EPIDEMIOLOGY OF SEPTORIA LEAF SPOT ON BLUEBERRY: TEMPORAL DYNAMICS, EFFECT OF LEAF AGE, AND RELATIONSHIP TO DEFOLIATION AND YIELD

by

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(Under the Direction of Harald Scherm)

ABSTRACT

Septoria leaf spot, caused by the fungus *Septoria albopunctata*, is a disease of increasing concern to the blueberry industry in Georgia and other southeastern states. Due to critical gaps in knowledge on disease ecology and epidemiology, current disease management guidelines are highly empirical. With this in mind, a series of field studies was carried out from 2001 to 2004 to generate key epidemiological information on temporal disease progression, seasonal inoculum dynamics, and the effects of disease on premature defoliation and yield. Additional studies were carried out to develop improved disease assessment and sampling procedures.

Disease onset on rabbiteye blueberries grown near Athens was observed between late April and mid-June, and disease severity increased rapidly and reached a maximum by mid- to late September; thereafter, disease severity decreased until the end of the season. Disease severity was highest on early-emerging leaves and on leaves located on shoots closer to the ground. Pycnidiospore inoculum was present throughout the season, and leaves became infected by *S. albopunctata* season-long. Disease severity, defoliation, flower bud set, and return yield

were found to be interrelated. Leaves with high disease severity at harvest abscised earlier in the fall than leaves with low disease severity, and shoots with severely diseased leaves and/or high levels of defoliation had a reduced potential to set flower buds. Furthermore, such shoots consistently had low return yields the following year. These results form the basis for identifying specific disease severity or defoliation levels that can be tolerated during specific periods of crop development without negatively impacting flower bud set and return yield. Based on the data collected in this multi-year field study, hierarchical sampling plans were developed for assessing disease severity and defoliation; these plans will be useful for obtaining reliable estimates of the two variables with the least expenditure of time.

INDEX WORDS: Blueberry, Defoliation, Disease assessment, Epidemiology, Inoculum dynamics, Sampling, *Septoria albopunctata*, Septoria leaf spot, Survival analysis, Temporal progress, *Vaccinium ashei*, *Vaccinium corymbosum*, Yield

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CHAPTER 1

INTRODUCTION

In 2003, Georgia was the fifth-largest producer of cultivated blueberries in the nation, behind the states of Michigan, New Jersey, Oregon, and North Carolina (Anonymous, 2004). After peach, blueberry is the second most important fruit crop in Georgia and is grown on more than 3,200 ha statewide, with a total volume of utilized production of about 10,000 tons and a revenue of about $27 million in 2003 (Boatright and McKissick, 2004). Production is concentrated in the southeastern parts of the state, with Bacon, Clinch, Appling, and Ware Counties accounting for more than 75% of the total production.

Among the different species of blueberry, the native rabbiteye blueberry (*Vaccinium ashei*) accounts for about 90% of the total production acreage. The remaining 10% is planted to the recently introduced southern highbush blueberry (*V. corymbosum* interspecific hybrids) (Scherm and Krewer, 2003). 'Brightwell', 'Climax', 'Premier', and 'Tifblue' are four of the most common rabbiteye cultivars, while 'Star', 'Bluecrisp', and 'Misty' are examples of common southern highbush cultivars (Scherm and Krewer, 2003).

Several diseases can affect blueberries in Georgia, the most important of which are mummy berry (*Monilinia vaccinii-corymbosi*), stem blight (*Botryosphaeria* spp.), twig blight (*Phomopsis vaccinii*), and leaf spots, mainly Septoria (*Septoria albopunctata*), anthracnose (*Gloeosporium minus* and *Colletotrichum* spp.), and rust (*Pucciniastrum vaccinii*) (Scherm *et al*., 2003). In a producer survey focusing on blueberry production problems within the state, about half of the growers reported that leaf spots were at least moderately important constraints (Scherm *et al*., 2001). A subsequent field survey showed that leaf diseases were prevalent on

both rabbiteye and southern highbush cultivars statewide, and that Septoria was the most commonly encountered leaf spot (Scherm *et al*., 2003).

Septoria leaf spot was first described in the United States by Cooke (1883) on *V. arboreum* in Florida and North Carolina (Demaree and Wilcox, 1947). The disease is widely distributed in the southeastern United States, with reports from Florida (Alfieri *et al*., 1984), South Carolina (Anonymous, 1960), North Carolina (Grand, 1985), and Georgia (Anonymous, 1960). Symptoms are characterized by small, circular leaf lesions with white to tan centers and purple margins (Milholland, 1995). Ostiolate pycnidia, usually one but occasionally up to four or five per lesion, occur on the upper leaf surface. Stem lesions are typically sunken, 5 to 6 mm in diameter, with tan or gray centers and reddish brown margins. The fungus overwinters asexually in stem lesions and in infected leaf litter on the ground (Milholland, 1995).

Leaf spot diseases combined are responsible for about 30% of the total disease-related blueberry losses in Georgia (Williams-Woodward, 2003). Given the high prevalence of Septoria leaf spot in the state (Scherm *et al*., 2003), it is likely that a large proportion of these losses is due to *S. albopunctata*. However, no studies have been conducted to quantify the actual effects of Septoria leaf spot on yield. There is also a lack of basic epidemiological information for *S. albopunctata*, such as data on disease progression and inoculum dynamics or how to assess the disease most efficiently. As a result, current management recommendations, which rely primarily on calendar-based applications of fungicides after harvest of the crop in summer and early fall (Brannen *et al*., 2001, 2002, 2003), are highly empirical and incorporate only very limited information about pathogen biology and disease ecology. With a better knowledge of the epidemiology of the disease, more targeted management recommendations could be developed.

A first step in studying the epidemiology of any plant pathogen is to characterize the temporal disease progress on its host. In the Septoria-blueberry pathosystem, the seasonal progress of the disease has not been documented previously. Key information as to when disease onset is observed and how fast the epidemic progresses on leaves that emerge at different times during the season can guide management decisions, for example, the number of fungicide sprays that may be required and when they need to be applied. Knowledge of the seasonal dynamics of inoculum, also unavailable for Septoria leaf spot, can explain temporal disease progress and provide supplementary information necessary for developing effective disease management strategies.

On blueberry, it is considered advantageous to retain leaves for as long as possible during the fall to enhance the ability of the plants to form flower buds (Darnell, 1991), thus increasing return yield the following year (Williamson and Miller, 2002). This suggests that leaf diseases which cause premature defoliation during summer and fall need to be controlled effectively during this period. Recent fungicide efficacy trials showed that control plots with severe levels of Septoria leaf spot also had the highest levels of fall defoliation (Brannen *et al*., 2002, 2003). However, there is no quantitative information on the relationship between defoliation and disease severity, or how the temporal dynamics of disease affects the timing and magnitude of premature defoliation.

In mechanical defoliation experiments on rabbiteye (Lyrene, 1992) and southern highbush blueberry (Williamson and Miller, 2002), premature leaf loss resulted in reduced flower bud set and return yield. If Septoria leaf spot can indeed induce premature defoliation, it is very likely that such a disease-induced leaf loss will also negatively affect these two yield parameters. In fact, the effects of disease-induced defoliation on flower bud induction and

subsequent yield may be even more pronounced than those documented for mechanical defoliation, given the negative effects of disease on photosynthesis of infected blueberry leaves prior to leaf abscission (Roloff *et al*., 2004). However, no studies have been carried out to determine the quantitative effects of Septoria leaf spot severity on flower bud set and return yield in blueberry. Establishing the effects of disease on yield would document to blueberry growers the economic importance of the disease and emphasize the need for adequate control to optimize yields. Further, studies on the effect of disease on yield parameters are critical to derive thresholds that indicate disease severity or defoliation levels that can be tolerated before resulting in negative effects on yield.

