicaceae, initiating new avenues for research on the way in which microenvironments within the silique influence seed development.

Plant/Pathogen Interactions

Initially developed to aid in the selection and introduction of disease resistance genes into economically important *Brassica* spp., the RCBr system is a useful model for understanding the genetic basis of

Table 2. Pathogens Associated with *Brassica* spp

Pathogen	Disease	Reference
Albugo candida	White rust	Edwards and Williams (1987)
Alternaria brassicicola	Black spot	Sigareva and Earle (1999a,b)
Aphanomyces raphani	Black root	Williams (1987)
Fusarium oxysporum	Fusarium yellows	Bosland and Williams (1988)
Leptoshaeria maculans	Black leg	Mengistu and others (1991)
Peronospora parasitica	Downy mildew	Williams (1987)
Plasmodiophora brassicae	Clubroot	Miller and Williams (1986)
Rhizoctonia solani	Damping off	Williams (1987)
TuMV	Turnip mosaic virus	Shattuck (1993)
		Fjellstrom and Williams (1997)
Verticillium dahliae	Verticillium wilt	Zeise and Buchmuller (1997)
Xanthomonas campestris	Black rot	Williams (1987)

plant disease. Williams (1987) described 36 diseases of brassicas, including nematodes and physiologic problems such as tipburn (caused by calcium deficiency). Bacterial, fungal, and viral pathogens are responsible for the diseases listed in Table 2. In addition, studies have confirmed the colonization of *Brassica* spp. by VA myccorhizae (Glenn and others 1985). Although glucosinolates influence the severity of pest interactions with *Brassica* spp., no relation between glucosinolate level and the colonization ability of VA myccorhizae was found (Glenn and others 1985).

Growth and Development

Research Applications with Plant Growth Regulators

RC *B. rapa* is an especially attractive model for the study of gibberellin control of internode elongation in *Brassica.* Endogenous gibberellic acids (GAs) in the vegetative and reproductive organs change after vernalization in winter canola as the crop moves into the flowering stage (Zanewich and Rood 1993, 1995). In the RC *B. rapa* and *napus* populations differing in height, some genotypes were insensitive to GA treatment, whereas others elongated (Zanewich and others 1991). Rood and colleagues (1989; 1990a; 1990b) quantified GAs in a dwarf rosette mutant (ros) and an elongated internode mutant (ein) of RC *B. rapa.* Cell length and number are affected in these lines (Rood and others 1990a), contributing to the described differences in leaf and reproductive development (Zanewich and others 1990). In the ein mutant it was possible to demonstrate convergent pathways for GA_1 biosynthesis (Rood and Hedden 1994).

Another GA-responsive tissue in RCBr is the seed, where lipase activity shows a negative correlation with GA_{20}, suggesting that GA turnover could be positively correlated with lipase activity (Imeson and others 1993). The seeds of the GA mutants described earlier demonstrated no difference in lipase activity, however, leading Imeson and colleagues to conclude that GA is not the sole regulatory factor. The existence of multiple sites for GA responses within the plant makes RCBr an attractive model for studying the mode of action of this growth regulator.

In the system just described, seed imbibition in the growth regulator, abscisic acid (ABA) decreased lipase activity (Imeson 1993). ABA is also implicated in the decreased growth of salt-stressed RCBr plants (He and Cramer 1996). Whole plant and callus ABA concentrations were lower in the salt-tolerant *B. napus* than in salt-sensitive *B. carinata* (He and Cramer 1996). Photosynthesis is not affected, suggesting a nonstomatal role for the mechanism of growth reduction by ABA.

RCBr studies with auxins and cytokinins are largely confined to the in vitro responses (next section). However, Schadler and others (1994) described hormone-induced parthenocarpy in pistils of RC *B. rapa* after treatment of the pistils with indoleacetic acid (IAA), indolebutyric acid (IBA), or naphthaleneacetic acid (NAA) in lanolin. Classic cytokinin responses of reduced internode elongation and delayed chlorophyll loss were observed in our laboratory in RC *B. rapa* plants sprayed with benzyladenine.

Studies of ethylene effects on horticultural and agricultural relatives of RCBr are numerous, owing to interest in the storage life of vegetables such as broccoli (Ku and Wills 1999) and factors controlling pod shatter in oil seed rape (Johnson-Flanagan and Spencer 1994). Concerns about ethylene buildup in tissue culture have been reported for cauliflower shoot cultures (Zobayed and others 1999) and shoot formation from cotyledon explants of RC *B. rapa* (Teo and others 1997). As with the other growth regulators described earlier, the RCBr system is underused for basic research on the mode of action of ethylene, especially given the ready applications for the research findings in agromonic and horticultural *Brassica* crops.

A new group of growth-promoting lipoidal hormones was discovered in extracts of *B. napus* pollen (Mitchell and others 1970). In 1979, a steroidal lactone called brassinolide was identified as the growth-promoting component in *B. napus* pollen (Mandava 1988). The mode of action of brassinosteroids is being studied extensively using *Arabidopsis* mutants (Clouse and others 1996; Li and others 1996; Szekeres and others 1996). RCBr would be a useful tool in this area of research.

In Vitro Culture

We found that the short life cycle of RCBr populations places unique constraints on the use of in vitro culturing systems. However, many investigators have developed successful in vitro systems, with a variety of interesting applications. For example, callus initiated from the leaves of 7-day-old seedlings of RC *B. napus* and *B. carinata* has been used to screen cellular responses of these species to salinity stress (He and Cramer 1993a).

