EPIDEMIOLOGY AND MANAGEMENT OF WHITE RUST OF SPINACH IN OKLAHOMA

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER II

CHAPTER I

INTRODUCTION

Spinach (*Spinacia oleracea* L.), a dioecious member of the Chenopodiaceae related to Swiss chard, sugar beet, table beet, pigweed, and saltbush, is an economically significant leafy vegetable crop in many countries and in the United States. Approximately 14,000 ha (35,000 acres) of spinach are grown every year throughout the United States for fresh and processed markets with an annual crop value of about $70 million (14). California, Texas, Arkansas, Oklahoma, Maryland, Virginia, New Jersey, and Colorado are the major spinach production states in the United States. Roughly 3,300 acres of spinach are planted annually in Oklahoma alone (4).

Spinach is usually grown as a direct seeded crop, with cultivar type, planting season, and disease control/management determined by the product's market destination (14). Cultivars vary from "flat-leaf" to "semi-savoy" to "highly-savoy" or wrinkled types. Most fresh market spinach is savoyed, and most processing spinach is flat-leaved. In some places spinach is grown all year, but in other locations, such as Oklahoma, spinach is a fall, winter, or spring crop. Fresh-market spinach may be hand-harvested, but both fresh-market and processing spinach in Oklahoma are usually mechanically harvested. Spinach also may be repeat-harvested after regrowth (14).

White rust, caused by the fungal pathogen *Albugo occidentalis* Wilson, is an important foliar disease of spinach in all United States production areas east of the Rocky Mountains (7, 14). White rust is a serious problem in spinach production areas of Texas, Arkansas and Oklahoma where it may occur at epidemic levels (51). It can cause

substantial yield losses through a reduction in the quality of both fresh and processed spinach (7). White rust does not occur on spinach in western production areas and has not been reported on spinach outside the United States (14).

History of the Disease:

White rust was first reported on *Chenopodium capitatum* (L.) Aschers, a weed closely related to spinach, from Colorado in 1903 (54). The first report of white rust on spinach was from Virginia in 1907 (14). During the next 25 years, white rust was mentioned only a few times on *Chenopodium* species before it suddenly became active in 1937 on spinach from the Winter Garden region of Texas[1]. Loads of spinach arriving at the New York market from this region contained plants showing varied amounts of infection. In one load, three-fourths of the examined plants bore from one to three leaves that were slightly to severely affected by the pathogen (46, 60, 61). After this discovery, white rust was regarded as an important and destructive disease of spinach.

Since the outbreak in Texas, the disease has spread considerably and has caused serious losses. Godfrey reported the first appearance of the disease in the Lower Rio Grande Valley and in the Coastal Bend area of Texas in 1941 (27). The first report of white rust in the Arkansas River Valley was near Muskogee, Oklahoma in 1943 (11). Later that year and in the spring of 1944, the disease was observed not only in Muskogee county, but also in Wagoner, Sequoyah, and LeFlore counties (37). In 1945, white rust was first reported in Arkansas near Van Buren (65). In 1970, white rust was considered

[1] Dimmit, Maverick, Uvalde, Zavala, and Frio counties comprise the Winter Garden region of Texas. The region is the location of about one-half of the acreage devoted to fresh market spinach in the United States

to be the primary disease problem for spinach in South Texas and in many production areas in the eastern United States (56). Today, white rust is regarded as one of the most economically important diseases of spinach, because with favorable environmental conditions, it can rapidly spread and cause dramatic decreases in crop marketability.

Symptoms:

White rust first appears as small chlorotic areas on the upper and lower surfaces of infected leaves. The chlorotic condition then becomes more pronounced on the upper surface as pustules (sori) develop as small, gray areas on the lower surface of the leaves. Although the pustules usually arise on the lower surface of the leaf, they occasionally can form on the upper surface (14, 46, 47).

After the pustules become visible on the leaf, they have a white, glassy appearance and resemble yeast colonies. Asexual sporangia are produced as the pustules mature. Pustules are blister-like and may be oval, irregularly oval, or elongated in shape. The size of pustules can range from about ½ to 2 mm in diameter and, if elongated, may be up to 3 to 4 mm in length (46, 47). Sometimes pustules appear in concentric rings, but more often they are found scattered on the leaf surface. The pustules are often so abundant that nearly the entire lower surface of the leaf is covered. Although the pustules are usually borne on leaves, they also are occasionally produced on petioles, side branches, and fruit coats (46, 47).

Oospores (sexual survival spore) are abundantly produced in mature pustules and often are favored under greenhouse conditions. Frequently, oospores are formed in such large numbers that the infected plant tissues appear nearly black. Oospores often give the pustule a grainy appearance prior to the beginning of leaf necrosis (46).

Occasionally, *A. occidentalis* becomes systemic in vegetative plants, but often it is systemic in plants that have bolted to seed. When systemic, the fungus produces both pustules and oospores on all infected parts of the spinach plant. It may cause a slight twisting of the stem and leaves, but there is little or no hypertrophy or hyperplasia as for other white rust diseases (46).

Under greenhouse conditions, both flat-leaved and savoyed varieties show equal amounts of infection. However, the symptoms are often more pronounced on the savoyed cultivars. The pustules tend to be larger and the leaves become more chlorotic and may become rolled when heavily infected (46).

Causal Fungus:

Though not much is known about the biology of *Albugo occidentalis*, it is thought to resemble that of *Albugo candida* (Pers.) Kunze, the more thoroughly studied white rust pathogen of crucifers (14). *A. occidentalis* is an obligate fungal pathogen in the Peronosporales, with its host range confined to *Spinacia* and several *Chenopodium* species. Unlike *A. candida*, however, physiological races of *A. occidentalis* have not been reported (14).

Sporangia are formed in chains, produced in pustules, and are released when the epidermal tissue covering the pustule ruptures (46). This sporangial release is responsible for the polycyclic nature of the disease. When dry, sporangia of *A. occidentalis* are hyaline, discoid, and measure approximately 10 x 14 μm. When hydrated, the sporangia become spherical to ellipsoid and measure 10 to 19 x 20 to 22 μm. Approximately 22^0 C is optimal for the production of sporangia (14, 46). Sporangia usually germinate indirectly to produce zoospores, but rarely can germinate directly. Sporangia can germinate

indirectly from 2 to 25^0 C, with an optimum temperature of 12 to 16^0 C (14, 46).

In indirect germination, the protoplasm of the sporangium swells and a bulge develops in the sporangial wall. When the weakened wall ruptures, 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, six to nine biflagellate zoospores begin to form and separate. After swimming for a short time, zoospores encyst and germinate to produce a germ tube, which may enter the host and initiate infection (46). Inside the host, the fungus produces a large, branched intercellular mycelium and acquires its nutrition through intracellular globular haustoria. The haustoria are usually produced in the cells of the spongy parenchyma and the palisade tissue, but also may form in epidermal cells (48).

The zoospores are shaped like "inverted dug-out canoes" and move by two flagella (each about 20 μm in length), one of which has a terminal knob (46). From the top, zoospores appear elliptical and measure approximately 7 x 10 μm. From the side, they are flat or slightly curved in on the bottom. They have a curved upper surface and are about 5 μm in thickness (46).

In addition to sporangia, the fungus also has a sexual stage, which results in the formation of oospores. Oospores are thick-walled resting spores that carry the fungus through adverse conditions. The oospores are spherical, yellowish-brown, and finely reticulate. These resting spores, which measure from 44 to 62 μm, are formed by the fusion of nuclei from the antheridia with those of the oogonia. Antheridia arise as terminal swellings, and oogonia arise as either terminal or intercalary swellings of the hyphae. The oogonia are spherical and measure about 100 μm; while the antheridia are elongated and

measure about 20 to 50 μm (14, 46, 48). Higher temperatures favor production of oospores over the production of sporangia (14).

Primary infection is thought to occur from soilborne oospores that are splashed onto plants by rainfall or overhead irrigation (14, 17, 18, 46, 56). The oospores are speculated to germinate and infect the plant through open stomata, however, their role in the disease cycle has not been determined (47). Oospores of *A. candida* can germinate directly or produce a sporangium to initiate infection (39, 58). However, there have not been any descriptions of oospore germination in *A. occidentalis* (14). It has been demonstrated that airborne sporangia can initiate primary infection (18). Secondary infection results from airborne sporangia discharged from pustules (46).

Environmental Factors:

Raabe and Pound (47) found that environmental conditions can affect the indirect germination (zoospore release) of sporangia of *A. occidentalis*. *In vitro*, germination was assayed at 4, 8, 12, 16, and 20^0 C to determine the role of temperature in germination. The optimum temperature was near 12^0 C and germination decreased by 5 to 20% with an increase or a decrease in temperature. In other trials, sporangia germinated at temperatures as low as 2^0 C and in the greenhouse at temperatures as high as 25^0 C. However, germination at these extremes was very poor (<1%). Chilling the sporangia at 12^0 C for 1.5 hours prior to incubation at a given temperature also increased germination at all temperatures (47). Although the optimum temperature for zoospore release was determined, there have been no reports on temperature requirements for plant infection.

Raabe and Pound (47) also studied the effect of the sporangial maturity and the moisture content of the leaves on indirect germination. Sporangia were collected from

very young unopened pustules, young unopened pustules, and pustules that had just opened. Sporangia removed from infected plants by shaking also were collected. Sporangia collected from very young unopened pustules failed to germinate. Sporangia collected from young unopened pustules and pustules that had just opened showed 3.2% and 16.6% germination, respectively. Sporangia removed by shaking not only had a higher germination percentage (24.6%), but also produced more disease than sporangia at any other stage of development (47). Therefore, the maturity of the sporangia was an important factor in indirect germination and plant infection. There was an increase in sporangial germination with increased water loss from the leaves, up to about 40% water loss. Germination subsequently dropped considerably (by 0.5 to 15 %) and in some experiments stopped (47).

Raabe and Pound (47) also examined the effect of light and pH on indirect sporangial germination. Light had little or no effect on sporangial germination, but pH was shown to have an important effect on germination. Sporangia were incubated in solutions with pH values of 3, 3.5, 4, 4.5, 5, 6.2, 7, 8, 9, 9.5, 10, 10.5, and 11. Although sporangia germinated over a wide pH range (from 3.5 to 11.0), near neutral pH resulted in an optimal germination level of 40%. Germination was 7.8% and 5.1% at the pH extremes of 3.5 and 11, respectively (47).

Disease Management:

Historically, white rust has been managed through the use of crop rotation, genetic resistance, and spray programs using protective fungicides such as the ethylene bisdithiocarbamates (EBDCs), maneb, zineb, and mancozeb (34). Populations of host-specific pathogens can sometimes be decreased in soil by rotating with non-host crops.

The length of an effective crop rotation varies from 1 to 4 years depending upon the pathogen. Satisfactory disease control through crop rotation is possible for pathogens that are soil invaders, but is much more difficult for pathogens that are soil inhabitants (1). Soil invaders survive only on or in living plants or saprophytically on plant debris until decomposition of the debris. However, soil inhabitants produce long-lived spores or can survive as saprophytes for more than 5 or 6 years. For these pathogens, the effectiveness of crop rotation is often limited, because they can survive even in the absence of a living host (1). *A. occidentalis* not only produces long-lived oospores, but sporangia can become airborne and be blown in from neighboring diseased fields (18). Crop rotation is also less effective for polycyclic diseases, such as white rust. White rust control through crop rotation, therefore, is often ineffective and in many cases long rotations are impractical (14, 16).

Horizontal (Polygenic)Resistance:

Resistance to white rust is inherited polygenically (6, 14, 17). The development of spinach cultivars with resistance to white rust was initiated in 1960 through the cooperative efforts of the U.S. Department of Agriculture and Texas A&M University. The resistance of the spinach cultivars "Wintergarden", "Jewel", and "Crystal" resulted in a significant reduction in white rust incidence compared to susceptible cultivars (7). A breeding program also was started in Arkansas in 1972. A field selection process was used to further develop field or horizontal resistance to white rust, as well as to select for resistance to other diseases. Each year individual plants with the highest levels of resistance were mass-crossed and the progeny were evaluated in a disease nursery (7). Several breeding lines have been developed and the cultivars "Fall Green", "Ozarka", and

"Greenvalley" have been released with varying levels of resistance to white rust (6, 28, 29).

When disease incidence is low, the use of partially resistant spinach cultivars can provide acceptable white rust control without fungicides for the entire season (18). However, such cultivars can be severely damaged under favorable environmental conditions and high disease pressure (14, 18). With partial resistance, latent periods are usually prolonged and both lesion development and sporulation are reduced but complete protection from infection does not occur (7, 14, 15). Effective levels of white rust resistance are lacking in long-standing spinach cultivars used for production in the spring. Many resistant cultivars are open-pollinated and do not grow as vigorously as hybrids, which can decrease yield (6).

Chemical Control:

Chemical control of white rust has involved repeated, preventive applications of protective fungicides, starting before disease appears. The EBDCs were registered by the Environmental Protections Agency (EPA) for use on spinach in 1955 (34). These compounds significantly reduced white rust compared to control plots and when applied on a 7-day schedule (10, 20, 34). Therefore, the EBDCs were widely used in disease management programs for spinach.

In 1977, the EPA issued a Rebuttal Presumption Against Registration (RPAR) of the EBDCs (34). A residue tolerance of 10 ppm was established in the United States in 1982, even though the RPAR was still unresolved. In 1980, Canadian markets restricted residues of EBDCs allowed on imported spinach to not exceed 0.1 ppm (any detectable quantity). Canada consumes about 50% of the fresh market spinach grown in the United

States and a significant proportion of the processing spinach produced in the Winter Garden region of Texas (34). Due to the restrictions imposed by the Canadian regulation, EBDCs were eliminated from many disease control programs for spinach.

Recently, EBDCs have been shown to break down into ethylene thiourea (ETU). Laboratory animals fed high levels of ETU have developed cancer, thyroid diseases, and birth defects. Therefore, the EPA classified ETU as a probable human carcinogen. Due to the pro-carcinogenic effects of the EBDCs, the registration of these compounds on spinach and other vegetables was revoked in 1992 by an EPA special review, and they now are no longer registered for use on spinach.

Control of white rust on spinach not only has been hampered by the loss of the registration for EBDC fungicides, but also by limitations of registered alternatives. At this time, the only fungicides registered for white rust control are the copper compounds (copper sulfate and copper hydroxide); metalaxyl; a pre-mix of metalaxyl and copper sulfate; and fosetyl-aluminum. Fosetyl-aluminum is not very effective in controlling the disease and is expensive. Copper compounds are phytotoxic, and injury symptoms may be just as damaging as the disease (19, 32). Soil applications of metalaxyl at planting may not control white rust later in the season and are expensive. There is a 21-day pre-harvest restriction for the pre-mix of metalaxyl with copper sulfate, and white rust development can occur during this period.

