ABSTRACT

Title of Thesis: MANAGEMENT OF WHITE RUST (CAUSED BY *ALBUGO OCCIDENTALIS*) OF SPINACH IN MARYLAND AND ITS IMPACT ON THE NONTARGET INVERTEBRATE COMMUNITY

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White rust, caused by the oomycete *Albugo occidentalis*, is a major foliar disease of spinach (*Spinacia oleracea*). Favorable environmental conditions are required for its initiation and development. A modified version of a weather-based spray advisory program was evaluated using chemicals with different modes of action. Entomopathogenic fungi may infect *Myzus persicae*, a major pest of spinach. The nontarget effects of chemicals used in the management of white rust on entomopathogenic fungi and on the invertebrate community were also investigated. Both acibenzolar-S-methyl initiated at the second true leaf stage and pyraclostrobin applied according to the advisory program and weekly, reduced disease incidence compared to untreated plots. Naiad sprayed weekly reduced the percentage of aphid infested leaves, however no entomopathogenic fungi were isolated. All three

chemicals caused population increases in predatory mites and phytophagous thrips. Actigard and Naiad caused increases in oribatid mites and beetle larvae populations

MANAGEMENT OF WHITE RUST (CAUSED BY *ALBUGO OCCIDENTALIS*) OF SPINACH IN MARYLAND AND ITS IMPACT ON THE NONTARGET INVERTEBRATE COMMUNITY

by

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INTRODUCTION

Spinach (*Spinacia oleracea* L.), is a member of the Chenopodiaceae family and is related to Swiss chard (*Beta vulgaris* L. Cicla group), sugarbeet (*B. vulgaris* L. Altissima group), and table beet (*B. vulgaris* L. Crassa group). Spinach is grown in many countries and in the United States. Approximately 35,000 acres are grown annually throughout the US, primarily in California, Texas, Arkansas, Oklahoma, Maryland, Virginia, New Jersey and Colorado, with an approximate crop value of $70 million (3). The states of Maryland and Delaware constitute the fourth leading spinach producing area of the United States with a total of 3500 acres planted and 2700 acres in Maryland alone (2).

White rust, caused by the oomycete, *Albugo occidentalis* G.W. Wils., is a major foliar disease of spinach grown in the eastern production areas of the United States (3). White rust reduces spinach quality, which causes a greater economic loss than the effect of white rust on yield. Spinach, for both fresh market and processing, is graded on the basis of the amount of leaf area with white rust lesions. The grade of the raw product, for processing, is determined by the percentage of spinach leaves with lesions of a certain size. A harvested load in which more than 5% of the leaves contain a white rust lesion of 1 cm or more in diameter is classified as grade B, and has less financial value than grade A. A processor may reject a load with more than 12% of the leaves containing a white rust lesion of 1 cm or more in diameter (16).

Biology and epidemiology of white rust

Albugo occidentalis is an obligate parasite, which attacks spinach as well as the weed strawberry blite (*Chenopodium capitatum* L. Aschers), on which it was first reported in 1903 in Colorado (3). Its host range is confined to *Spinacia* and several species of *Chenopodium*. Formerly a member of the kingdom Fungi, *A. occidentalis* is now considered a member of the kingdom Stramenopila or Chromista. The biology of the oomycete *A. occidentalis* is similar to that of the more thoroughly studied *A. candida* (Pers.), which causes white rust of crucifers. However, unlike *A. candida*, physiological races of *A. occidentalis* have not been reported (3).

Initial symptoms of white rust appear as small chlorotic areas on the upper leaf surface. As the disease progresses, small white and shiny blister-like pustules called sori are produced on the underside of the leaf and occasionally on the upper leaf surface (3). The pustules are also occasionally produced on petioles, branches, and fruit coats (28). Pustules may be oval, irregularly oval, or elongated and their size can vary from 0.5 to 2 mm in diameter to 3 to 4 mm in length (28).

Pustules contain large numbers of sporangia borne on sporangiophores. Sporangia, which are asexual spores, are formed in chains and released when the epidermal tissue covering the sori ruptures (3). They are carried by wind or splashing rain to plants where they may germinate, causing secondary infections (27). Thus, white rust is a polycyclic disease. Sporangia can also initiate primary infections (27). Sporangia are mainly dispersed by wind, but dispersal by rain and insects can also occur (6).

When dry, the sporangia are hyaline and discoid, measuring around 10 x 14 μm. When hydrated, they become both spherical and ellipsoid and measure 10-19 x 20-22 μm (3). Sporangia rarely germinate directly (29). Typically, they germinate indirectly producing six to nine biflagellate motile zoospores that encyst and produce a germ tube that directly penetrates the spinach leaf (3). In indirect germination, the protoplasm swells and a bulge develops (29). The weakened wall ruptures, and the contents flow out as a protoplasmic mass in which cleavage planes are sometimes visible. This mass immediately begins oscillating and after a short time the zoospores begin to form and separate from each other. After swimming a short time, they encyst and germinate to produce a germ tube, which may enter the stoma or directly penetrate the leaf, and initiate infection. Inside the host, the fungus produces an abundance of intercellular mycelium in the spongy parenchyma where it gives rise to the sporangiophores and sporangia, which make up the sori. Sporangiophores are borne in solid layers just beneath the epidermis. Sporangia are formed in chains with occasional interstitial spacer cells. This characteristic distinguishes the Albuginaceae from all related families. The fungus acquires its nutrition through small intracellular globular haustoria, which are produced in the cells of the spongy parenchyma and the palisade tissue, but also form in epidermal cells (29).

As the lesions mature, dark oospores are abundantly produced, giving the lesion a grainy appearance prior to necrosis (3). Oospore formation creates prominent symptoms, particularly under greenhouse conditions, and oospores are formed in lesions on leaves, petioles, main stem, side branches, and fruit coats (28). These sexual spores serve as the overwintering structure and probable source of primary inoculum to initiate epidemics the following season (3,28). Primary infections occur on the lower leaves that are in direct contact or are close to the soil (33). Oospores splashed onto plant surfaces by rain or overhead irrigation are thought to germinate and infect the plant through open stomata (27).

Oospores are spherical, yellowish-brown, finely reticulate and measure 44-62 μm. Oospores are formed by the fusion of nuclei from the antheridia with those of the oogonia (27). Antheridia arise as terminal swellings, and oogonia arise as either terminal or intercalary swellings of the hyphae (29). Oospores of *A. candida* can germinate directly or produce a sporangium to initiate infection but there are no descriptions of oospore germination of *A. occidentalis* (3).

The fungus can become systemic in plants that have bolted to seed, but rarely in vegetative plants. When systemic, both sporangia and oospores are produced on all infected parts of the plant. Systemic infection of the fungus may cause a slight twisting of the stem and leaves, but unlike other white rust diseases, little or no hypertrophy or hyperplasy is induced (28).

Management of white rust in spinach

Management practices for white rust have included the use of resistant cultivars, crop rotation, and protective fungicides.

Resistance to white rust is inherited polygenically (5). Several cultivars with moderate or partial resistance to white rust have been released. When different spinach cultivars were tested in Maryland, ‘Fall green’ had less white rust than ‘Seven R’, and ‘Vancouver’ was intermediate in white rust severity (15). Partially resistant cultivars can provide acceptable white rust control without fungicides during the entire season when disease pressure is low (6). However, severe losses can occur with such cultivars, under favorable environmental conditions and high disease pressure in the absence of fungicides (3,6).

A three-year crop rotation and the deep plowing of diseased plant residues will reduce pathogen inoculum (19). However a long rotation is often not practical and crop rotation is generally less effective in reducing damage due to polycyclic than monocyclic diseases. *Albugo occidentalis* produces long-lived oospores and sporangia can become airborne and be blown in from neighboring diseased fields (5). Therefore white rust may become a serious problem even when initial inoculum is low.

EBDC (ethylene bisdithiocarbamate) fungicides were used for white rust management in spinach in the U.S. until EPA issued a Rebuttal Presumption Against Registration (RPAR) on this class of fungicides, in 1977 (22). Although the RPAR was unresolved as of August 1982, a residue tolerance of 10 ppm was established for the fresh market. In 1980, Canada, which consumed about 50% of the fresh market spinach and a significant proportion of processing spinach produced in Wintergarden, Texas, restricted the importation of spinach with residues exceeding 0.1 ppm of EBDC. This restriction made the chemical control of white rust in spinach very difficult because the predominant fungicide used during the time was the EBDC, maneb (22). Maneb was registered on spinach until 1989. In 1992, the EPA terminated the registration of EBDCs on spinach on the basis of pro-carcinogenic effects.

Metalaxyl was another fungicide previously registered for use on spinach. Metalaxyl was used at planting to control damping-off, and was effective against white rust when used alone or at reduced rates in tank mixes with chlorothalonil and maneb (22). Metalaxyl in-furrow soil applications at planting can delay initiation of white rust epidemics by delaying or reducing the primary infection from oospores (6). Recently the company that marketed metalaxyl further refined the chemical to its effective isomer, mefenoxam. Mefenoxam has replaced metalaxyl and has the same efficacy as metalaxyl. There is, however, a concern that multiple foliar applications of metalaxyl, and now mefenoxam will result in the development of resistance. Strains of *Pseudoperonospora cubensis* as well as other downy mildews were found to be resistant to metalaxyl (18).

Other currently registered fungicides include copper-based products and fosetyl-Al. Copper fungicides can cause phytotoxicity (8, 9, 15, 21*)*. Fosetyl-Al provides insufficient control and is expensive (30). Dodine, which is not registered on spinach, has been shown to provide excellent control of white rust at the rates of 1.46 to 2.13 kg/ha, but was also phytotoxic (9). Reducing the rates from 0.73 kg/ha to 0.22 kg/ha reduced leaf injury but also the extent of white rust control.

