Chapter 18
Challenges in Controlling Verticillium Wilt by the Use of Nonchemical Methods

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Abstract Verticillium wilt is one of the most serious soilborne diseases worldwide. Three non-fumigant control methods that appear to have great potential for reducing losses due to wilt and other soilborne pathogens are detailed here. High nitrogen organic amendments and products containing volatile fatty acids (VFAs) can significantly reduce disease severity and inoculum density but only under specific soil conditions. Identification of the modes of action for these products provides new avenues to improve their efficacy. Broccoli amendments also effectively reduce Verticillium wilt and have great potential for use on a large scale where economics allow. Grafting susceptible cultivars onto Verticillium resistant root stocks has become widely adopted in many countries. Eggplants and tomatoes provide a good model system for testing this technology. Promising results have been obtained under diverse disease pressure and soil and climatic conditions.

Keywords Soilborne diseases • Organic amendments • Modes of action • Grafting • Volatile fatty acids • Ammonia • Nitrous acid • Swine manure • Fish emulsion • Brassicas • Green manures • Glucosinolates • Root stocks

18.1 Introduction

Verticillium wilt, caused by the fungus *Verticillium dahliae* Kleb, remains one of the most important soilborne plant diseases worldwide. Since the fungus infects dozens of crop and ornamental plants, losses incurred to this pathogen likely

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amount to billions of dollars worldwide (Pegg and Brady, 2002). Actual dollar values however, are difficult to assess and are likely underestimated as most often infections result in reduced plant growth rather than the outright death of plants. The best known and most important causal agents of Verticillium wilt are *Verticillium dahliae* and *V. albo-atrum. V. dahliae* produces only microsclerotia (MS) as resting structures and *V. albo-atrum* produces only dark resting mycelium (Pegg and Brady, 2002). A third species, *V. longisporum* that predominantly affects the members of Brassicaceae is now emerging as an important pathogen (Karapapa et al., 1997) although the validity of this new species has not been universally accepted (Barbara and Clewes, 2003; Collins et al., 2003). In this paper therefore all MS-producing, plant pathogenic species will be referred to as *V. dahliae*.

Disease management has focused on reducing the populations of MS in soil, which are hardy resting structures that are produced in the plant by the millions. MS can survive in soils and plant debris for decades and once introduced into soil are nearly impossible to eradicate. The most successful technology for eliminating MS from soil has been the use of broad spectrum soil fumigants. However, due to numerous negative health and environmental concerns associated with these pesticides and increasing urban sprawl, their use has been greatly curtailed. Disease resistance for *Verticillium* spp. in crop plants has been difficult to identify. For tomato plants, the *Ve* resistance gene was the primary method for disease control for decades but the evolution of biotypes that overcame resistance has now made this crop highly susceptible to wilt again. Efforts are now being placed into finding non-chemical control methods for control of Verticillium wilt and other soilborne diseases. In this article, we describe three approaches that have found limited success but are seen to have tremendous future potential.

18.2 Organic Amendments

18.2.1 Disease Reductions in the Field

Almost 60 years ago Wilhelm (1951) tested a spectrum of chemical pesticides and organic amendments for the control of Verticillium wilt of tomato, and found that only blood meal and fishmeal reduced the incidence of wilt to zero. Why these products controlled Verticillium wilt, however, was never questioned or explained. Subsequent studies also found that a number of similar amendments provided some level of disease control but the efficacy was variable and difficult to reproduce. Over the last decade, the Lazarovits laboratory examined how these products may be controlling plant diseases. Products tested included organic by-products derived from animal and plant processing industries, including blood meal, meat and bone meal, feather meal, fish emulsion (Abbasi et al., 2006), soymeal, lignosulfonates from the pulp and paper industry (Lazarovits et al., 2008), condensed distillers solubles from alcohol production (Abbasi et al., 2007), and several types of manures. The products were selected for their potential use at the field scale. Summaries of the results of such work have been previously published (Lazarovits, 2001, 2004; Lazarovits et al., 2001, 2005; Bailey and Lazarovits, 2003). As most of the products were considered waste products by the industries that generated them, they were mostly inexpensive, reasonably consistent from batch to batch, deemed environmentally safe, and had fertility values that enhanced crop growth.