A whole suite of in vitro techniques is available for culture of different parts of rapid-cycling plants:

- In vitro pollen germination methods (Sato and others 1998; also effective in the rapid-cycling population)
- Standard embryo culture techniques (RC *B. rapa;* Kuang and others 2000a)
- Plant regeneration from cotyledon explants through direct shoot regeneration (Teo and others 1997; takes 40 days from explant to flowering)
- Protoplast culture (RC *B. oleracea;* Hansen and Earle 1994a), which makes possible gene transfer by direct DNA uptake and by protoplast fusion (Time to flower for the regenerants is similar to that of plants grown from seed.)
- Production of embryoids by cultured anthers of RC *B. rapa* and *B. napus* (Aslam and others 1990b)
- Transformation procedures for RC *B. oleracea* var. *capitata* using *Agrobacterium tumefaciens* (Berthomieu and others 1994) and *A. rhizogenes* (Berthomieu and others 1992).

Both intrageneric and intergeneric somatic hybridization can be accomplished using *Brassica,* and this makes RCBr an exciting tool for the study of complex traits under nuclear and somatic genetic control. For example, protoplast fusions between RC-*B. rapa* and *B. oleracea* gave rise to a somatic hybrid *B. napus* with increased vigor and novel seed fatty acids (Hansen and Earle 1994b). Intergeneric somatic hybrids were formed between *Sinapis alba* and RC *B. oleracea* to transfer *Alternaria* resistance to *B. oleracea* (Hansen and Earle 1997). Intertribal somatic hybrids between *B. oleracea* and *Capsella bursa-pastoris* (Sigareva and Earle 1999b) and between *B. oleracea* and *Camelina sativa* (Hansen 1998; Sigareva and Earle 1999a) were also created as a source of *Alternaria* resistance.

Genetics

Through the work of the Crucifer Genetics Cooperative (CrGC; Williams 1987), many useful genetic lines derived from the original rapid-cycling base populations have been catalogued and maintained. These include cytoplasmic traits such as cytoplasmic triazine resistance (see later), cytoplasmic male sterility, and somatic variegation. In addition, growth form variants of RC *B. rapa* (such as ein, ros, and dor) have been identified with specific physiologic traits, in this case conferring different endogenous GA-producing capabilities. Anthocyanin-deficient mutants have been useful tools for understanding the role of this pigment in oxidative stress responses.

Apart from these allelic traits, quantitative genetic variation has been examined in foliar glucosinolate production (Stowe 1998a) and flowering time (Bohuon and others 1998). Gurevitch and others (1996) and Miller and Schemske (1990) investigated the genetic correlation for plant performance in different competition regimens in RCBr. The phenotypic expression of genetic differences depends on density of neighbors. Inbreeding in the RCBr population significantly postponed germination and flowering (Lascoux and Lee 1998) and led to a decrease in pollen viability over three generations, as well as many developmental abnormalities and a marked reduction in the number of seeds set (Aslam and others 1990a).

Self-incompatibility

Brassica species demonstrate self-incompatibility (SI), a phenomenon that involves the recognition of self versus non-self pollen and the rejection of self-related pollen, thus preventing self-fertilization. In *Brassica,* SI is sporophytic and is determined genetically by alleles at the S-locus. The SI system occurs naturally in diploid *Brassica* species but is introduced into the amphidiploid species by interspecific breeding; in both cases the heterosis that results carries the potential of improved traits (Cheung and others 1997).

The female (stigmatic) components of this self-incompatibility recognition reaction are a polymorphic transmembrane receptor protein kinase (Letham and others 1999; Nasrallah 1997; Schopfer and others 1999), and a soluble cell wall–localized glycosylated protein (Cheung and others 1997; Letham and others 1999; Schopfer and others 1999). Both are encoded at the S-locus. The male determinant of self-incompatibility in *Brassica* is encoded at the S-locus by an anther-expressed gene, SCR (Schopfer and others 1999).

Recent progress in genomic analysis has initiated a study of intraspecific mating incompatibility in crucifers. Mutational analysis is clarifying which loci are needed for functional SI (Nasrallah and others 2000). Because no sequences similar to the *Brassica* S-locus genes that are known to be required for the SI response have been found within the *Arabidopsis* genome, Conner and colleagues (1998) have posited that the self-fertile character in the *Arabidopsis* genus is a result of deletion of these self-recognition genes during evolution.

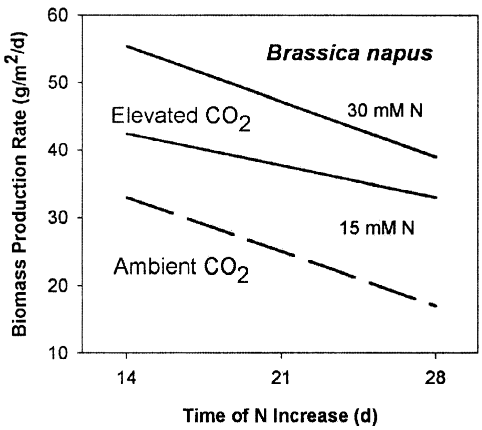

Figure 2. Although developed for rapid life cycles and compact growth form, rapid-cycling populations of *Brassica* can also become quite large when grown without restricted root zones and under high nutrient conditions. Frick and others (1994) grew rapid-cycling *Brassica napus* hydroponically (initially at 2 mM N) and compared growth under ambient and elevated (1,000 ppm) CO_2. At times corresponding to preflowering(14d), flowering (21d), and postflowering (28d), N was increased to full (15 mM) or double-strength (30 mM) Hoagland's concentrations. The graph demonstrates the increase in biomass production caused by elevated CO_2 in full-strength Hoagland's and the additional increase in biomass when the nutrient supply is doubled under elevated CO_2 conditions. Adapted from Frick and others (1994).