Researchers have thus begun evaluating alternative fungicides and more effective use patterns for registered compounds to effectively control white rust. For example, Chambers et al. (10) evaluated three foliar fungicides for white rust control when applied in a regular 5-day spray program. Chlorothalonil gave good disease control, but this

fungicide is not cleared for use on spinach. Dodine controlled white rust effectively but is phytotoxic and is not labeled for use on spinach. Benomyl gave little disease control (10).

Resistance to metalaxyl has developed in strains of *Pseudoperonospora cubensis*, as well as other downy mildews (9, 25, 35, 50). Anti-resistance strategies have resulted in the marketing of metalaxyl in combination with other fungicides, i.e. mancozeb (Ridomil MZ 58), and alternating sprays of metalaxyl with other fungicides (17). The identification of downy mildews that are resistant to metalaxyl and increasing yield losses attributed to new races of blue mold (*Peronospora effusa* Grev. ex. Desm.), another important spinach pathogen, prompted Jones and Dainello (34) to conduct field tests to examine the efficacy of metalaxyl alone and in combination with chlorothalonil and maneb (reduced-rate tank mixes) on white rust and blue mold. Metalaxyl (Ridomil 2E), chlorothalonil (Bravo 500 F), and tank mixes of metalaxyl with maneb or chlorothalonil were applied on 14-day intervals to spinach beginning at the four to six-leaf stage. Maneb also was applied at 7-day intervals beginning at the same time. Metalaxyl and maneb significantly improved yield over the control. Chlorothalonil reduced yield loss from white rust, but did not control blue mold. Reduced-rate tank mixes of metalaxyl with maneb were as effective as full rates of the individual fungicides in controlling white rust and blue mold. A reduced-rate tank mix of metalaxyl with chlorothalonil was as effective as the full rate of the individual compounds in controlling white rust, but not blue mold (34). Metalaxyl and chlorothalonil were shown to provide effective control of white rust when applied alone or in combination with effective tank mix partners. However, neither fungicide combination is registered for use on spinach.

Dainello and Jones (17) further evaluated use patterns for metalaxyl including seed

treatment, in-furrow granular application at planting, and application to the soil surface before planting. Disease incidence was assessed during two harvests of the same planting. The incidence of white rust in the control plots at 60 days after planting/harvest was 10.3% and 77.6%, respectively. Metalaxyl at 1g/kg applied as a seed treatment reduced incidence to 0.05% at the first cutting but did not reduce disease incidence at the second cutting. Metalaxyl at 1 kg/ha applied in-furrow reduced incidence to 0.0% and 49.1% at the first and second cuttings, respectively. The bed-spray treatment was evaluated in a separate planting from the seed and in-furrow treatment. The incidence of white rust in the control plots was 32.0 and 62.6% for the first and second cuttings, respectively. Metalaxyl at 3.67 kg/ha applied as a pre-plant bedspray reduced incidence to 1.1% at the first cutting and to 36.3% at the second cutting. The in-furrow and bed spray treatments controlled white rust for 48 to 60 days after planting. Seed treatments with metalaxyl controlled white rust up to 30 days after planting. Therefore, white rust can be reduced with seed, soil, or in-furrow application of metalaxyl in first-harvest spinach, but repeated applications or supplemental foliar sprays may be necessary to provide control in subsequent harvests or during seasons when conditions are optimal for disease development (17).

In Oklahoma, a single application of metalaxyl (Ridomil 2E) alone at planting did not reduce white rust incidence and severity compared to the control (19). Disease incidence and severity in the control were 76.7% and 25.1%, respectively. A single application of metalaxyl at planting followed by four foliar applications of mancozeb (Dithane 75 DF) reduced incidence and severity to 12.5% and 0.5%, respectively. A single application of metalaxyl at planting followed by a foliar application of metalaxyl and

mancozeb (Ridomil MZ 72W) and three foliar applications of copper hydroxide (Kocide 61DF) reduced incidence and severity to 14.2 and 0.6%, respectively. A single application of metalaxyl at planting followed by three foliar applications of copper hydroxide and a foliar application of metalaxyl and copper sulfate (Ridomil Copper 70W) reduced incidence and severity to 25.8% and 2.4% (19). In another Oklahoma study, disease incidence and severity in the control was 45.8% and 4.4%, respectively. A single foliar application of metalaxyl and copper sulfate did not reduce incidence or severity when compared to the control (20). A single application of metalaxyl and copper sulfate followed by three foliar applications of copper hydroxide (Kocide 101) completely controlled white rust, but was phytotoxic (20).

Because repeated sprays may be necessary to adequately control white rust, Dainello et al. (18) examined the efficacy of multiple soil applications of metalaxyl for season-long control of white rust. The contribution of partial resistance of the cultivar to disease control also was assessed. Soil applications of metalaxyl (1, 2, or 3) were applied to resistant (HY-R) and susceptible (HY-S) cultivars. However, the authors did not include the actual cultivar names. Disease incidence was assessed during two harvests of the same planting. For untreated plots, the cultivar HY-R had 30% lower disease incidence than the cultivar HY-S at the first cutting. One or two applications of metalaxyl to the cultivar HY-S resulted in a significant reduction in disease incidence from 38% in the control to 24% and 18%, respectively. For the cultivar HY-R, 1, 2, or 3 metalaxyl applications did not significantly reduce disease incidence. During the second harvest, similar results were obtained. For the cultivar HY-S, a single application of metalaxyl significantly reduced disease incidence from 29% for the control to 14%. However,

additional applications did not reduce disease incidence. For the cultivar HY-R, disease incidence was low (<1%) and was not affected by metalaxyl application. Results showed that partial resistance accounted for a greater reduction in disease than did applications of metalaxyl. Disease incidence for the partially resistant cultivar without metalaxyl was lower at every harvest than for the susceptible cultivar with 1, 2, or 3 applications of metalaxyl (18).

Azoxystrobin, a recently developed systemic broad-spectrum fungicide, is effective in controlling major Ascomycete, Basidiomycete, and Oomycete plant pathogens on various crops, including spinach (5, 66). The EPA has classified it as a reduced-risk pesticide because of its favorable toxicological and environmental profiles (5, 66). Azoxystrobin has provided equal or better control than other registered fungicides on fungal diseases of bean (3, 23), broccoli (43), cantaloupe (2, 42), corn (22), muskmelon (44), potato (13, 24, 55), tomato (21, 40), and wheat (57). For white rust of spinach, Johnston and Phillips (33) showed that azoxystrobin provided excellent disease control (<1.0% severity) with no phytotoxic effects on the crop and performed better than other registered fungicides in the study. Damicone and Bostian (20) obtained similar results with azoxystrobin in Oklahoma.

Disease Forecasting:

Calendar-based programs for fungicides generally consist of sprays applied on 7 to 14-day intervals. While disease control is effective, fungicides may be used during periods when environmental conditions are unfavorable for infection (31). Extended periods of leaf wetness (free surface moisture or high relative humidity) are necessary for many plant pathogens to infect their hosts (64). Typically the infection process proceeds more

quickly at higher temperatures, so the temperature during the wetting period as well as the duration of the wetting period must be considered (64). Often the two factors are combined to construct an index to predict infection or a weather-based advisory program to predict the need for fungicide sprays (26, 31, 36, 38, 41, 49, 52, 59). The practical motivation for the development of such advisory programs is to limit fungicide applications only to times when conditions favor disease development (31). The desired result is a reduction in the number of sprays during the growing season from a calendar-based program with potential for savings in production and environmental costs (64).

In an attempt to develop a spray advisory for white rust of spinach, Dainello and Jones (16) used continuous hours of leaf wetness (CHLW) as a weather-based threshold for scheduling fungicide applications. All spray thresholds were arbitrarily chosen and were not based on empirical data. For one experiment using chlorothalonil, spray programs consisted of an unsprayed control, a 10-day schedule, and sprays following 6, 12, and 18 CHLW. Disease incidence in the control was 40%. Incidence for the spray programs ranged from 2% to 8% for the 10-day schedule, from1% to 13% for the 6 CHLW, from 1% to 8% for the 12 CHLW, and from 0.8% to 49% for the 18 CHLW. By making chlorothalonil applications after 12 CHLW, a 25% reduction in the number of sprays was achieved without significantly reducing control compared to the 10-day schedule. For the other experiment, spray programs consisted of a control, a 7-day schedule, and applications of metalaxyl following 12, 18, and 24 CHLW. Disease incidence in the control was 13.1%. Incidence for metalaxyl on a 7-day schedule was 0%; while incidence for the 12, 18, and 24 CHLW thresholds was 0.3%, 0.0%, and 3.2%, respectively. A 39% reduction in the number of sprays was achieved by making

applications following 12 CHLW compared to the 7-day schedule (16). Although fungicide treatments were applied after 12 CHLW, no attempt was made to determine the minimum duration of moisture needed for infection or the effect of temperature. Monitoring the wetness duration may prove to be a useful tool in spinach disease control, and the potential exists to refine the system by superimposing other climatic variables such as temperature to develop a weather-based spray advisory for white rust control.

For efficient management of white rust, it is important to understand the biology of the pathogen and epidemiology of the disease. By altering even one stage of the disease cycle via cultural practices or applying fungicides only during periods favorable for infection, the disease can be better managed with less chemical input and lower expense. Raabe and Pound (47) quantified the effects of several environmental parameters on zoospore release from *A. occidentalis* sporangia. However, environmental conditions favoring infection by zoospores have not been identified. The disease can not develop if conditions are not favorable for infection, even in the presence of inoculum. Determining the infection requirements for *A. occidentalis* is, therefore, necessary to more efficiently manage the disease and is vital to developing a weather-based spray advisory. The advisory program developed by Dainello and Jones (16) should be empirically verified and other variables required for infection, such as temperature, should be incorporated. A weather-based spray advisory could further decrease fungicide input without reducing control, which has environmental incentives for everyone and reduced costs for producers.

Systemic fungicides can provide disease control when applied to plants that have already been infected and have "curative" or "postinfection" activity, while protectant fungicides must be applied preventatively before infection (12). Metalaxyl is a systemic

fungicide that controls late blight of tomato (*Phytophthora infestans* De Bary) when applied either before or within 2 days of inoculation. Metalaxyl also inhibits sporulation of the downy mildew pathogen of grape (Plasmo*para viticola* (Berk. and Curt)) and disease development when applied within 3 days of inoculation (8, 62). Similar activity has been reported with sterol-inhibiting fungicides, i.e. triforine and fenarimol, against Ascomycetes such as *Venturia inequalis*, *Monilinia fructicola* (Wint) Honey, and *Monilinia vacinii-corymbosi* (Reade) Honey (30, 45, 53, 63). The effectiveness of azoxystrobin against white rust has been documented (20, 33). However, nothing is known about its postinfection activity. By quantifying postinfection activity, the maximum time after infection that a fungicide can be applied for effective disease control would be identified. Such knowledge would permit efficient use of azoxystrobin in conjunction with a weather-based spray advisory. By monitoring infection periods, growers should be able to schedule postinfection applications of these fungicides and likely reduce the number of sprays required in some years. By quantifying the post-infection activity of azoxystrobin and other promising fungicides, white rust can be better managed by further reducing fungicide applications and increasing the time between applications.

Three chapters of this thesis are written in journal manuscript format. Chapter II, entitled "The Effects of Temperature and Wetness Period on Infection of Spinach by *Albugo occidentalis*", describes the post-inoculation effects of temperature and duration of relative humidity≥95% (wetness) on infection and disease development in a susceptible spinach cultivar. Chapter III, entitled "Development of a Weather-Based Advisory Program for Scheduling Fungicide Applications to Control White Rust of Spinach", describes field studies conducted to develop a weather-based spray advisory for white rust

using temperature and wetness thresholds for infection identified in Chapter II. Chapter IV, entitled "Postinfection activity of Maneb, Azoxystrobin, and BAS 500 against White Rust of Spinach", describes studies under controlled conditions where the activity of these fungicides was evaluated at various periods after infection.

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CHAPTER II

The Effects of Temperature and Wetness Period on Infection of Spinach by *Albugo occidentalis*

ABSTRACT

Controlled environment experiments were conducted to determine the influence of temperature and duration of wetness on infection of spinach *by Albugo occidentalis* and development of white rust. Plants of the susceptible cultivar "Kent" were exposed to temperatures of 6 to 28^0 C and interrupted wetness periods (hours of relative humidity ≥95%) that totaled 3 to 84 h following inoculation. Disease severity, the proportion of leaf area with white rust, was visually estimated following further incubation in a greenhouse at 20 to 30^0 C. Disease was observed at all temperatures and increased with wetness duration. Disease did not occur at the 0-h wetness period at all temperatures. The optimum temperature for infection ranged from 12 to 18^0 C, and as little as 3-h of wetness were required for infection at these temperatures and at 20 and 22^0 C. For the 84-h wetness period, maximum disease severity occurred at 12^0 C and approached 90%; while severity at 14 to 18^0 C ranged from 70 to 80%. Disease severity declined to <1% at 6 and 28^0 C for all wetness periods. A minimum wetness period of 6 to 12 h was required for infection at 8, 10, 24, and 26^0 C. Regression analysis was used to determine the functional relationship between disease severity and temperature and wetness duration. A multiple regression model describing the response surface of disease severity was developed that had significant quadratic wetness effects, cubic temperature effects, and interaction between temperature and wetness. The resulting polynomial model provided a close fit to the observed data with a coefficient of determination (R^2) of 0.89

and an adjusted R^2 of 0.89. Oospores were tested for infectivity to assess their importance as inoculum. It was not possible to verify the infectivity of the oospores and their role in the epidemiology of white rust remains unclear.

INTRODUCTION

Albugo occidentalis Wilson is an obligate Oomycete pathogen that causes white rust, an economically important foliar disease of spinach (*Spinacia oleracea* L.) in production areas of the United States east of the Rocky Mountains (5). White rust is a serious problem for spinach production in the Arkansas River Valley of Oklahoma where it was first reported in 1943 (4, 19). The disease causes substantial yield losses through a reduction in the quality and marketability of fresh and processed spinach (2).

Symptoms of white rust begin as chlorotic lesions on the upper leaf surface. As the lesions develop, small white pustules (sori) are produced on the underside of the leaf and occasionally on the upper leaf surface (16, 17). The pustules often are so abundant that nearly the entire leaf surface is covered. Sporangia are produced as the pustules mature. The optimum temperature for the production of sporangia is 22^0 C (5). Primary infection is thought to occur from soilborne oospores that are splashed onto plants by rainfall or overhead irrigation, or by airborne sporangia (7, 8, 23). It is speculated that oospores germinate and infect plants through open stomata (17). However, oospore germination in *A. occidentalis* has not been described, and its role in the disease cycle has not been defined (5). Secondary infection results from airborne sporangia discharged from pustules (16). Sporangia usually germinate indirectly to produce six to nine biflagellate zoospores which initiate infection, but occasionally sporangia germinate directly (16, 17).