In field trials on sandy soils, such products as poultry manure and soymeal reduced the incidence of Verticillium wilt and potato scab, and populations of plant pathogenic nematodes to the extent seen by Wilhelm (1951) in his pot trials (Conn and Lazarovits, 1999). In many cases, the disease reduction persisted for two crop seasons. The efficacy of these products, however, was often site- and product-specific. Overall, the level of control was lower than that found with chemical treatments but the added fertility resulted in increased plant vigor and yield. Field testing of organic products proved to be more complicated than testing of chemicals as there were many unknowns associated with the use of the products including the rates required, the effect of soil type, the impact of climatic conditions such as moisture and temperature, the nature and source of the amendment, the methods and times of application, etc. To examine even a few of these factors in the field would have required hundreds of plots to be established. Thus, studies were moved into the laboratory and model systems developed to simplify evaluating these products' impact on pathogen survival and to learn how best to deploy them in the field.

18.2.2 Mechanisms of Action by Which High Nitrogen Containing Amendments Reduce Pathogen Populations

Conversion of Ammonium to Ammonia A microcosm assay was developed to assess the survival of MS buried in amended soils and the result used as an indicator of inoculum reduction (Lazarovits et al., 2005). This assay allowed the identification of several modes of action for the various amendment types. In soils amended with high nitrogen containing material, mortality of MS was observed within one week after incorporation and was caused by the production of ammonia (Tenuta and Lazarovits, 2002a,b, 2004). When microbes degrade proteins, NH_4^+ ions are released and as they accumulate in the soil, an increase in soil pH occurs. When soil pH increases above 8, NH_4^+ is converted into NH_3 with the equilibrium (pK_a) between $NH_4^+ \leftrightarrow NH_3$ occurring at pH 9.3. NH_4^+ is non-toxic even at high concentrations, but NH_3 is very toxic (Warren, 1962). This mechanism becomes progressively less effective with increasing levels of soil organic matter primarily because the high populations of microorganisms in such soils rapidly convert NH_4^+ into nitrate (NO_3^-) (Tenuta and Lazarovits, 2004). In soils with high organic matter content, such amendments often fail to be pathogen-suppressive (Tenuta and Lazarovits, 2002a,b, 2004).

Conversion of Nitrite to Nitrous Acid The second mechanism by which high nitrogen amendments reduce pathogen populations occurs 4–6 weeks after incorporation of the amendments (Tenuta and Lazarovits, 2002a). When NH_4^+ is converted to nitrite (NO_2^-), $4H^+$ ions are freed up and soil pH drops. When the pH drops below 5.5, NO_2^- is converted to HNO_2 (nitrous acid). The equilibrium (pK_a) between $NO_2^- \leftrightarrow HNO_2$ occurs at pH 3.3. Nitrous acid is about 300–500 times more toxic to MS than NH_3 (Tenuta and Lazarovits, 2002a) and is also toxic to many plant pathogens including *Streptomyces scabies*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, as well as to crop and weed seeds. The formation of HNO_2 is most influenced by soil buffering capacity (Tenuta and Lazarovits, 2004) as nitrification lowers soil pH only in poorly buffered soils. The toxicity of ammonia and nitrous acid to plant pathogens was predicted by Tsao and Oster (1981) from their work on controlling soilborne pathogens with poultry manure.

18.2.3 Mechanism of Action of Materials Containing Volatile Fatty Acids

Single applications of liquid swine manure (LSM) significantly reduced the severity and incidence of Verticillium wilt and potato scab but disease reductions were site specific even when the same manure was used (Conn and Lazarovits, 1999). Laboratory assays using manure and soil from a field where disease reductions were optimal revealed that MS were killed within 1–2 days after manure application. The mechanism for this was shown to be caused by the presence of high concentrations of volatile fatty acids (VFA) in the manure, with acetic acid representing 60% of the active ingredients, and propionic, butyric, isobutyric, valeric, isovaleric and caproic the remainder (Tenuta et al., 2002). When commercially purchased VFAs were adjusted to the identical concentration found in LSM they reproduced all the LSM killing activity. It is important to note that only the acidic forms of VFA molecules are toxic and therefore disease control can only occur in acidic soils (Conn and Lazarovits, 2000). This explains most of the soil-specific activity of LSM. Most VFAs are at equilibrium with their non-ionic counterpart at pH 4.7 (pK_a). However, LSM quality can also vary, and of the dozens of LSM tested from different farms, only about 50% had sufficient VFAs to kill MS (Conn et al., 2005, 2007). Chemical analysis of manure can be used to predict the disease control potential of different batches and this can be used to spike the manures with deficient VFAs to provide consistent results.

High concentrations of VFAs have also been identified in fish emulsion (Abbasi et al., 2009), molasses (G. Lazarovits, unpublished), and composts (Bailey and Lazarovits, 2003). In some cases, the disease suppressive and phytotoxic activity of composts may be related to VFA content but this has not been examined. Immature composts that were phytotoxic to crop plants were found to have high concentrations of VFAs (Bailey and Lazarovits, 2003). Anaerobic decomposition of wheat straw resulted in the phytotoxicity due to production of acetic acid (Lynch, 1977, 1978).