Environmental Physiology

Nutrient Responses

Small stature and rapid life cycle are genetically fixed traits that occur under the specific environmental conditions that were used during the RCBr selection regimen. When grown with restricted root space and restricted nutrients, RC *B. rapa* reaches a final height of about 12 cm (base population) and flowers 14 days after planting. Larger root zones and more generous nutrient supplies yield plants with very different characteristics. This can either prove detrimental to the usefulness of the model (Xiao and others 1996) or could conceivably be used to maximize tissue production in plants being harvested for extraction purposes. Similarly, when sufficient root zone and nutrients are supplied, RCBr responds to CO_2 enrichment with greatly enhanced growth (Figure 2). Frick and others (1994) examined the effects of N level, time of N increase, planting density, and CO_2 enrichment (1000 µmol/mol) on yield and seed oil content when RC *B. napus* (CrGC#5-2) was grown in a solid-matrix hydroponic system in a controlled environment. CO_2 enrichment reduced seed yield per unit biomass by stimulating vegetative shoot growth and by delaying flowering and senescence. Seed yield was optimized by lower N applied later in the growth cycle and without CO_2 enrichment. The great plasticity of RCBr with regard to nutrient supply is an underexploited feature of this model plant.

Stress Tolerance

For stress physiologists, a logical extension of the original concept behind the development of RCBr is to mine the rapid-cycling base populations for extremes in stress responses and create new populations on the basis of the differential response to stress. This approach worked well for the study of waterlogging tolerance. Starting with the unselected base population (which is uniform for life cycle time but heterogeneous for other traits [Williams and Hill 1986]) of RC *B. rapa,* Daugherty and Musgrave (1994) documented the range in leaf chlorophyll content in individual plants under normal drainage (E_h = 350 mV) or when waterlogged to the soil surface (E_h = 200 mV). The range in chlorophyll concentrations in the waterlogged plants was large, permitting selection and interbreeding among individuals of the extreme groups. Recurrent selection and breeding over seven generations led to distinct tolerant and sensitive populations, with no significant loss of chlorophyll after waterlogging in the tolerant plants and a 67% decrease in the sensitive population. Physiologic differences of waterlogging tolerant RC *B. rapa* included delayed accumulation of carbohydrates when waterlogged (Daugherty and others 1994) and a delay in alcohol dehydrogenase response (Daugherty and Musgrave 1994). Growth analysis confirmed the more favorable net assimilation rate and relative growth rate of the waterlogging tolerant plants under waterlogged conditions when compared with either the sensitive or unselected populations (Daugherty and Musgrave 1994).

Stress responses in RCBr to environmental pollutants make them suitable subjects for bioassay material. The use of a RCBr life-cycle testing protocol for assessing the effects of surfactants in sludge-amended soils avoids the methodologic challenges in risk assessments posed by (1) different modes of exposure and (2) different types of soil (Kloepper-Sams and others 1996). Sheppard and others (1992; 1993) compared a plant life-cycle bioassay for metal-contaminated soil using RCBr with other bioassays and found RCBr growth responses to be very sensitive to mercury, zinc, and uranium.

Kopsell and Randle (1999) have proposed RCBr populations as a model for investigations into selenium accumulation and metabolism and its genetic control. Organically bound Se holds promise for the delivery of beneficial Se in mammalian diets. RC *B. oleracea* accumulates high amounts of Se in its tissues in response to high Na_2SeO_4 concentrations in the root zone. The unique reproductive relationship of RCBr populations with *Brassica* vegetable and forage crops makes possible the ready transfer of improved Se accumulation traits.

The salt-tolerant features of rapeseed make it one of the first crops grown during low-lying polder land reclamation in the Netherlands (Williams and Hill 1986). Rapid-cycling *B. napus* is more tolerant of salinity than RC *B. carinata,* both at the callus and whole plant level (He and Cramer 1993a, b). In all six RC *Brassica* species, the K/Na ratio in the shoots is greatly reduced by seawater salinity. Neither K/Na ratio nor K-Na selectivity was correlated with the relative salt tolerance of these species (He and Cramer 1993b). The differences in salt tolerance between *B. napus* and *B. carinata* are not related to specific ion effects but to some factor that reduces the net assimilation rate of *B. carniata* during early stages of growth (He and Cramer 1993c, 1996).

Other types of stress responses that have been studied in RCBr include desiccation tolerance acquisition in seeds and anthocyanin-mediated protection from ultraviolet radiation (UV) damage. Stress proteins (HSP, LEA proteins) accumulate in RC *B. rapa* seeds during the late stages of maturation, hastened by water stress to the maternal plant (Bettey and others 1998). Klaper and others (1996) compared growth responses of RC *B. rapa* genotypes differing in anthocyanin content after elevated levels of shortwave ultraviolet radiation (UVB, 280–325 nm). Plants with low levels of anthocyanin showed twice the reduction in flowering capacity after UVB treatment as did plants with normal or elevated levels of anthocyanin. The wide range of RCBr genetic material available with differential responses to environmental factors makes this system ideally suited for research applications in stress physiology.