White rust is managed through crop rotation, partial genetic resistance, and fungicide programs (6, 15). For efficient disease management with fungicides, knowledge of the environmental conditions required for infection by *A. occidentalis* and for development of white rust would be beneficial. Specific temperatures and durations of free surface moisture are required for infection by many plant pathogenic fungi (3, 11, 14, 20, 21, 25). Severe white rust epidemics in Texas reportedly were favored by cool nights with heavy dew alternating with warm, dry sunny days (16). The environmental factors affecting sporangial germination have been determined (16, 17). Raabe and Pound (17) found that temperature influenced the indirect germination of sporangia. Sporangia germinated indirectly at temperatures from 2 to 25^0 C (17). The optimum temperatures for sporangial germination, however, ranged from 12 to 16^0 C, and germination decreased sharply with an increase or decrease in temperature (17). Although the optimum temperature for zoospore release was identified, temperature requirements for infection and disease development were not defined.

Raabe and Pound (17) also observed that free surface moisture was required for the indirect germination of sporangia. This observation indicated that leaf wetness might be a useful parameter for predicting disease outbreaks. In an attempt to assess the utility of wetness duration for forecasting white rust outbreaks, Dainello and Jones (6) used continuous hours of leaf wetness as a parameter for scheduling fungicide applications. Efficient disease control was achieved for sprays applied following 12 continuous hours of leaf wetness. However, the duration of wetness that is required by *A. occidentalis* for infection and disease development has not been empirically defined.

It may be possible to better forecast white rust outbreaks by refining the system of Dainello and Jones, by verifying the 12-h wetness threshold, and by incorporating other variables that are required for infection, such as temperature. Therefore, the objective of this study was to quantify the effects of temperature and wetness on infection of spinach by *A. occidentalis* and development of white rust.

MATERIALS AND METHODS

Pathogen maintenance and inoculation. Spinach seedlings were grown in a greenhouse at 20 to 30^0 C in plastic pots (10-cm diameter) containing an soilless growing medium (65% peat moss, 20% vermiculite, 10% perlite, and 5% hort sand) for 40 to 60 days. Plants were watered as needed and nutrients were supplied by applying fertilizer (0.2, 0.08, and 0.03 g/L N/P/K, respectively) weekly.

An isolate of *A. occidentalis*, obtained from diseased plants collected in a field in Oklahoma, was maintained on plants. Sporangial suspensions were prepared by agitating pieces of leaves with pustules in distilled water. The suspensions were strained through cheesecloth and adjusted to $1x10^5$ sporangia/ml with a hemacytometer. Suspensions were sprayed onto the upper leaf surface of plants to run-off using a hand-held spray bottle. Sets of plants were spray-inoculated every two weeks for the duration of the study and placed in a dew chamber (Model I-60DL, Percival, Boone, IA) at 15^0 C with a 12-h wet cycle each day for 2 to 3 days. Plants were removed from the dew chamber and further incubated in the greenhouse at 20 to 30^0 C until symptom development, usually about 14 days after inoculation.

Cultivar and inoculum density assays. The cultivars "Avon", "Kent", "Melody", and "Bloomsdale" were inoculated as described above. There was no

difference among cultivars (P>0.1) for disease severity, which ranged from 79% for "Bloomsdale" to 83% for "Avon". The cultivar "Kent" was readily available and was used in all further experiments.

A detached-leaf method was used to determine the minimum density of sporangia that would yield maximum levels of disease. For the detached-leaf method, two fully expanded, detached leaves were placed in a petri dish containing water agar amended with benzyladenine (0.5 μg/ml). The upper surface of the leaves was then inoculated to run-off with 10 drops of sporangial suspension using a sterile pipette at concentrations of 0, 10, 10^2, 10^3, 10^4, 10^5, and 10^6 sporangia/ml. The plates were placed in a dew chamber at 15^0 C for 3 days, removed, and incubated at room temperature. Disease severity, the percentage of leaf area with symptoms, was visually estimated 14 days after inoculation, and the experiment was repeated. The percentage of leaf area was divided by 100. Disease severity was expressed as a decimal and ranged from 0 to 1. Analysis of variance was conducted with the SAS GLM procedure (version 6.11, SAS Institute, Cary, NC) to determine if there was a difference between experiments. Regression analysis was performed with the SAS REG procedure to assess the relationship of inoculum concentration to disease severity.

Oospores were tested for infectivity to assess their importance as inoculum. Oospores were extracted from symptomatic leaves of plants 14 days after inoculation that were produced as described above. For the extraction procedure (26), 8 to 10 spinach leaves were surface sterilized for 4 minutes in a 1% sodium hypochlorite suspension. Leaves were then rinsed in sterile distilled water and comminuted for 5 minutes in 130 ml sterile distilled water and 20 ml of ice in a blender. The suspension was cooled to 10^0 C

in an ice bath and then sonicated twice for 5 minutes. The suspension was sieved through screens of mesh sizes 75 and 38 μm. The residue in the 38 μm sieve was suspended in sterile distilled water, and the suspension was treated with cellulase to digest the plant material. Equal volumes of the suspension and a buffered solution of cellulase (4 mg/ml in 0.1 M acetate buffer, pH 4.6) were mixed together, resulting in a final concentration of 2 mg cellulase/ml in 0.05 M acetate buffer, and incubated for 2 hours at 20^0 C. After washing the suspension four times in sterile distilled water by centrifugation at 2000 g for 3 minutes, it was practically free of plant material.

Oospore suspensions ($1x10^3$ oospores/ml) were used immediately and spray-inoculated onto plants as described above. The plants were placed in a dew chamber at 12^0 C for 3 weeks and then incubated in a greenhouse at 20 to 30^0 C for 7 days. Plants were visually examined for symptom development 4 weeks after inoculation. Oospores were stained for viability with 2, 5 diphenyl tetrazolium bromide (MTT), a stain commonly used to stain oospores of *Phytophthora spp*. (18). Approximately 0.5 ml of 0.1% MTT in 0.1 M phosphate buffer, pH 5.8 was then added to 0.5 ml of extracted oospores in a vial. The oospore and MTT suspension was incubated in a water bath at 35^0 C for 2.5 hours and then examined microscopically. The color of viable oospores ranges from dark blue to deep red (18). The oospore suspensions were also tested for germinability on water agar and potato dextrose agar containing 100 μg/ml ampicillin and 10 μg/ml rifamcin, and on soil extract agar. The soil was collected from a field that was currently cropped to spinach. Each experiment was repeated.

Temperature and wetness effects. Plants were exposed to various temperatures and periods of RH≥95%, hereafter referred to as wetness period, using dew chambers.

Because only two dew chambers were available, experiments were conducted over time by temperature. The order of temperature treatments was assigned at random and was repeated. Plants (40 to 60 day old) were spray-inoculated as described above and placed in a dew chamber set from 6 to 28^0 C at 2^0 C increments following inoculation. Within each temperature treatment, plants were exposed to cumulative wetness periods of 0, 3, 6, 12, 24, 36, 48, 60, 72, and 84 h in increments of 12 h per day. Each dew chamber was set for a 12-h night period of RH≥95%, which supported infection, and a 12-h day period of RH 70 to 75%. The regime was used to simulate the cyclic nature of wetness periods in the field. Temperature and RH in each chamber were monitored with a seven-day recording hygrothermagraph (5020-A, Weathertronics). After exposure to a specified wetness period, three plants from each chamber (a total of six plants per temperature and wetness combination) were removed at random and transferred to a greenhouse at 20 to 30^0 C and maintained until symptoms developed. Disease severity, the percentage of leaf area with symptoms, was visually estimated on 8 to 10 leaves per plant at 14 days after inoculation as described above. The percentage of leaf area was divided by 100. Disease severity was expressed as a decimal and ranged from 0 to 1.

The experimental design was a split-plot with dew chamber temperature as the whole-plot treatment and wetness period as the split-plot treatment. The whole-plot was arranged in a randomized complete block design with chamber as the blocking factor. An observation used in statistical analysis was the mean value of the 8 to 10 leaves evaluated per plant, for a total of six observations per temperature and wetness combination. The arc sine square root transformation was used to stabilize variances. Analysis of variance was performed with the PROC MIXED procedure of SAS (version 6.11), which

considers random effects. This procedure is appropriate for a split-plot experimental design when the whole-plot error term and the blocking factor are random (22). Multiple regression analysis, performed with the PROC REG procedure of SAS, was used to determine the functional relationship of wetness period and temperature on disease severity. A regression equation also was computed using the PROC REG procedure. An F-test, the coefficient of determination (R^2), and the adjusted R^2 for degree of freedom evaluated the goodness of fit to the regression model.

RESULTS

Cultivar and inoculum density assays. There was no difference between experiments ($P>0.2$) conducted to determine sporangial density to disease severity relationships. Therefore, data were combined over experiments. A minimum concentration of 10^2 sporangia/ml was required for disease development (Fig. 1). Disease severity increased with sporangial concentration up to 10^5 sporangia/ml, where it leveled off and achieved the maximum level of disease. Therefore, this inoculum concentration was chosen for use in all further experiments. The following logistic model best defined the relationship between inoculum concentration (X) and disease severity (Y):

$$\log_e Y / (1-Y) = b_o + b_1X \quad \text{or} \quad Y = \exp (b_o + b_1X) / 1 + \exp (b_0 + b_1X) \qquad (1)$$

where b_o and b_1 are the parameter estimates –5.7046 and 1.3527, respectively for $\log_{10}$ transformed Y. The regression was significant at $P\leq0.01$ and fit the observed data well with an R^2 of 0.97.

In assessing the inoculum potential of oospores, results were consistent for each experiment. Oospores did not take up the viability stain. Oospores also did not

germinate on either artificial media or soil extract agar. Inoculated plants did not develop white rust symptoms, even after three weeks at 12^0 C and one week in a greenhouse at 20 to 30^0 C. Therefore, sporangia were used as inoculum for experiments on temperature and wetness effects.

Temperature and Wetness Effects. Results from the two experiments were similar. Blocks were non-significant and pooled with the whole-plot error term. Analysis of variance indicated that there was no effect ($P>0.5$) of experiment, chamber, or the interaction between experiment and chamber on disease severity. There also were no significant interactions between experiment and chamber and temperature, and between experiment and chamber and wetness period at the $P>0.5$ level. Therefore, data by experiment and chamber were combined. In the combined analysis, the interaction between temperature and wetness period was significant ($P\leq0.0001$), and the main effects of temperature and wetness exposure period were significant ($P\leq0.0001$).

Disease was observed at all temperatures evaluated, and generally, there was a unimodal response of disease severity with temperature for all wetness periods (Fig. 2). The maximum disease severity occurred at 12^0 C, where disease severity approached 90% for wetness periods of 48 to 84-h. Disease severity also was high at 14 to 18^0 C and ranged from 70 to 80% at the 72- and 84-h wetness period. The optimum temperature for infection, therefore, ranged from 12 to 18^0 C. Higher and lower temperatures resulted in lower levels of disease severity. Disease severity was <1% at 6 and 28^0 C for all wetness periods. Disease severity increased from 6 to 10^0 C, peaked at 12^0 C, leveled off from 14 to 18^0 C, and declined from 20 to 28^0 C (Fig. 2).

Disease was observed at all wetness periods, and increased with wetness duration sigmoidally for all temperatures except the two extremes (Fig. 3). Disease did not develop at the 0-h wetness period for all temperatures evaluated. Although maximum disease severity occurred at the 84-h wetness period for all temperatures, the minimum wetness duration required for infection was 3 h at 12 to 22^0 C. As the temperature increased or decreased, a longer wetness period was needed for infection. A minimum wetness period of 6 to 12 h was required for infection at the sub- and super-optimal temperatures of 8, 10, 24, and 26^0 C. A minimum of 72 and 60 h of wetness were required for infection at 6 and 28^0 C, respectively (Fig. 3).

In regression analysis, there were significant linear, quadratic, and cubic relationships between disease severity and temperature (T) averaged across wetness period ($P \leq 0.0001$). There also were significant linear and quadratic relationships between disease severity and wetness period (W) averaged across temperature ($P \leq 0.0001$). The W-linear x T-linear, W-quadratic x T-linear, W-linear x T-quadratic, W-quadratic x T-quadratic, W-linear x T-cubic, and W-quadratic x T-cubic interactions also were significant ($P \leq 0.0001$).

The following model best defined the relationship of temperature (T) and wetness period (W) on disease severity (Y):

$$Y = b_o + b_1W + b_2W^2 + b_3T + b_4T^2 + b_5T^3 + b_6WT + b_7W^2T + b_8WT^2 + b_9W^2T^2 + b_{10}WT^3 + b_{11}W^2T^3 \quad (2)$$

where b_o to b_{11} are the parameter estimates (Table 1). The R^2 and adjusted R^2 were 0.8917 and 0.8909, respectively. The curves generated by the polynomial model fit the observed means at each temperature (Fig. 4) and at each wetness period well (Fig. 5). There was an increase in disease severity with an increase in W until 60 h at all values of

T, except at 6 and 28^0 C (Fig. 4). Unimodal curves were produced when disease severity was plotted against temperature (Fig. 5). The curves flattened with decreasing values of wetness duration. According to the model, disease severity was at maximum at 14^0 C and 60 h of leaf wetness. The optimum temperature range was 12 to 18^0 C, and disease severity increased with an increase in wetness duration until 60 h (Fig. 6).

DISCUSSION

Temperature and wetness period were clearly important factors in infection of spinach by sporangia of *A. occidentalis* and white rust development. In this study, temperatures of 12 to 18^0 C favored foliar infection of spinach and disease development when accompanied by a minimum of 3 h of wetness. Disease was not observed at the 0-h wetness period even in the presence of inoculum and optimal temperatures. This indicates that wetness is a requirement for plant infection. These results were similar to those of Raabe and Pound (17) who determined that the optimum temperature for indirect germination of sporangia was from 12 to 16^0 C and that free surface moisture was required for germination. Because zoospores initiate plant infection, the temperature requirements for zoospore release appear to be similar to those for plant infection.

Disease was observed at all temperatures evaluated in this study, although disease severity at the extremes of 6 and 28^0 C was <1% nearly zero at all wetness periods. Disease severity also decreased when temperature increased or decreased from the optimum range of 12 to 18^0 C. Zoospore release from sporangia of *A. occidentalis, A. tragopogi* and *A. candida* can occur over a relatively wide temperature range (from 2 to 25^0 C) (9, 12, 17). However, germination levels decreased when temperature was above or below the optimal range of 10 to 16^0 C (9, 12, 17).