In field trials involving Septoria leaf spot, disease severity has been assessed routinely by counting the number of spots per leaf (Brannen *et al*., 2001, 2002, 2003). This measure of disease severity has two key advantages: 1) it is based on a count and is therefore unbiased and highly reproducible; and 2) because spore-producing pycnidia bearing the spores are harbored within the spots, the number of spots may provide an epidemiologically relevant estimate of inoculum pressure. However, when conducting disease surveys or evaluating disease in epidemiological or crop loss studies, counting of spots is often too time-consuming. Other disease assessment methods, such as visually estimating the proportion of necrotic leaf area, are potentially more efficient (Campbell and Madden, 1990), but their reliability has not been established for Septoria leaf spot of blueberry. Furthermore, optimum sample sizes for assessing disease severity and defoliation have not been derived in this pathosystem. Such calculations ensure that reliable estimates of disease are obtained with the least expenditure of time.

Based on the above considerations, the overall goal of this study was to fill critical knowledge gaps regarding the epidemiology of Septoria leaf spot as a basis for improved management of the disease. The specific objectives were to:

1. Characterize the temporal progress of Septoria leaf spot in the field and determine the effect of inoculum dynamics and selected leaf attributes on disease progression.
2. Determine the relationship between premature defoliation and Septoria leaf spot severity, and establish how the temporal dynamics of disease affect the timing and magnitude of defoliation.
3. Quantify the effect of Septoria leaf spot on flower bud set and return yield in the field.
4. Determine the relationship between different measures of disease severity and derive optimum sample sizes for assessing Septoria leaf spot severity and associated premature defoliation.

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CHAPTER 2

LITERATURE REVIEW

Septoria leaf spot, caused by the Deuteromycete *Septoria albopunctata*, was first described in the United States by Cooke (1883) on *Vaccinium arboreum* (tree sparkleberry) in Florida and North Carolina (Demaree and Wilcox, 1947). The disease is widely distributed in the southeastern United States, including Florida (Alfieri *et al*., 1984), South Carolina (Anonymous, 1960), North Carolina (Grand, 1985), and Georgia (Anonymous, 1960). The optimum temperature for pathogen growth and disease development is 24 to 28°C (Milholland, 1995), which may explain why the disease has not been reported from cooler, northern blueberry production regions in the United States.

On blueberry, symptoms of Septoria leaf spot consist of small circular leaf lesions with white to tan centers and purple margins. Young, expanding leaves have been reported to be more susceptible to infection than older, fully expanded leaves with waxy surfaces (Demaree and Wilcox, 1947). The pathogen is also capable of causing stem lesions; these are typically sunken, 5 to 6 mm in diameter, with tan or gray centers and reddish-brown margins. In early spring, stem lesions on vigorous shoots are dark purple, while later, white spots appear in the purple areas. The fungus is thought to overwinter asexually in stem lesions and in infected leaf litter on the ground (Milholland, 1995).

The fruiting body of *S. albopunctata* is a well-developed pycnidium on the upper leaf surface. Upon maturity, the ostiole breaks through the epidermis. Pycnidia are ovoid measuring on average 118 × 90 µm and walls are 4 to 6 µm thick and composed of two to four layers of

cells (Demaree and Wilcox, 1947). The pycnidiospores are hyaline, straight or curved, 5- to 11-celled, filiform, and obclavate to spindle-shaped. They vary in size from 42 to 96 µm long and 3.0 to 4.8 µm wide, averaging about 70 × 3.6 µm (Demaree and Wilcox, 1947; Milholland, 1995). Unlike other *Septoria* species that infect woody, perennial hosts (Sinclair *et al*., 1987), *S. albopunctata* does not have a known teleomorph stage.

Apart from the basic mycological description of the pathogen (Cooke, 1883) and a few articles summarizing field observations on the disease (Demaree and Wilcox, 1947; Milholland, 1995), the scientific literature on *S. albopunctata* is extremely limited. In particular, there are no studies on the epidemiology of the disease, including aspects such as seasonal disease onset and progression, inoculum dynamics, and the effects of disease on yield. Due to the limited literature on *S. albopunctata*, the following section focuses on the epidemiology of *Septoria* spp. in other pathosystems, primarily on perennial, woody hosts, in an attempt to identify key features from these pathosystems that could provide insight in the epidemiology and management of *S. albopuctata* on blueberry. While there is considerable literature on *Septoria* spp. on annual hosts such as wheat or tomato, the relevance of this information for understanding and managing Septoria leaf spot of blueberry is questionable. Perennial hosts provide more opportunities for pathogen survival and overwintering, their complex canopies affect pathogen dispersal and disease spread in different ways, and they differ in their propensity for causing yield losses, given the potential for carry-over effects on yield from one year to the next.

Several dozen species of *Septoria* infect the leaves of trees and shrubs in North America, most of which cause brown spots and premature defoliation (Sinclair *et al*., 1987). *Septoria exotica* causes necrotic spots on leaves of shrubs in the genus *Hebe* and on herbaceous plants in the related genus *Veronica* (Beaumont, 1950). In conditions of high disease pressure, *S. exotica*

causes premature defoliation of leaves of the two plant genera. *Septoria azaleae* causes brown, usually angular spots with yellow halos on leaves of evergreen azalea (*Rhododendron* spp.). The disease usually appears late in the growing season and intensifies slowly during cool weather in regions with a mild climate. Water from rain and overhead irrigation disperses the pycnidiospores (Hemmi and Kurata, 1931). Temperatures of 16 to 28°C favor spore germination and growth of the fungus, but up to 2 months may elapse between infection and appearance of symptoms. This long incubation period may explain the late-season development of the disease. *Septoria azaleae* occasionally causes economic damage by inducing defoliation, which, in turn, leads to death of terminal buds (Sinclair *et al*., 1987).

Septoria musiva (teleomorph *Mycosphaerella populorum*) and *S. populicola* (teleomorph *M. populicola*), both of which infect poplar (*Populus* spp.), are perhaps the most widely studied and most damaging *Septoria* species on woody plants (Ostry, 1987). *Septoria musiva* causes leaf spots on most poplar species but can also cause cankers on eastern cottonwood (*P. deltoides* var. *deltoides*) and on natural hybrids of North American poplars (Krupinsky, 1989). *Septoria populicola* affects only a few species and is less virulent than *S. musiva*, usually causing only leaf spots. Hosts of *S. populicola* include balsam poplar and narrowleaf cottonwood (*P. angustifolia*). Leaf infections by *S. musiva* usually precede stem infections and are initiated annually either by airborne ascospores from pseudothecia or by pycnidiospores from overwintered pycnidia in cankers (Ostry, 1987). Ascospores are discharged in greatest numbers at 22 to 26°C during moist weather. Leaves become infected soon after they unfold, and lesions develop 1 to 2 weeks later. Leaf spots caused by *S. musiva* are most numerous on the foliage of lower branches and increase rapidly both in size and number in favorable environmental conditions (Ostry, 1987). Both *S. musiva* and *S. populicola* overwinter in infected leaf litter on

the ground. *Septoria musiva* is especially serious in poplar nurseries and in coppiced plantations where high levels of disease result in premature defoliation and multiple branch and stem cankers (Zalasky, 1978). Premature defoliation of trees severely infected by *S. musiva* begins as early as mid-June when disease is most severe (Ostry, 1987). Application of three benomyl sprays on poplar has been reported to effectively reduce leaf spot caused by *S. musiva* (Ostry, 1987).

It appears that the above studies allow little generalization with regard to the epidemiological patterns of *Septoria* on perennial hosts. For example, in some pathosystems (*S. populicola* on poplar), survival is primarily in stem cankers, while in others (*S. musiva* on poplar), fallen leaves appear to be more important in the survival of the pathogen. In some pathosystems such as *S. azaleae* on evergreen azaleas, the fungus has a very long incubation period on leaves (up to 2 months), while in others (e.g., *S. musiva* on poplar) the disease is characterized by short incubation periods (1 to 2 weeks). This broad range in epidemiological behavior is not surprising in light of recently presented molecular evidence showing that the genus *Septoria* is polyphyletic (Feau *et al*., 2004; Verkley *et al*., 2004). This emphasizes the need to develop species-specific epidemiological information for members of the genus *Septoria*. Such information will be derived here for *S. albopunctata* on blueberry.

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CHAPTER 3

TEMPORAL PROGRESS OF SEPTORIA LEAF SPOT ON RABBITEYE BLUEBERRY[1]

[1]Ojiambo, P.S., and H. Scherm. 2004. To be submitted to *Plant Disease*.