Herbivore Defenses

RCBr and other members of the Brassicaceae family are excellent models for investigating the evolution of herbivore defenses because

- Both generalists and specialists feed on these species
- They all produce glucosinolates or mustard oil glycosides, a group of chemicals involved in the acceptance or rejection of these plants as suitable oviposition and/or feeding sites
- Glucosinolates are well characterized and easily quantified

Stowe (1997, 1998a) selected lines of RC *B. rapa* with high (14.0 mg/g) or low (5.5 mg/g) concentrations of foliar glucosinolates, on the basis of high performance liquid chromatography analysis of extracts from the first true leaves. Although oviposition was not affected by glucosinolate concentration, the most leaf area was damaged in lines with low glucosinolate concentrations, less damage occurred in the intermediate control line, and the least damage occurred in the high glucosinolate line. Interestingly, both polyphagous and oligophagous larvae responded similarly to the glucosinolates (Stowe 1998a). The metabolic cost of mounting this defense was reduced tolerance to mechanical defoliation stress (Stowe 1998b). Lines with less glucosinolate showed a higher fitness after mechanical defoliation than did lines with higher amounts of the defense compound. This may demonstrate a genetic tradeoff between defense and tolerance and suggests that the cost of glucosinolate production in *B. rapa* could constrain the evolution of increased defense (Stowe 1998b).

Herbicide Resistance

The chloroplast of a triazine-resistant weed biotype of *B. rapa* (bird's rape) was transferred by repeated backcrossing to an agriculturally important strain of *B. napus* to form a triazine-resistant cultivar of canola (Xiao and others 1986). Cytoplasmic triazine resistance has been transferred to RC *B. rapa,* and this material has been used in educational activities (AgriScience Institute and Outreach Program 1994). Plowman and Richards (1997) compared the effect of light and temperature on competition between triazine-susceptible and triazine-resistant *B. rapa.* Relatively small variations in both light and temperature, well within the range encountered during British summers, can have large effects on the relative competitiveness of triazine-resistant and triazine-sensitive biotypes in this species, with implications for the spread of resistance genes through seminatural communities with global warming trends. In vitro selection of rapid-cycling *B. napus* embryos produced by haploid culture of UV-irradiated microspores revealed heritable resistance to the herbicide "Glean" (active ingredient chlorsulfuron) (Ahmad and others 1991a, b). The close relationship between RCBr and both weedy and economically important *Brassica* species makes this model a good choice for herbicide resistance research.

Gravitational and Space Biology

What makes a plant system successful as a model? The first step is connecting the need for an experimental vehicle and the knowledge that a plant with the needed traits exists. For many people who now use RCBr as a research tool, this connection was forged by Paul Williams, who has tirelessly educated the community about the potential of RCBr for research and education.

As an example, in 1986 at the annual meeting of the American Society for Gravitational and Space Biology, Paul affixed four preflowering RC *B. rapa* horizontally to his poster to demonstrate the rapid response of the flowering stalk to gravity (1°/min) and to propose it as a model plant for use in gravitational and space biology. The small size and minimal light and root space requirements of the plants meshed well with the constraints imposed by space and power limitations on the US space shuttle. As described later, RC *B. rapa* has subsequently become one of the commonly used plant materials for space biology research. Most recently, Porterfield and others (2000) used *B. rapa* cv. "Astroplant" and super dwarf wheat (Bugbee 1999) as model species for testing nutrient delivery technologies for spaceflight applications.

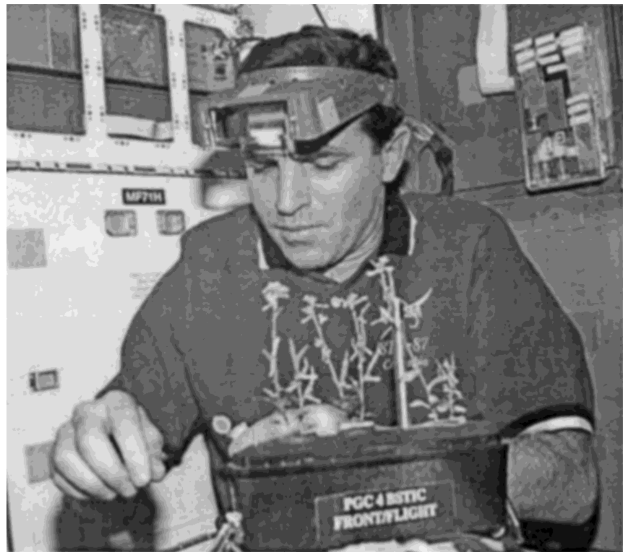

Figure 3. Rapid cycling *B. rapa* flowers were marked and pollinated by a payload specialist on successive days during an experiment on the US space shuttle in 1997 (STS-87) to produce cohorts of siliques of different ages. Pollen collected each day on bee sticks (Williams 1980; as shown) was stored desiccated for subsequent study. Small size, low light requirements, short life cycle, and self-incompatibility of the flowers were key features for selecting RCBr as a model plant for studying the role of gravity in plant reproductive development.

After three spaceflight experiments on plant reproduction with *Arabidopsis* (Kuang and others 1996a, b; Kuang and others 1995) our laboratory switched to RC *B. rapa* as a model to study events of pollination, fertilization, and early seed development. The requirement for manual pollination in *Brassica* (Williams 1980) made it superior to *Arabidopsis,* because by marking flowers to distinguish their date of pollination, we could compare embryos in spaceflight and ground control on the basis of days after pollination (Figure 3). Having control over the initiation of the process also allowed us to assess microgravity effects on different components of the reproductive process. Pollen viability, pollen transfer, pollen germination, pollen tube growth, and fertilization, and different stages of embryo development could thus be assessed despite remote access by the investigator (Kuang and others 2000a, b).