Fungicides with new chemistries, and modes-of-action, have been developed and found to be very effective at managing white rust. Azoxystrobin is a systemic broad-spectrum fungicide and a member of the new class of chemicals called strobilurins. This recently developed product is registered under the tradename of Quadris (Syngenta Crop Protection Inc., Greensboro, NC) and labeled on spinach. Azoxystrobin provided excellent control of white rust in several studies

(10,11,12,13). It was also found to control white rust better than other registered fungicides tested (21). However, there is now evidence of widespread resistance to azoxystrobin in several plant pathogens, including *Didymella bryoniae,* which causes gummy stem blight of watermelon and *Podosphaera xanthii,* which causes powdery mildew in cucurbits (17, 24). Resistance to strobilurins in *A. occidentalis* has not yet been reported. However to reduce the probability of resistance development, Syngenta Crop Protection, Inc. has restricted the number of sequential and total applications that can be applied to spinach in one cropping season.

Pyraclostrobin is also a strobilurin and interferes with fungal cell respiration and energy production. It provided excellent control of white rust when tested in Oklahoma in three different experiments (10,11,12). Pyraclostrobin was used for the control of white rust in overwintered spinach in 1999, at 0.45 l/ha and 0.88 l/ha. At both rates, pyraclostrobin reduced disease incidence and severity to 0% compared to the control, which had 67.5% incidence and 21.5% severity. In the spring of 1999, pyraclostrobin sprayed at 0.45 l/ha reduced incidence to 1.7 % and severity to 0.03% compared to the control, which had 53.3% incidence and 15.8% severity (11). In the spring of 2000, field trials were conducted in Bixby, Arkansas in a field previously cropped to soybean and in Stillwater Arkansas in a field previously cropped to spinach. In plots sprayed with pyraclostrobin at the rate of 0.67 l/ha, disease incidence and severity were 0% for both locations. Disease incidence for the control at Bixby and Stillwater was respectively, 82.7% and 92.2%, while disease severity was 33.7% and 49.1% (12).

Acibenzolar-S-methyl is a benzothiadiazole compound, which contributes to the management of plant diseases by stimulating the plant's natural defense mechanisms. It mimics the biological induction of systemic activated resistance (SAR). Acibenzolar-S-methyl was shown to provide good control of white rust in spinach (1, 10,11). Two experiments were conducted with spring and overwintered spinach in Bixby, Oklahoma. Disease incidence and disease severity were reduced, compared to the control in both experiments (10,11). Acibenzolar-S-methyl sprayed at 0.07 l/ha (1 oz per acre) resulted in disease incidences of 2.5% and 27% in the overwintered and spring spinach respectively. Disease severities were, respectively, 0.4% and 3.10% for the overwintered and the spring spinach. However, Dainello et al. found that acibenzolar-S-methyl did not reduce lesion severity and caused some phytotoxicity in the form of upward leaf cupping (7). Acibenzolar-S-methyl was also found to cause leaf tip curling and yellowing (20). Acibenzolar-S-methyl is labeled for use in spinach in a limited number of counties in California and Texas. It currently is available for use in New Jersey and Virginia under a Special Local Needs label.

Field and greenhouse experiments have been conducted to test the efficacy of surfactants in controlling white rust. Most of the surfactants tested were found to cause rapid zoospore lysis (20). Naiad was as effective as azoxystrobin in reducing disease incidence and severity in several trials. In two greenhouse tests, 40 ml of Naiad was applied to the susceptible cultivar St Helens, 30 min prior to inoculation with white rust. Disease severity was low overall and Naiad reduced severity from 5.6% and 5.4% for the controls to 0% and 0.2%, respectively. Field experiments were conducted in Kibler, Arkansas and in Crystal City, Texas. In Kibler, Naiad reduced incidence and severity compared to the control for both the susceptible cultivar St. Helens and the partially susceptible cultivar F380. Naiad reduced disease incidence compared to the controls by 60%, 38%, and 87% for St. Helens in the spring 1999, the fall of 1999, and the spring of 2000, respectively. Disease severity was low (less than 1%) for Naiad treated and nontreated St. Helens, in all three experiments. In the Texas field experiments, Naiad reduced disease incidence to 25% compared to 54% for the control. Disease severity was 0.8% for the control and 0.3% for the Naiad treatment. Sodium Dodecyl Sulfate (SDS) was also found to be very effective and both Naiad and SDS showed little or no phytotoxicity (20).

Environmental factors in white rust epidemiology

Development and spread of white rust occurs only under favorable environmental conditions. White rust development is favored by clear and relatively warm and dry days, followed by cool nights with heavy dew formation (28). Free moisture is necessary for spore germination (28)

Raabe and Pound tested the effect of temperature on germination, by placing sporangia in temperature chambers, at 4, 8, 12, 16 and 20°C (28). The optimum temperature for germination was 12°C; germination decreased sharply with increase or decrease in temperature. In other trials, sporangia germinated at temperatures as low as 2°C, and in the greenhouse at temperatures as high as 25°C. However, germination was poor at these temperature extremes. Chilling the spores for 1.5 hours at 12°C before incubation at different temperatures increased germination (28).

Spores germinated equally well in light or dark (28). The maturity of sporangia was an important factor in germination (28). Sporangia from very young unopened pustules did not germinate, while 3.2% of sporangia from young unopened pustules and 16.6% of just opened pustules germinated. In contrast, 24.6% of just fallen sporangia germinated while germination failed for old fallen sporangia.

Sporangia collected from wilted plants germinated better than sporangia from well-watered plants, indicating that desiccation affected germination (28). This was confirmed in the fields, where it was observed that in spite of abundant inoculum, very little disease development occurred during periods of rainfall and high humidity, while white rust developed abundantly during periods of relatively warm and dry days, followed by nights cool enough for dew formation (27). Warm days and low humidity affect desiccation of sporangia while the moisture condensation and cool temperatures at night favor germination and host penetration (28).

The effect of pH on spore germination was also tested (27). Although the sporangia germinated over a wide range of pH, germination was poor at extremes and the zoospores that were formed did not develop germ tubes. Germination was optimum near the neutral point (27).

The effect of environmental factors on sporangia production, sporulation, pustule formation, and oospore production was also studied (28). Disease developed more rapidly at 28°C than at 16°C. However, cool temperatures cause increases in the amount of sporangia produced, and as the fungus grows, more pustules are produced. Pustules formed at cool temperatures were larger than those formed at higher temperatures. Oospores were produced more rapidly, and more abundantly, at warm temperatures (28). Sporulation occurs optimally at 22°C (3).

The maturity of the plant also affects disease development. Oospore production is more abundant on seed plants than on vegetative plants (27).

Weather based advisory spray programs

In the management of white rust, fungicides are usually applied preventively on a calendar basis at intervals of 7 to 14 days, regardless of whether disease occurs or not. Yet, white rust, as with many diseases, only develops when environmental conditions are favorable. If fungicides are applied only when environmental conditions are favorable, fewer fungicide sprays may be applied compared to a calendar-based program.

Dainello and Jones (4) used continuous hours of leaf wetness (CHLW) as a parameter for scheduling fungicide applications to control white rust in spinach. When chlorothalonil was applied only after 12 hours of CHLW, a 25% fungicide spray reduction was observed compared to a 10-day schedule, without a significant reduction in control. When metalaxyl was applied after 12 hours of CHLW, a 39% reduction in spray applications was observed compared to a 7-day schedule (4).

In an effort to improve the CHLW system, a weather based advisory program was developed incorporating temperature (32). In experiments conducted in controlled environments, Sullivan found that the optimum temperature for disease development ranged from 12 to 18°C. At the temperatures of 12 to 22°C, infection occurred with a minimum of 3 hours of wetness. At suboptimal temperatures, at least 6 to 12 hours of wetness were required for disease development (31). Sullivan also developed a weather based advisory program using the protectant EBDC (maneb) and azoxystrobin. Maneb and azoxystrobin were applied after 3, 6, 12, 24 and 36 cumulative hours of favorable temperature, T, and wetness, W, (T/W). Maneb was less effective in controlling disease, but all T/W programs reduced the number of sprays compared to the 7-day schedule. The 6- and 12-h T/W programs were most efficient based on spray reductions and disease control for the EBDCs and azoxystrobin, respectively (32).

The impact of chemicals on entomopathogenic fungi and the nontarget invertebrates

Infestations of the green peach aphid, *Myzus persicae* are another limiting factor to the production of spinach. Foliar sprays of mevinphos or dimethoate have failed to control aphids and registration of mevinphos was cancelled. Currently, imidacloprid, and delayed planting in fall are used for aphid management (25).

The entomopathogenic fungus, *Erynia neoaphidis* (Remaudiere & Hennebert), infects *M. persicae* and is a potential management tool for this aphid (14). In the Arkansas River Valley, *E. neoaphidis* was found to contribute to aphid population regulation in spinach, although epizootics were observed at or past the harvest period (25).

Several studies have shown that fungicides used to protect crops from foliar pathogens can have a negative effect on entomopathogenic fungi, which in turn may cause increases in insect pest populations (23, 26). In a study conducted in Minnesota, several fungicides used to protect potatoes from foliar pathogens reduced entomophtoran pathogens and resulted in an increase in the green peach aphid populations (23).

In Arkansas, the protectant fungicide, maneb, as applied on spinach, had little effect initially, but the percentage of aphid infections by *E. neoaphidis* was significantly reduced in samples taken 21 and 41 days after the initial application. However, it was noted that, under normal spinach management practices, fungicide applications are less frequent than those in the study, and the negative effects on entomopathogenic fungi may be less important (26).

The objectives of this research were to evaluate the weather-based spray advisory program developed by Sullivan (32) using a continuous method of computing favorable hours of temperature and leaf wetness, and determine the influence of chemicals with different modes of action on white rust epidemics. A second objective was to evaluate the impact of these chemicals on entomopathogenic infections of the green peach aphid in spinach, and on the nontarget invertebrate community.