Temporal Progress of Septoria Leaf Spot on Rabbiteye Blueberry

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ABSTRACT

Septoria leaf spot, caused by *Septoria albopunctata*, is an important disease on blueberry in the southeastern United States whose epidemiology is largely unknown. Disease severity and densities of rain-dispersed pycnidiospores were monitored from 2002 to 2004 in a planting of susceptible 'Premier' rabbiteye blueberry to characterize the temporal progress of the disease and determine the effect of inoculum dynamics and selected leaf attributes on disease development. Disease onset was observed between late April and mid-June, and this was followed by a rapid increase in severity until mid- to late September reaching a maximum of ~17 to 60 spots per leaf on average; thereafter, disease severity decreased until the end of the season, presumably due to abscission of severely infected leaves. A logistic model was fitted to disease severity data using nonlinear regression, and parameter estimates were used to compare the effects of leaf position, canopy location, and time of leaf emergence on disease progress. Based on this model, the highest absolute rate of disease increase and the highest upper asymptote were predicted for lower leaves on a shoot, leaves on shoots in the lower canopy, and leaves that emerged early in the season. Data collected with funnel spore samplers showed that pycnidiospores of *S. albopunctata* were present throughout most of the period from April through late October. Apart from spore densities being lowest early and late in the season, there were no consistent seasonal patterns or trends in the 3 years. Final disease severity on individual leaves was more strongly

correlated with cumulative spore densities available throughout the entire season (from leaf emergence to the end of the assessment period in November) than with cumulative spore densities during shorter periods around the time of leaf emergence; this suggests that leaves at all developmental stages can become infected by *S. albopunctata* season-long. Disease incidence on potted trap plants exposed to natural inoculum in the field during rain events in 2003 and 2004 was >70% irrespective of leaf developmental stage at the time of exposure. Taken together, the results of this study indicate inoculum of *S. albopunctata* is present throughout most of the growing season and that infection can occur season-long on leaves of any age.

INTRODUCTION

Septoria leaf spot, caused by *Septoria albopunctata*, is the most prevalent foliar disease of blueberry (*Vaccinium* spp.) in Georgia (Scherm *et al*., 2001) and in other southeastern states (Cline, 2002). Most southern highbush blueberry (*V. corymbusum*) cultivars and a number of rabbiteye blueberry (*V. ashei*) cultivars are highly susceptible to the disease. Symptoms appear as small circular leaf lesions with white to tan centers and purple margins. The optimum temperature range for pathogen growth and disease development is 24 to 28°C (Milholland, 1995). In Georgia, symptoms of Septoria leaf spot do not become widespread in the field until early to late summer when the crop has already been harvested (Ojiambo, unpublished).

With the increasing acreage and intensity of blueberry production, Septoria leaf spot is emerging as an important production constraint. Based on field studies in both rabbiteye and southern highbush blueberry cultivars, Ojiambo *et al*. (2005; Chapter 4) showed that high levels of the disease in the summer and fall can reduce flower bud set and return yield in the following year. Thus, there is a need to devise management guidelines for the disease in order to minimize

its yield-reducing effects. This requires knowledge of the epidemiology of the disease, but such information is presently lacking in the Septoria-blueberry pathosystem. For example, very limited information is available on the temporal progress of the disease or how leaf attributes such as leaf age or position on a shoot affect this process. Demaree and Wilcox (1947) reported that young leaves that are still expanding are more susceptible to infection by *S. albopunctata* than fully expanded, mature leaves, but this seems inconsistent with the rapid disease increase observed in the field in summer and early fall (Ojiambo and Scherm, 2005), which is well past the period of leaf expansion for the first flush of leaves. Pycnidiospores of *S. albopunctata* are thought to be produced on new leaf lesions throughout the season (Milholland, 1995), but the seasonal availability of inoculum and the influence of inoculum dynamics on disease progress have not been examined. As a result, current guidelines for Septoria leaf spot management are highly empirical, and where fungicides have been used for disease control, their application has been based on a calendar approach (Brannen *et al*., 2001, 2002, 2003; Cline, 2002).

Based on the above considerations, the objectives of this study were to 1) characterize the temporal progress of Septoria leaf spot in the field and determine the effect of selected leaf attributes on disease progression; 2) monitor the seasonal availability of inoculum using spore samplers; and 3) establish whether leaves are infected season-long or only during the short period of leaf expansion.

MATERIALS AND METHODS

Field site. The study was carried out in an experimental blueberry planting at the University of Georgia Horticulture Farm near Athens from 2002 to 2004. The planting was established in 1988 and consisted of alternating rows of the rabbiteye blueberry cultivars

'Premier' and 'Climax'. Maintenance of the planting, including fertilization, pruning, and weed control, followed generally recommended practices (Austin, 1994). Supplemental overhead irrigation was applied when needed, primarily during the fruit maturation phase in the dry 2002 growing season. Plants remained untreated with fungicides throughout the 3-year period. Daily temperature and precipitation records were obtained from a Georgia Automated Environmental Monitoring Network weather station located about 500 m from the planting (Hoogenboom and Gresham, 1997).

Evaluation of disease progress. On 'Premier', which is highly susceptible to Septoria leaf spot (Scherm *et al*., 2003), 50 shoots were selected arbitrarily from 12 bushes during the period of leaf expansion (NeSmith *et al*., 1998) of the first (spring) flush of leaves on 22 April, 24 March, and 27 March in 2002, 2003, and 2004, respectively. Each shoot was tagged at its base, and the distance from the base to the ground was measured to the nearest cm to provide a measure of leaf location in the canopy. Each leaf present or subsequently developing on the distal 20 cm of the selected shoots was tracked individually during each year of the study. No new leaves emerged on selected shoots after 31 May 2002 and 14 May 2004, and a total of 410 and 416 leaves was monitored in these 2 years. In 2003, no new leaves emerged on selected shoots after 13 June, but 20 additional shoots with later-emerging leaves were tagged after harvest in August (second flush), resulting in a total population of 663 leaves being monitored that year. Each leaf was assigned a number between 0 and 1 to indicate its position on the shoot, whereby the extreme values of 0 and 1 corresponded to the lowest and uppermost leaves, respectively.

Leaves were assessed for Septoria leaf spot severity beginning immediately after shoots were tagged in early spring, expressing disease severity as number of spots per leaf. For the first

6 to 8 weeks, disease severity was assessed at 3- to 5-day intervals, after which assessments continued every 7 to 10 days for the remainder of the season. The last disease assessment was made on 15 November 2002, 8 November 2003, and 1 November 2004, after which leaf spot counts became impossible due to necrosis associated with natural leaf senescence. Throughout the entire study period, Septoria leaf spot was the only noticeable foliar disease on 'Premier'.

To obtain an estimate of leaf developmental stage, the length of each leaf on selected shoots was measured on each disease assessment date for about 2 months after leaf emergence, by this time leaf expansion had ceased. Measurements were made to the nearest millimeter using a ruler.

Inoculum dynamics. *Septoria albopunctata* does not have a known sexual stage producing ascospores (Milholland, 1995). Seasonal availability of asexual pycnidiospores was monitored using funnel samplers similar to those described by Bertrand and English (1976). Each year, a total of six traps were installed in the planting to collect rain splash and runoff from the shoots and branches. Funnels measuring 15 cm in diameter were directly attached to 1.0-liter plastic bottles containing 10 ml of 5% $CuSO_4$ solution. Each assembly was partially buried in the ground underneath a blueberry plant close to the crown where it was firmly secured. The traps were positioned such that the height of the funnel above the ground was about 15 cm. After each rain event, the bottles were emptied by filtering the spore suspension through two layers of cheesecloth into a graduated cylinder to record the amount of rain water collected. A 50-ml subsample of the spore suspension was centrifuged at 3000 min^{-1} for 10 min, concentrated five-fold, and agitated for 5 sec using a Vortex mixer. The number of pycnidiospores per milliliter in each sample was determined based on an average of four hemacytometer counts and converted to

number per unit area based (spore density) on the amount of rain collected and the area of the funnel.