The flower parts are large enough for manipulation without special equipment, and individual flowers were fixed on orbit at different intervals after pollination. The same user-friendly characteristics that make RCBr work in the classroom made them suitable for manipulations by nonbiologists in the astronaut/payload specialist cadre. The nonshattering character of the siliques made it possible to devise a seed-to-seed experiment with *Brassica* that had not been feasible with *Arabidopsis* (Musgrave and others 2000). Seeds produced on orbit by hand pollination were later separated from dried siliques and replanted to provide totally gravity-näive plants. The finding that seed quality is compromised in RC *B. rapa* in microgravity despite equivalent performance by the maternal plants in space and on the ground suggests a role for gravity in seed development (Kuang and others 2000b).

The usefulness of RCBr as candidate species in Controlled Ecological Life Support Systems (CELSS) envisioned for future lunar or planetary outposts has also been examined. Again, the short life cycle, low light requirement, and potential for high productivity per unit volume in a controlled environment are key elements (Bugbee 1999). Frick and colleagues (1994) found that in hydroponic culture, low to moderate N levels (up to 15 mM) favor seed yield and oil content; higher N decreases these. They concluded that RC *B. napus* seems competitive for a CELSS compared with other potential oilseed crops (peanut and soybean). Seed oil of *B. napus* is higher in monounsaturated fatty acids than either peanut or soybean oil, and it is lower in polyunsaturated fatty acids than soybean oil. In addition to oil production, brassica leaves are readily edible as a calcium-rich fresh salad vegetable, so a mixed harvest and culture strategy may allow this species to be used to its fullest potential (Frick and others 1994).

Weaknesses of this Model System

The short life cycle and small size of RCBr have several consequences for stress physiology investigations. In the previous example that described selection of waterlogging-tolerant and -sensitive populations of RCBr, the developmental window permitting meaningful comparisons of foliar carbohydrates was very narrow. To avoid obfuscation by changes surrounding the transition to flowering, sampling had to occur by 14 days after planting, but the waterlogging treatment could not begin until the first true leaves appeared 7 days after planting. Similarly, Kleier and others (1998) found that biomass losses in RCBr caused by ozone treatment (60%) greatly exceeded those in related brassicas (turnip, spring rape) and radish (12%). They suggest that RCBr does not have the capacity to respond to stress by partitioning resources from other parts of the plant because of its short life cycle and small size.

Although the self-incompatibility system in *Brassica* is a useful model for studying the genetic control of pollen recognition, it presents an obstacle for other types of research applications. Bud pollination, the transfer of pollen to immature pistils 2 days before anthesis, is the conventional means of overcoming this problem in *Brassica* (Williams, 1987). Other means of bypassing the SI system in *Brassica* include direct pollination of exposed ovules in vitro (Zenkteler and others 1987) and treatment of the stigma with a NaCl solution (15 g/L) (Monteiro and others 1988).

Through recurrent selection from the RC base population, Williams created a self-compatible line of RC *B. rapa* (CrGC#66) that is now commercially available. However, this line still requires mechanical pollination (Williams 1980), although selection efforts are underway to obtain the floral architecture that would be necessary to make this line self-pollinating and self-fertile (Williams, personal communication). These developments would provide *Brassica* genetic material with the same ease of seed production as is found in the *Arabidopsis* model. As mentioned previously, fecundity and size of the RCBr plant is easily increased by providing additional rootzone space and nutrients to create a larger, branching plant.

Needs and Future Roles for this Versatile Model System

The major element needed for this model system to be used more widely is recognition by investigators of the ways that RCBr can facilitate the bridge from basic to applied research. As interest in new applications for basic research grows within the scientific community, RCBr use will increase. In addition to this attitude shift, increased knowledge of RCBr genetic markers and development of a genomic map would improve use of the model system.

In contrast to other model systems reviewed in this issue, the RCBr system has a unique standing because of its facile relationship with crop plants (Figure 1) and its close phylogenetic relationship with *Arabidopsis*. As study of the *Arabidopsis* genome yields new approaches to crop improvement, RCBr materials offer a convenient intermediary for investigating genetic modifications in an integrative way, even assessing possible changes in pathogen and herbivore interactions with the modified plant.

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REFERENCES

AgriScience Institute and Outreach Program. 1994. Using fast plants and bottle biology in the classroom. Reston, VA: National Association of Biology Teachers.

Ahmad I, Day JP, MacDonald MV, Ingram DS. 1991b. Haploid culture and UV mutagenesis in rapid-cycling *Brassica napus* for the generation of resistance to chlorsulfuron and *Alternaria brassicicola.* Ann Bot 67:521–526.

Ahmad I, MacDonald MV, Ingram DS. 1991a. Chlorosulfuron tolerance of primary embryos derived from UV-treated microspores of rapid cycling *Brassica napus.* Sarhad J Agric 7:507–511.

Aslam FN, MacDonald MV, Ingram DS. 1990b. Rapid-cycling *Brassica* species: anther culture potential of *Brassica campestris* L. and *Brassica napus* L. New Phytol 115:1–10.

Aslam FN, MacDonald MV, Loudon P, Ingram DS. 1990a. Rapid-cycling *Brassica* species: inbreeding and selection of *Brassica campestris* for anther culture ability. Ann Bot 65:557–566.

Berthomieu P, Beclin C, Charlot F, Dore C, Jouanin L. 1994. Routine transformation of rapid cycling cabbage (*Brassica oleracea*): molecular evidence for regeneration of chimeras. Plant Sci 96:223–235.

Berthomieu P, Jouanin L. 1992. Transformation of rapid cycling cabbage (*Brassica oleracea* var. *capitata*) with *Agrobacterium rhizogenes.* Plant Cell Rep 11:334–338.