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CHAPTER II

THE USE OF A WEATHER-BASED SPRAY ADVISORY PROGRAM IN THE MANAGEMENT OF WHITE RUST OF SPINACH IN MARYLAND

ABSTRACT

A modified version of a spray advisory program for scheduling fungicide applications for white rust of spinach was evaluated using chemicals with different modes of action. Experiments were conducted over three cropping seasons and included fields that had not been previously planted to spinach for three years and where spinach was double cropped within one year (rotated and non-rotated plots). Spinach was treated with pyraclostrobin on a weekly schedule or according to a spray program based on temperature and leaf wetness. In a second set of experiments, pyraclostrobin, acibenzolar-S-methyl and Naiad, a surfactant, were applied weekly or according to the weather-based spray advisory program. Naiad and acibenzolar-S-methyl caused phytotoxicity. Both acibenzolar-S-methyl initialized at the second true leaf stage and pyraclostrobin applied according to the weather-based spray program and weekly, reduced white rust incidence compared to untreated plots. Naiad sprayed according to the weather-based spray program did not reduce white rust incidence. Pyraclostrobin did not always reduce the percent weight of infected leaves compared to the control, but maintained processing grading threshold levels below 5% or 12% (with the exception of the non-rotated plots). Acibenzolar-S-methyl initiated at the

second true leaf stage and applied weekly or according to the weather-based spray advisory program reduced the percent weight of infected leaves compared to the control and maintained the levels below 12%. Only weather-based applications of Naiad reduced the percent weight of infected leaves compared to the untreated plots, but not below the 12% maximum level for a grade B.

INTRODUCTION

White rust, caused by the obligate oomycete pathogen, *A. occidentalis* G.W. Wils, is a major foliar disease of spinach (*Spinacia oleracea.* L) in the United States, which only occurs east of the Rocky Mountains (2). Soilborne oospores are thought to serve as primary inoculum to initiate epidemics (2,18). Initial symptoms are small chlorotic lesions that develop on the upper leaf surface. As disease progresses, small white pustules called sori appear on the underside of the leaves, and sometimes on the upper leaf surface, frequently in concentric rings (2). Sori contain large numbers of sporangia, which are released when the pustules mature, and are blown to, or splashed, on nearby leaves, causing secondary infections (12). Lack of information on primary oospore infections and rapid secondary spread makes white rust a difficult disease to manage.

White rust is currently managed through the use of resistant cultivars, crop rotation and fungicides. White rust resistance is inherited polygenically and cultivars with moderate levels of resistance are commercially available (2). Partially resistant cultivars can provide acceptable white rust control without fungicides only when disease pressure is low; severe losses occur with such cultivars, under favorable environmental conditions and high disease pressure (2). A three-year crop rotation and deep plowing of diseased residue are practiced to reduce white rust inoculum. However, oospores survive long periods and sporangia can be airborne from nearby fields. White rust management in the mid-Atlantic region, even in moderately resistant cultivars, requires the use of fungicides. Several chemicals, with new chemistries, have recently been registered or will soon be registered on spinach, such as azoxystrobin, pyraclostrobin and acibenzolar-S-methyl.

Fungicides are currently applied to manage white rust, on a calendar basis at intervals of 7 to 14 days, even when weather does not favor disease. Yet, white rust develops only when environmental conditions are favorable and like many pathogens, requires extended periods of leaf wetness (free surface moisture or relative high humidity) during favorable temperatures to infect its host. White rust development is favored by cool nights, and heavy dew alternating with warm, dry sunny days, conditions that result in free moisture required for sporangia germination and zoospore development (13). Sporangia germinate between 2 and 25°C with an optimum temperature of 12 to 16°C (13). Germinating sporangia produce zoospores, which are released, encyst and produce a germ tube that directly penetrates the spinach leaf (12). Sporulation occurs optimally at 22°C and lesion development is favored by higher temperatures (2).

Fungicide application advisory programs that are based on an understanding of environmental conditions required for the development of white rust have been developed. Dainello and Jones (3) used continuous hours of leaf wetness (CHLW) as a parameter to schedule fungicide applications. Fungicides applied following 12 continuous hours of leaf wetness provided adequate control of white rust (3). Sullivan improved the CHLW system by incorporating temperature. She conducted experiments in a controlled environment to determine the effect of temperature and wetness periods on white rust development (16). The optimum temperature for disease development was found to range from 12 to 18°C. Sullivan also found that at the temperatures ranging from 12 to 22°C, a minimum of 3 cumulative hours of wetness were required for disease development, while 6 to 12 hours were required at suboptimal temperatures (16).

These data were used to identify categories of T/W units that incorporated cumulative hours of wetness and temperature. At the optimum temperatures of 12 to 18°C, each hour of wetness was counted as one T/W hour. At sub-optimal temperatures of 10 to 11°C and 19 to 21°C, each hour of wetness was counted as 0.75 T/W hour; at 6 to 9°C and 22 to 26°C, each hour was counted as 0.50 T/W hour. At temperatures less than 6°C or greater than 26°C, each hour of wetness was multiplied by 0 (14). There were thus four broad categories of T/W units. Weather-based advisory programs based on 3, 6, 12, 24, and 36 cumulative hours of favorable temperature and wetness (T/W) were evaluated in the field with an EBCD fungicide (maneb) and azoxystrobin (17). Azoxystrobin applied after 12 hours of T/W was the most efficient based on spray reduction and disease control (17).

The first objective of this study was to evaluate the modified temperature wetness based spray advisory program. The modified program was based on the same data as the original model and developed by computing cumulative hours of

T/W on a more continuous scale. The optimum temperature was set at 12°C and the endpoints below 8°C and above 26°C, where disease doesn't occur and beyond which no T/W units accumulated. In between these endpoints, where disease occurs, each degree was given a specific value based on data collected by Sullivan in controlled experiments (14). The threshold for a spray advisory in the modified weather-based advisory spray program was made when there was an accumulation of 12 hours of favorable temperature and leaf wetness.

The second objective of this study was to evaluate the influence of chemicals with different modes of action on white rust epidemics, when applied on a calendar schedule or according to the modified weather-based program. Pyraclostrobin is a broad-spectrum fungicide, and member of a new class of chemicals, strobilurins or, more broadly, quinone outside inhibitors (QoI). Pyraclostrobin provided excellent control of white rust in several trials (6,7,8,9). This fungicide interferes with fungal cell respiration and energy production, reducing mycelial growth. It was found to have a postinfection activity of 4 days (15). Pyraclostrobin was used in this study because of its excellent control of white rust and its postinfection activity. Fungicides with postinfection activity are likely to perform better than protectant fungicides when applied according to an advisory program, as sprays are often recommended during or immediately following periods of weather conducive to infection and disease development (17). For example, azoxystrobin, which was found to have a one-day postinfection activity on white rust (15), was more efficient than maneb when sprayed according to weather-based advisory programs (17).

Acibenzolar-S-methyl and Naiad were the two other chemicals tested in this study. Both products have different modes of action. The label for pyraclostrobin limits sequential applications to two prior to application of a chemical with different mode of action, for resistance management. Acibenzolar-S-methyl, a benzothiadiazole compound that controls plant diseases by stimulating the plant's natural defense mechanisms, provided good control of white rust in spinach (1,7,8). 2It mimics the biological induction of systemic activated resistance (SAR). Because acibenzolar-S-methyl elicits a general plant defense response, it is less likely to result in resistance development. Naiad, a surfactant, causes zoospore lysis (11). It reduced white rust incidence and severity when applied to spinach in both greenhouse and field experiments (11).

MATERIALS AND METHODS

Agronomic practices

Field experiments were conducted in both the spring and fall of 2002. Field trials were conducted in the spring at the University of Maryland Wye Research and Education Center (WREC) near Queenstown and at the University of Delaware Research and Education Center (UD REC) near Georgetown. All experiments were arranged as a randomized complete block design with 4 replications for each treatment.

At the WREC, lime at 2812 kg/ha and fertilizer, 0-0-60 NPK, at 168 kg/ha were incorporated on 4 April. Additional fertilizer, NH_4NO_3, was incorporated on 10

April at 224 kg/ha. Cycloate (Ro-Neet 6E, Helm Agro US, Inc., Memphis, TN) was incorporated to control weeds on 10 April at the rate of 4 l/ha. Spinach, cultivar 'Tyee', was planted on 11 April in plots that consisted of a 5-row bed, 7.6 m long. S-metolachlor (Dual Magnum, Syngenta Crop Protection, Inc., Greensboro, NC) was applied pre-emergence at 0.438 l/ha on 11 April. Imidacloprid (Admire 2F BayerCropScience, Research Triangle Park, NC) was applied on 11 April at 1.46 l/ha. The spinach was topdressed with NH_4NOS at 168 kg/ha on 24 May.

At UD REC, lime (dolomitic) was broadcast and incorporated at the rate of 1235 kg/ha on 16 April. Fertilizer, 0-0-60 NPK, was incorporated at 140 kg/ha and 30% UAN at 280 l/ha was applied on 16 April. Cycloate was incorporated for weed control on April 16 at 4.09 l/ha. Spinach cultivar Tyee was planted on 23 April at 11.2 kg/ha in plots that consisted of a 5-row bed, 7.6 m long. The spinach was topdressed with 30% UAN at 44.8 kg/ha on 7 June.

In the fall of 2002, experiments were conducted at WREC. Procedures were the same as in the spring except for the following. Fertilizer, NH_4NO_3, was incorporated on 6 September at 224 kg/ha (78 kg nitrogen/ha). Ro-Neet 6E at 3.51 l/ha in 561 liter of water/ha was incorporated with a field cultivator to control weeds on 8 September. Spinach, cultivar 'Seven R', was planted on 9 September with a 5-row planter JR seeder, 0.30 m between rows. The seeder was calibrated to 50 seeds/m. Dual Magnum at 0.58 l/ha and Ridomil Gold 4E at 1.17 l/ha were applied pre-emergence on 9 September. Phenmedipham (SpinTor 2SC, Dow Agrosciences LLC, Indianapolis, IN) was applied on 13 September at the rate of 0.35 l/ha to control armyworms. The chemical experiment was also used in an insect experiment, therefore carbaryl (Sevin XLR Plus, BayerCropScience, Research Triangle Park, NC) was applied at 1.17 l/ha on 7 October to suppress natural enemies and promote aphid populations.