Free surface moisture is a requirement for development of other diseases caused by Oomycetes. For downy mildew of cantaloupe caused by *Pseudoperonospora cubensis*, 4 to 6 h of free surface moisture was required for infection and disease development (10, 24). Infection by *Phytophthora infestans* takes place most readily when zoospores are formed and this occurs only when there is a film of moisture on the leaf surface (1). High relative humidity (≥95%) favors outbreaks of downy mildew of crucifers, caused by *Peronospora parasitica*, and promotes sporulation (13).

In this study, there was a general trend for disease severity to increase with an increase in wetness period duration. Since a continuous film of moisture is required for the indirect germination of sporangia, for the germination of zoospores, and for the penetration of leaf tissues, the longer a film of moisture persists the greater the chance of infection (1, 10). According to Gross et al. (11), the effects of temperature are more pronounced at longer wetness duration periods than shorter periods indicating that leaf wetness is perhaps the critical environmental variable for infection, but temperature regulates its rapidity and level. Differences between temperature treatments were easier to observe as wetness period increased.

The interaction between temperature and wetness period significantly influenced infection of spinach by *A. occidentalis*. No disease developed until 72 and 60 h of wetness at 6 and 28^0 C, respectively. For more favorable temperatures, disease severity increased following about 12 h of wetness, which contributed to the interaction.

A high proportion of the variability of mean disease severity was accounted for by the components of the polynomial model. The model fit the observed data well with the following exceptions. According to the model, disease severity should be maximum at

14^0 C and 60 h of wetness. Maximum disease severity, however, occurred at 12^0 C and at 84 h of wetness. The model also predicted that disease severity should increase with an increase in wetness duration until 60 h. Disease severity, however, did not level off or decrease after 60 h of wetness. In the observed data, the disease severity peaked at 12^0 C for all wetness periods. Data with a distinct peak is often difficult to model. Even though the observed and predicted maximum disease severity did not match at 12 ^{0}C, the model adequately describes the observed data. The model does predict the optimal temperature range of 12 to 18^0 C and that disease increases with an increase in wetness period.

This studied verified that the 12-h wetness threshold found in the field by Dainello and Jones supported infection and disease development (6). Disease severity ranged from 0 to nearly 70% at this wetness period depending on temperature. However, results of this study indicated that as few as 3 h of wetness were required for infection at optimum temperatures, while 6 to 12 h were necessary at 8, 10, 20, and 22^0 C. Three hours of wetness exposure often occurs in a single dew period in the field, thus making the development and use of a weather-based spray advisory difficult. The 3 to 12-h thresholds need to be verified in the field to determine the practicality of developing a weather-based spray advisory. Although interrupted wetness periods were applied, temperature was kept constant in the chamber. Humidity and temperature varies in the field, and there could be differences in the susceptibility of plants grown in the field and in the greenhouse.

There have been no descriptions of germination of *A. occidentalis* oospores (4). In this study, oospores did not germinate and disease did not develop following

inoculation with oospores. Many factors, such as oospore maturity, light, pH, temperature, etc., play a role in oospore germination (18). According to Ribeiro (18), oospore germination is largely a matter of trial and error in trying to find the precise conditions for a particular isolate; even isolates of the same species can differ in their requirements; and no one method of oospore germination has been found to be universally applicable. Oospores of *Phytophthora spp.* require a maturity period of at least 30 days (18). *Albugo* oospores may have a similar requirement, and these experiments only ran for a total of 30 days. The main component of the cell wall of Oomycetes is cellulose. Perhaps the cellulase enzyme used in the extraction procedure to degrade excess plant material disrupted the cell wall and caused oospores to become inviable. Viability could not be verified with the oospore stain. Since it was not possible to verify the infectivity of the oospore, its role in the epidemiology of white rust remains unclear.

A model was developed that describes the influence of temperature and wetness duration on infection of spinach by *A. occidentalis* and on white rust development. The defined relationship between white rust disease development and environmental conditions might be useful for timing fungicide sprays in the field. The response surface will be used as a framework for developing and testing a weather-based spray advisory program for white rust in the field.

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Table 1. Estimated parameter values from the polynomial regression equation 2 (see text) describing the relationship of postinoculation wetness period (W) and temperature (T) on severity of white rust of spinach (Y)[a].

Parameter[b]	Parameter estimate	Standard Error
b_0 (intercept)	-0.912896	0.09920274
b_1	-0.065962	0.00669859
b_2	0.000593	0.00008146
b_3	0.199248	0.02106211
b_4	-0.010643	0.00133817
b_5	0.000168	0.00002607
b_6	0.014963	0.00142220
b_7	-0.000131	0.00001730
b_8	-0.000717	0.00009036
b_9	6.363×10^{-5}	0.00000110
b_{10}	9.642×10^{-5}	0.00000176
b_{11}	-8.739×10^{-8}	0.00000002

[a] Data from two experiments did not differ significantly ($P \leq 0.5$) and were combined for analysis. Regression analysis was performed on arc sine square root transformed values of disease severity.

[b] All parameters were significant at $P \leq 0.0001$ level.

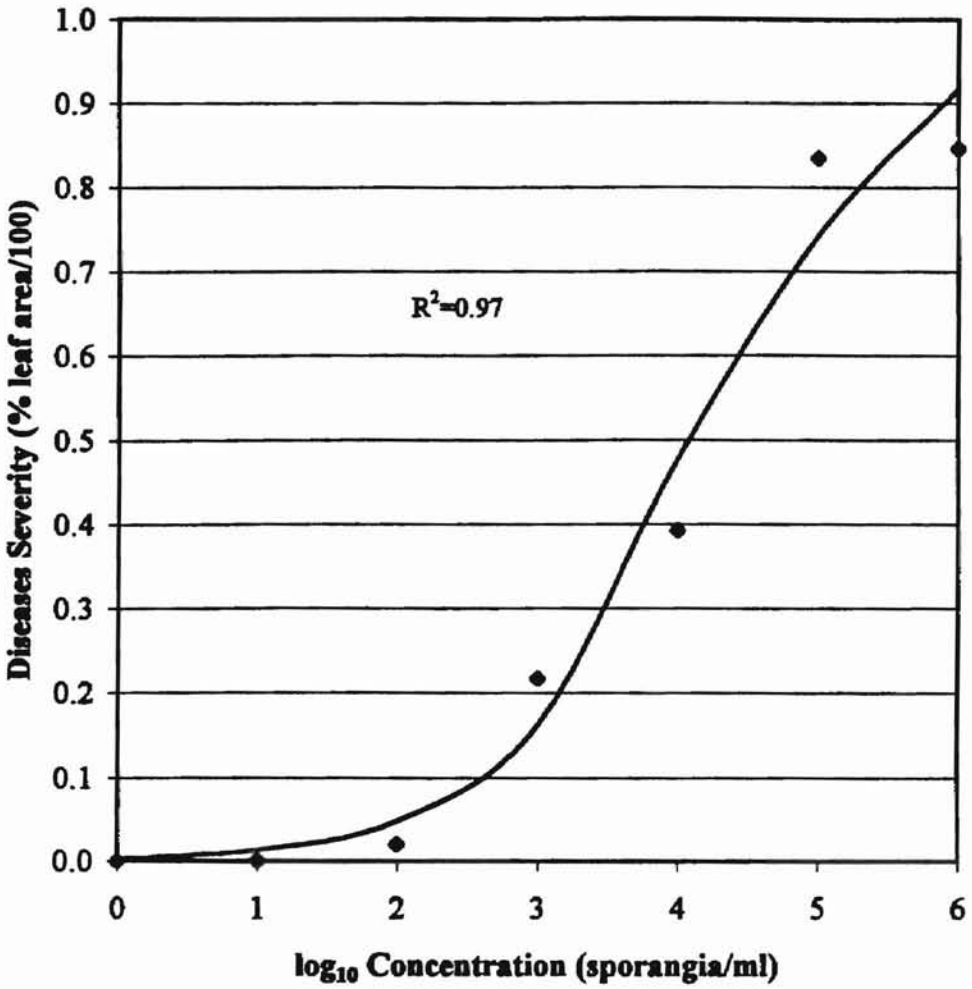

Figure 1. The influence of inoculum concentration on severity of white rust for the spinach cultivar "Kent". Data points are observed values averaged over two experiments and twelve leaves per experiment. The regession line was generated by the logistic equation $Y = \exp(b_o + b_1X) / 1 + \exp(b_o + b_1X)$.

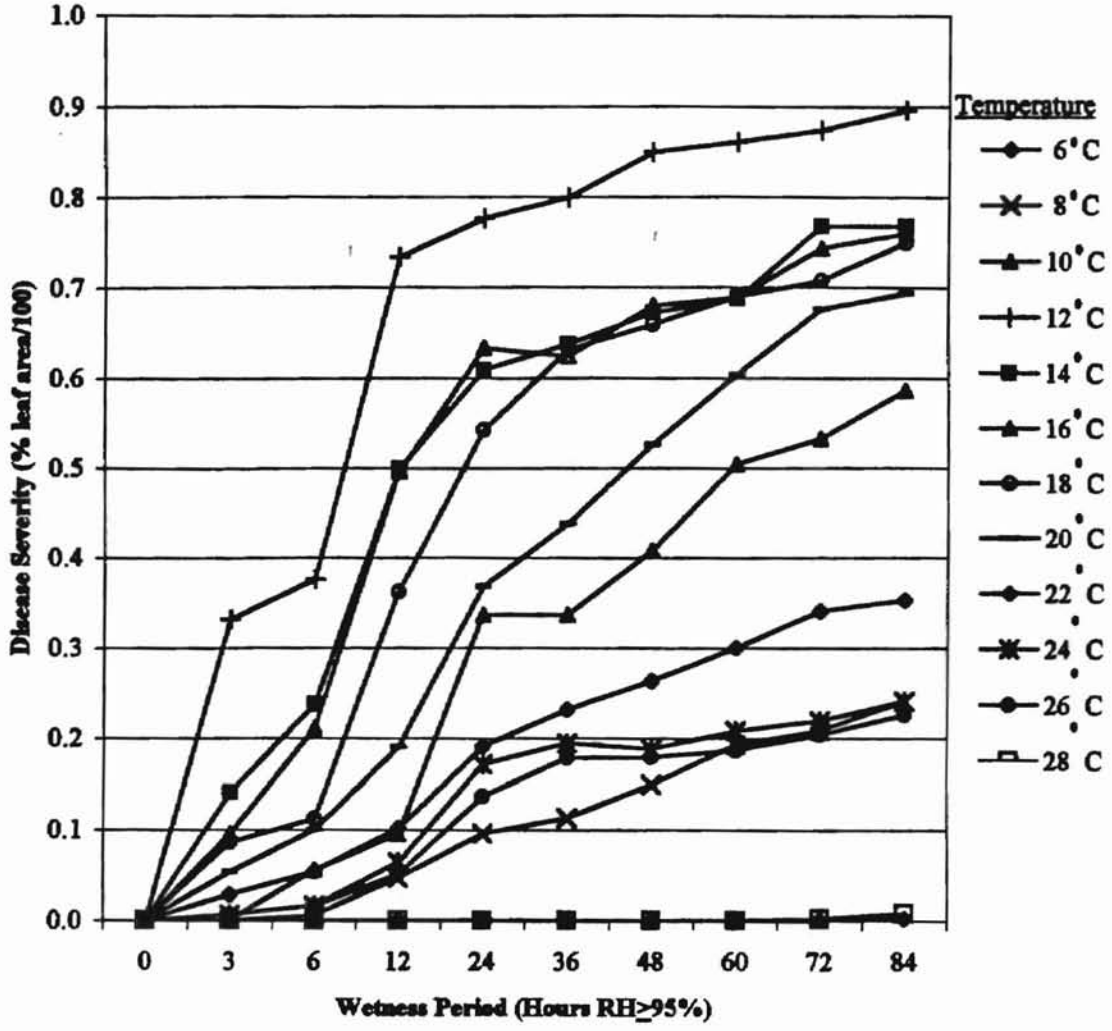

Figure 2. The observed disease severity of white rust of spinach plotted against temperature for various wetness periods. Data points represent the mean values at 14 days after inoculation averaged over two experiments and six plants per wetness and temperature combination.

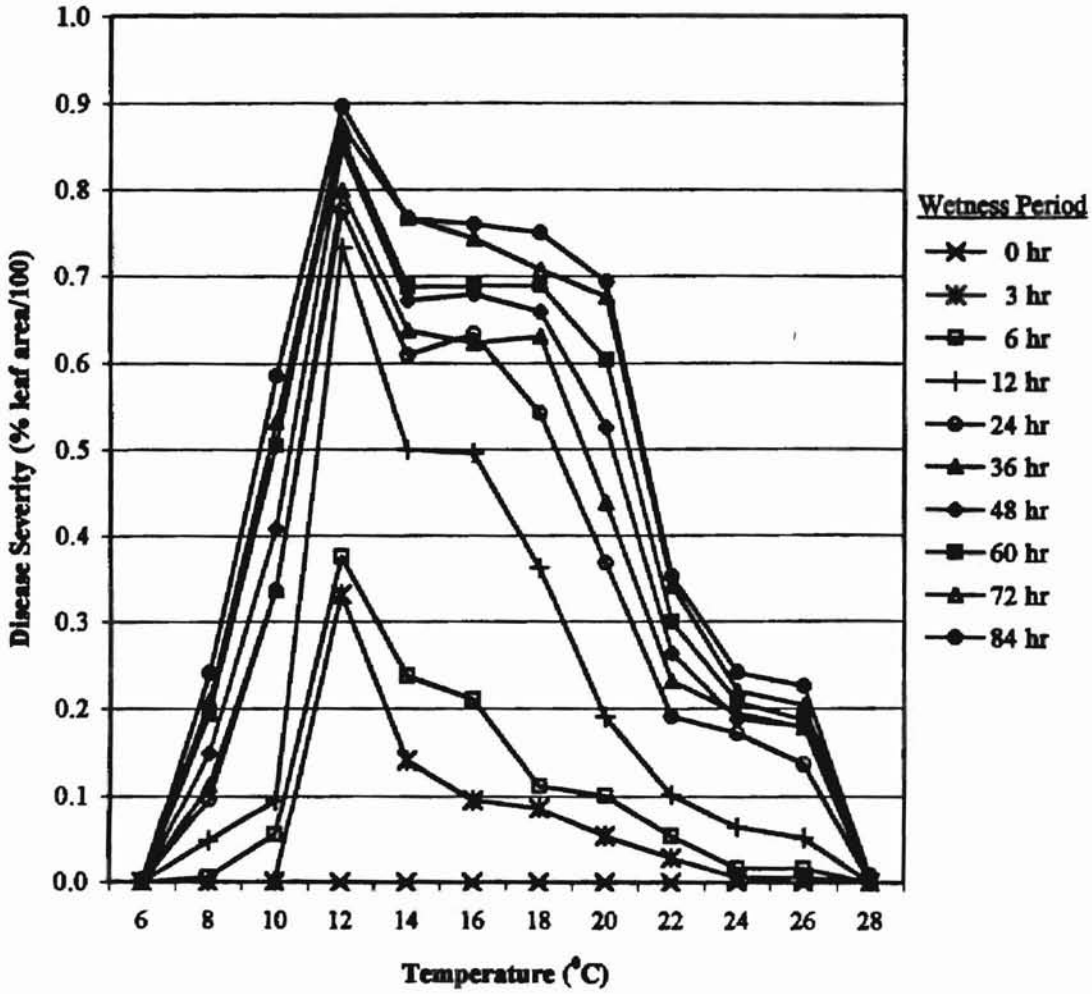

Figure 3. The observed disease severity of white rust of spinach plotted against wetness period for various temperatures. Data points represent the mean values at 14 days after inoculation averaged over two experiments and six plants per temperature and wetness combination.