Bettey M, Sinniah UR, Finch-Savage WE, Ellis RH. 1998. Irrigation and seed quality development in rapid-cycling *Brassica:* Accumulation of stress proteins. Ann Bot 82:657–663.

Bohuon EJR, Ramsay LD, Craft JA, Arthur AE, Marshall DF, Laydiate DJ, Kearsey MJ. 1998. The association of flowering time quantitative trait loci with duplicated regions and candidate loci in *Brassica oleracea.* Genetics 150:393–401.

Bosland PW, Williams PH. 1988. Pathogenicity of geographic isolates of *Fusarium oxysporum* for crucifers on a differential set of crucifer seedlings. J Phytopathol 123:63–68.

Bugbee B. 1999. Engineering plants for spaceflight environments. Gravita Space Biol Bull 12(2):67–74.

Cheung WY, Champagne G, Hubert N, Tulsieram L, Charne D, Patel J, Landry BS. 1997. Conservation of S-locus for self-incompatibility in *Brassica napus* (L.) and *Brassica oleracea* (L.). Theoret Appl Gen 95:73–82.

Clouse SD, Langford M, McMorris TC. 1996. A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. Plant Physiol 111:671–678.

Conner JA, Conner P, Nasrallah ME, Nasrallah JB. 1998. Comparative mapping of the *Brassica* S locus region and its homeolog in *Arabidopsis:* implications for the evolution of mating systems in the Brassicaceae. Plant Cell 10(5):801–812.

Daugherty CJ, Matthews SW, Musgrave ME. 1994. Structural changes in populations of rapid-cycling *Brassica rapa* L. selected for differential waterlogging tolerance. Can J Bot 72:1322–1328.

Daugherty CJ, Musgrave ME. 1994. Characterization of populations of rapid-cycling *Brassica rapa* L. selected for differential waterlogging tolerance. J Exp Bot 45:385–392.

Edwards MD, Williams PH. 1987. Selection for minor gene resistance to *Albugo candida* in a rapid-cycling population of *Brassica campestris.* Phytopathol 77:527–532.

Fjellstrom RG, Williams PH. 1997. Fusarium yellows and turnip mosaic virus resistance in *Brassica rapa* and *B. juncea.* Hortscience 32:927–930.

Frick J, Nielsen SS, Mitchell CA. 1994. Yield and seed oil content response of dwarf, rapid-cycling *Brassica* to nitrogen treatments, planting density, and carbon-dioxide enrichment. J Am Soc Hort Sci 119(6):1137–1143.

Glenn MG, Chew FS. 1985. Hyphal penetration of *Brassica* (Cruciferae) roots by a vesicular-arbuscular mycorrhizal fungus. New Phytol 99:463–472.

Gurevitch J, Taub DR, Morton TC, Gomez PL, Wang IN. 1996. Competition and genetic background in a rapid-cycling cultivar of *Brassica rapa* (Brassicaceae). Am J Bot 83:932–938.

Hansen LN. 1998. Intertribal somatic hybridization between rapid cycling *Brassica oleracea* L. and *Camelina sativa* (L.) Crantz. Euphytica 104(3):173–179.

Hansen LN, Earle ED. 1994a. Regeneration of plants from protoplasts of rapid-cycling *Brassica oleracea*L. Plant Cell Rep 13(6):335–339.

Hansen LN, Earle ED. 1994b. Novel flowering and fatty-acid characters in rapid-cycling *Brassica napus* L. resynthesized by protoplast fusion. Plant Cell Rep 14:151–156.

Hansen LN, Earle ED. 1997. Somatic hybrids between *Brassica oleracea* L. and *Sinapis alba* L. with resistance to *Alternaria brassicae* (Berk) Sacc. Theor Appl Gen 94(8):1078–1085.

He T, Cramer GR. 1993a. Cellular-responses of 2 rapid-cycling *Brassica* species, *Brassica napus* and *B. carinata,* to seawater salinity. Physiol Plant 87(1):54–60.

He T, Cramer GR. 1993b. Salt tolerance of rapid-cycling *Brassica* species in relation to potassium-sodium ratio and selectivity at the whole plant and callus levels. J Plant Nutr 16(7):1263–1277.

He T, Cramer GR. 1993c. Growth and ion accumulation of two rapid-cycling *Brassica* species differing in salt tolerance. Plant Soil 153(1):19–31.

He T, Cramer GR. 1996. Abscisic acid concentrations are correlated with leaf area reductions in two salt-stressed rapid-cycling *Brassica* species. Plant Soil 179(1):25–33.

Imeson HC, Rood SB, Weselake J, Zanewich KP, Bullock BA, Stobbs KE, Kocsis MG. 1993. Hormonal control of lipase activity in oilseed rape germinants. Physiol Plant 89:476–482.

Johnson-Flanagan AM, Spencer MS. 1994. Ethylene production during development of mustard (*Brassica juncea*) and canola (*Brassica napus*) seed. Plant Physiol 106:601–606.

Klaper R, Frankel S, Berenbaum MR. 1996. Anthocyanin content and UVB sensitivity in *Brassica rapa.* Photochem Photobio 63(6):811–813.

Kleier C, Farnsworth B, Winner W. 1998. Biomass, reproductive output, and physiological responses of rapid-cycling *Brassica* (*Brassica rapa*) to ozone and modified root temperature. New Phytol 139(4):657–664.

KloepperSams P, Torfs F, Feijtel T, Gooch J. 1996. Effects assessments for surfactants in sludge-amended soils: a literature review and perspectives for terrestrial risk assessment. Sci Total Environ 185:171–185.

Kopsell DA, Randle WM. 1999. Selenium accumulation in a rapid-cycling *Brassica oleracea* population responds to increasing sodium selenate concentrations. J Plant Nutr 22(6):927–937.