Descriptive analyses. Disease severity values were used to calculate the area under the disease progress curve (AUDPC) for each leaf (Campbell and Madden, 1990). Based on data from individual leaves, the PROC CORR procedure of SAS (v. 8.2; SAS Institute, Inc., Cary, NC) was used to assess whether significant ($\alpha = 0.05$) associations existed among disease variables (final disease severity and AUDPC) and leaf attributes (leaf position on the shoot and shoot height above the ground). Since six correlation coefficients were assessed for significance simultaneously each year (Table 1), the Bonferroni correction (Legendre and Legendre, 1998) was used to obtain an overall significance level of $P = 0.008$ ($\alpha/6$) for each test.

Disease progress curves. Fitting of disease progress curves was based on disease progress data corrected for leaf abscission; that is, disease severity of a leaf that abscised mid-season was carried along until the end of the season in these calculations. This correction was necessary because premature abscission of severely diseased leaves resulted in a drop in average disease severity toward the end of the season (Fig. 3.1).

Individual leaves were categorized into groups to fit disease progress curves for leaves with similar attributes. For leaf position, leaves were assigned to three categories depending on their location on the shoot, with position values (defined above) of 0 to 0.33, 0.34 to 0.66, and 0.67 to 1.0 assigned to lower, intermediate, and upper leaf position categories, respectively. Depending on the height of the shoot above the ground, three categories were constructed corresponding to distances from the ground to the base of the shoot of <70, 70 to 110, and >110 cm, respectively. Three categories related to the date of leaf emergence were also assigned. The first category (early first flush and fully expanded) consisted of leaves that already had attained

95% of their final lengths on the first disease assessment date. The second category (early first flush, not fully expanded) was made up of leaves of the first flush whose lengths were <95% of their final leaf lengths on the first assessment date. The third category consisted of leaves that emerged late during the flush after mid-May.

Separate disease progress curves were fitted for each category of leaf position, shoot height, and leaf emergence date each year. A nonlinear logistic model of the form $y = K / (1+ \exp[- r_L(t - m)]$ was fitted to the disease progress data using Genstat version 5.1 (Payne *et al*., 2002), where y = disease severity (number of spots per leaf); K = upper asymptote of the disease progress curve; r_L = logistic rate of disease increase; and m = parameter indicating the location of the inflection point of the curve in relation to time, t, in days. The appropriateness of the model in describing disease progress was assessed by correlation analysis of observed versus predicted y-values. The product $r_L K$, an overall (mean) measure of the absolute rate of disease increase (Campbell and Madden, 1990), was calculated and used to compare disease progress among the different leaf categories.

Temporal infection windows. To determine empirically whether leaves become infected season-long or primarily during the period of leaf expansion when tissues are most susceptible (Demaree and Wilcox, 1947), three potential infection windows were constructed for each leaf: 1) a 2-week period centered on the date of full leaf expansion (referred to as ±1-week window henceforth), whereby the date of full leaf expansion is defined here as the assessment date when the leaf attained 95% of its final length; 2) a 2-week period beginning at the date of full expansion; and 3) the entire season from full leaf expansion to the last disease assessment (Fig. 3.2). Cumulative spore densities during the three windows were computed for each leaf based on the spore sampling study described above. For each year, correlation analyses were

conducted to determine the association between final disease severity on individual leaves and cumulative spore density potentially available in each window. All statistical analyses were performed using SAS.

Trap plants. Two- or three-year-old southern highbush blueberry plants of cultivar 'Star', which is highly susceptible to Septoria leaf spot (Scherm *et al*., 2003), were used to provide complementary data on disease incidence in relation to leaf developmental stage. Plants were grown in a sheltered area on the Athens campus of the University of Georgia in 3.8-liter containers in a potting mix containing sand and pine bark in a 1:3 vol/vol ratio. Shortly before exposure of the plants in the blueberry planting described above, nodes having leaves at three developmental stages were tagged; the stages were: I = leaves recently emerged and not completely unfolded, II = young leaves completely unfolded but not waxy, and III = old leaves with waxy cuticles. Trap plants were exposed to natural field inoculum on three to four occasions between 17 April and 20 September in 2003 and 2004, whereby each exposure period encompassed two to five plants with 10 to 26 leaves per stage. Exposure was timed to coincide with predicted rainfall, and exposure periods ranged from 2 to 3 days with 11 to 50 mm of rain. Trap plants were subsequently removed from the field and placed in a greenhouse (25/18 ±5°C average day/night temperatures) where their foliage was kept dry. Each exposure period included one unexposed control plant transferred into the greenhouse at the time when the exposed plants were returned from the field. Symptom development was monitored on the tagged nodes for about 1 month, and the incidence of infected leaves for each of the three developmental stages was determined for each exposure period. Control plants that were not exposed to field inoculum were also monitored for symptom development on leaves at the three growth stages.

RESULTS

Descriptive analyses. Significant correlations were observed among final disease severity, AUDPC, and leaf attributes (Table 3.1). In all 3 years, disease severity was negatively correlated with shoot height, indicating that leaves on shoots in the lower part of the canopy had higher levels of Septoria leaf spot. Disease severity was also negatively correlated with leaf position (i.e., lower leaves were more severely infected), but this correlation was significant only in 2002 and 2004. AUDPC was highly correlated with final severity; not surprisingly, therefore, correlations between AUDPC and leaf attributes were similar to those between final disease severity and leaf attributes (Table 3.1).

Disease progress curves. Disease progress followed a similar pattern in all 3 years, although the time of disease onset and final disease severity differed among years (Fig. 3.1). In 2002, symptoms were first observed at the end of April; this was followed by an approximately exponential increase in disease severity up to late September, reaching a maximum of ~60 spots per leaf on average (Fig. 3.2A). Thereafter, disease severity decreased toward the end of the disease assessment period, presumably due to abscission of severely infected leaves. In 2003 and 2004, disease onset occurred nearly 2 months later (Fig. 3.2B and C), reaching a maximum of about 60 and 17 spots per leaf in 2003 and 2004, respectively. In the latter 2 years, disease severity increased rapidly between late July and late September, compared with slower disease increase in 2002 during the same period. Progress of Septoria leaf spot on the second flush of leaves tagged after harvest in 2003 was negligible (Fig. 3.1B). When disease progress curves were corrected for defoliation, disease severity increased sigmoidally and reached an average maximum of ~24 to 80 spots per leaf at the end of the season (Fig. 3.1).

The logistic model provided a good description of the corrected disease progress curves within each class of leaf position, shoot height, and time of leaf emergence, except for the second flush of leaves (included only in 2003) where the optimization process did not converge for the 2003 data set (Table 3.2, Fig. 3.3). Across the 3 years, the highest estimated values of the absolute rates of disease progress ($r_L K$) and of the upper asymptote K were observed on leaves in intermediate positions on the shoots and on shoots located in the lower canopy. In 2002 and 2004, $r_L K$ and K were highest on leaves of the early first flush that were already fully expanded at the first disease assessment date, while in 2003 estimated values of both parameters were higher on early first-flush leaves that were still expanding at the beginning of the assessment period (Table 3.2, Fig. 3.3).

Inoculum dynamics. Pycnidiospores of *S. albopunctata* were present in rainwater splash and runoff from blueberry bushes in all 3 years throughout most of the period from April through late October. However, relative spore densities varied from year to year, and were highest in 2003 and lowest in 2004 (Fig. 3.4A, B, and C). Apart from spore densities being lowest early and late in the season, there were no consistent seasonal patterns or trends across the 3 years. Since funnel traps were used for spore monitoring, spore dispersal events were always associated with rain events; however, not all rain events resulted in the dissemination of spores, nor was there a clear association between spore density and precipitation amount (Fig 3.4D, E, and F).

Temporal infection windows. Final severity of Septoria leaf spot and cumulative densities of *S. albopunctata* pycnidiospores within the three potential infection windows were positively associated, albeit with different levels of statistical significance (Table 3.3). Consistently across the 3 years, correlation coefficients were highest and statistically significant

($P \leq 0.05$) for the entire-season window. In 2003, the association involving cumulative spore densities during the 2-week window after full leaf expansion was also statistically significant, but the level of significance was weaker and the correlation coefficient considerably smaller than that for the entire-season window.