Computing hours of leaf wetness temperature units

The temperature/wetness-based spray advisory program was modified to compute T/W on a continuous scale based on controlled experiments by Sullivan for 12 hours of leaf wetness (14). In between 8°C and 26°C, each degree was assigned a specific value (T/W unit). At the temperature of 12°C, one hour of leaf wetness was counted as one T/W unit, and counted as zero below 8°C and above 26°C. But for the degrees between endpoints, each degree was assigned a value equal to the ratio of the disease severity at that temperature and the severity at the value of reference 12°C. For odd numbered temperatures, the average of the values for the adjacent two even numbers was computed. A comparison of the initial spray advisory program developed by Sullivan (32) with the modified advisory program is given in Table 1.

Table 1. Comparison of parameters of a weather-based fungicide application program for white rust developed in Oklahoma and a modified version.

INITIAL MODEL*	**MODIFIED MODEL**
	Below 8°C, 1h W = 0 h T/W
	At 8°C, 1h W = 0.07 h T/W
	At 9°C, 1h W = 0.37 h T/W
6 to 9°C and 22 to 26°C, 1h W = 0.5 h T/W	At 10°C, 1h W = 0.68 h T/W
	At 11°C, 1h W = 0.84 h T/W
12 to 18°C, 1h W =1h T/W	At 12°C, 1h W = 1 h T/W
	At 13°C, 1h W = 0.84 h T/W
10 to 11°C and 19 to 21°C, 1h W = 0.75 h T/W	At 14°C, 1h W = 0.68 h T/W
	At 15°C, 1h W = 0.68 h T/W
Below 6°C and above 26°C, 1h W = 0 h T/W	At 16°C, 1h W = 0.68 h T/W
	At 17°C, 1h W = 0.59 h T/W
	At 18°C, 1h W = 0.5 h T/W
	At 19°C, 1h W = 0.38 h T/W
	At 20°C, 1h W = 0.26 h T/W
	At 22°C, 1h W = 0.13 h T/W
	At 24°C, 1h W = 0.1 h T/W
	At 26°C, 1h W = 0.06 h T/W

*Sullivan, M.J., Damicone, J.P. and Payton, M.E. 2003. Development of a weather-based advisory program for scheduling fungicide applications for control of white rust of spinach. Plant Dis. 87:923-928.

Weather-based advisory spray experiments

The 12-h T/W spray program was tested at the WREC and at UD REC in the spring of 2002. In the fall of 2002, two 12-h T/W spray program experiments were conducted at the WREC. The first experiment was on ground that had been rotated out of spinach for two years, while the second experiment was in the field that had been cropped to spinach the previous spring; and therefore had no rotation. In all experiments, the broad-spectrum fungicide pyraclostrobin (Cabrio 20WG, BASF, North Mount Olive, NJ) was used at 0.55 kg/ha. Applications were initiated at the first sign of disease. Subsequent applications were applied either weekly or after 12 accumulated hours of favorable temperature and leaf wetness (i.e. 12 T/W units were accumulated). In the weather-based advisory spray experiments conducted at UD REC in the spring of 2002 and at the WREC in the fall of 2002, an additional treatment was included where applications were initiated at scouting and subsequent applications were scheduled 4 days after the 12 T/W units were accumulated. Four days is the post infection activity of pyraclostrobin (15).

Chemical experiments

Experiments were conducted, at UD REC in the spring of 2002 and at the WREC in the fall of 2002, to evaluate the efficacy of spray programs using acibenzolar-S-methyl (Actigard, Syngenta Crop Protection Inc., Greensboro, NC) at 0.035 kg/ha, pyraclostrobin (Cabrio 20WG, BASF, North Mount Olive, NJ) at 0.55 kg/ha and a surfactant, NAIAD (2-hydroxyethyl ammonium alkyl benzene sulfonate, alkyl phenoxy poly (ethylene oxy) ethanol, DI (2-hydroxy ethyl) ammonium CIS-9-octadecadonate octyl alkyldamide, Naiad Company, Shelton, CT) at 25 ml/m^2. In the spring of 2002, all chemical applications were initiated at the first sign of disease and subsequent applications were either weekly or after accumulation of 12 T/W units. In the fall of 2002, the timing of the initial and subsequent applications was the same for pyraclostrobin and Naiad; however for acibenzolar-S-methyl, applications were either initiated at the 2^{nd} true leaf stage, as recommended by the manufacturer, or at the first sign of disease. Subsequent applications were either weekly or after 12 T/W units.

Disease rating

The total number of infected and uninfected plants were counted in each plot before harvest to estimate disease incidence. The average percentage of leaf area covered with white rust, on infected plants, was visually estimated for all plants in each plot. Four 1-m row segments were harvested at random from the center two or three rows of each plot. All harvested leaves were examined and the weight of infected leaves (leaves with a lesion of more than 1 cm in diameter) and uninfected leaves were determined and used to calculate a percent weight of infected leaves for each plot. The economic losses due to white rust result primarily from its impact on quality rather than its effect on yield. Spinach leaves with lesions are not acceptable for the fresh market and processed spinach is graded on the basis of the amount of leaf area with lesions. A single 1 cm diameter lesion per leaf is the damage threshold for a spinach leaf. The grade of a load is determined by the percentage of leaves with lesions. A load with more than 5% of the leaves containing a white rust lesion of 1 cm in diameter or more is classified as a grade B, and has less financial value than a grade A. A load with more than 12% of the leaves infected by white rust can be rejected by the processor (10).

In the spring of 2002, disease incidence and disease severity were assessed for the weather-based advisory spray program experiments on 13 June at the WREC and 19 June at the UD REC. The percent weight of infected leaves was evaluated on 13 June at the WREC and 25 June at UD REC. For the chemical experiment at UD REC, disease incidence and disease severity were assessed on 25 June, and the percent weight of infected leaves on 26 June.

In the fall of 2002, disease incidence and severity were assessed on 9 November and the percent weight of infected leaves on 14 November for the weather-based advisory spray experiment where there had been no rotation. Disease incidence and severity were assessed on 9 November and the percent weight of infected leaves on 20 November for the weather-based advisory spray experiment on new ground. For the chemical experiment, disease incidence and severity and the percent weight of infected leaves were assessed on 20 November.

Statistical analyses

Analysis of variance (ANOVA) was performed on disease incidence, disease severity and percent weight of infected leaves using the Mixed procedure, (SAS, Cary, NC) followed by Fischer's Least Significant Difference (LSD) mean separation test ($P<0.05$) when allowed by the ANOVA results.

RESULTS

Weather-based advisory spray experiments

Disease pressure was high at the WREC and low at UD REC for all experiments. In the spring of 2002, at the WREC, disease incidence was 61% for the untreated plots, 24.1% for plots sprayed with pyraclostrobin weekly and 13.2% for plots sprayed with pyraclostrobin according to the 12 T/W program (Table 2). Both pyraclostrobin spray programs reduced incidence and severity compared to the control. The percent of weight of infected to non-infected leaves was 13.3% for the control. It was not reduced in treated plots compared to the control, but was less than 5% for both pyraclostrobin spray programs.

Table 2. Comparison of white rust incidence, severity and weight of infected leaves of spinach sprayed weekly or according to a weather-based spray program at UM Wye Research and Education Center, Queenstown, MD – Spring 2002.

Treatment [a]	Number of sprays	Incidence [b,c] (%)	Severity [b,d] (%)	Weight of infected leaves [b,e] (%)
Weekly (1,2) [f]	2	24.1 b	2.1 b	4.3 a
12-h T/W (1,3)	2	13.2 b	0.8 b	1.2 a
Control	0	61 a	8.5 a	13.3 a

[a] Weekly = sprays applied on a weekly schedule, 12-h T/W = sprays applied after the accumulation of 12 hours of favorable temperature and leaf wetness units (Table 1)
[b] Means in a column followed by the same letter are not significantly different at *P*= 0.05 according to the Fischer's protected LSD test. Arc sine transformed data were used for statistical analysis.
[c] Percentage of plants within a plot with symptoms of white rust from each of four replicates.
[d] Percentage of leaf area of infected plants with visible white rust symptoms from each of four replicates.
[e] Percent weight of leaves with a lesion of more than 1 cm in diameter.
[f] Application dates were: 1=2 June, 2=9 June, 3= 13 June. Pyraclostrobin (Cabrio 20WG) was applied at 0.55 kg/ha. Applications initiated at the first sign of disease.

In the spring of 2002 at UD REC, disease incidence and severity were, respectively, less than 3% and 2% for all treatments. None of the treatments reduced disease incidence or severity compared to the control (Table 3). Both weekly and 12-h T/W spray programs resulted in 2 applications, although the second application was performed later for the 12-h T/W program.

Table 3. Comparison of white rust incidence, severity, and weight of infected leaves of spinach sprayed weekly or according to a weather-based spray program at UD Research and Education Center, Georgetown, Delaware – Spring 2002

Treatment [a]	Number of sprays	Incidence [b,c] (%)	Severity [b,d] (%)	Weight of infected leaves [b,e] (%)
Weekly (1,3) [f]	2	0.0 a	0.3 a	0.0 a
12-h T/W (1,4)	2	0.0a	0.0 a	0.0 a
12-h-T/W + 4 days (2)	1	1.6 a	1.5 a	0.1 a
Control	0	2.9 a	1.0 a	0.3 a

[a] Weekly = sprays applied on a weekly schedule, 12-h T/W = sprays applied after the accumulation of 12 hours of favorable temperature and leaf wetness units (Table 1), 12-h T/W + 4 days= sprays applied 4 days after the accumulation of 12-h T/W sprays. The postinfection activity of pyraclostrobin is 4 days.

[b] Means in a column followed by the same letter are not significantly different at *P*= 0.05 according to the Fischer's protected LSD test.

[c] Percentage of plants within a plot with symptoms of white rust from each of four replicates.