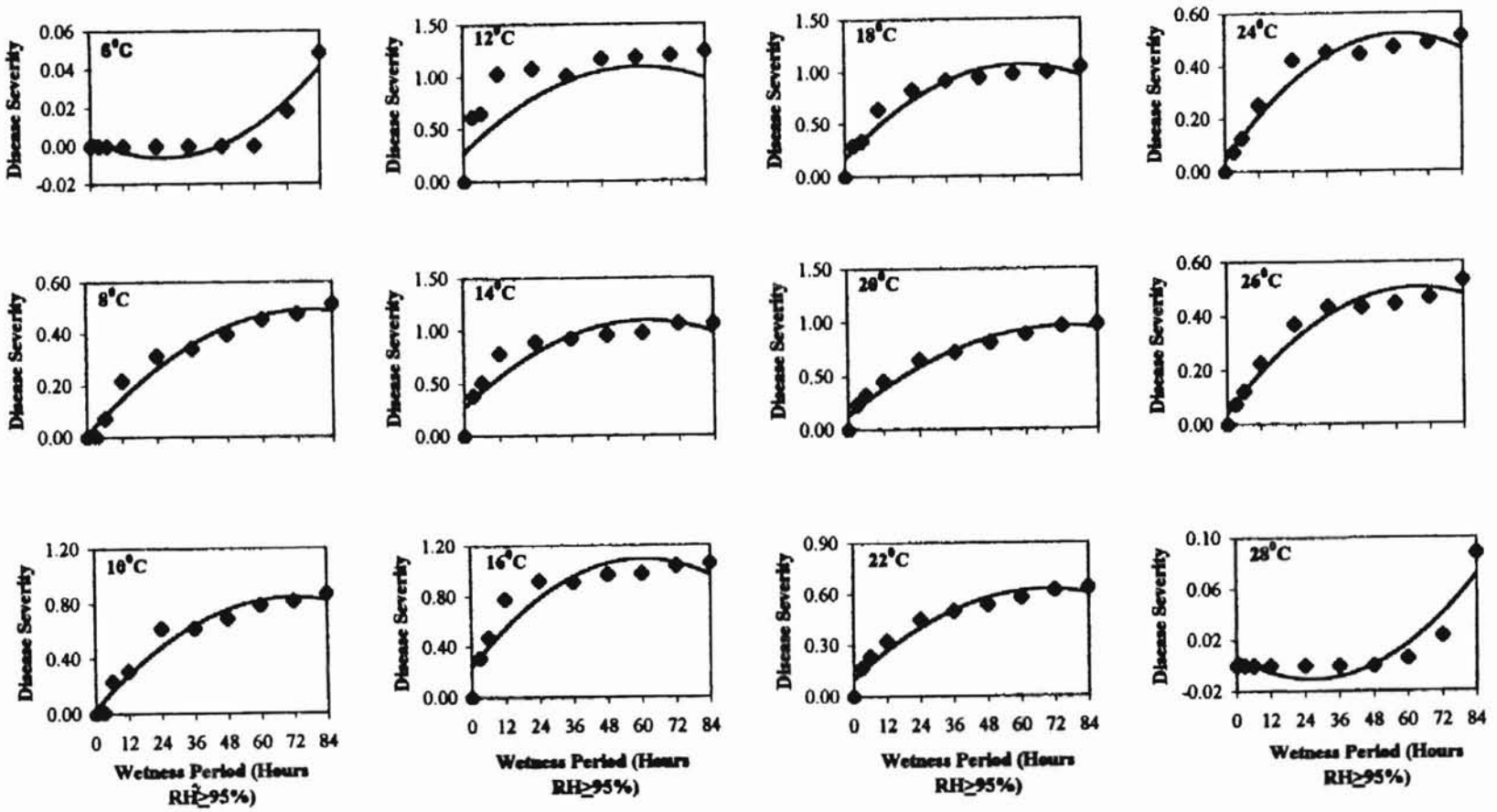

Figure 4. Observed and predicted severities (Y) of white rust of spinach plotted against wetness period (W) by temperature. Lines represent predicted values generated by the polynomial model described in the equation 2 (see text) and the parameter estimates in Table 1. Data points represent the observed values (arc sine square root transformed) 14 days after inoculation averaged over two experiments and six plants per wetness and temperature combination.

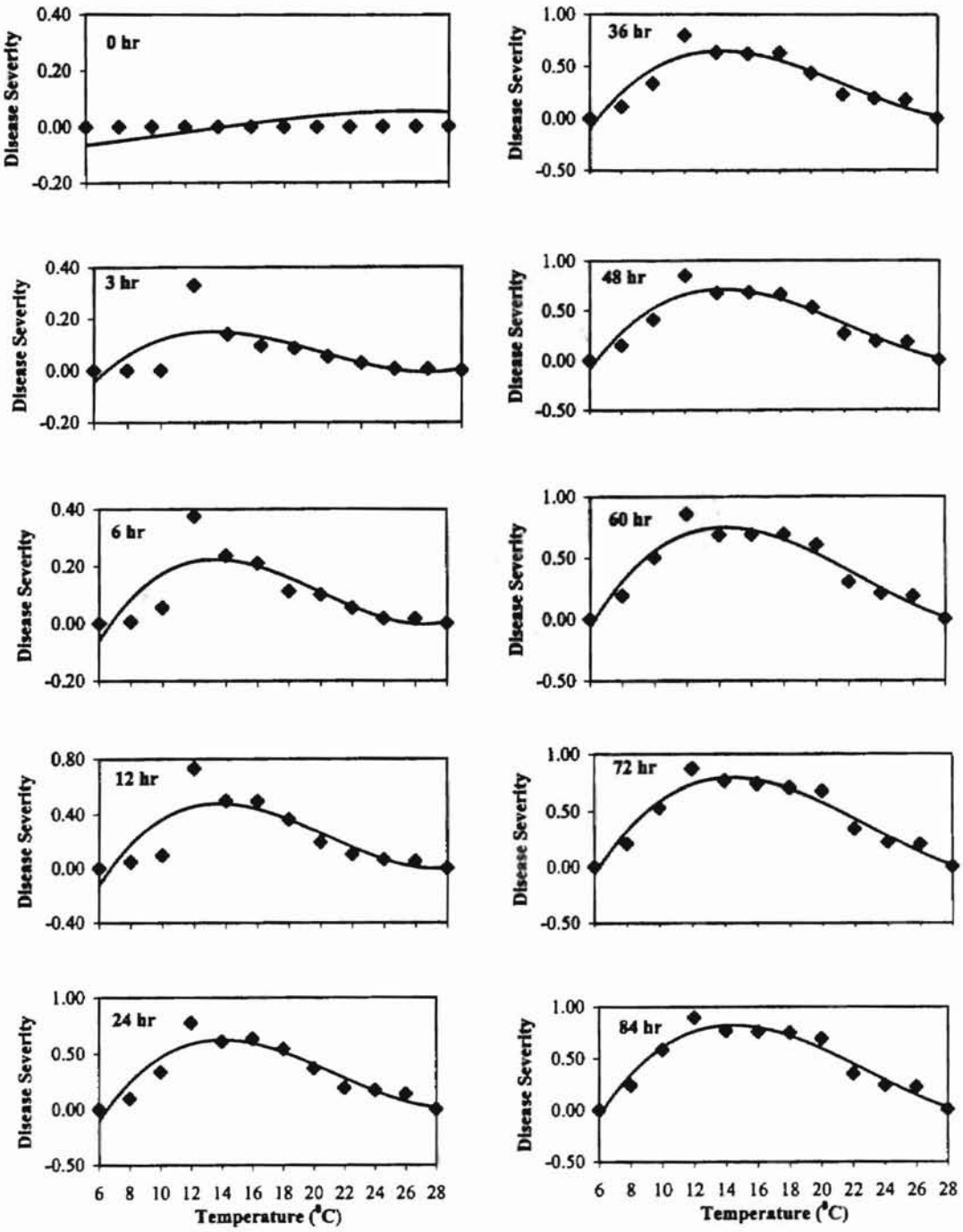

Figure 5. Observed and predicted severities (Y) of white rust of spinach plotted against temperature (T) by wetness period. Lines represent predicted values generated by the polynomial model described in equation 2 (see text) and the parameter estimates in Table 1. Data points represent the observed values (arc sine square root transformed) 14 days after inoculation averaged over two experiments and six plants per temperature and wetness combination.

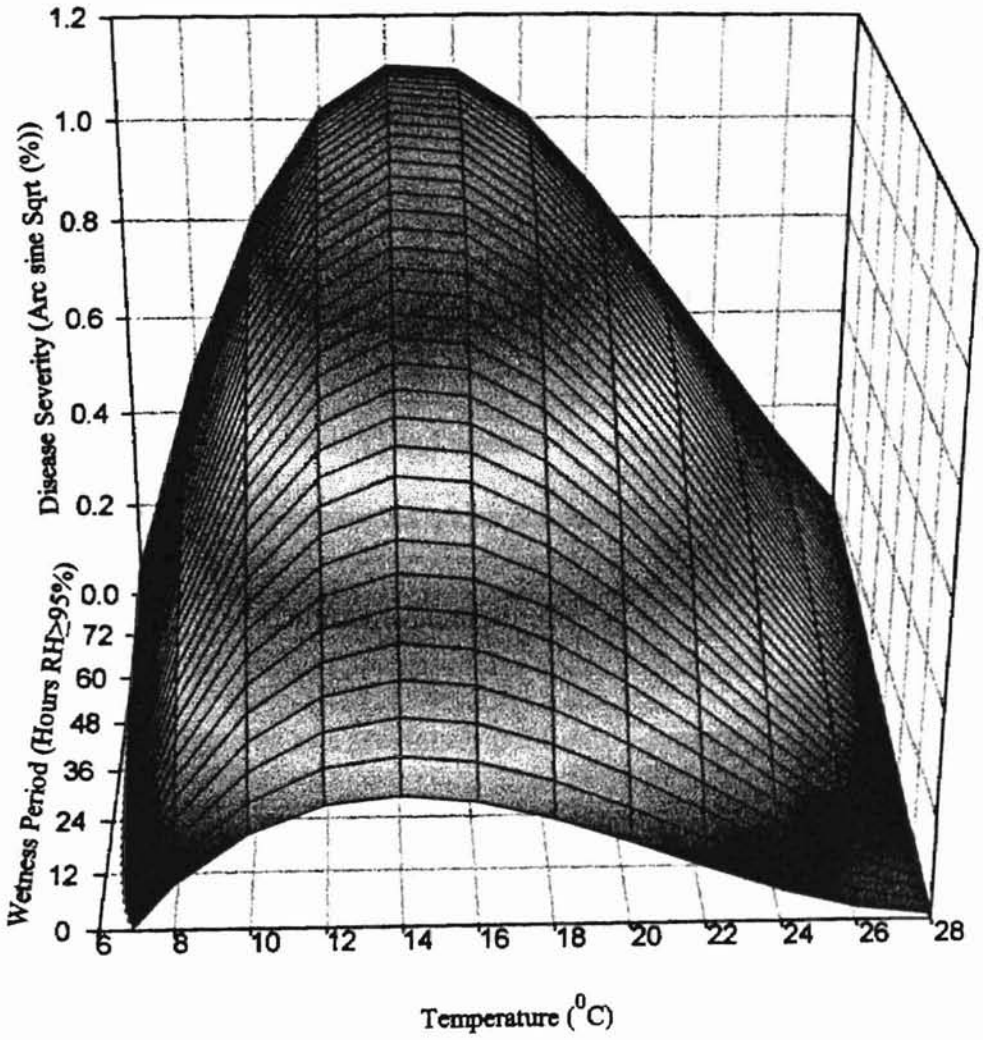

Figure 6. Response surface based on the polynomial model (equation 2) describing the influence of temperature and wetness period on the severity of white rust of spinach

CHAPTER III

Development of a Weather-Based Advisory Program for Scheduling Fungicide Applications to Control White Rust of Spinach

ABSTRACT

Weather-based advisory programs were developed and evaluated for timing of fungicide applications to control white rust of spinach caused by *Albugo occidentalis* in three field trials using the susceptible cultivar "Melody". Advisory programs were based on the periods of temperature (T) and wetness (relative humidity ≥90%, W) that favored infection in other studies conducted under controlled conditions. The protectant fungicides mancozeb or maneb (EBDCs) and the systemic fungicide azoxystrobin were applied after 3, 6, 12, 24, and 36 cumulative hours of favorable T and W (T/W). T/W programs were compared to a previously published advisory program of 12 continuous hours of wetness (12-h W), a 7-d program, and an unsprayed control. An average of 5.7 sprays were applied for the 7-d program. Sprays were reduced to 4.3 for the 3-h T/W program; 3.0 for the 6-h T/W program; 2.7 for the 12-h T/W program; and 2.0 for the 24- and 36-h T/W and 12-h W programs. For the control, disease incidence (percentage of leaves with symptoms) averaged 47% and severity (percentage of leaf area with symptoms) averaged 89%. For azoxystrobin, all spray programs reduced ($P=0.05$) incidence and severity compared to the control. The 7-d, 3-h T/W, 6-h T/W, and 12-h T/W programs provided the greatest reductions in disease incidence (<20%) and severity (<2%). The 24-h T/W program was intermediate in performance while the 12-h W and 36-h T/W programs had the highest levels of disease with incidence (<80%) and severity (<25%). Disease control was less effective for EBDC fungicides for all spray programs.

For EBDCs, all spray programs except the 36-h T/W and 12-h W programs reduced (P=0.05) disease incidence and severity in each trial compared to the control. The 7-d, 3-h T/W, and 6-h T/W programs generally provided the greatest reductions in disease incidence (<50%) and severity (<15%). Disease control was less effective for the 12-h T/W and 24-h T/W programs. Based on spray reductions and disease control, the 6-h T/W and 12-h T/W programs were most efficient for the EBDCs and azoxystrobin, respectively.