Trap plants. Incidence of Septoria leaf spot on trap plants exposed to natural inoculum in the field was high, with values ranging from 70 to 100% (Table 3.4). Disease symptoms were observed on all leaves on the tagged nodes irrespective of their developmental stage at the time of exposure. Symptoms first appeared between 20 and 32 days after exposure. No symptoms were observed on control plants that not exposed to inoculum in the field.

DISCUSSION

This report provides the first quantitative description of the temporal progress of Septoria leaf spot of blueberry and how leaf attributes such as position on the shoot and location of shoots above the ground influence disease development. Progress of Septoria leaf spot over time was typical of a polycyclic epidemic, and leaves in intermediate positions on a shoot, leaves on shoots in the lower part of the canopy, and those that emerged early in the season were more severely infected than upper or late-emerging leaves. A drop in disease severity was observed towards the end of the season, presumably due to the abscission of severely diseased leaves. Rain-dispersed pycnidiospores were present throughout the season, and based on the analysis of potential infection windows combined with the results of the trap plant experiments, we conclude that leaves at all developmental stages can become infected by *S. albopunctata* season-long.

The earlier disease onset in 2002 (late April) compared with 2003 and 2004 (mid-June) may have been associated with warmer spring temperatures. For example, the monthly mean temperature for April was 18.1°C in 2002 but only 15.5°C in 2003 and 16.1°C in 2004. In contrast, the more rapid disease progression during 2003 was likely due to increased summer precipitation. The total amount of rain recorded between July and September was 482 mm in 2003 compared with 236 mm in 2002 and 130 mm in 2004. The low levels of disease observed in 2004 may have been due to low levels of disseminated inoculum. In addition, the 2004 season was generally dry apart from four late peaks of rainfall that were associated with three hurricanes that struck the southeastern coast during that period. The late-season decrease in disease severity was likely due to the abscission of severely infected leaves (Chapter 4; Ojiambo and Scherm, 2005).

Analysis of disease progress curves showed that leaves in intermediate positions on a shoot, leaves on shoots in the lower part of the canopy, and those that emerged early in the season were more severely infected by *S. albopunctata*. The high disease severity on leaves in intermediate positions on the shoot compared with those in upper or lower position may be due to the greater leaf area available for infection. Leaves in the former category typically were larger in size than those in the latter two groups (*data not shown*).

Leaves located on shoots in the lower part of the canopy consistently had higher levels of disease than leaves on shoots in the upper canopy. The higher disease severity in lower parts of the canopy could be related to more favorable surface moisture retention and cooler temperatures due to its protection from direct sunlight, creating a microclimate more conducive for infection. Since *S. albopunctata* can overwinter in fallen leaves infected during the previous season (Milholland, 1995), the proximity of the lower canopy to this inoculum source likely contributes

further to the higher levels of disease severity observed in the lower canopy. Furthermore, studies in other Septoria pathosystems have documented the inhibition spore germination in conditions of high light intensity (Elmer and Ferrandino, 1995; Ferrandino and Elmer, 1996; Hood *et al*., 2002); thus, it is possible that the lower disease levels observed in the upper canopy where radiation is greater may have been due to direct inhibition of *S. albopunctata*. In addition, upper leaves are probably not exposed to much of the secondary inoculum originating from leaf or stem lesions and carried downward by rain, similar to what has been documented for *Phomopsis amygdali* on peach (Lalancette and Robison, 2001) and *Septoria* on wheat (Lovell *et al*., 2004). Regardless of the mechanism, the higher disease levels on leaves in the lower canopy underscore the importance of adequate application of fungicides to lower parts of the plant. In addition, regular pruning of the bushes may reduce disease severity by altering the microclimate. Additional control may be achieved through sanitation by destruction of leaf litter on the ground, although this needs to be investigated further, particularly since the relative importance of leaf residue vs. stem lesions in providing inoculum in the Septoria-blueberry pathosystem is still unclear.

Given that inoculum of *S. albopunctata* was present season-long, higher disease levels on early-emerging leaves could simply be due to the fact that these leaves were exposed to inoculum for a longer period than later-emerging leaves. In addition, there was the potential for more secondary cycles of infection given the longer presence of the former leaves. Our spore sampling data did not indicate the presence of large spore peaks early in the season, showing that later-emerging leaves did not simply escape inoculum exposure. Given a reported temperature optimum of 24 to 28^{o}C for *S. albopunctata* (Milholland, 1995) and the erratic distribution of rainfall during the season in our study, it is also unlikely that environmental conditions were

consistently more favorable for infection on leaves that emerged early (when temperatures were considerably cooler than the reported optimum). Relative to disease management, the higher levels of Septoria leaf spot on early-emerging leaves indicates the need for implementing early-season control tactics for effective management of the disease. Current recommendations for fungicidal control focus primarily on summer and fall applications after harvest of the crop (Brannen *et al*., 2001, 2002, 2003).

The lack of a strong and significant association between final disease severity and cumulative spore densities in the ±1-week and 2-week infection windows relative to the time of full leaf expansion suggests that infection by *S. albopunctata* is not restricted to young leaves in a relatively short period during leaf expansion. Instead, the consistent and significant association between final disease severity and cumulative spore densities in the entire-season window shows that leaves can become infected continuously throughout the season. This conclusion is supported by results of the trap plant experiments, in which leaves of all developmental stages became infected by *S. albopunctata* during all exposure periods. Further, the polycyclic shape of the disease progress curves observed in the field suggests that multiple cycles of infection occur on leaves season-long. Relative to disease management, this indicates that multiple fungicide applications are needed for adequate disease control, and that these applications should begin early when symptoms first appear on the first flush of leaves.

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Table 3.1. Pearson's correlation coefficients among Septoria leaf spot-related variables and leaf attributes on 'Premier' rabbiteye blueberry

Year	Variable 1	Variable 2[a]		
		Disease severity[b]	AUDPC[c]	Shoot height[d]
2002	AUDPC	0.802*	-	-
	Shoot height	–0.315*	–0.344*	-
	Leaf position[e]	–0.170*	–0.184*	0.003
2003	AUDPC	0.861*	-	-
	Shoot height	–0.413*	–0.419*	-
	Leaf position	–0.010	–0.074	0.006
2004	AUDPC	0.986*	-	-
	Shoot height	–0.210*	–0.185*	-
	Leaf position	–0.162*	–0.173*	0.013

[a] Asterisks indicate significant correlation coefficients at $P = 0.008$. Sample size (n) = 410, 663, and 416 in 2002, 2003, and 2004, respectively, except for shoot height (n = 396, 633, and 411, respectively).

[b] Number of spots per leaf on the last disease assessment date.

[c] Area under the disease progress curve (spot-days).

[d] Distance (cm) between the ground and the base of the tagged shoot segment.

[e] Leaf position on the tagged shoot, where 0 = lowest (oldest) and 1 = highest (youngest) leaf.

Table 3.2. Results of nonlinear regression analysis of temporal progress of Septoria leaf spot on 'Premier' rabbiteye blueberry obtained using the logistic model for different classes of leaf position, shoot height and leaf emergence

Year/variable	Rate (r_L)	S.E. for r_L	Maximum disease (K)	S.E. for K	Absolute rate (r_LK)	r^2[a]	Sample size (n)
2002							
Leaf position[b]							
Lower	0.0374	0.0015	88.6	1.730	3.31	0.997	97
Intermediate	0.0415	0.0023	116.8	3.140	4.85	0.996	155
Upper	0.0515	0.0032	61.8	1.560	3.18	0.995	154
Shoot height[c]							
<70 cm	0.0443	0.0019	137.7	2.570	6.10	0.996	89
70-110 cm	0.0407	0.0024	84.9	2.590	3.45	0.995	209
>110 cm	0.0361	0.0028	54.2	2.270	1.96	0.991	108
Leaf emergence[d]							
1st flush (EP)	0.0403	0.0021	112.6	2.840	4.54	0.993	239
1st flush (NE)	0.0460	0.0027	74.7	2.020	3.43	0.995	125
2nd flush	0.0396	0.0019	4.7	0.137	0.18	0.994	42
2003							
Leaf position							
Lower	0.0537	0.0027	34.6	0.528	1.85	0.995	174
Intermediate	0.0562	0.0023	66.1	0.775	3.71	0.996	242
Upper	0.0621	0.0029	41.7	0.512	2.59	0.996	244
Shoot height							
<70 cm	0.0546	0.0013	66.5	0.479	3.63	0.999	94
70-110 cm	0.0601	0.0022	52.7	0.521	3.16	0.997	395
>110 cm	0.0586	0.0046	30.5	0.656	1.78	0.993	171
Leaf emergence							
1st flush (EP)	0.0710	0.0024	41.7	0.328	2.96	0.998	106
1st flush (NE)	0.0607	0.0017	63.1	0.483	3.83	0.998	383
2nd flush	….[e]	….	….	….	….	….	171