[d] Percentage of leaf area of infected plants with visible white rust symptoms from each of four replicates.

[e] Percent weight of leaves with a lesion of more than 1 cm in diameter.

[f] Application dates were: 1=8 June, 2=12 June, 3= 15 June, 4=17 June. Pyraclostrobin (Cabrio 20WG) was applied at 0. 0.55 kg/ha. Applications initiated at the first sign of disease.

White rust incidence in untreated plots, and the 12-h T/W + 4 days spray program was 100% in a nonrotated field following a spinach crop (fall, 2002). Only pyraclostrobin sprayed weekly, reduced incidence to 74.2% (Table 4). Disease severity was 35% for the control, and less than 10% for both pyraclostrobin sprayed weekly and according to the 12-h T/W program. The percent of weight of infected to non-infected leaves was above 25% for all treatments.

Table 4. Comparison of a spray program scheduled weekly and a weather-based spray program on non-rotated ground at UM Wye Research and Education Center, Queenstown, MD – Fall 2002.

Treatment [a]	Number of sprays	Incidence [b,c] (%)	Severity [b,d] (%)	Weight of infected leaves [b,e] (%)
Weekly (1,3,5,6) [f]	4	74.2b	9.5 c	29.8 c
12-h T/W (1,3,5,7)	4	98.8a	10.0 c	34.1 bc
12-h-T/W + 4 days (2,4,6)	3	100a	21.3 b	45.0 ab
Control	0	100a	35.0 a	52.7 a

[a] Weekly = sprays applied on a weekly schedule, 12-h T/W = sprays applied after the accumulation of 12 hours of favorable temperature and leaf wetness units (Table 1). 12-h T/W + 4 days= sprays applied 4 days after the accumulation of 12-h T/W sprays. The postinfection activity of pyraclostrobin is 4 days.

[b] Means in a column followed by the same letter are not significantly different at *P*= 0.05 according to the Fischer's protected LSD test

[c]. Percentage of plants within a plot with symptoms of white rust from each of four replicates.

[d] Percentage of leaf area of infected plants with visible white rust symptoms from each of four replicates.

[e] Percent weight of leaves with a lesion of more than 1 cm in diameter.

[f] Application dates were: 1=19 Oct, 2= 23 Oct, 3=27 Oct, 4=1 Nov, 5=3 Nov, 6=10 Nov, 7=14 Nov. Pyraclostrobin (Cabrio 20WG) was applied at 0.55 kg/ha. Applications were initiated at the first sign of disease.

Disease incidence was 78.9% in untreated plots in the experiment planted on rotated ground WREC (Table 5). Both pyraclostrobin sprayed weekly and according to the 12-h T/W program reduced disease to less than 25%. However, pyraclostrobin application at either schedule did not reduce disease severity. The percent of weight of infected to non-infected leaves was 13.5% in untreated plot. Pyraclostrobin spray programs did not significantly reduce the percent weight of infected to non-infected leaves compared to the control, however it was less than 5% for both programs. Two sprays were applied in plots scheduled weekly and according to the 12-h T/W program, although the second application was performed later for the 12-h T/W program in both fall weather-based spray advisory experiments.

Table 5. Comparison of a spray program scheduled weekly and a weather-based spray program on rotated ground at UM Wye Research and Education Center, Queenstown, MD – Fall 2002

Treatment [a]	Number of sprays	Incidence [b,c] (%)	Severity [b,d] (%)	Weight of infected leaves [b,e] (%)
Weekly (1,3,5,6)[f]	4	13.3 b	3.3 a	3.4 a
12-h T/W (1,3,5,7)	4	21.1b	3.3 a	2.9 a
12-h-T/W + 4 days (2,4,6)	3	94.4a	7 a	5.3 a
Control	0	78.9 a	7.3 a	13.5 a

[a] Weekly = sprays applied on a weekly schedule, 12-h T/W = sprays applied after the accumulation of 12 hours of favorable temperature and leaf wetness units (Table 1), 12-h T/W + 4 days= sprays applied 4 days after the accumulation of 12-h T/W sprays. The postinfection activity of pyraclostrobin is 4 days.
[b] Means in a column followed by the same letter are not significantly different at *P*= 0.05 according to the Fischer's protected LSD test.
[c] Percentage of plants within a plot with symptoms of white rust from each of four replicates.
[d] Percentage of leaf area of infected plants with visible white rust symptoms from each of four replicates.
[e] Percent weight of leaves with a lesion of more than 1 cm in diameter.
[f] Application dates were: 1=19 Oct, 2= 23 Oct, 3=27 Oct, 4=1 Nov, 5=3 Nov, 6=10 Nov, 7=14 Nov. Pyraclostrobin (Cabrio 20WG) was applied at 0.55 kg/ha. Applications were initiated at the first sign of disease.

Disease incidence was low, 7.7%, in untreated plots of the Chemical experiment conducted at UD REC in the spring of 2002. All treatments reduced disease incidence but not severity compared to the untreated control (Table 6). All weekly and 12-h T/W spray programs resulted in 2 applications, although the second application was performed later for the 12-h T/W programs.

Table 6. Comparison of efficacy of pyraclostrobin, Naiad and acibenzolar-S-methyl sprayed weekly or according to a weather-based application spray program at UD Research and Education Center, Georgetown, Delaware – Spring 2003.

Treatment [a]	Number of sprays	Incidence [b,c] (%)	Severity [b,d] (%)	Weight of infected leaves [b,e] (%)
Pyraclostrobin Weekly (1,2) [f]	2	0.6 b	1.3 a	0.0 a
Pyraclostrobin 12-h T/W (1,3)	2	0.6 b	0.3 a	0.0 a
Acibenzolar-S-methyl Weekly (1,2)	2	2.5 b	3.5 a	0.2 a
Acibenzolar-S-methyl 12-h T/W (1,3)	2	0.8 b	3.8 a	0.0 a
Naiad Weekly (1,2)	2	0.6 b	0.3 a	0.1 a
Naiad 12-h T/W (1,3)	2	0.0 b	0.0 a	0.0 a
Control	0	7.7 a	2.5 a	0.5 a

[a] Weekly = sprays applied on a weekly schedule, 12-h T/W = sprays applied after the accumulation of 12 hours of favorable temperature and leaf wetness units (Table 1)
[b] Means in a column followed by the same letter are not significantly different at *P*= 0.05 according to the Fischer's protected LSD test.
[c] Percentage of plants within a plot with symptoms of white rust from each of four replicates.
[d] Percentage of leaf area of infected plants with visible white rust symptoms from each of four replicates.
[e] Percent weight of leaves with a lesion of more than 1 cm in diameter.
[f] Application dates were: 1=8 June, 2=14 June, 3=17 June. Pyraclostrobin (Cabrio 20WG) was applied at 0.55 kg/ha, acibenzolar-S-methyl (Actigard) at 0.035 kg/ha, and Naiad at the rate of 25 m l/m^2. Applications initiated at the first sign of disease.

Chemical experiments

In the chemical experiment conducted at the WREC in the fall of 2002, pyraclostrobin sprayed weekly and according to the 12-h T/W program provided the greatest reduction in white rust incidence (Table 7). Acibenzolar-S-methyl initiated at the 2nd true leaf stage, sprayed weekly or according to the 12-h T/W program also reduced white rust incidence compared to the control. Acibenzolar-S-methyl caused some phytotoxicity. Both Naiad treatments caused phytotoxicity in the form of chlorosis, and did not reduce white rust incidence or severity compared to the control. Pyraclostrobin sprayed weekly or according to the 12-h T/W program and acibenzolar-S-methyl initiated at the 2nd true leaf stage, reduced white rust severity compared to the control (Table 7). Acibenzolar-S-methyl initiated at the 2nd true leaf stage, pyraclostrobin spray programs, and Naiad sprayed according to the 12-h T/W program reduced the percent of weight of infected to non infected leaves compared to the control. However, acibenzolar-S-methyl initiated by scouting and sprayed weekly, and Naiad sprayed weekly did not reduce the percent of weight of infected to non-infected leaves. Acibenzolar-S-methyl initiated at the second true leaf stage and sprayed weekly, was the only treatment to reduce the percent of weight of infected to non-infected leaves to less than 5%, pyraclostrobin, acibenzolar-S-methyl and Naiad 12-h T/W spray programs reduced it to less than 12%. All weekly and 12-h T/W spray programs using pyraclostrobin, acibenzolar-S-methyl initiated at the first sign of disease, and Naiad resulted in 4 sprays, although the last 12-h T/W application was sprayed later than the last weekly spray.

Table 7. Comparison of efficacy of pyraclostrobin, Naiad and acibenzolar-S-methyl sprayed weekly or according to a weather-based application spray program at UM Wye Research and Education Center, Queenstown, MD – Fall 2002.

Treatment [b]	Number of sprays	Incidence [c,d] (%)	Severity [c,e] (%)	Weight of infected leaves [c,f] (%)
Pyraclostrobin Weekly (3,4,5,6) [a]	4	30.0 d	1.0 d	13.5 bc
Pyraclostrobin 12-h T/W (3,4,5,7)	4	19.6 d	1.5 cd	11.9 bc
Acibenzolar-S-methyl 2nd true leaf and weekly (1,2,3,4,5,6)	6	66.7c	3.5 bcd	3.2 c
Acibenzolar-S-methyl 2nd true leaf 12-h T/W (1,3,4,5,7)	5	79.1bc	4.0 bc	10.4 bc
Acibenzolar-S-methyl Weekly (3,4,5,6)	4	94.1ab	5.3 ab	15.6 b
Naiad 12-h T/W (3,4,5,7)	4	85.0 ab	5.3 ab	8.9 bc
Naiad Weekly (3,4,5,6)	4	84.2 ab	5.8 ab	18.7 ab
Control	0	95.8 a	7.0 a	28.7 a

[a] Weekly = sprays applied on a weekly schedule, 12-h T/W = sprays applied after the accumulation of 12 hours of favorable temperature and leaf wetness units (Table 1)
[b] Means in a column followed by the same letter are not significantly different at *P*= 0.05 according to the Fischer's protected LSD test.
[c] Percentage of plants within a plot with symptoms of white rust from each of four replicates.
[d] Percentage of leaf area of infected plants with visible white rust symptoms from each of four replicates.
[e] Percent weight of leaves with a lesion of more than 1 cm in diameter.
[f] Application dates were: 1=4 Oct, 2=13 Oct, 3=19 Oct, 4=27 Oct, 5=3 Nov, 6=10 Nov, 7=14 Nov. Pyraclostrobin (Cabrio 20WG) was applied at 0.55 kg/ha, acibenzolar-S-methyl (Actigard) at 0.035 kg/ha, and Naiad at 25 ml/m^2. Applications were initiated at the first sign of disease, with the exception of two acibenzolar-S-methyl treatments initiated at the 2nd true leaf stage.