Additions of large quantities of organic matter were used to create anaerobic conditions, and possibly to VFA formation, to reduce inocula of *F. oxysporum*, *Rhizoctonia solani*, and *V. dahliae* (Blok et al., 2000) and populations of *Meloidogyne hapla* and *M. incognita* (Browning et al., 2006). As VFAs are components of edible products and have very low toxicity to mammals with no mutagenic potential, they can be used in organic farming systems. VFAs however, persist in soil for only short times as they are readily metabolized by bacteria and fungi. In our tests, VFAs rarely lasted in soil for more than a week and in fact, rapid degradation may explain why they do not work at some locations.

18.2.4 Formulation for Site Specific Activity

The increasing cost of synthetic fertilizers makes the use of organic amendments more attractive to growers and if disease control can also obtained their utilization will greatly escalate. Through a greater understanding of mechanisms involved in disease reduction, we can select materials for optimal benefits to plant health based on soil and other conditions that favor their chemical and biological activity. There are also numerous options available for modifying the composition of such amendments for increased disease control. By anaerobically digesting LSM, Xiao et al. (2007) enriched both VFAs and NH_4^+ levels in the manure after 4 weeks of incubation. The enriched manure was superior to untreated manure for reducing egg production of the soybean cyst nematode. VFA-containing mixtures can be easily generated from almost any organic byproduct of agriculture or forestry. By acidifying the materials prior to treatment or by applying them in the fall when soil pH levels are lowest, their disease reduction activity can be enhanced (Conn and Lazarovits, 2007). There is also potential for formulating these to extend efficacy. Combining fish emulsion with biological control agents increased its disease control efficacy (El-Tarabily et al., 2003). Several companies are looking to generate such custom amendments and to commercially market them in the near future.

Combining organic amendments with solarization often results in a synergistic affect which improves the efficacy of both treatments for control of soilborne pathogens, although not many studies have focused specifically on the control of Verticillium (Gamliel and Stapleton, 1993a,b; Oka et al., 2007). The combination of the two treatments allows for reductions in the quantity of organic matter applied, as well as the time needed for effective solarization for reduction of pathogen populations that would normally take 6–8 weeks to as low as 3 weeks. This can be partially attributed to the efficacy by which plastic traps and enhances the toxicity of the volatiles formed, especially when residues of brassicas and Compositae are used. The presence of organic matter also helps to stabilize or increase the soil microbial biomass, which may have a disease suppressive impact (Scopa and Dumontet, 2007). A component that likely has been overlooked is the interaction of heat and organic amendments in the formation of ammonia and nitrous acid. With increasing temperature the concentrations of these toxicants is likely to greatly increase as the equilibrium constant for both (pKa) shifts toward the neutral soil pH range (Lazarovits et al., 2005). Increased levels of ammonium are known to form in solarized soils (Oka et al., 2007) and thus its toxic forms would also likely be present. The combined technologies thus would greatly increase the range of soils where either technology alone would not produce desired control levels.

Organic products offer a technology for growers who wish to incorporate it as part of a sustainable and holistic crop production system to keep populations of key pathogens to levels that are below crop loss thresholds. The only potato grower in Ontario that did not have high levels of Verticillium wilt applied five tons of poultry manure every fall to his soil. It is likely that this practice not only accelerated the degradation of the potato plant debris that releases microsclerotia from the plant tissues into the soil but also produced sufficient nitrous acid to kill most of these MS such that Verticillium inoculum levels were kept at below disease threshold levels. For many growers, the lack of availability of manure or manure of uniform quality or other by-products has prevented adoption of this disease-suppressive tool. There are also substantial costs for moving such products long distances and incorporating them into soil, and the technology for application is still not available. Populations of microorganisms always increase in the soil following applications of organic amendments but the role these microbes play in disease reduction has not yet been clearly defined. Some amendment may increase microorganism populations that lead to disease-suppressive conditions (Trankner, 1992; Mazzola, 2004; Mazzola et al., 2007). We need to refine this selective management of microbiological ecosystems for agriculture to the same extent as is now occurring in the probiotic movement found in human health protection. Here, disease is prevented by products that enrich the native microflora, thereby keeping pathogens from becoming established either directly, or indirectly. We already know that disease suppressive soils exist. The goal is to learn how create them at will.