INTRODUCTION

White rust, caused by *Albugo occidentalis* Wilson, is a serious foliar disease of spinach (*Spinacia oleracea* L.) in Oklahoma, Texas, Arkansas, and many other spinach production areas in the eastern United States (5). Symptoms of white rust begin as chlorotic lesions on the upper leaf surface (12, 13). As the lesions develop, small white blister-like pustules (sori) are produced on the underside of the infected leaves rendering them unmarketable (5, 12, 13). Yield losses from 30 to 100% have been reported due to a reduction in the quality and marketability of fresh and processing spinach (2, 6).

Integrated practices, such as crop rotation, the use of partially resistant cultivars, and the use of fungicides, have been used in the management of white rust of spinach (5). Growing partially resistant cultivars can provide acceptable levels of white rust disease control without fungicides when disease incidence is low (7). Partially resistant cultivars, however, can be severely damaged under favorable environmental conditions (5, 7). Resistance also is lacking in long-standing spinach cultivars useful for production in the spring. As a result, fungicides are an important component of disease management.

Chemical control of white rust was based on protective programs with the EBDCs maneb and zineb until their registration was revoked in 1992 (3, 6, 8, 10). Currently, only coppers, metalaxyl, and fosetyl-Al are registered for use on spinach. Phytotoxity, label restrictions, low or erratic efficacy, and cost have limited their effectiveness and adoption by growers.

Azoxystrobin is a recently developed, broad-spectrum fungicide that is effective against several Ascomycete, Basidiomycete, and Oomycete pathogens on various crops (1, 16). Johnston and Phillips (9) showed that azoxystrobin provided excellent control of white rust (<1.0% severity) with no phytotoxic effects on the crop and performed better than other registered fungicides used in the study. In a trial in Oklahoma where levels of disease were moderate, azoxystrobin provided complete disease control with no phytotoxic effect (8). Azoxystrobin is being evaluated for use on spinach by IR-4 and was registered for use on spinach in Oklahoma through an emergency exemption in 1999.

Weather variables have been important factors that affect pathogen and disease development. Temperature (T) and wetness (W) regulate indirect germination (zoospore release) of sporangia of *A. occidentalis* under controlled conditions (12, 13). Sporangia germinate indirectly from 2 to 25°C with an optimum temperature of 12 to16°C. The presence of free surface moisture is also a requirement for indirect germination (12, 13).

The T and W requirements for infection by *A. occidentalis* and development of white rust under controlled conditions were identified (Chapter II). Plants of a susceptible cultivar were exposed to temperatures from 6 to 28° C and interrupted wetness periods (hours RH≥95%) that totaled 3 to 84 hours following inoculation. The optimum temperature for infection was 12 to 18°C. Although some disease was observed

at all temperatures examined, the severity decreased with an increase or decrease in temperature from the optimum. At the optimal T of 12 to 18° C, severity ranged from 70 to 90% with 84 h of wetness. As T declined to 6° C or increased to 28° C, disease severity approached 0%. The minimum wetness duration needed for infection was from 3 to 12 h depending upon temperature. However, disease increased sigmoidally with an increase in wetness duration. A multiple regression model describing the response surface of disease severity to T and W was developed, which had significant quadratic W effects, cubic T effects, and interactions between T and W.

Leaf wetness has been used to predict the need for fungicide sprays to control white rust (6). Applications of chlorothalonil and metalaxyl were made after various periods of continuous leaf wetness. Disease incidence in plots sprayed after 12-h continuous leaf wetness did not differ from the calendar spray program, and 1 and 1.6 fewer sprays were applied on average per season for chlorothalonil and metalaxyl, respectively (6).

Knowledge of the role of environmental factors in disease initiation and development is important so that the efficiency of spray programs can be optimized. Although use of leaf wetness was effective, the influence of temperature, found to be important under controlled conditions, may also be important. The objective of this study was to develop and evaluate several weather-based advisory programs that were based on the infectivity studies under controlled conditions. In addition, spray programs using a protectant and systemic fungicide were compared.

MATERIALS AND METHODS

Spray programs were compared in three field trials at the Plant Pathology Research Farm in Stillwater, OK. The susceptible cultivar "Melody" was used in all trials (Chapter II). For trial 1, granular fertilizer (20-0-0 kg/ha N-P-K) was broadcast and incorporated prior to direct seeding on 18 Sep 1998 in a field of Norge loam previously cropped to winter peas. Metolachlor (Dual 8E) at 2.24 kg/ha was broadcast on 18 Sep after planting to control weeds. Additional fertilizer at 26-0-0 kg/ha N-P-K was broadcast three weeks after emergence. For trial 2, the spinach from trial 1 was overwintered, mowed, and then allowed to regrow. Plots were top-dressed with 9-0-0 kg/ha N-P-K fertilizer after mowing on 17 Feb 1999. For trial 3, fertilizer at 85-0-0 kg/ha N-P-K was broadcast and incorporated prior to direct seeding on 24 Feb 1999 in a field of Norge loam previously planted to wheat. Metolachlor as described above was broadcast for weed control. Additional fertilizer at 26-0-0 kg/ha N-P-K was broadcast on 1 Apr. The fields received sprinkler irrigation as necessary to prevent moisture stress.

Treatments (spray programs) were applied to plots randomized in four complete blocks. For trials 1 and 2, plots consisted of four 4.6-m rows spaced 0.36 m apart. For trial 3, plots consisted of four 12.2-m rows spaced 0.36 m apart. Alleys (1.5, 1.5, and 2.1 m) were planted with the cultivar "Melody" between blocks to serve as spreader rows in each trial. Alleys were inoculated at dusk on 22 Oct 1998 with a sporangial suspension ($1x10^5$ sporangia/ml) of *A. occidentalis* in trial 1, but plots became naturally infected in trials 2 and 3.

Foliar sprays of the protectant fungicides mancozeb (Dithane 75 DF), maneb (Manex 4F), and maneb (Maneb 75 DF) at 2.24 kg a.i./ha were used for all spray programs in trials 1, 2, and 3, respectively. The systemic fungicide azoxystrobin (Quadris 2.08 F) at 0.16, 0.16, and 0.23 kg/ha was used for all spray programs in trials 1, 2, and 3, respectively. Sprays were broadcast to all four rows of a plot with a wheelbarrow sprayer equipped with three 8003kv flat-fan nozzles beginning on 23 Oct 1998, 17 Mar 1999, and 19 Mar 1999 for trials 1, 2, and 3, respectively. The sprayer was calibrated to deliver 314, 338, and 338 liters per ha at 290 kPa for trials 1, 2, and 3, respectively.

After examining the observed response of disease severity to T and W (Chapter II), five-weather based spray thresholds of T/W were developed. The T/W program accumulated hourly periods of favorable weather for infection, herein called T/W hours, that consisted of W (RH≥90%) while T is 6 to 26^0 C. Hours of W (RH≥90%) were used instead of hours of RH≥95% to insure that all periods of favorable wetness were included, because peanut leaf spot work has shown that the OKLAHOMA MESONET RH sensors are not accurate above 90%. Due to the T and W interaction effect in the model, each hour of W was weighted by a factor that accounted for the effect of temperature. At the optimum temperatures of 12 to 18^0 C, each hour of W was counted as one T/W hour. At sub-optimal temperatures of 10 to11^0 C and 19 to 21^0 C, each hour of W was multiplied by 0.75; while at 6 to 9^0 C and 22 to 26^0 C each hour of W was multiplied by 0.50. At temperatures of 2 to 5^0 C and 27 to 30^0 C, each hour of W was multiplied by 0, because little or no infection occurs at these temperatures. Spray thresholds of 3, 6, 12, 24, and 36 accumulated T/W hours were tested in trials 1, 2, and 3.

Advisory programs were compared with a 7-d program, an unsprayed control, and the previously published program of 12 continuous hour of leaf wetness. The original program measured leaf wetness directly with a leaf wetness hygrothermograph. In this study, the threshold was measured as 12 continuous hours of RH≥90% (12-h W).

Sprays for the 7-d program and accumulation of weather variables were initiated when the first true leaves were fully expanded for trials 1 and 3, and one month after mowing for trial 2. The first sprays for all T/W programs and the 12-h W program were made when the respective spray thresholds were exceeded. Sprays for the advisory programs were made as soon as possible after thresholds were first exceeded, usually within two days, but not within seven days of the previous spray. All spray programs were maintained until seven days before anticipated harvest.

W (hours of RH≥90%) and T were monitored continuously via the OKLAHOMA MESONET, a network of automated, computer-linked weather stations with a station within 0.5 km of the test site. Readings of T and RH were taken every 5 min and a 15-min mean was output. The 15-min mean data were processed with a SAS (version 6.11 SAS Institute Cary, NC) program that provided advisory program outputs for a 24-hour period beginning at 1200 CST. Program output was the cumulative number of T/W hours and number of consecutive W hours.

Plots were evaluated for disease incidence, the percentage of leaves with symptoms, and severity, the percentage of leaf area with symptoms, at the end of the cropping season on 16 Dec 1998, 22 Apr 1999, and 26 Apr 1999 for trials 1, 2, and 3, respectively. Six, 0.31-m row segments were harvested arbitrarily from the middle two rows of each plot. The harvested leaves were bulked, mixed, and 40 leaves were blindly

sampled. The percentage of leaf area covered with white rust was visually estimated on each sampled leaf.

Analysis of the incidence and severity data was performed on the mean of the 40 subsamples per plot. Analysis of variance (ANOVA) was performed on the incidence and severity data using the SAS GLM procedure. The effects of trial, fungicide, treatment (spray program), and the trial x fungicide, trial x treatment, fungicide x treatment, and trial x fungicide x treatment interactions were tested. Where the trial x treatment interactions were significant, the ANOVA was performed by trial and fungicide. Treatment means were separated using Fisher's Least Significant Difference (LSD) Test as indicated by significant effects in the ANOVA. Unless otherwise indicated, only significant ($P<0.05$) differences between means are described below.

RESULTS

Weather conditions that favored development of white rust, the only foliar disease encountered in this study, were recorded in each trial. Rainfall from emergence to harvest totaled 31 cm in trial 1, 16 cm in trial 2, and 27 cm in trial 3. All trials received two supplemental 3-cm irrigations. T/W hours totaled 182 in trial 1, 192 in trial 2, and 230 in trial 3. Continuous W hours totaled 50 in trial 1, 12.25 in trial 2, 25.25 in trial 3.

The number of sprays for the 7-d program averaged 5.7 over the three trials (Tables 1 and 2). All advisory programs reduced the number of sprays per trial compared to the 7-d program. For the T/W programs, spray reductions increased with T/W duration (Tables 1 and 2). Spray reductions ranged from 24% for the 3-h T/W program to 65% for the 36-h T/W program. The 12-h W program also reduced the number of sprays by 65%.

For the combined ANOVA, the main effects of fungicide, treatment, and the trial x fungicide x treatment interactions were significant for disease incidence and severity ($P \leq 0.01$). For the ANOVA by fungicide, trial x treatment interactions also were significant for disease incidence and severity ($P \leq 0.01$). Therefore, means were separated by trial and fungicide.

Levels of white rust were severe in each trial (Tables 1 and 2). Over all treatments, disease severity was 25% and incidence was 74% for the control in trial one. Disease levels in trials 2 and 3 were greater than for trial 1. For trials 2 and 3, disease severity averaged over 50% and nearly all leaves had symptoms.

Over the spray programs, azoxystrobin provided better disease control than the EBDCs in each trial. Averaged over treatments, disease incidence was 7% and 44% and severity was 4% and 11% for azoxystrobin and EBDC, respectively in trial 1. In trial 2, incidence increased to 35% and 56%, while severity increased to 6% and 19% for azoxystrobin and EBDC, respectively. Disease incidence was 33% and severity was 9% for azoxystrobin in trial 3. Disease incidence was 68% and severity was 27% for EBDC in trial 3.

For azoxystrobin, all advisory programs in each trial reduced disease incidence and severity compared to the control (Table 1). In all trials, the most effective spray programs were the 7-d; and the 3-, 6-, and 12-h T/W programs which had <20% disease incidence and <2% severity. The 24-h T/W program was intermediate in effectiveness among the advisory programs. Incidence and severity for the 36-h T/W program in each trial and the 12-h W program in trials 1 and 3 were highest among advisory programs. Generally disease levels increased with the T/W duration from 12- to 36-h T/W

programs. In trial 1, disease incidence and severity increased from 8 to 12% and 5 to 12% for the 24 and 36-h T/W programs, respectively. For trial 2, incidence increased from 45 to 79% and severity from 9 to 19% with T/W durations of 24 to 36 h, respectively. In trial 3, disease incidence increased from 35 to 71% and severity from 11 to 24% for T/W programs of 24 to 36 h. Incidence and severity for the 12-h W program did not differ from the 36-h T/W program in trials 1 and 3, but were similar to the 24-h T/W program in trial 2 (Table 1).

For the EBDCs, all advisory programs in each trial except the 36-h T/W program and the 12-h W program reduced disease incidence and severity compared to the control (Table 2). In trial 1, the most effective spray programs were the 7-d, and 3-, 6-, and 12-h T/W programs which had <32% disease incidence and <4% severity. In trial 2, only the 3- and 6-h T/W programs provided disease control equivalent to the 7-d program. Disease incidence was <42% and severity <4% for the best programs in trial 2. In trial 3, only the 3-h T/W program provided disease control similar to the 7-d program. Disease incidence was nearly 50% and severity <15% for the most effective programs in trial 3. Generally disease levels increased with the T/W duration for other less effective T/W programs. In trial 1, disease incidence and severity increased from 47 to 71% and 14 to 24% for the 24 and 36-h T/W programs, respectively. For trial 2, incidence increased from 44 to 90% and severity from 10 to 56% with T/W durations of 12 to 36 h, respectively. In trial 3, disease incidence increased from 50 to 95% and severity from 14 to 57% for T/W programs of 6 to 36 h. Incidence and severity for the 12-h W program did not differ from the 36-h T/W program in trials 1 and 3, but were similar to the 24-h T/W program in trial 2 (Table 2).

DISCUSSION

Several weather-based advisory programs, based on the disease response to T and W under controlled conditions, were developed to improve the efficiency of fungicide usage to control white rust of spinach. Results indicated that T/W programs reduced applications of azoxystrobin and EBDCs without reducing white rust disease control compared to a calendar schedule. However, the level of spray savings and disease control differed among T/W programs.

The T/W programs provided the most consistent control of white rust over the three trials and most were superior to the 12-h W program. The most efficient threshold for the T/W program, however, varied with fungicide. For azoxystrobin, the 12-h T/W was the most efficient spray program and provided disease control equivalent to the 7-d program. While T/W programs were more variable for EBDC fungicides, the 6-h T/W program was the most efficient program in two of three trials, and provided nearly the same control as the 7-d schedule in the third trial. While longer T/W thresholds provided good control, particularly for azoxystrobin, disease control was less effective than for the calendar program, which may limit their adoption.