DISCUSSION

Disease pressure was high in all experiments conducted at the WREC. In the spring of 2002, plots sprayed weekly and according to the 12-h T/W program received the same number of fungicide applications. However, in plots sprayed the

12-h T/W program, the second application occurred 4 days after the weekly spray and white rust incidence was still reduced. This may have occurred because the 12-h T/W program applications were better timed. Pyraclostrobin sprayed according the 12-h T/W program reduced disease incidence compared to the control, in all experiments conducted at the WREC, except in the absence of rotation. In the no-rotation experiment conducted in the fall of 2002 at the WREC, the weekly spray program, but not the 12-h T/W reduced white rust incidence. White rust incidence was the highest in the no rotation experiment compared to all the experiments conducted. Incidence was 74% for the weekly spray program and 99% for the model spray program in the absence of rotation, but was less than 22% in the new ground experiment, in both spray programs.

In the experiments conducted on rotated ground, neither pyraclostrobin spray programs reduced the percent weight of infected leaves compared to the control, however the percent weight of infected leaves was below 5%, which is the processing spinach threshold for grade A. In untreated plots the overall level of the percent weight of infected leaves was above 13%, which exceeds grade B, and a level that may be rejected by the processor. In the absence of rotation, both pyraclostrobin programs reduced the percent weight of infected leaves to greater than 29%, but the level still exceeded grade B and would have been unacceptable for the processor.

These results confirm the importance of crop rotation in the management of white rust. In this experiment, the rotation was only for two years, and yet disease pressure was significantly lower than in the adjacent non-rotated field. The results also indicate the importance of over-wintering inoculum, presumably, soilborne oospores. Airborne sporangia may have played a role in infections in the experiment on non-rotated ground, the fact that disease pressure was much greater than on rotated ground, shows the importance of infections from the over-wintering inoculum. Airborne sporangia probably initiated epidemics in the experiment conducted on ground that was rotated. The weather-based advisory program is based on the temperature and leaf wetness conditions that favor sporangial germination and secondary spread of white rust. In the absence of rotation, primary inoculum is high and the 12-h T/W program will not succeed in managing white rust.

Although the post infection activity of pyraclostrobin is 4 days (15), results show that, applications should be triggered by the 12-h T/W program, and not four days after. Use of products with post infection activity when timing sprays appear to be important to use of a weather-based program. Sullivan found that maneb, with no post infection activity, should be applied according to a 6-h T/W threshold, but azoxystrobin, with post infection activity, could be timed with a 12-h T/W threshold (17). Use of a product with post-infection activity would allow some flexibility to growers that could not apply a fungicide immediately when the threshold was reached.

Results of the chemical experiment conducted in the fall of 2002 at the WREC, showed that acibenzolar-S-methyl applications must be initiated at the 2nd true leaf stage, as recommended by the manufacturer. In field tests conducted in Arkansas and Texas, Naiad reduced both disease incidence and severity compared to the control and phytotoxicity was not observed (11). However, in our field study, not only did Naiad programs fail to reduce incidence and severity, but phytotoxicity was observed. However, Naiad applications were initiated later in our field experiments than in Arkansas and Texas. Pyraclostrobin provided the greatest reduction in white rust incidence of the chemicals used here, however, not of disease severity, like several other studies (7,8,9). Acibenzolar-S-methyl provided similar control for disease severity. One explanation could be that because of its mode of action, acibenzolar-S-methyl does initially reduce infection as well as pyraclostrobin, but with time, when the plant's defenses are activated, it is as efficient in limiting lesion development or expansion. Acibenzolar-S-methyl provided the greatest reduction in percent weight of infected to non-infected leaves in the chemical experiment conducted at WREC. Again, the host defense response may limit lesion development or expansion. Alternatively, the phytotoxicity observed with acibenzolar-S-methyl , although not as nearly severe as with Naiad may have influenced the ratings when determining the percent weight of infected to non-infected leaves, at harvest.

Disease pressure was very low for all experiments conducted at UD REC. In the chemical experiment, all spray programs reduced white rust incidence compared to the control, while in the advisory spray experiment, none of the treatments reduced incidence. Because disease pressure was so low, few conclusions came be made from these results

In our field studies, we observed few spray reductions. In the three different trials, Sullivan conducted using the EBCDs, mancozeb, maneb, and the strobilurin, azoxystrobin, she was able to save up to 60% in sprays scheduled with the 12h T/W spray program, compared to the 7 day program. All sprays were initiated when the first true leaves were truly expanded (14). However, our sprays were initiated later at scouting, fewer overall applications occurred, and fewer sprays were saved.

Different methods of computing T/W units were compared. T/W units computed with the four broad categories, and based on calculated leaf wetness, consistently reached the spray threshold earlier than computation with the more continuous method (Table 8). So besides the fact that the more continuous method of computing T/W is more reflective of the data from controlled experiments, from which the spray advisory program is based, it impacts the length of time required to reach the spray threshold.

Table 8. Comparison of three methods of computing hours of leaf wetness for a weather-based advisory spray program. UM Wye Research and Education Center, Queenstown, MD – Spring 2002 and Fall 2002.

	Method I[a]	Method II[b]	Method III[c]
		Spring 2002	
N. of days to predicted 2nd T/W application	3	4	7
		Fall 2002	
N. of days to predicted 2nd T/W application	3	4	4
N. of days to predicted 3rd T/W application	2	6	4
N. of days to predicted 4th T/W application	4	6	9

[a] Method I: 4 broad categories of computing T/W units, based on leaf wetness
[b] Method II: Continuous method of computing T/W units, based on leaf wetness
[c] Method III: 4 broad categories of computing T/W units, based on relative humidity

Site-specific weather data (SkyBit, Inc., Bellefonte, PA) was used for the WREC experiments. When calculated leaf wetness was used (Method II) instead of relative humidity (RH) (Method III), in two cases, the spray threshold was reached earlier for the leaf wetness, in one case, for the RH, and in another case, it was reached at the same time (Table 8). As the data from the controlled experiments are based on leaf wetness, measured leaf wetness seems to be a better parameter than relative humidity to use in calculation of T/W units. However, we are unable to say whether calculated leaf wetness used in this study is a better parameter than relative humidity. Some of the recordings of calculated leaf wetness were not accompanied with RH>90%. In those cases, RH was usually greater than 75% and never less than 50%. This explains the instances where the spray threshold was reached earlier with Method II than Method III. Instances where this threshold was reached later with Method II were explained by the fact that T/W units were computed continuously.

We noticed that in the fall of 2002, rains were so frequent that it was difficult to save on sprays. Experiments were conducted in a particularly wet year.

Further experiments are needed to verify the efficacy of this 12-h T/W spray program in managing white rust in Maryland. This work indicates that the program should be refined prior to use in Maryland. Changes could include the use of measured, instead of calculated, leaf wetness, and initiating application after 4 weeks, instead of scouting, as preplant applications of metalaxyl, which are commonly used in the mid-Atlantic region, provide protection early in the season.

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CHAPTER III

THE IMPACT OF CHEMICALS ON ENTOMOPATHOGENIC FUNGI AND THE NONTARGET INVERTEBRATE COMMUNITY

ABSTRACT

The green peach aphid (*Myzus persicae*) is a major pest of spinach. Entomopathogenic fungi may infect this aphid and are a potential biological control agent. Some fungicides impair entomopathogenic fungi and may in turn enhance pest populations. The nontarget effects of chemicals used in the management of white rust on entomopathogenic fungi and on the invertebrate community were investigated. Plots where Naiad was applied weekly had significantly fewer leaves infested with aphids than nontreated plots. No entomopathogenic fungi were isolated from aphids collected from the field. Pyraclostrobin, acibenzolar-S-methyl and Naiad caused populations increases in predatory mites and phytophagous thrips. Acibenzolar-S-methyl and Naiad caused increases in oribatid mites and beetle larvae populations.

INTRODUCTION

The green peach aphid, *Myzus persicae* (Sulzer), is a major insect pest of spinach. Heavy infestations can stunt seedlings and reduce yields by direct feeding and indirectly by vectoring several viruses. However, the major concern resulting from high aphid numbers is contamination of the spinach product. The broad and crinkled leaves make aphid removal during processing extremely difficult (6). Aphid parts in processed spinach result in consumer complaints and litigation (2).

Imidacloprid and delayed planting in fall are currently used for aphid management (6). An entomopathogenic fungus, *Erynia neoaphidis* (Remaudiere & Hennebert), also causes epizootics in *M. persicae* and is a potential management tool for the green peach aphid (2). This fungus is the most common pathogen of aphids and distributed worldwide (12). One study in Arkansas reported that, *E. neoaphidis* contributed to aphid population regulation in spinach, although epizootics were often observed at or after the harvest period (6). Interestingly, epizootics occurring at harvest, often decrease the crop value, as diseased aphids attached to the spinach leaves become contaminants in the raw product. Epizootics may be more valuable in areas where spinach is harvested more than once (6).