18.2.5 Crop Rotation and Green Manures

The use of crop rotation and green manure crops for the control of *V. dahliae* has been used interchangeably although the two techniques differ both conceptually and in their effects on MS survival and populations, as wells as to their overall benefits and limitations. The practice of growing a sequence of taxonomically different, economically valuable plant species on the same piece of ground is known as crop rotation. Continuous cropping with susceptible hosts can result in the build-up in soil of populations of specific plant pathogens, resulting in a decline in crop yield and quality. Crop rotation is effective for limiting the increase of soilborne pathogen populations that have a limited host range, but is less effective when pathogen population densities are high. In contrast, incorporating into soil green biomass of a plant species that may not be an economically important crop or crop residue brought in from elsewhere, is referred to as green manuring. *Brassica* species are particularly well suited as green manures because of their large taproot with a dense network of fine surface roots (Matthiessen and Kirkegaard, 2006). These species reduce wind erosion, improve water infiltration of the soil and soil structure, and are effective at mineralizing soil nitrogen and thus reducing leaching, etc. (Thorup-Kristensen et al., 2003).

Crop rotation, cover cropping and green manures are tactics that provide multiple benefits and are therefore of vital importance to agroecosystems (Haramoto and Gallandt, 2004) and their benefits in general soilborne disease suppression well known. However, their success in the management of *V. dahliae* has been less clear primarily because of the unique survival characteristics of the MS (Isaac and MacGarvie, 1966; Hoes, 1971). *V. dahliae* is able to infect over 200 plant species, including high value annual and perennial crop species (Subbarao et al., 1995; Bhat and Subbarao, 1999a) and the list of new hosts infected is continually expanding, with cauliflower and lettuce two major crops in California being prime examples (Koike et al., 1994; Subbarao et al., 1995; Bhat and Subbarao, 1999a,b). This capability of *V. dahliae* to infect numerous crop plants, as well as natural flora and weeds which sometimes remain asymptomatic (Vallad et al., 2005a), and to contaminate seeds of various plants make *V. dahliae* a chronic disease problem (Bhat and Subbarao, 1999a; Qin et al., 2006; Vallad et al., 2005a, b). Reducing MS populations prior to planting to below the crop-dependent critical threshold for crop loss to occur is essential for disease management. However, the successful implementation of a crop rotation strategy has been confounded by long survival times of MS, the bewildering array of hosts the fungus infects, and that it can also be a seed resident (Vallad et al., 2005a, b). Even when rotations included resistant or immune crops, *V. dahliae* can colonize roots and maintain soil population levels with no apparent impact on the crop; even on non-hosts such as graminaceous crops (Krikun and Bernier, 1990). The use of *Brassica* spp. as green manure crops for reducing plant diseases was thoroughly reviewed by Matthiessen and Kirkegaard (2006) and this section focuses on crop rotations for the control of *Verticillium* species.

18.2.6 Attributes of Successful Rotation Crops

The basic criteria for developing successful rotation crops to minimize the impact of *V. dahliae* include: (i) the crop should result in a reduction of MS in soil and a concomitant reduction of wilt in the susceptible crop, (ii) be compatible with current production practices, and (iii) result in grower acceptance of the crop for rotation. Identifying crops that fit these criteria is difficult for the reasons given previously. During the evaluation of Verticillium wilt on cauliflower Koike et al. (1994) observed that Verticillium wilt did not affect a related host, broccoli. *V. dahliae* isolates from cauliflower were either weakly-pathogenic or non-pathogenic on broccoli and no MS developed on inoculated plants (Subbarao et al., 1995). The broccoli plants suffered no yield losses even when planted into highly infested fields; nor could the pathogen be isolated from mature plants from these fields (Koike et al., 1994). Broccoli and cauliflower however, are closely related taxonomically as both are

Brassica olearacea var. *botrytis* L., but broccoli and cauliflower are separated into the subvarieties *cymosa* and *cauliflora,* respectively. Most other cultivated *Brassica* species are susceptible to *V. dahliae* but a few exhibit varying levels of resistance (Ciccarese et al., 1987; Subbarao et al., 1995).