In this study, the 12-h W program did not provide the most efficient disease for EBDC sprays. Disease severity and incidence in plots sprayed according to the 12-h W program often did not differ significantly from the control for EBDC and results were similar to less effective T/W thresholds for azoxystrobin. Levels for this program would not be adequate control for commercial spinach production. Results of this study differed compared to those of Dainello and Jones (6), who showed that fungicide applications made after 12-h W were as effective as calendar programs. Differences in measuring

wetness duration occurred between the two studies. Leaf wetness was measured directly with a leaf wetness hygrothermograph in the Dainello and Jones study. In this study, leaf wetness was not measured directly, but continuous hours of RH≥90% were used as an approximation of leaf wetness. Twelve continuous hours of RH≥90% occurred only three times in trial 1, once in trial 2, and twice in trial 3. The susceptible cultivars "Iron Duke" and "Chinook" were used in the Dainello and Jones study, while "Melody" was used in this study. Perhaps the duration of RH≥90% was not a good estimate of leaf wetness or the cultivars used in each study differed in susceptibility.

In this study, disease development was less in trial 1 than in trials 2 and 3. This was attributed to the fewer T/W hours that accumulated in trial 1 compared to trials 2 and 3. Rainfall was near normal during the three trials, and differences in levels of disease were not associated with rainfall or accumulated duration of W.

The systemic fungicide azoxystrobin performed better in the weather-based programs than did the protectant EBDCs. Disease severity and incidence were higher in EBDC-treated plots than for azoxystrobin regardless of the spray program. The difference between the performance of azoxystrobin and the EBDCs were attributed to the post-infection activity of azoxystrobin. Although the duration of post-infection activity of azoxystrobin has not been reported, the results of this study indicate that post-infection activity is an important aspect in the control of white rust with an advisory program.

BLITECAST is a well-known weather-based advisory program for potato late blight, caused by the Oomycete pathogen *Phytophthora infestans* (11, 14). As in this study, systemic fungicides have performed better with BLITECAST than protectant fungicides (14). BLITECAST, like our T/W programs, recommends sprays during or

after weather conditions have been conducive to disease development, when applications of protectant fungicides may be less effective (14).

According to Thomas (15), fungicide application programs to manage foliar diseases of vegetable crops can be better implemented by monitoring meteorological conditions that prevail in a production area rather than by crop phenology. For the protectant EBDC fungicides, an average of 2.7 fungicide applications were saved over the three trials by using the 6-h T/W threshold. Similar results might be expected for other protectant fungicides that may be registered for use on spinach in the future. Because 6 h of T/W could occur in a single dew period in the field, a rapid response might be required for implementing this program commercially. For azoxystrobin, the 12-h T/W threshold is more practical for improving the efficiency of spray programs. The results of this study indicate disease forecasting using the T/W programs developed in this study has the potential for reducing the number of fungicide applications required for white rust disease control in Oklahoma. However, the program is better adapted for use with fungicides that have postinfection activity, like azoxystrobin. Quantification of the postinfection activity of azoxystrobin and other fungicides should be useful in optimizing the performance of advisory programs for white rust management.

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Table 1. Comparison of spray programs with azoxystrobin for control of white rust on a susceptible spinach cultivar for three trials (1-3) during 1998-1999[1].

	Sprays (no.)				Severity (%)[3]				Incidence (%)[4]			
	Trial				Trial				Trial			
Spray program[2]	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
7-d	8	5	4	5.7	0.1 d[5]	1.0 d	1.3 d	0.8	2.0 e	15.8 d	13.8 d	10.5
3-h T/W	6	3	4	4.3	0.1 d	1.0 d	1.4 d	0.9	2.8 e	20.0 d	13.8 d	12.2
6-h T/W	5	2	2	3.0	0.4 d	1.1 d	1.7 d	1.1	4.0 de	20.0 d	15.0 d	13.0
12-h T/W	4	2	2	2.7	0.4 d	1.5 d	1.9 d	1.3	4.0 de	20.0 d	15.8 d	13.3
24-h T/W	3	1	2	2.0	4.7 c	9.0 c	11.4 c	8.4	8.3 cd	45.3 c	34.8 c	29.4
36-h T/W	3	1	2	2.0	12.2 b	18.5 b	24.0 b	18.3	12.0 bc	79.0 b	71.3 b	54.1
12-h W	3	1	2	2.0	12.2 b	9.3 c	24.3 b	15.2	15.8 b	47.8 c	70.0 b	44.5
Control	0	0	0	0.0	25.5 a	57.1 a	60.0 a	47.5	73.8 a	94.5 a	98.3 a	88.8
LSD (P=0.05)					1.7	1.3	3.0		5.5	7.6	6.3	

[1] Values are from 40 leaves per plot and 4 replicate plots per treatment.

[2] Azoxystrobin was applied at 0.16 kg/ha in trials 1 and 2 and at 0.23 kg/ha in trial 3. 7-d = calendar program, N-hr = advisory programs using N cumulative hours of favorable temperature and wetness (T/W) and 12-h W = 12 continuous hours of wetness (see text for details).

[3] Percentage of leaf area with symptoms.

[4] Percentage of leaves with symptoms.

[5] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference (LSD) Test.

Table 2. Comparison of spray programs with EBDC fungicides for control of white rust on a susceptible spinach cultivar for three trials (1-3) during 1998-1999[1].

	Sprays (no.)				Severity (%)[3]				Incidence (%)[4]			
	Trial				Trial				Trial			
Spray program[2]	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
7-d	8	5	4	5.7	3.5 c[5]	2.8 d	10.3 f	5.5	29.0 c	39.5 c	47.8 c	38.8
3-h T/W	6	3	4	4.3	3.3 c	2.9 d	12.5 ef	6.2	29.0 c	39.0 c	49.5 c	39.2
6-h T/W	5	2	2	3.0	3.4 c	3.9 d	14.4 e	7.2	31.5 c	42.0 c	50.0 c	41.2
12-h T/W	4	2	2	2.7	3.6 c	9.7 c	18.4 d	10.6	31.5 c	44.0 c	67.8 b	47.8
24-h T/W	3	1	2	2.0	14.1 b	27.3 b	26.3 c	22.5	47.0 b	75.3 b	71.3 b	64.5
36-h T/W	3	1	2	2.0	24.4 a	56.3 a	56.9 ab	45.9	71.5 a	90.0 a	95.3 a	85.6
12-h W	3	1	2	2.0	24.7 a	28.4 b	53.7 b	35.6	70.8 a	73.3 b	94.0 a	79.3
Control	0	0	0	0.0	25.5 a	57.1 a	60.0 a	47.5	73.8 a	94.5 a	98.3 a	88.8
LSD (P=0.05)					1.2	4.5	3.7		8.9	8.8	6.0	

[1] Values are from 40 leaves per plot and 4 replicate plots per treatment.

[2] EBDC fungicides were applied at 2.24 kg/ha in all trials. 7-d = calendar program, N-hr = advisory programs using N cumulative hours of favorable temperature and wetness (T/W and 12-h W = 12 continuous hours of wetness (see text for details).

[3] Percentage of leaf area with symptoms.

[4] Percentage of leaves with symptoms.

[5] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference (LSD) Test.

CHAPTER IV

Postinfection Activity of Maneb, Azoxystrobin, and BAS 500 against White Rust of Spinach

ABSTRACT

The postinfection activity of maneb, azoxystrobin, and BAS 500 against white rust of spinach was determined in dew chambers. Applications of each fungicide were applied one day before, immediately before, and one to four days after inoculation. Disease incidence (the percentage of leaves with symptoms) and severity (the percentage of leaf area with symptoms), estimated 14 days after inoculation, were 100% and 83% for the unsprayed control. No disease developed on plants sprayed one day or immediately before inoculation for any fungicide. BAS 500 showed the greatest postinfection activity of the three fungicides. No disease occurred when BAS 500 was applied within three days after inoculation. Incidence and severity for BAS 500 at four days after inoculation were only 19 and 0.86%, respectively. Azoxystrobin also showed postinfection activity. No disease developed when the fungicide was applied within one day after inoculation. Severity increased from 4% at two days after inoculation to 15% at four days, but was lower than the control at all application timings. Incidence also was lower than the control at all application timings (42 to 85%), except at four days after inoculation (100%). Maneb showed the least postinfection activity. For maneb, disease severity was lower than the unsprayed control at each spray timing, except at four days after inoculation. Disease severity increased from 20% at one day after inoculation to 79% at four days after inoculation. However, incidence was 100% at all spray timings after inoculation and did not differ from the control. These results indicate that applications of azoxystrobin up to one day after infection and of BAS 500 at least four days after

infection should be effective. Results explain the improved control for azoxystrobin observed in the field when fungicides are applied according to weather-based advisory programs.

INTRODUCTION

White rust of spinach, caused by the fungus *Albugo occidentalis* Wilson, is an economically important foliar disease of spinach in Oklahoma, Texas, and spinach production areas in the eastern United States (8). Yield losses from 30 to 100% have been reported and are attributed to a reduction in the quality and marketability of both fresh and processing spinach (2, 9). Symptoms of white rust begin as chlorotic lesions on the upper leaf surface (18, 19). As the lesions develop, small white blister-like pustules are produced on the underside of the leaves, which renders them unmarketable (8, 18, 19).

Control of diseases with protectant fungicides has involved repeated applications, starting before the disease appears (7). White rust of spinach has been managed historically by foliar protectant fungicides, particularly the ethylene bisdithiocarbamates (EBDCs) maneb, zineb, and mancozeb (4, 9, 14). Changes in pesticide regulations in the United States and in Canada eliminated these compounds from disease control programs for spinach in 1991 (8, 10). However, the discovery of systemic fungicides with "curative" or "postinfection" activity against Oomycetes has enabled disease control when applications were made to plants that had already been infected (7).

The postinfection activity of metalaxyl against late blight of potato and tomato, and downy mildew of grape has been studied (3, 6, 22). Metalaxyl was shown to control late blight when applied within two days of inoculation and to inhibit sporulation of the downy mildew pathogen of grape and disease development when applied within three

days of inoculation (6, 22). Other work has examined the curative action of the sterol-inhibiting fungicides, i.e. triforine and fenarimol, against apple scab, brown rot, and mummy berry (12, 17, 20, 23). Sterol-inhibiting fungicides prevented the formation of visible apple scab lesions when applied within three days after inoculation and only chlorotic flecks or spots developed if they were applied later than 3 days after the onset of infection, but before symptoms were visible (17, 20).

The strobilurin fungicide azoxystrobin is a recently developed, broad-spectrum fungicide with activity against diseases caused by Ascomycetes, Basidiomycetes, and Oomycetes, including white rust of spinach (1, 5, 11, 13, 24). Disease control with azoxystrobin and other strobilurins is achieved through their protectant and systemic properties (5, 15, 16, 24). Azoxystrobin is a potent inhibitor of spore germination and mycelial growth (5). Azoxystrobin acts as a specific inhibitor of respiration by binding to the center Q_p of cytochrome b and thus blocks electron transport in fungal mitochondria (5, 15).

A weather-based spray advisory program based on the duration of favorable temperature (T) and wetness (W) (Chapter II) was developed and evaluated (Chapter III). In three field trials, applications of EBDC fungicides was most effective when applied after following shorter durations of favorable T and W than for azoxystrobin. Results suggest that improved disease control with azoxystrobin may be due to its postinfection activity. However, the postinfection activity of azoxystrobin against spinach white rust has not been quantified.

BAS 500 (BASF Corporation) is an experimental fungicide being developed in North America for broad-spectrum disease control in turf, ornamentals, and numerous

food crops. The proposed common name for BAS 500 is methoxycarbamate. Information on the chemistry and mode of action of BAS 500, however, will not be available for public disclosure until 2000. In field studies during 1999 in Oklahoma, BAS 500 was highly effective in controlling white rust and gave better disease control than other fungicides used in the studies, including azoxystrobin and maneb (J. Damicone unpublished data). BAS 500 is, therefore, a promising fungicide for white rust control, but further research on its postinfection activity against *A. occidentalis* would be beneficial.

The objectives of this study were to quantify the postinfection activity of azoxystrobin and to compare its postinfection activity with that of the protectant fungicide maneb and the experimental fungicide BAS 500. Quantification of the postinfection activity of these fungicides will be beneficial for their use in management of white rust in conjunction with weather-based advisory programs for white rust of spinach.

MATERIALS AND METHODS

"Kent" spinach plant were grown in a greenhouse at 20-30° C in plastic pots containing a medium (65% peat moss, 20% vermiculite, 10% perlite, and 5% hort sand) for 40 to 60 days. Plants were watered as needed and nutrients were supplied by applying liquid fertilizer (0.2, 0.08, and 0.03 g/L N/P/K, respectively) weekly.

An isolate of *A. occidentalis*, obtained from diseased plants in a field in Oklahoma, was maintained on plants. Sporangial suspensions were prepared by agitating pieces of leaves with pustules in double distilled water. The suspensions were strained though cheesecloth and adjusted to $1x10^5$ sporangia/ml with a hemacytometer (Chapter

II). Sets of plants were spray-inoculated every two weeks for the duration of the study and placed in a dew chamber (Model I-60DL, Percival, Boone IA) at 15° C with a 12-h wet cycle each day for 2 to 3 days. Suspensions were sprayed to runoff onto the upper leaf surface of plants using a hand-held spray bottle. Plants were removed from the dew chamber and further incubated in the greenhouse at 20-30° C until symptom development, usually about 14-days after inoculation.

The fungicides maneb, azoxystrobin, and BAS 500 were applied to plants at six application; one day before inoculation, immediately before inoculation, and one, two, three, and four days after inoculation. The experimental design was a split-plot with fungicide as the whole-plot treatment and application timing as the split-plot treatment. There were four plants for each combination of fungicide and application timing, and the experiment was repeated.

Plants (40 to 60 day-old) were spray-inoculated as described above and placed in a dew chamber set at 12° C, the optimum temperature for infection and disease development (Chapter II). The chamber was set for a 12-h night period of relative humidity (RH) ≥95%, which supported infection, and a 12-h day period of RH 70 to 75%. Fungicides, maneb (Maneb 75 DF) (2.24 kg/ha), azoxystrobin (Quadris 2.08 F) (0.23 kg/ha), and BAS 500 (0.12 kg/ha), were added to 0.3 L of water at a rate equivalent to the per ha rate in 935 L. Individual plants were sprayed to runoff with maneb (2.4 g/L), azoxystrobin (0.92 g/L) and BAS 500 (0.46 g/L) using a hand-held spray bottle. To determine the protectant activity of the fungicides, applications were made immediately before or one day before inoculation. To determine the postinfection activity of the fungicides, plants were randomly removed from the chamber, and fungicides were

applied one, two, three, and four days after inoculation. The plants were returned to the chamber following fungicide application. Six days after inoculation, all plants were removed from the chamber and incubated in a 20 to 30° C greenhouse. Fourteen days after inoculation, disease severity (the percentage of leaf area with symptoms) was visually estimated. Eight leaves were evaluated per plant for a total of 32 leaves per fungicide and application timing combination.