Several studies have shown that fungicides used to protect crops from foliar pathogens can have a negative effect on entomopathogenic fungi, which in turn may enhance insect pest populations (4,7). In a study conducted in Minnesota, certain fungicides used to protect potatoes from foliar pathogens reduced entomopathogenic fungi and increased green peach aphid populations (4). *Erynia neoaphidis* and

Entomophtoran planchoniana accounted for 66.7% and 22.3% of the mycoses in field collected green peach aphids.

In direct toxicity laboratory studies, all lower labeled rates of fungicides, except chlorothalonil, significantly reduced germination of all entomopathogenic fungi. Copper hydroxide was intermediate. Metalaxyl + mancozeb, mancozeb, and captafol were strongly inhibitory of germination of conidia even at 0.1X rates. Triphenyltin hydroxide, benomyl, metalaxyl + mancozeb, and mancozeb strongly inhibited mycelial growth of all entomopathogenic fungi at concentrations of 1X, while copper hydroxide inhibited *C. obscurus*, and *E. planchoniana*, but not *E. neoaphidis*. Chlorothalonil did not have any significant effect on mycelial growth of any of the entomopathogenic fungi, even at 2X concentrations. Benomyl was highly toxic to the green peach aphid, while copper hydroxide and chlorothalonil were intermediate. The green peach aphid population was greatest during late season in potatoes sprayed with metalaxyl + mancozeb, captafol or mancozeb, and lowest with benomyl, triphenyltin hydroxide, chlorothalonil or copper hydroxide (4).

In Arkansas, the fungicide, maneb, had little effect initially, but the percentage of aphid infections by *E. neoaphidis* was significantly reduced in samples taken 21 and 41 days after the initial application. However, under normal spinach management practices, fungicide applications are less frequent than those used in the study and therefore should have little effect on epizootic occurrence (7).

A study evaluated the effect of 20 fungicides on the infectivity of conidia of *E. neoaphidis* (5). Conidia, applied to broad bean leaves, were treated with fungicides applied at their recommended field rate. The infectivity of the conidia was tested using an aphid bioassay. Some of the products tested reduced infectivity by 50% to 100%. Benzimidazoles were the least toxic for *E. neoaphidis*, while the morpholines were the most toxic. Reduction in infectivity ranged from 37% to 100% for triazoles and from 17% to 68% for strobilurines. Azoxystrobin caused a 68% reduction in conidia infectivity (5).

This study was part of an experiment to evaluate the efficacy and timing of chemicals with different modes of action, used in the management of white rust, a disease caused by *A. occidentalis* in spinach. The objective was to examine the impact of pyraclostrobin, Naiad, and acibenzolar-S-methyl on entomopathogenic infections of the green peach aphid, and on the abundance and diversity of nontarget invertebrates. Pyraclostrobin is a broad-spectrum fungicide, belonging to the class of strobilurines. Pyraclostrobin is effective against plant pathogens from all classes of fungi, and, like other strobilurines such as the azoxystrobin, it could have a negative effect on entomopathogenic fungi. Naiad is a surfactant, which causes zoospore lysis (3). Acibenzolar-S-methyl is a benzothiadiazole compound that controls plant diseases by stimulating the plant's natural defense mechanisms. It mimics the biological induction of systemic activated resistance (SAR).

MATERIALS AND METHODS

Impact of chemicals on entomopathogenic fungi

The experiment was conducted during the fall of 2002, at the University of Maryland Wye Research and Education Center (WREC), in Queenstown, Maryland. Eight treatments were arranged as a randomized complete block design with four replications. Pyraclostrobin (Cabrio 20WG, BASF, North Mount Olive, NJ) at 0.55 kg/ha, Naiad (Naiad Company, Shelton, CT) at 25 ml/m^2, and acibenzolar-S-methyl (Actigard 50WG, Syngenta Crop Protection Inc., Greensboro, NC) at the rate of at 0.035 kg/ha, were applied, either weekly or according to a modified weather based advisory program. All pyraclosytrobin and Naiad treatments were initiated at the first sign of white rust. Advisory program-based acibenzolar-S-methyl applications were initiated either at the 2nd true leaf stage or at the first sign of disease. Ten plants were randomly sampled for each of the plots, shortly before harvest. For each sample, individual leaves were examined for the presence of aphids, and the percentage of leaves infested and the total number of aphids were recorded. Aphids with symptoms of fungal infection were removed from the leaves and counted. Fifty aphids were randomly picked from each sample and held for three days. These aphids were then examined to see if signs of infection had developed and infective fungi were identified. The percentage of diseased aphids was determined from the total number of aphids.

Impact of chemicals on nontarget invertebrates

Experiments were conducted during two cropping seasons of spinach (fall 2002 and spring 2003). Each experiment was arranged as a randomized complete block design with 4 replications for each treatment. The plots were sprayed (weekly or according to a modified weather based advisory program) with either the fungicide pyraclostrobin, the surfactant NAIAD, or the plant activator acibenzolar-S-methyl, as described in the previous experiment. At harvest, one sample of 1 m of row was randomly taken from each plot and processed through Berlese funnels for three days to extract all invertebrates. The collected organisms were identified and counted to the family or order level.

Statistical analyses

An analysis of variance (PROC Mixed, SAS, Cary, NC) was used to test for treatment differences in the percentage of leaves infested and the percentage of diseased aphids. If the F test was significant, Tukey's mean separation test ($P<0.05$) adjusted for experiment-wise comparison.

For the impact of the chemicals on nontarget invertebrates, data were pooled over weekly and the weather advisory program-based scheduling regimes for each chemical treatment. Principal response curve (PRC) analysis was used to distill the community-level effects of the chemical treatments into a graphical form (11). This multivariate analysis used linear models similar to those underlying regression analyses and summarized all information on the recorded taxa simultaneously. The principal response, expressed as a canonical coefficient, is a weighted sum of the abundances of the taxa and reflects the response of the chemical-treated community relative to the untreated control. The analysis also generated taxa weights, which reflect the relative contribution of each invertebrate group to the principal responses. Taxa with high positive weights are expected to behave the same as the patterns indicated by the response curves, whereas taxa with high negative weights behave in the opposite way. A Monte-Carlo permutation method was used to determine if the treatment effects were statistically different from the control community. Treatment effects on mean abundance of key invertebrate groups were also tested by ANOVA for each experiment.

RESULTS

Impact of chemicals on entomopathogenic fungi

We were not able to isolate entomopathogenic fungi from the field. Fungi isolated from aphids with signs of infection were secondary saprophytic fungi that apparently invaded cadavers that succumbed to other causes. Naiad applied weekly, but no other treatment, reduced the number of leaves infested with aphids compared to untreated plots (Table 1). Naiad caused high levels of phytotoxicity in the form of leaf chlorosis and necrosis.

Table 1. Percentage of leaves infested with aphids

Treatment [a]	Number of sprays	Percent infested leaves [b] (%)
Pyraclostrobin Weekly (3,4,5,6) [c]	4	69.5 a
Pyraclostrobin Model (3,4,5,7)	4	57 ab
Acibenzolar-S-methyl 2nd true leaf and weekly (1,2,3,4,5,6)	6	64.4 a
Acibenzolar-S-methyl 2nd true leaf and model (1,3,4,5,7)	5	68 a
Acibenzolar-S-methyl Weekly (3,4,5,6)	4	61.2 a
Naiad Model (3,4,5,7)	4	56.4 ab
Naiad Weekly (3,4,5,6)	4	40.3 b
Control	0	61.3 a

[a] Weekly = sprays applied on a weekly schedule, Model = sprays applied according to a weather- based advisory spray program.
[b] Means in a column followed by the same letter are not significantly different at *P= 0.05* according to Tukey's.
[c] Application dates were: 1=4 Oct, 2=13 Oct, 3=19 Oct, 4=27 Oct, 5=3 Nov, 6=10 Nov, 7=14 Nov. Pyraclostrobin (Cabrio 20 EC at 0.55 kg/ha), acibenzolar-S-methyl (Actigard at 0.035 kg/ha), and Naiad at 25 ml/m2 were applied.

Impact of chemicals on nontarget invertebrates

Sixty % of the invertebrates collected were herbivores, 33.8% were saprophytes, and 6.2% were natural enemies. The herbivores were mainly dominated by green peach aphids (Aphididae, 66.8%) and phytophagous thrips (Thripidae, 29.1%), which feed on the spinach leaves. Saprophytes were mainly composed of fly larvae (Diptera, 46.7%), springtails (Entomobryidae, 26.1%), various fungus beetles (Coleoptera, 6.8%), psocids (Psocoptera, 6.5%), beetle larvae (Coleoptera, 5.4%) and oribatid mites (Oribatida, 2.4%). These saprophytes feed on the senescing lower leaves. Natural enemies mainly consisted of spiders (Araneae, 31.2%), minute pirate bugs (Anthocoridae, 20.4%), rove beetles (Staphylinidae,16.1%), ladybird beetles (Coccinellidae, 11.7%), predatory mites (Acari, 6.9%) and big-eyed bugs (Lygaeidae, 1.7%). The frequency of occurrence of the collected invertebrates and their average densities per meter of row are presented in table 2. Overall, the most abundant taxa were the herbivorous invertebrates aphids and thrips, and the saprophytic fly larvae and springtails (Table 2).