2004							
Leaf position							
Lower	0.0900	0.0036	18.2	0.1640	1.64	0.995	125
Intermediate	0.0833	0.0013	44.8	0.1710	3.73	0.997	137
Upper	0.0671	0.0051	9.6	0.2170	0.64	0.995	154
Shoot height							
<70 cm	0.0807	0.0017	33.5	0.1740	2.70	0.992	139
70-110 cm	0.0818	0.0024	23.8	0.1700	1.95	0.997	133
>110 cm	0.0837	0.0041	14.1	0.1660	1.18	0.996	144
Leaf emergence							
1st flush (EP)	0.0838	0.0016	30.5	0.1430	2.55	0.993	174
1st flush (NE)	0.0827	0.0029	17.8	0.1540	1.47	0.996	130
2nd flush	0.0726	0.0054	8.6	0.1940	0.62	0.994	112

[a] Linear correlation coefficient between observed and predicted disease severity values.

[b] Leaves on shoot segments were assigned values between 0 and 1, with 0 to 0.33, 0.34 to 0.66, and 0.67 to 1.0 representing leaves in the lower, intermediate, and upper positions, respectively.

[c] Distance between the ground and the base of the tagged shoot segment.

[d] NP and NE refer to fully expanded and not fully expanded leaves, respectively, at the time of the first disease assessment in early spring. Leaves were considered fully expanded when they had attained 95% of their final length (Fig. 3.2).

[e] Optimization process failed to converge.

Table 3.3. Pearson's correlation coefficients between final Septoria leaf spot severity on leaves of 'Premier' rabbiteye blueberry and the cumulative density of *Septoria albopunctata* pycnidiospores collected in funnel samplers during different potential infection windows

Temporal window[a]	Correlation coefficient (*r*)	Sample size (*n*)	*P*-value
2002			
± 1 week	0.22	43	0.1522
+ 2 weeks	0.29	43	0.0634
Entire season	0.36	43	0.0229
2003			
± 1 week	0.04	403	0.4071
+ 2 weeks	0.12	611	0.0274
Entire season	0.44	611	<0.0001
2004			
± 1 week	0.09	181	0.1846
+ 2 weeks	0.11	243	0.0816
Entire season	0.21	243	0.0012

[a] Relative to time of full leaf expansion; leaves were considered fully expanded when they had attained 95% of their final length (Fig. 3.2). The entire-season window refers to the cumulative spore densities from the time of full leaf expansion until the end of the study period. Similarly, the 2-week window refers to spores collected during the first 2 weeks after full leaf expansion. The ±1-week temporal window represents spores collected during the period from 7 days prior to 7 days after leaves were fully expanded.

Table 3.4. Incidence of Septoria leaf spot of blueberry on leaves of potted 'Star' trap plants at different leaf developmental stages following exposure to natural inoculum of *Septoria albopunctata* in the field

Year/plant group	Date exposed	Date of first symptoms	Leaf stage[a]	Total no. of leaves[b]	No. of leaves infected	Disease incidence (%)
2003						
1	4/17/03	5/19/03	I	21	18	85.7
			II	24	22	91.6
			III	26	23	88.5
2	5/14/03	6/12/03	I	20	18	90.0
			II	24	23	100.0
			III	21	21	100.0
3	6/06/03	6/29/03	I	16	13	81.2
			II	18	17	94.4
			III	20	17	85.0
4	7/01/03	7/27/03	I	18	13	72.2
			II	19	16	84.2
			III	19	15	75.0
2004						
1	6/26/04	7/18/04	I	20	15	75.0
			II	23	17	73.9
			III	17	13	76.5
2	8/20/04	9/15/04	I	14	10	71.4
			II	12	9	75.0
			III	11	8	72.7
3	9/20/04	10/10/04	I	10	7	70.0
			II	11	8	72.7
			III	12	9	76.7

[a] I = leaves recently emerged and not completely unfolded, II = young leaves completely unfolded but not waxy, and III = old leaves with waxy cuticle.

[b] Number of leaves based on three shoots selected from three to four plants exposed on each date.

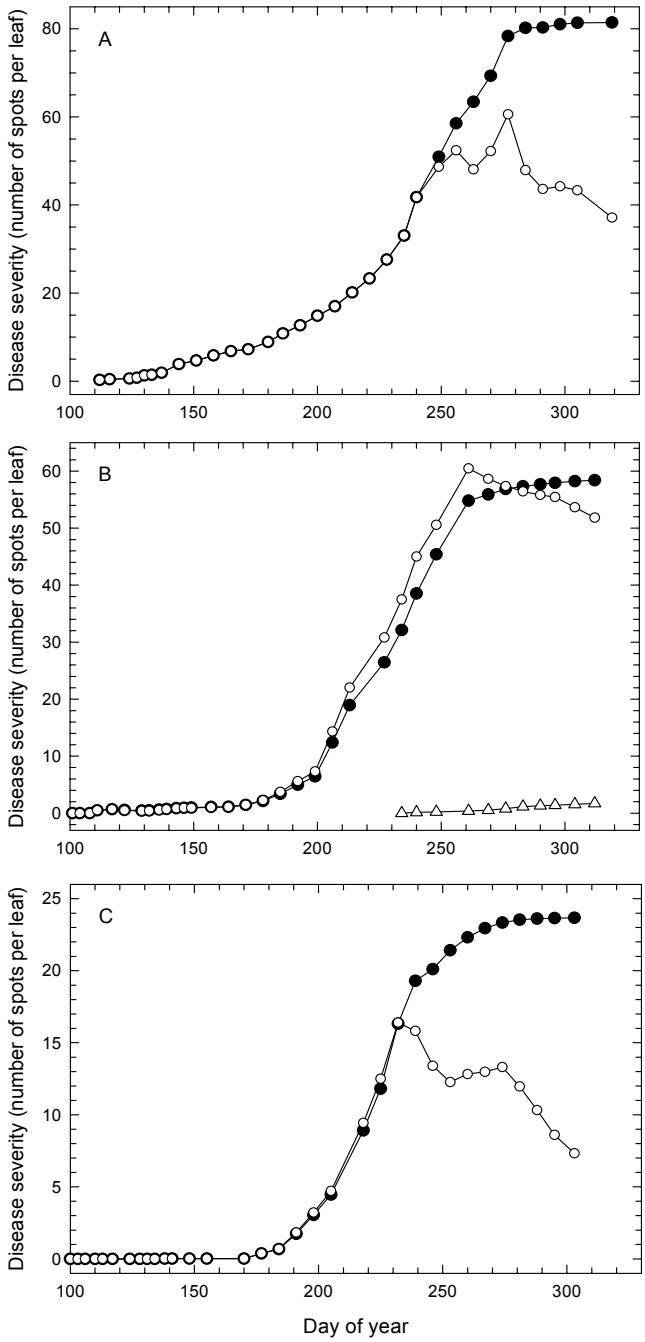

Fig. 3.1. Temporal progress of Septoria leaf spot on 'Premier' rabbiteye blueberry in 2002 (**A**), 2003 (**B**), and 2004 (**C**). The circles (● and ○) refer to disease progress on leaves emerging on shoots selected for assessment in early spring, while the triangles (Δ) correspond to leaves on late-emerging shoots selected for assessment after harvest in late summer (included in 2003 only). The open circles (○) show disease progress as observed in the field, including a drop in disease severity toward the end of the season due to leaf abscission. The solid circles show the same disease progress curves corrected for defoliation, i.e., disease severity of a leaf that abscised mid-season was carried along until the end of the season.