A detailed analysis of the host range of *V. dahliae* isolates from artichoke, cabbage, cotton, pepper, potato, strawberry, tomato, watermelon and two virulent isolates from cauliflower when tested on all of the above crops, lettuce, and other crucifer crops showed that there was no host specificity associated with *V. dahliae* (Subbarao et al., 1995). Isolates from cauliflower were only weakly pathogenic on broccoli and Brussels sprouts and were non-pathogenic on lettuce. To clarify if this was cultivar-specificity or a more general reaction of broccoli, a number of commercial cultivars of broccoli were evaluated against *V. dahliae* isolates from 15 different hosts as also against *V. albo-atrum* from alfalfa. All cultivars evaluated were resistant to *V. albo-atrum* and *V. dahliae* from all hosts except from crucifer hosts against which they were weakly susceptible (Bhat and Subbarao, 2002). Strains re-isolated from internally discolored broccoli plants were unable to cause symptoms on broccoli but caused severe wilt on cauliflower. The immunity of broccoli against *V. dahliae* isolates from non crucifer hosts and resistance against crucifer isolates and *V. alboatrum,* coupled with its importance as a commercial vegetable, makes broccoli an attractive rotation crop for the management of Verticillium wilt.

While broccoli as a rotational crop possessed all attributes listed above, it was necessary to determine if fresh broccoli residue is as effective as dry broccoli and the temperature at which the benefits of broccoli are maximized; this information was also useful in determining the timing of broccoli crop planting and residue incorporation. Fresh broccoli residue suppressed *V. dahliae* MS more than dry broccoli residue over the entire temperature range tested (10°C to 35°C) (Subbarao and Hubbard, 1996). Furthermore, the greatest reductions in MS occurred at soil temperatures above 20°C with both fresh and dry broccoli residue (with fresh broccoli significantly more effective than dry broccoli) and most of this reduction occurred within 15 days after incorporation. In multiple greenhouse experiments cauliflower plants grown in fresh broccoli treatments were consistently taller, had greater root and shoot biomass, and had the least number of infected plants than in other treatments. These studies demonstrated that for maximal reductions of soilborne *V. dahliae* MS and subsequent lower wilt incidence in cauliflower, the broccoli residue incorporation should occur when the soil temperatures are at least 20°C.

Following these laboratory studies, rotations of susceptible cauliflower with broccoli as well as incorporating post-harvest residue was evaluated in an experimental field as well as a grower's field. The experiment in a grower's field involved the comparison of broccoli in a highly infested field with treatments of methyl bromide plus chloropicrin, Vapam, broccoli residue with tarp, broccoli residue without tarp, control with tarp, and control without tarp, and was arranged in a randomized block design with four replications. Approximately 11 Kg chopped broccoli per m^2 was uniformly spread and incorporated into the corresponding plots by disking. For treatments with tarping, clear plastic was spread over the plots and sealed at the edges. Tarps were removed after two weeks. The numbers of *V. dahliae* propagules in broccoli-treated plots was lower than in control plots and were comparable to those in fumigated plots. Similarly, plant height, marketable heads, and head weight were significantly higher in broccoli treatments than in control plots. Tarping alone did not reduce propagule numbers. These results suggested that broccoli residue has the potential for Verticillium wilt control. The ideal means of exploiting this, however, was by rotating cauliflower with broccoli (Subbarao et al., 1999).

In a follow-up experiment in an experimental field, actual broccoli–cauliflower rotations were tested for their efficacy to control Verticillium wilt on cauliflower (Xiao et al., 1998). Treatments tested were a factorial combination of three main plots (broccoli crop grown, harvested, and residue incorporated in *V. dahliae*-infested plots, no broccoli crop or residue in infested plots, and fumigated control plots), two sub-plots (furrow and subsurface-drip irrigation), and three sub-sub-plots (deficit, moderate, and excessive irrigation regimes) arranged in a split-split-plot design with three replications. Number of propagules in all broccoli plots declined significantly after residue incorporation and continued to decline throughout the cauliflower season. The overall reduction in propagule numbers after two broccoli crops was 94%, in contrast to the fivefold increase in infested plots without broccoli after two cauliflower crops. Disease incidence and severity were both reduced approximately 50% in broccoli treatments compared with no broccoli treatments. Differences between furrow and subsurface drip irrigation were not significant, but incidence and severity were significantly lower in the deficit irrigation regime compared to the other two regimes. MS of *V. dahliae* on infected cauliflower roots 8 weeks after cauliflower harvest was significantly lower in treatments with broccoli compared to treatments without broccoli. Rotating broccoli would be successful regardless of the irrigation methods or regimes followed on susceptible crops for Verticillium wilt control. These two studies proved the efficacy of broccoli rotations to control Verticillium wilt.