Analysis of the disease incidence, the percentage of leaves with symptoms) and severity data was performed on the mean of 8 leaves per plant. Analysis of variance (ANOVA) was performed on the incidence and severity data using the SAS GLM procedure (version 6.11, SAS Institute, Cary, NC). The effects of experiment, fungicide, application timing, and their interactions were tested. However, heterogeneity of variances between combinations of fungicide and application timing was severe. Therefore, disease incidence and severity data were analyzed using contingency tables (21). Where the ANOVA indicated a significant fungicide x application timing interaction, contingency tables were constructed by fungicide. To examine the effect of application timing on disease severity and incidence, three 2 x 6 contingency tables were constructed with SAS by fungicide. To examine the effect of fungicide on disease severity and incidence, six 1 x 2 contingency tables were constructed by application timing. To construct the contingency tables, a value of 0 was assigned to disease severity and incidence values < 10%, while a value of 1 was assigned to disease severity and incidence values $\geq$ 10 %. The frequency, or the number of plants with values of 0 and 1 in each treatment and fungicide combination, was displayed in each table. Fisher's exact test was then used with SAS to determine if the frequency data observed in each table

was due to chance or was due to significant treatment or fungicide differences. Unless otherwise indicated, only significant ($P \leq 0.01$) differences between treatment means are described below.

RESULTS

In the ANOVA, over experiments effects of experiment, and the experiment x fungicide, experiment x application timing and the experiment x fungicide x application timing interactions were not significant for disease incidence or severity ($P > 0.20$). Therefore, the combined analyses were retained. In the combined analysis, the main effects of fungicide and application timing and the interaction between fungicide x application timing were significant, and the main effects of fungicide and application timing were significant ($P < 0.0001$).

Disease severity (83%) and incidence (100%) were severe in the unsprayed control. Protectant treatments of maneb, azoxystrobin, and BAS 500, applied either one day prior to or immediately before inoculation, resulted in complete disease control compared with the control (Table 1). According to contingency table analyses, there was no difference in the frequency of plants with disease incidence or severity $\geq$ 10% between the three fungicides for these two application timings (Table 2).

BAS 500 showed the greatest postinfection activity of the three fungicides evaluated. BAS 500 provided complete disease control through three days after inoculation (Table 1). Disease incidence (19%) and severity (<1%) were low even for four days after inoculation. Azoxystrobin also showed postinfection activity when applied within three days of infection. There was complete disease control when applications of azoxystrobin were made one day after inoculation. Disease severity was

lower than the control for all four post-inoculation timings. Therefore, there was postinfection activity when azoxystrobin was applied within four days after inoculation for disease severity. Disease incidence was also lower than the unsprayed control until four days after inoculation when 100% of leaves were symptomatic. Maneb showed the least postinfection activity. For maneb, disease severity increased with an increase in post-inoculation application timing (Table 1). Disease severity was high (from 20 to 79%) for all times periods after inoculation. Disease severity was lower than the control at all application timings, except four days after inoculation. Disease incidence, however, was 100% at all timings from one to four days after inoculation (Table 1).

According to contingency table analyses, the effect of application timing on disease control was dependent on the fungicide used (Table 2). The number of maneb-treated plants with disease severity $\geq$ 10% was significantly higher than those with azoxystrobin and BAS 500 treatments at one and two days after inoculation. Maneb-treated plants with disease incidence $\geq$ 10% also was higher than the azoxystrobin and BAS 500 treatments one day after inoculation. The frequency of plants with disease severity $\geq$ 10% in the BAS 500-treated plants was lower than the azoxystrobin and maneb treatments three to four days after inoculation, while disease incidence $\geq$10% was significantly lower two to three days after inoculation (Table 2).

According to contingency table analyses, the efficacy of all fungicides decreased with increasing time after inoculation (Table 3). The number of maneb-treated plants with disease severity $\geq$ 10% was significantly higher when applied from one to four days after inoculation than when applied before inoculation. The number of azoxystrobin-treated plants with severity >10% was significantly higher when applied from three to

four days after inoculation than when applied before or within two days after inoculation. There was no difference in the frequency of plants with disease severity ≥ 10% for all six timings with BAS 500. The number of plants with disease incidence ≥ 10% was significantly higher in plants sprayed with maneb one to four days after inoculation, with azoxystrobin two to four days after inoculation, and with BAS 500 four days after inoculation than for other application timings with the respective fungicide (Table 3).

DISCUSSION

BAS 500 and azoxystrobin provided postinfection activity when applied within four days and three days following inoculation, respectively. The EBDC fungicide maneb had the least postinfection activity. All fungicides had protectant activity when the fungicides were applied immediately before or one day before inoculation. The protectant activity of the fungicides indicate that the difference between maneb and azoxystrobin observed in the field (Chapter III) was not due to differences in fungicide efficacy. Maneb is a protectant fungicide whose use is recommended prior to infection (14). Maneb would, therefore, be expected to have protectant activity and little or no activity once infection had taken place.

The results obtained in this study with BAS 500 and azoxystrobin are similar to those reported previously for other systemic fungicides. BAS 500, like metalaxyl and the sterol-inhibiting fungicides against other diseases (6, 17, 20, 22), completely controlled white rust when applied up to three days after inoculation. Only low levels of disease were evident four days of inoculation. When sterol-inhibiting fungicides were applied to plants/trees one to two days after inoculation with *Monilinia fructicola* or *Monilinia vaccinii-corymbosi*, disease onset and sporulation were delayed and disease incidence

was low indicating excellent postinfection activity (12, 23). Azoxystrobin must be applied within one day of inoculation to obtain complete disease control. However, disease development, as measured by severity, was reduced as compared to the control when azoxystrobin was applied within three days of inoculation.

The duration of favorable T and W necessary for infection of spinach by *A. occidentalis* and disease development was determined in dew chambers (Chapter II). At 12^0 C with a minimum of 3 hours of favorable W (RH>95%), infection occurred and disease developed. Because dew chambers in this study were set at 12^0 C with a 12-h wet cycle (RH>95%) and a 12-h dry cycle (RH 70 to 75%), only 12-h of favorable W occurred each day. The fungicidal activity of azoxystrobin stems from its ability to inhibit spore germination, fungal growth, and fungal respiration (5). Because many infections o occurred by the first 12-h period of favorable T and W, the postinfection activity of azoxystrobin against *Albugo occidentalis* at one to three days after inoculation shown in this study is most likely due to inhibition of fungal growth. The protectant activity can likely be attributed to inhibition of sporangial germination (zoospore release) and mycelial growth.

Results explain the improved control for azoxystrobin over EBDCs observed in the field when fungicides were applied according to weather-based advisory programs (Chapter III). In the field studies, EBDC (maneb or mancozeb) had to be applied within 3 to 12 hours of favorable T and W, while azoxystrobin could be applied as late as after 12 hours of favorable T and W to achieve control equivalent to a 7-day schedule. In this study, maneb showed protectant but little post-infection activity. Azoxystrobin showed protectant and postinfection activity when applied before or within three days of

inoculation. When maneb was applied within one day after inoculation, disease severity was 20% and incidence was 100%, which indicates that some infection had occurred after the first 12 h period of favorable T and W. When azoxystrobin was applied within two days after inoculaton, severity was only 4% and incidence was 43%, which indicates additional infection had occurred after 24 hours of favorable T and W. As in the field study, azoxystrobin could be applied after a longer duration of favorable T and W than EBDCs with lower disease levels, because of its postinfection activity. The improved control of azoxystrobin over EBDCs observed in the field is, therefore, is most likely due to the greater postinfection activity of azoxystrobin.

BAS 500 completely controlled disease when applied up to three days after inoculation, and only low levels of disease were evident four days after inoculation. Disease would be expected to increase with application timing after inoculation. At some point, disease levels would be expected to reach those of the control It would be interesting to extend application timings to five or more days after inoculation to determine its maximum postinfection activity.

Quantification of the postinfection activity of azoxystrobin and BAS 500 will aid in the management of white rust of spinach in conjunction with a weather-based spray advisory by identifying periods that the fungicides can be applied while maintaining disease control. By monitoring periods of favorable T and W for infection, it should be possible to schedule postinfection applications of these fungicides and likely reduce the number of sprays required in some years. These results suggest that azoxystrobin and BAS 500 are better suited for use in predictive, weather-based spray programs for white rust than protectant fungicides such as maneb.

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Table 1. Effects of fungicide and application timing on control of white rust of spinach.

Fungicide and Rate	Timing[1]	Disease incidence (%)[2]	Disease severity (%)[3]
Maneb (2.4 g/L)	-1	0.00[4]	0.00
	0	0.00	0.00
	1	100.00	20.00
	2	100.00	38.83
	3	100.00	71.56
	4	100.00	78.71
Azoxstrobin (0.92 g/L)	-1	0.00	0.00
	0	0.00	0.00
	1	0.00	0.00
	2	42.25	3.81
	3	84.50	9.30
	4	100.00	15.08
BAS 500 (0.46 g/L)	-1	0.00	0.00
	0	0.00	0.00
	1	0.00	0.00
	2	0.00	0.00
	3	0.00	0.00
	4	19.13	0.86
Control		100.00	83.13

[1] Maneb, azoxystrobin, and BAS 500 were sprayed to runoff: one day before (-1), immediately before (0), and one to four (1-4) days after after inoculation with sporangia of *Albugo occidentalis*.

[2] The percentage of leaves with symptoms.

[3] The percentage of leaf area with symptoms.

[4] Values represent the mean disease levels at 14 days after inoculation averaged over two experiments and four plants per fungicide and application timing combination for each experiment.

Table 2: Contingency table analyses for the effect of fungicide on control of white rust of spinach for various application timings.

	Disease incidence[2]						Disease severity[3]					
	-1[1]	0	1	2	3	4	-1	0	1	2	3	4
Fungicide												
Maneb	0/8 a[4]	0/8 a	8/8 a	8/8 a	8/8 a	8/8 a	0/8 a	0/8 a	8/8 a	8/8 a	8/8 a	8/8 a
Azoxystrobin	0/8 a	0/8 a	0/8 b	8/8 a	8/8 a	8/8 a	0/8 a	0/8 a	0/8 b	0/8 b	5/8 a	8/8 a
BAS 500	0/8 a	0/8 a	0/8 b	0/8 b	0/8 b	8/8 a	0/8 a	0/8 a	0/8 b	0/8 b	0/8 b	0/8 b

[1] Fungicides were applied to plants: one day before (-1), immediately before (0), and one to four (1-4) days after inoculation with sporangia of *Albugo occidentalis*.

[2] The percentage of leaves with symptoms.

[3] The percentage of leaf area with symptoms.

[4] The fraction of plants in two experimental repetitions with ≥10% disease incidence or severity. Fractions within each column followed by the same letter are not significantly different at $P \leq 0.01$ according to Fisher's exact test.

Table 3: Contingency table analyses for the effect of application timing on control of white rust of spinach for various fungicides.

	Disease incidence[2]			Disease severity[3]		
Timing[1]	Maneb	Azoxystrobin	BAS 500	Maneb	Azoxystrobin	BAS 500
-1	0/8 a[4]	0/8 a	0/8 a	0/8 a	0/8 a	0/8 a
0	0/8 a	0/8 a	0/8 a	0/8 a	0/8 a	0/8 a
1	8/8 b	0/8 a	0/8 a	8/8 b	0/8 a	0/8 a
2	8/8 b	8/8 b	0/8 a	8/8 b	0/8 a	0/8 a
3	8/8 b	8/8 b	0/8 a	8/8 b	5/8 b	0/8 a
4	8/8 b	8/8 b	8/8 b	8/8 b	8/8 b	0/8 a

[1] Fungicides were applied to plants: one day before (-1), immediately before (0), and one to four (1-4) days after inoculation with sporangia of *Albugo occidentalis*.

[2] The percentage of leaves with symptoms.

[3] The percentage of leaf area with symptoms.

[4] The fraction of plants in two experimental repetitions with ≥10% disease incidence or severity. Fractions within each column followed by the same letter are not significantly different at $P \leq 0.01$ according to Fisher's exact test.

SUMMARY

Controlled environment experiments were conducted to determine the influence of temperature (T) and wetness duration (W) on infection of spinach by *Albugo occidentalis* and on development of white rust. Plants were exposed to post-inoculation temperatures of 6 to 28^0 C and interrupted wetness periods that totaled 3 to 84 hours. T and W were found to be important factors in infection of spinach. Temperatures of 12 to 18^0 C favored infection when accompanied by a minimum of 3 hours of wetness. A model describing the response surface of disease to T and W was developed. Weather-based advisory programs, based on the periods of T and W that favored infection, were developed and evaluated for timing fungicide applications to control white rust in three field trials. The protectant EBDC fungicides and the systemic fungicide azoxystrobin were applied after 3, 6, 12, 24, and 36 cumulative hours of favorable T and W (T/W). T/W programs were compared to a previously published program of 12 continuous hours of wetness (12-h W), a 7-d program, and an unsprayed control. Under field conditions, all advisory programs reduced the number of fungicide sprays compared to the 7-d. Disease control was less effective for protectant fungicides for all spray programs. Based on spray reductions and disease control, the 6- and 12-h T/W programs were most efficient for the EBDCs and azoxystrobin, respectively. The postinfection activity of maneb, azoxystrobin, and BAS 500 against white rust was determined in dew chambers. Applications of each fungicide were made immediately or one day before and from one to four days after inoculation. In the study, no disease developed on plants sprayed before inoculation for any fungicide. BAS 500 showed the greatest postinfection activity of the fungicides, followed by azoxystrobin. Maneb showed little postinfection activity.

APPENDIX

APPENDIX A

ANALYSIS OF VARIANCE TABLE FOR TEMPERATURE AND WETNESS EFFECTS STUDY

Random Effects[a]	Ratio	Estimate	Std. Error	Z	Pr>|Z|
Exp.	8.1×10^{-3}	7.2×10^{-6}	1.4 x 10-5	0.52	0.5996
Chamber	6.7×10^{-4}	6.2×10^{-7}	5.1 x 10-6	0.12	0.9036
Exp. x Chamber	2.3×10^{-3}	2.0×10^{-6}	6.3 x 10-6	0.32	0.7509
Exp. x Temp. x Chamber	0.0	0.0	-	-	-
Exp. x Temp. x Wet x Chamber	0.0	0.0	-	-	-

Fixed Effects	NDF	DDF	F	Pr>F
Temp.	11	1317	11727.06	0.0001
Wet	9	1317	9529.74	0.0001
Temp. x Wet	99	1317	270.13	0.0001

[a] Exp. = experiment, Temp. = temperature, Wet = wetness period

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