Ku VVV, Wills RBH. 1999. Effect of 1-methylcyclopropene on the storage life of broccoli. Postharvest Biol Technol 17:127–132.

Kuang A, Musgrave ME, Matthews SW. 1996a. Modification of reproductive development in *Arabidopsis thaliana* under spaceflight conditions. Planta 198:588–594.

Kuang A, Musgrave ME, Matthews SW, Cummins DB, Tucker SC. 1995. Pollen and ovule development in *Arabidopsis thaliana* under spaceflight conditions. Am J Bot 82:585–595.

Kuang A, Popova A, Xiao Y, Musgrave ME. 2000a. Pollination and embryo development in *Brassica rapa* L. in microgravity. Int J Plant Sci 161(2):203–211.

Kuang A, Xiao Y, McClure G, Musgrave ME. 2000b. Influence of microgravity on ultrastructure and storage reserves in seeds of *Brassica rapa* L. Ann Bot 85(6):851–859.

Kuang A, Xiao Y, Musgrave ME. 1996b. Cytochemical localization of reserves during seed development in *Arabidopsis thaliana* under spaceflight conditions. Ann Bot 78:343–351.

Lascoux M, Lee JK. 1998. One step beyond lethal equivalents: characterization of deleterious loci in the rapid cycling *Brassica rapa* L. base population. Genetica 104:161–170.

Letham DLD, Blassard GW, Nasrallah JB. 1999. Production and characterization of the *Brassica oleracea* self-incompatibility locus glycoprotein and receptor kinase in a baculovirus infected insect cell culture system. Sex Plant Reprod 12(3):179–187.

Li J, Nagpal P, Vitart V, McMorris T, Chory J. 1996. A role for brassinosteroids in light-dependent development of *Arabidopsis.* Science 272:398–401.

Mandava NB. 1988. Plant growth-promoting brassinosteroids. Annu Rev Plant Physiol Plant Molec Biol 39:23–52.

Mengistu A, Rimmer SR, Koch E, Williams PH. 1991. Pathogenicity grouping of isolates of *Leptosphaeria maculans* and *Brassica napus* cultivars and their disease reaction profiles on rapid-cycling Brassicas. Plant Dis 75:1279–1282.

Miller CB, Williams PH. 1986. Selection for resistance to *Plasmodiophora brassicae* in oriental subspecies of *Brassica rapa.* Euphytica 35:583–592.

Miller TE, Schemske DW. 1990. An experimental study of competitive performance in *Brassica rapa* (Cruciferae). Am J Bot 77:993–998.

Mitchell JW, Mandava NB, Worley JF, Plimmer JR, Smith MV. 1970. Brassins: a new family of plant hormones from rape pollen. Nature 225:1065–1066.

Monteiro AA, Gabelman WH, Willaims PH. 1988. Use of sodium chloride solution to overcome self-incompatibility in *Brassica campestris.* HortSci 23:876–877.

Musgrave ME, Kuang A, Xiao Y, Stout SC, Bingham GE, Briarty LG, Levinskikh MA, Sychev VN, Podolski IG. 2000. Gravity-independence of seed-to-seed cycling in *Brassica rapa.* Planta 210 (3):400–406.

Nasrallah JB. 1997. Evolution of the *Brassica* self-incompatibility locus: a look into S-locus gene polymorphisms. Proc Nat Acad Sci USA 94(18):9516–9519.

Nasrallah ME, Kandasamy MK, Chang MC, Stadler Z, Lim S, Nasrallah JB. 2000. Identifying genes for pollen-stigma recognition in crucifers. Ann Bot 85 (suppl A):125–132.

Plowman AB, Richards AJ. 1997. The effect of light and temperature on competition between atrazine susceptible and resistant *Brassica rapa.* Ann Bot 80(5):583–590.

Porterfield DM, Dreschel TW, Musgrave ME. 2000. A ground-based comparison of three nutrient delivery technologies originally developed for growing plants in the spaceflight environment. HortTechnol 10(1):179–185.

Porterfield DM, Kuang A, Smith PJS, Crispi ML, Musgrave ME. 1999. Oxygen-depleted zones inside reproductive structures of *Brassicaceae:* implications for oxygen control of seed development. Can J Bot 77(10):1439–1446.

Robbelen G, Thies W. 1980. Biosynthesis of seed oil and breeding for improved oil quality of rapeseed. In: Tsunoda S, Hinata K, Gomez-Campo C, editors. Brassica crops and wild allies, biology and breeding. Tokyo: Japan Scientific Societies Press. p 253–284.

Rood SB, Hedden P. 1994. Convergent pathways of gibberellin A-1 biosynthesis in Brassica. Plant Growth Regul 15:241–246.

Rood SB, Pearce D, Williams PH, Pharis RP. 1989. Gibberellin-deficient *Brassica* mutant. Plant Physiol 89:482–487.

Rood SB, Williams PH, Pearce D, Murofushi N, Mander LN, Pharis RP. 1990b. A mutant gene that increases gibberellin production in *Brassica.* Plant Physiol 93:1168–1174.

Rood SB, Zanewich KP, Bray DF. 1990a. Growth and development of *Brassica* genotypes differing in endogenous gibberellin content: II. Gibberellin content, growth analyses and cell size. Physiol Plant 79:679–685.

Sato S, Katoh N, Iwai S, Hagimori M. 1998. Establishment of reliable methods of in vitro pollen germination and pollen preservation of *Brassica rapa* (syn. *B. campestris*). Euphytica 103(1):29–33.