18.2.7 Mode of Action

As early as the late 1930s, the toxicity of mustard oils and their breakdown products were demonstrated on *Colletotrichum circinans*, *Botrytis alii*, *Aspergillus niger*, *A. alliaceus*, and *Gibberella saubinetii* (Subbarao and Hubbard, 1996) in laboratory studies. Subsequent studies identified that this broad spectrum toxicity to plant pathogens and pests resulted from the release of toxic products from crucifer residues (Ramirez-Villapadua and Munnecke, 1988; Gamliel and Stapleton, 1993b). The efficacy of disease suppressive effects was related to the degree of dryness of the crucifer residue at the time of soil amendments (Ramirez-Villapadua and Munnecke, 1988) and to the glucosinolate content in a crop (Mayton et al., 1996). However, the use of this technology has not been widely adapted by growers for disease management primarily owing to the difficulty of obtaining dry crucifer residue and the potential cost of application. Subbarao and Hubbard (1996) demonstrated that fresh broccoli residue is more effective than dry broccoli powder at equivalent amounts at all temperatures tested. They also established the temperature at which maximal pathogen suppression occurred. A comprehensive review by Matthiessen and Kirkegaard (2006) focused on the bioactive compounds in Brassicaceae members as the mechanism responsible for the pathogen/pest suppression observed with these plants. Alternative mechanisms are likely because pathogen suppression has been observed with isothiocyanates from plants with relatively low antimicrobial activity (Mancini et al., 1997). Furthermore, the suppression of soilborne pathogens and pests by Brassicaceae residues continued long after isothiocyantes had been volatilized or degraded (Lewis and Papavizas, 1971) or was observed independent of the glucosinolates content (Mazzola et al., 2001). The suppression observed with Brassicaceae and other plant products was also attributed to the depletion of oxygen in soil through anaerobiosis (Blok et al., 2000), accumulation of acetic and butyric acid (Momma, 2008) and hydrogen cyanide (Bjarnholt et al., 2008) in amended soils, oxidation of N in soil amendments to nitric oxide by soil bacteria (Cohen et al., 2005) which is known to stimulate plant defense pathways (Durner et al., 1998).

With broccoli specifically, attempts to isolate *V. dahliae* from inoculated plants or plants collected from fields heavily infested with MS were unsuccessful (Koike et al., 1994). Inoculated broccoli plants seldom showed symptoms consistently across isolates from 14 hosts (Subbarao et al., 1995), all commercial broccoli cultivars responded similarly to isolates of *V. dahliae* (Bhat and Subbarao, 2002). Despite the apparent lack of foliar symptoms and few root symptoms, broccoli root cortex was still colonized *V. dahliae* to the same degree as cauliflower (Shetty et al., 2000). Under high inoculum density however, colonization rates of cauliflower roots were 1.5-fold higher than in broccoli. Empirical data evaluating the mechanisms of broccoli-induced *V. dahliae* suppression are only now becoming available. Although bacterial populations, especially actinomycetes, increased by as much as three orders of magnitude following the incorporation of broccoli residue, identifying an actual cause and effect relationship has proven difficult (K.V. Subbarao, unpublished data). Instead, the ontogenic changes in the type and levels of glucosinolates, structural components such as lignin and phenolic compounds explain why broccoli is resistant to *V. dahliae* relative to cauliflower. Colonization patterns of *V. dahliae* in cauliflower and broccoli were compared using immunohistochemical staining (Shetty et al., 2000) and a green-fluorescent-protein (GFP)-tagged *V. dahliae* isolate from cauliflower (Njoroge et al., 2008a). Minimal differences in the colonization of cortex were observed between broccoli and cauliflower (Shetty et al., 2000) but the vascular tissue in broccoli was uncompromised in contrast to the extensive colonization in cauliflower (Njoroge et al., 2008b). The type of glucosinolates and the range of their catabolic products have been associated with the suppressive effects of crucifer crops in general (Matthiessen and Kirkegaard, 2006) but it is clear from the high susceptibility of many crucifer crops to *V. dahliae*, including strains from crucifer crops, that not all glucosinolates and their catabolic products are involved in pathogen suppression. However, *V. dahliae* suppression by broccoli remains effective long after the volatilization of the isothiocyanates and is independent of glucosinolate content suggesting that the suppression is due more to biological than chemical reasons. The reduction in *V. dahliae* soil population was perhaps caused by the combined effects of broccoli acting as a trap crop to force the germination of MS, and the activation of resident microflora with an ability to degrade lignin-rich broccoli residue in addition to the melanized MS of *V. dahliae* (Shetty and Subbarao, 1999). Microorganisms with melanolytic activity may be selectively enhanced by the incorporation of broccoli residue in soil (Butler and Day, 1998; Shetty and Subbarao, 1999). Interestingly, broccoli rotations were also suppressive to *S. minor*, another pathogen producing melanized sclerotia (Hao et al., 2003).