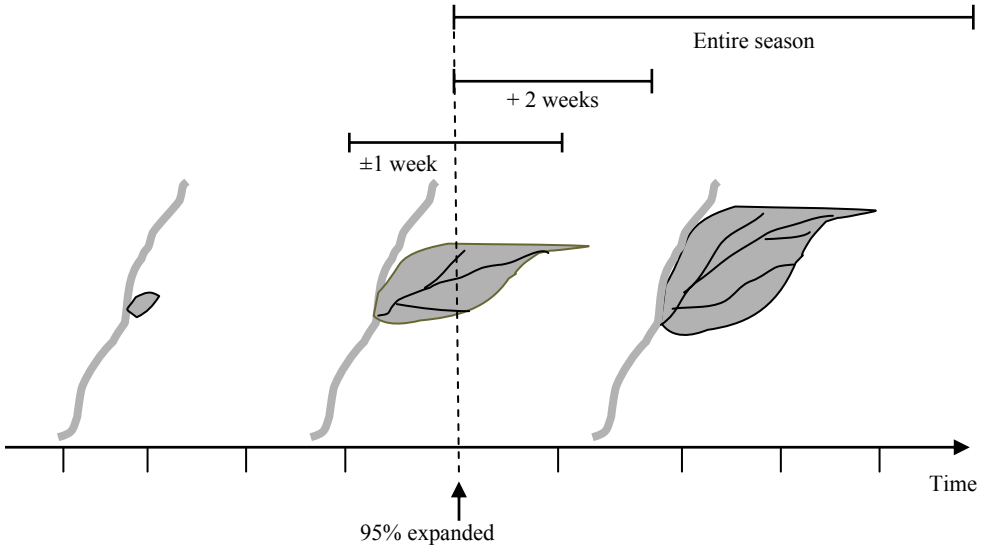

Fig. 3.2. Schematic depiction of the temporal windows used to summarize the cumulative densities of *Septoria albopunctata* pycnidiospores collected in funnel samplers based on relative times after full leaf expansion; leaves were considered fully expanded when they had attained 95% of their final length. The entire-season window refers to the cumulative spore densities from the time of full leaf expansion until the end of the study period. Similarly, the 2-week window refers to spore densities collected during the first 2 weeks after full leaf expansion. The ±1-week temporal window represents spore densities collected during the period from 7 days prior to 7 days after leaves were fully expanded.

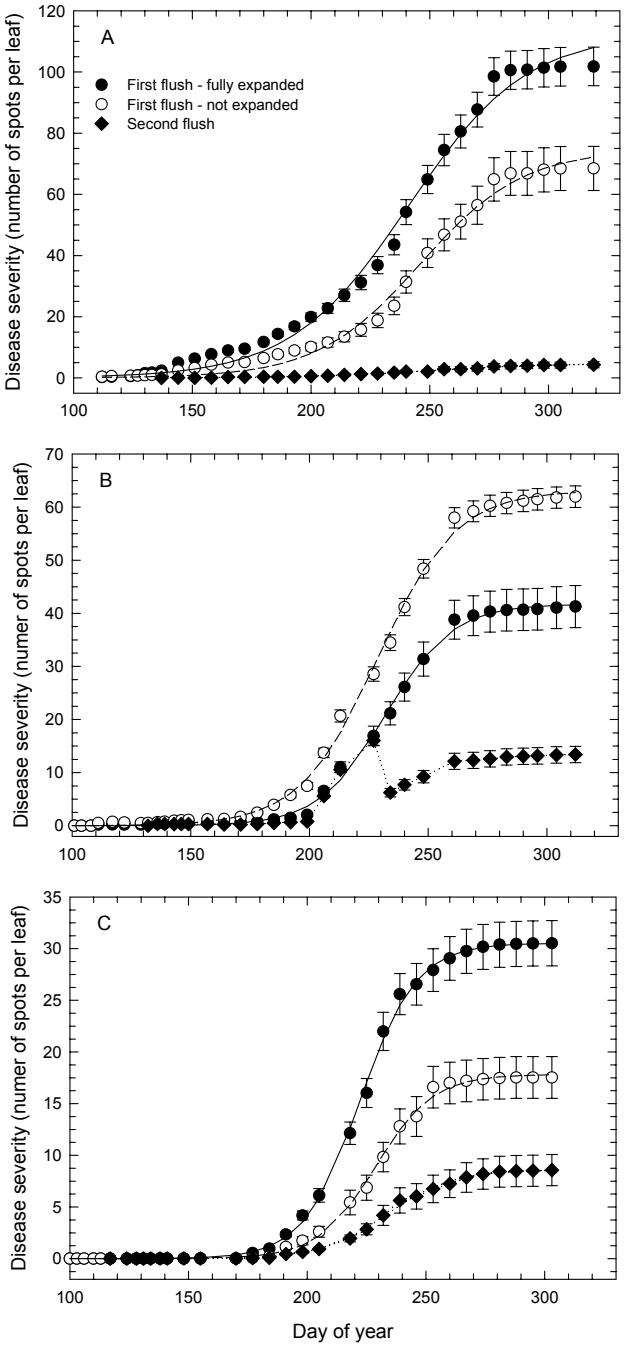

Fig. 3.3. Effect of time of leaf emergence on the temporal progress of Septoria leaf spot on 'Premier' rabbiteye blueberry in 2002 (**A**), 2003 (**B**), and 2004 (**C**). Predicted disease progress curves are based on the logistic model ($y = K / (1+ \exp[- r_L(t - m)])$. No regression line is shown for the second flush of leaves in **B** due to failure of the optimization process to converge.