Schadler DL, Bushnell IW, Moddy BL, Corum JW. 1994. Hormone-induced parthenocarpy in wildtype rapid-cycling *Brassica rapa.* Wisconsin Fast Plants Notes 7(1):6–8.

Schopfer CR, Nasrallah ME, Nasrallah JB. 1999. The male determinant of self-incompatibility in *Brassica.* Science 286(5445):1697–1700.

Shattuck VI. 1993. Glucosinolates and glucosinolate degradation in seeds from turnip mosaic-virus-infected rapid cycle *Brassica campestris* L. plants. J Exp Bot 262:963–970.

Sheppard SC, Evenden WG, Abboud SA, Stephenson M. 1993. A plant life-cycle bioassay for contaminated soil, with comparison to other bioassays: mercury and zinc. Arch Environ Contamin Toxicol 25:27–35.

Sheppard SC, Evenden WG, Anderson AJ. 1992. Multiple assays of uranium toxicity in soil. Environ Toxicol Water Qual 7:275–294.

Sigareva MA, Earle ED. 1999a. Camalexin induction in intertribal somatic hybrids between *Camelina sativa* and rapid-cycling *Brassica oleracea.* Theor Appl Gen 98(1):164–170.

Sigareva MA, Earle ED. 1999b. Regeneration of plants from protoplasts of *Capsella bursa-pastoris* and somatic hybridization with rapid cycling *Brassica oleracea.* Plant Cell Rep 18(5):412–417.

Sinniah UR, Ellis RH, John P. 1998a. Irrigation and seed qulity development in rapid-cycling *Brassica:* Soluble carbohydrates and heat-stable proteins. Ann Bot 82:647–655.

Sinniah UR, Ellis RH, John P. 1998b. Irrigation and seed quality development in rapid-cycling *Brassica:* Seed germination and longevity. Ann Bot 82:309–314.

Stowe KA. 1997. Experimental evolution of resistance in *Brassica rapa:* correlated responses of life history traits, tolerance, and realized defense to selection for foliar glucosinolate content. PhD dissertation. St. Louis, University of Missouri-St. Louis.

Stowe KA. 1998a. Realized defense of artificially selected lines of *Brassica rapa:* effects of quantitative genetic variation in foliar glucosinolate concentration. Environ Ent 27(5):1166–1174.

Stowe KA. 1998b. Experimental evolution of resistance in *Brassica rapa:* correlated response of tolerance in lines selected for glucosinolate content. Evolution 52(3):703–712.

Szekeres M, Nemeth K, Koncz-Kalman Z, Mathur J, Kauschmann A, Altman T, Redel GP, Nagy F, Schell J, Koncz C. 1996. Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis.* Cell 85:171–182.

Teo W, Lakshmanan P, Kumar P, Goh CJ, Swarup S. 1997. Direct shoot formation and plant regeneration from cotyledon explants of rapid-cycling *Brassica rapa.* In Vitro Cell Develop Bio Plant 33(4):288–292.

Williams PH. 1980. Bee-sticks, an aid in pollinating Cruciferae. HortSci 15:802–803.

Williams PH. 1987. Crucifer Genetics Cooperative resource book. Madison, WI: Crucifer Genetics Cooperative. 309 p.

Williams PH. 1989. Rapid-cycling Brassicas. Carolina Tips 52(2):5–7.

Williams PH. 1990. Rapid cycling brassicas—a context for plant biotechnology. Biotechnol Educ 1(3):111–114.

Williams PH. 1993. Bottle biology. Dubuque: IA, Kendall/Hunt Publishing Co. 127 p.

Williams PH, Hill CB. 1986. Rapid-cycling populations of *Brassica.* Science 232:1385–1389.

Wisconsin Alumni Research Foundation. 1989. Wisconsin fast plants manual. Burlington, NC: Carolina Biological Supply Co.

Xiao W, Reith M, Erickson LR, Williams JP, Straus NA. 1986. Mapping the chloroplast genome of triazine resistant canola. Theoret Appl Gen 71:716–723.

Xiao Y, Kuang A, Porterfield DM, Musgrave ME. 1996. Substrates for growth of *Brassica rapa* in the PGF and SVET. Gravita Space Biol Bull 10:40

Zanewich KP, Rood SB. 1993. Distribution of endogenous gibberellins in vegetative and reproductive organs of *Brassica.* J Plant Growth Regul 12:41–46.

Zanewich KP, Rood SB. 1995. Vernalization and gibberellin physiology of winter canola: Endogenous gibberellin (GA) content and metabolism of $(^3H)GA_1$ and $(^3H)GA_{20}$. Plant Physiol 108:615–621.

Zanewich KP, Rood SB, Southworth CE, Williams PH. 1991. Dwarf mutants of Brassica: Responses to applied gibberellins and gibberellin content. J Plant Growth Regul 10:121–128.

Zanewich KP, Rood SB, Williams PH. 1990. Growth and development of Brassica genotypes differing in endogenous gibberellin content: I. Leaf and reproductive development. Physiol Plant 79:673–678.

Zeise K, Buchmuller M. 1997. Studies on the susceptibility to *Verticillium dahliae* KLEB var *longissporum* STARK of six related *Brassica* species. Z Pflanzenkr Pflanzensch 104:501–505.

Zenkteler M, Maheswaran G, Willaims EG. 1987. In vitro placental pollination in *Brassica campestris* and *Brassica napus.* J Plant Physiol 128:245–250.

Zobayed SMA, Armstrong J, Armstrong W. 1999. Cauliflower shoot-culture: effects of different types of ventilation on growth and physiology. Plant Sci 141:209–217.