A related area that was thoroughly researched is the employment of cyanogenic green manure crops for pathogen suppression. Davis et al. (1996) determined that incorporating sudangrass and corn residues increased potato yields in fields infested by *V. dahliae*. The release and accumulation of hydrogen cyanide in amended soils is believed to be responsible for the pathogen suppression in these crops (Matthiessen and Kirkegaard, 2006).

18.2.8 Successes and Frustrations

Rotations with broccoli have proven successful in not only experimental plots but also in grower fields in repeated large scale studies in the management of Verticillium wilt and diseases caused by *Sclerotinia* spp. Based on these successful studies, there has also been an encouraging adaptation of this procedure by vegetable and strawberry growers in both conventional and organic production systems. Nevertheless, the wider adaptation of this technique has been less than total due to the low economic returns from broccoli crops that have also depressed the overall broccoli production, and the very high land costs in coastal California (>$100,000 per ha) with rentals costing as high as $30,000 per year. Ultimately, the economics of crop production trumps all other factors and there is little researchers could do to alter this reality.

18.2.9 Grafted Plants

Until 2005, when methyl bromide (MB) was banned as a soil fumigant, Italy ranked first in Europe and second in the world in its use of MB for horticultural crop production. Preplant soil fumigation was practiced in Southern Italy for the protection of solanaceous plants where Verticillium wilt was a serious problem. Grafting commercial cultivars susceptible to Verticillium onto resistant rootstocks was developed as a replacement for fumigation. However, the practice of growing grafted vegetables started in Japan and Korea in the late 1920s (Lee, 1994). Grafting vegetables onto resistant rootstocks represents a technically and economically feasible alternative particularly in Japan and in Korea where 54% and 81%, respectively, of vegetables grown are grafted (Rivero et al., 2003). In the

Mediterranean region, grafting represented an opportunity to maintain productivity of crops such as watermelon, cantaloupe, tomato, pepper, and eggplant (Bletsos et al., 2003; Diánez et al., 2007). It was rapidly adopted and for instance in Greece, 90–95% of watermelon, 40–50% of cantaloupe, 5–8% of tomato and 5–8% and 2–4% of cucumber and eggplant are now grafted (Traka-Mavrona et al., 2000); in Spain 98% of watermelon and 10% of tomato, in Morocco and Netherlands more than 25% and 50% of protected tomatoes and in Cyprus 80% of watermelon (Diánez et al., 2007). Over 5 million eggplants and 5.8 million tomato plants were produced from grafted seedlings in Italy in 2005 (Minuto et al., 2007).

Grafting vegetables onto resistant rootstocks offers numerous advantages including: resistance to soil pathogens, specifically *Verticillium* and *Fusarium* (Lockwood et al., 1970; Lee, 1994; Bletsos et al., 2003), improved yield in infested soils (Bletsos et al., 2003), greater tolerance against low and high temperatures and salt stress (Rivero et al., 2003) and higher plant vigour that can support longer crop cycles under adverse climatic conditions. Bletsos et al. (2003) found that grafted eggplants had not only increased fruit yield of up to 79% over non-grafted plants in Verticillium-infested soil (Bletsos et al., 2003) but they also produced fruit a week earlier (Khahm, 2005). Fruits from grafted eggplants contain fewer seeds than from non-grafted plants (Khahm, 2005) and this is regarded as another qualitative benefit to the consumer.

Several rootstocks are available for grafting of both tomato and eggplant, the most common being tomato hybrids (Energy, Kyndia) and interspecific hybrids of *L. esculentum* and *L. hirsutum* (He Man, Beaufort, Maxifort, Trifort). For grafted eggplant *Solanum torvum* was introduced and now represents more than the 70% of the total market of eggplant rootstocks in the south Italy (Minuto et al., 2007). Other *Solanum* species could be adopted for grafting eggplant including *S. sisymbriifolium*, but *S. torvum* guarantees the highest resistance against Verticillium wilt (Bletsos et al., 2003) and also carries traits of resistance to the most serious disease of eggplant namely bacterial wilt (*Ralstonia solanacearum*) and nematodes (Gousset et al., 2005).

18.2.10 Limitations of Adoption of Grafted Plants

Among the major constraints and limitations of grafted rootstocks is that resistance may break down under high pathogen population pressure, that new races of the pathogen may evolve, and under some environmental stresses such as high temperature and salinity, the plants may prematurely collapse. Furthermore, pathogens generally considered minor can become major pathogens on the rootstocks in the absence of soil fumigation. As an example, novel root rots caused by *Colletotrichum coccodes* were repeatedly observed on rootstocks currently used for grafting tomatoes and eggplant (Garibaldi and Minuto, 2003). Although *C. coccodes* was previously reported to infect *L. hirsutum* rootstocks, it was never observed on *L. lycopersicum* × *L. hirsutum* hybrids, the most widely used rootstocks.