Table 2. Frequency and abundance of invertebrates in spinach recorded from plant extractions using Berlese funnels. Data pooled over treatments for two cropping seasons. Taxa are grouped by ecological role and ranked by most abundant

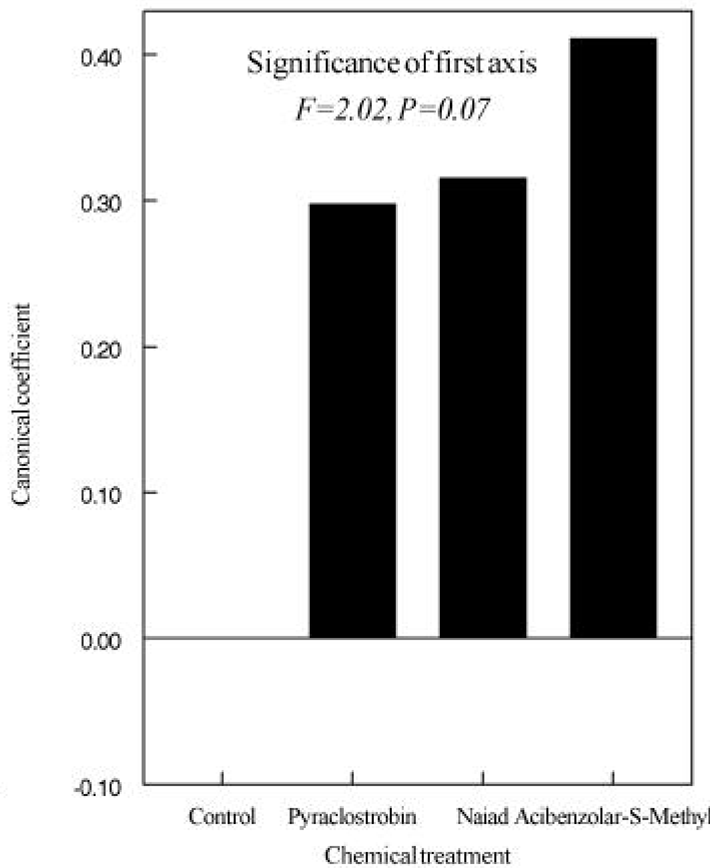
Invertebrate group	Taxa	% Frequency of occurrence	Average density per 1 meter row
	Herbivores		
Green peach aphids	Aphididae	100	194.8
Phytophagous thrips	Thripidae	97	84.8
Caterpillars	Noctuidae	94	4.8
Mirid plant bugs	Miridae	84	4.6
Misc. beetles	Coleoptera	72	2.0
Leaf/plant hoppers	Cicadellidae	31	0.7
	Saprophytes		
Fly larvae/pupae	Diptera	100	76.5
Springtails	Entomobryidae	100	42.9
Misc. fungus beetles	Coleoptera	91	11.2
Psocids	Psocoptera	91	10.7
Beetle larvae	Coleoptera	97	8.9
Flies	Diptera	100	5.8
Oribatid mites	Oribatida	91	4.0
Minute fungus beetles	Corylophidae	59	2.9
Shining mold beetles	Phalacridae	31	1.2
	Predators		
Spiders	Araneae	94	9.4
Minute pirate bugs	Anthocoridae	84	6.1
Rove beetles	Staphylinidae	91	4.8
Ladybird beetles	Coccinellidae	84	3.5
Predatory mites	Acari	81	2.1
Big-eyed bugs	Lygaeidae	56	1.7
Carabid beetles/larvae	Coleoptera	25	0.5
Lacewing larvae	Chrysopidae	13	0.4
Ants	Formicidae	19	0.2
	Parasites		
Parasitic wasps	Hymenoptera	56	1.2

The PRC analysis (Figure 1) did not indicate a significant treatment effect on the community as a whole ($P=0.07$), although the probability level was very close to

5%. However, a preponderance of positive weight factors for taxa seem to indicate increases in certain populations due to the treatments.

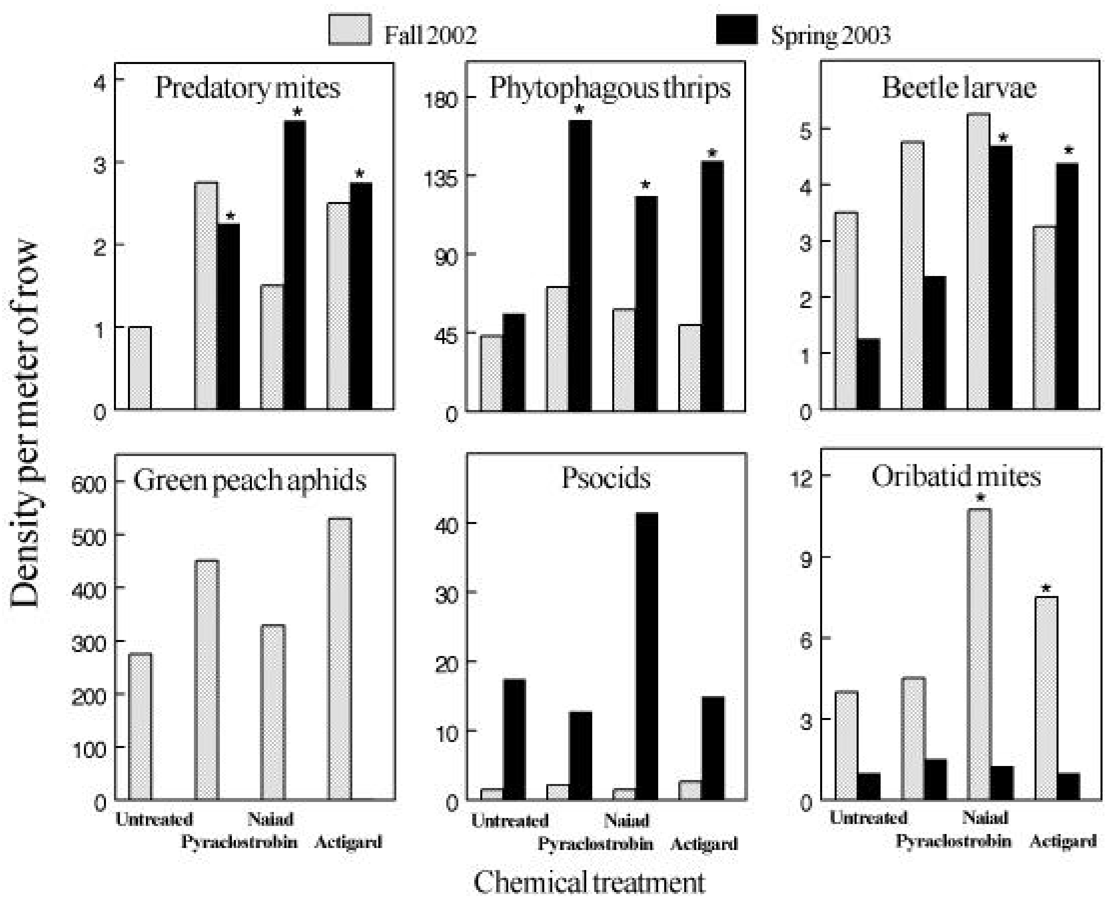
Figure 1. Principal response curve (PRC) and weights for each taxonomic group of the collected invertebrates

Taxon weight	Taxon group
2.316	Predatory mites
2.172	Phytophagous thrips
2.013	Beetle larvae
1.591	Green peach aphids
1.344	Psocids
1.316	Oribatid mites
1.138	Caterpillars
1.093	Minute pirate bugs
0.832	Rove beetles
0.709	Leaf/plant hoppers
0.444	Flies
0.273	Shining mold beetles
0.250	Minute fungus beetles
0.201	Ants
0.170	Mirid plant bugs
0.158	Spiders
0.097	Ladybird beetles
0.063	Big-eyed bugs
-0.170	Parasitic wasps
-0.173	Fly larvae/pupae
-0.195	Springtails
-0.201	Lacewing larvae
-0.249	Carabid beetles/larvae
-0.441	Misc. fungus beetles
-0.530	Misc. beetles



Treatment effects were tested on the abundance means of the six invertebrates with the highest taxon weights (Figure 2). In the fall of 2002, acibenzolar-S-methyl and Naiad caused increases in populations of oribatid mites. In the spring of 2003, all three chemicals caused increases in predatory mites and phytophagous thrips, while Naiad and acibenzolar-S-methyl caused increases in beetle larvae populations.

Figure 2. Average densities of the taxonomic groups with highest taxon weight. Asterisks indicate significant difference from untreated control. Actigard = Acibenzolar-S-methyl.



DISCUSSION

Weekly applications of Naiad, but not other chemicals, significantly reduced the percentage of leaves infested with aphids. The mechanism that contributed to this result is not known, the Naiad plots may have been less attractive to colonizing aphids due to the occurrence of high levels of phytotoxicity. Symptoms of phytotoxicity were initial flecks that expanded and became chlorotic, then necrotic. Naiad which is a surfactant, could have a direct effect on aphids by reducing the lipid compounds on their cuticles, which provide a barrier against desiccation. .

All three chemicals caused significant population increases in several invertebrates collected in this study. The exact cause of these population increases is not known, however one hypothesis is that the chemicals suppressed the spread of entomopathogenic fungi, which naturally infect and kill invertebrates, and resulted in population increases. Azoxystrobin, another member of the strobilurin class, was found to be highly toxic to *E. neoaphidis* (4). Direct toxicity tests on known entomopathogenic fungi of nontarget invertebrates in spinach could provide insights to these results.

Azoxystrobin applied at twice the recommended concentration was also found to cause accelerated larval development of two coccinellids species (*Cycloneda sanguinea* and *Harmonia axyridis*) exposed to the treated leaf residues (8). Azoxystrobin also altered the behavior responses of the adult beetles. *Cycloneda sanguinea* was found more often on azoxystrobin treated surfaces than on nontreated filter papers. However, it was noted that there was no evidence of ‘attraction’ to this chemical, since there the beetles did not orient toward such treated surfaces from any distance (8). Further research could include testing the power of any of these chemicals as attractants.

Acibenzolar-S-methyl may have affected nontarget invertebrates by altering plant defense response. Acibenzolar-S-methyl, elicits systemic acquired resistance through the salicylic acid pathway, which may result in inhibition of the jasmonic acid pathway responsible for plant resistance to herbivores. Laboratory studies have shown that salicylic acid inhibits the biosynthesis of jasmonic acid and the subsequent chemical responses (9, 1). A field experiment conducted on tomato provided evidence for a trade-off between salicylic acid induced resistance to the bacterial speck pathogen, *Pseudomonas syringae* pv. tomato, and jasmonic acid induced resistance to *Spodoptera exigua* (10). Jasmonic acid inhibition could explain the increase of herbivores in acibenzolar-S-methyl-treated communities.

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