Grafted hybrids of *L. lycopersicum* × *L. hirsutum* ("Beaufort", "He Man") and of *L. lycopersicum* ("Energy") were infected by *Phytophthora nicotiane* and *Rhizoctonia solani* accompanied by some plant stunting (Minuto et al., 2007). Finally, eggplants (cv Black Bell and Mirabell) grafted onto rootstock of *S. torvum* that confer a high degree of nematode tolerance exhibited low levels of Verticllium wilt in several greenhouses in Sicily (Garibaldi et al., 2005) that in subsequent crops increased to about 10 times in the same greenhouses. *S. torvum* exhibited partial tolerance to *V. dahliae* in artificially inoculated conditions (20–27% of infected plants) compared with non grafted Black Bell eggplants (87–100% of infected plants).

The relatively low tolerance of *S. torvum* to *V. dahliae* was known previously known (Ginoux and Laterrot, 1991; Gousset et al., 2005). Ginoux and Laterrot (1991) confirmed the resistance of *S. torvum* against *V. dahliae* particularly under mild climate conditions and in sandy soils and when 70–80-days-old grafted plants were transplanted. In trials carried out with 15 day old *Solanum spp* seedlings belonging to 14 different species vertical resistance to *V. dahliae* was not found but there was only tolerance to wilt symptoms (Nothamann and Ben-Yephet, 1979). Experiments conducted in highly infested fields confirmed that *S. torvum* conferred only partial wilt resistance (30–50% infection plants compared with non grafted eggplant 80–100% infection), while *L. lycopersicum* × *L. hirsutum* and *L. lycopersicum* hybrid rootstocks always showed low infection (7–10% infected plants) (Minuto et al., 2007).

18.2.11 Physiological Disorders Caused by Grafting Adoption

In Northern Italy since 1997, sudden collapse of grafted plants in protected and open field tomatoes (cv Cuore di Bue and cv Marmande-Raf) grafted on "He-Man" and on "Energy" rootstocks were observed (Garibaldi and Minuto, 2003). The collapse before or after fruit setting during spring and summers was in the 15–70% range. Sudden collapses were also observed on cv Iride, Naomi, Cuore di Bue, and Marmande-Raf grafted on "He-Man", "Energy" and sometimes on "Beaufort", regardless of the season or phenological stage of plants in Southern Italy (Garibaldi and Minuto, 2003). This collapse appears to be a direct consequence of the incompatibility between scion and rootstock or the climatic conditions during fruit setting and ripening.

Similar collapses were observed on eggplant grafted on tomato rootstocks (Ginoux and Laterrot, 1991) demonstrating the importance of rootstock selection. *S. torvum* performs best as an eggplant rootstock during warm seasons, but may reduce plant vigor during other seasons. Tomato rootstocks should be more vigorous and possess cold tolerance, graft incompatibility may reduce cold tolerance (Minuto et al., 2007). With tomato rootstocks grafted onto eggplant one often finds that the diameter of the rootstocks is double that of the scion but this is not the main reason for graft incompatibility. This is inferred from the fact that plants with tomato rootstocks transplanted in late spring to early summer do not show signs of damage although the size differences between rootstock and scion are present.

Catara et al. (2001) found a widespread dieback of eggplant (Mission Bell), grafted onto the interspecific hybrid (Beaufort) and on tomato hybrid (Energy) during winter cultivation. Bacteria isolated from symptomatic tissues were identified as *Pectobacterium carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *atrosepticum* and confirmed to be pathogenic. Ginoux and Laterrot (1991) recognized these same symptoms as a graft incompatibility enhanced by low temperature and by heavy leaf guttation and water soaked leaf areas and lesions. Since the wide scale adoption of *S. torvum* for eggplant grafting, this type of plant dieback is no longer considered important.

18.2.12 Potential for Future

Since the use of grafting is likely to increase in the future (Edelstein, 2004), especially following the ban of MB for soil disinfestation, the adoption of grafting robots and healing chambers (Kurata, 1994) has assumed major importance. The cost of grafted plants is still the major constraint to their wide adoption together with the risks of unexpected plant dieback caused by biotic and/or abiotic factors. Grafting robots, healing chambers, and quick predictive methods for graft compatibility–incompatibility will improve and increase the adoption of grafting as a tool to reduce many soilborne diseases.

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