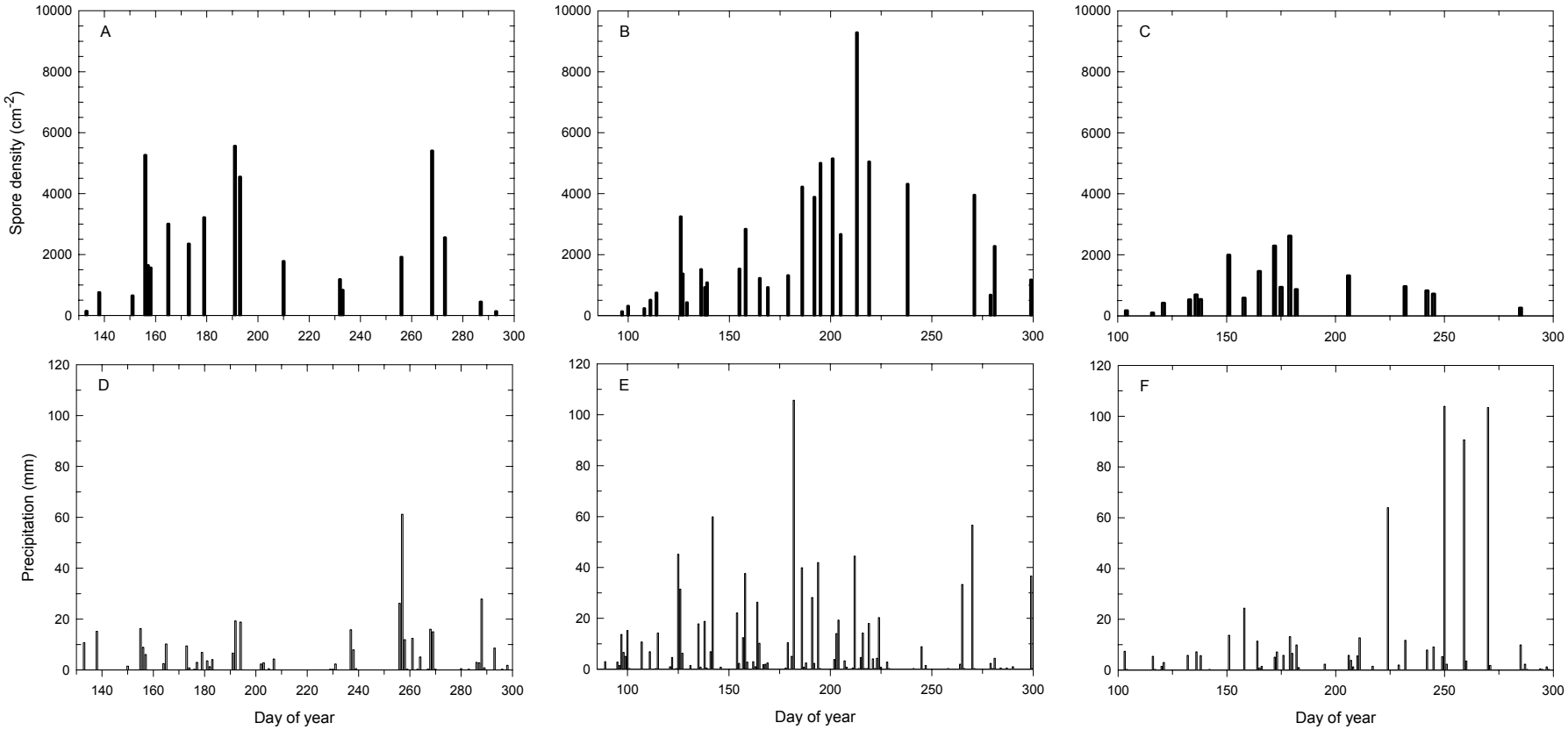

Fig. 3.4. Density of pycnidiospores of *Septoria albopunctata* captured in funnel samplers (**A**, **B**, and **C**) and daily precipitation (**D**, **E**, and **F**) at the experimental site in 2002 (**A** and **D**), 2003 (**B** and **E**), and 2004 (**C** and **F**).

CHAPTER 4

SURVIVAL ANALYSIS OF TIME TO ABSCISSION OF BLUEBERRY LEAVES AFFECTED BY SEPTORIA LEAF SPOT [1]

[1]Ojiambo, P.S., and Scherm. H. 2005. *Phytopathology* 95: (*in press*). Reprinted here with permission of publisher, 10/27/2004.

Survival Analysis of Time to Abscission of Blueberry Leaves Affected by Septoria Leaf Spot

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ABSTRACT

In the southeastern United States, Septoria leaf spot, caused by *Septoria albopunctata*, can result in premature defoliation of blueberry plants during summer and fall, thereby reducing yield potential for the following year. The effects of disease severity and plant attributes (leaf age and leaf location in the canopy) on the dynamics (timing and extent) of defoliation were quantified in field plots of 'Premier' rabbiteye blueberry in 2002 and 2003. In each year, 50 shoots were selected for assessment in early spring, and all leaves on these shoots (n = 410 and 542 in 2002 and 2003, respectively) were monitored individually for disease progress and time of abscission at 3- to 10-day intervals throughout the season. In both years, disease progress was characterized by a rapid increase in disease severity up to late September, followed by a decline toward the end of the assessment period in late November. Defoliation was sporadic up to late August, followed by more rapid and sustained levels of leaf loss. Abscission of severely infected leaves could explain the decline in disease severity toward the end of the season. Final disease severity (i.e., disease severity on the last assessment date before leaf drop) was highest for leaves that abscised early and lowest for leaves that had not abscised by the end of the assessment period. Survival analysis revealed that older leaves (located on the lower halves of shoots) and leaves with high levels of disease (≥ 5 spots/leaf at the time of fruit harvest in mid-June) abscised

significantly ($P < 0.0001$) earlier than younger leaves and leaves with lower disease severity. Relative to their respective reference groups, mean times to abscission were ~2 weeks shorter for the older leaf group and ~3 weeks shorter in the leaf group afflicted by high disease severity. When an accelerated failure time model was fitted to the data, the resulting parameter estimates indicated that each additional leaf spot present at harvest accelerated time to leaf abscission (expressed using late August as a starting point) by 1.9 and 4.5% in 2002 and 2003, respectively. Leaf location in upper or lower portions of the canopy had no significant effect on time to abscission ($P > 0.05$).

INTRODUCTION

Blueberry is the second most important fruit crop in Georgia and is currently grown on 3,220 ha statewide (Boatright and McKissick, 2003). About 90% of the area is planted to rabbiteye blueberry (*Vaccinium ashei*), with the remainder devoted to production of southern highbush blueberry (*V. corymbosum* interspecific hybrids) (Scherm and Krewer, 2003). Both species can be affected by foliar diseases, with disease susceptibility being strongly cultivar-dependent (Scherm *et al*., 2003). Among these foliar diseases, Septoria leaf spot, caused by *Septoria albopunctata*, is of particular concern to blueberry producers in Georgia and other southeastern states (Cline, 2002; Scherm *et al*., 2003). Symptoms are characterized by small circular leaf lesions with white to tan centers and purple margins. The optimum temperature for pathogen growth and disease development is 24 to 28°C (Milholland, 1995). In Georgia, foliar symptoms of Septoria leaf spot appear first around early May and then increase rapidly between June and September (Chapter 3).

When left uncontrolled, Septoria leaf spot can result in premature defoliation (henceforth referred to simply as defoliation) in late summer or early fall (Brannen, *et al*., 2002; Brannen *et al*., 2003). In blueberry, it is advantageous to retain leaves for as long as possible during the fall to enhance the ability of the plants to set flower buds (Darnell, 1991). Indeed, in field experiments involving mechanical leaf removal treatments, Lyrene (1992) demonstrated that early fall defoliation of rabbiteye blueberry resulted in a significantly lower percentage of nodes that produced flower buds the following spring. Similar results were obtained with southern highbush blueberry (Williamson and Miller, 2002). The reduction in flower bud set associated with defoliation may be due to the elimination of photoreceptors on removed leaves and/or a lowering of carbohydrate reserves during critical periods in the fall (Lyrene, 1992).

The effect of Septoria leaf spot-induced (as opposed to mechanical) fall defoliation on reproductive development of blueberry has not been studied previously. Thus, there is a need for quantitative information on the dynamics of defoliation and how it relates to disease progress. Studies with mechanical leaf removal showed that both the extent and timing of defoliation affected return yield (Lyrene, 1992; Williamson and Miller, 2002) and that defoliation is affected by leaf attributes such as age (older leaves abscise earlier) and leaf location in the canopy (leaf drop on shoots in the lower canopy of the bush occurs earlier) (Ojiambo *et al*., 2002; Scherm *et al*., 2003). Based on these considerations, the goal of this study was to model the timing and extent of abscission of blueberry leaves in relation to Septoria leaf spot severity, leaf age, and leaf location in the canopy in order to provide estimates of the probability of leaf loss at given times during the growing season.

MATERIALS AND METHODS

Field site and data collection. The study was carried out in an experimental blueberry planting at the University of Georgia Horticulture Farm near Athens in 2002 and 2003. The planting was established in 1988 and consisted of alternating rows of *V. ashei* cultivars 'Premier' and 'Climax'. Maintenance of the planting, including fertilization, pruning, and weed control, followed commercially recommended practices (Austin, 1994). Supplemental overhead irrigation was applied as needed, primarily during the fruit maturation phase in the dry 2002 growing season. No fungicides were applied during the 2-year study period. Records of daily air temperature and amounts of precipitation were obtained from an on-site electronic weather station that is part of the Georgia Automated Environmental Monitoring Network (Hoogenboom and Gresham, 1997).

On 'Premier', which is highly susceptible to Septoria leaf spot (Scherm *et al*., 2003) but not to other foliar diseases, 50 shoots were selected arbitrarily from 12 bushes during the period of leaf expansion (NeSmith *et al*., 1998) on 22 April and 24 March in 2002 and 2003, respectively. Each shoot was tagged at its base, and its position above the ground was measured to the nearest cm to provide a measure of leaf location in the canopy. Each leaf present on the distal 20 cm of these shoots was assigned a unique number for tracking during the respective year of the study. New leaves that emerged on the selected shoots as a result of shoot growth as the season progressed were tracked similarly. No new leaves emerged on selected shoots after 31 May 2002 and 13 June 2003, resulting in a population of 410 and 542 leaves that were monitored in 2002 and 2003, respectively. A distinction was made between older leaves (on the lower halves of the shoot segments) and younger leaves (those on the upper halves of the shoots), to which values of 0 and 1 were assigned, respectively.

Leaves were assessed individually for severity of Septoria leaf spot beginning immediately after shoots were tagged in early spring, whereby disease severity was expressed as number of spots per leaf. Disease severity was assessed at 3- to 5-day intervals for the first 6 to 8 weeks and every 7 to 10 days thereafter for the remainder of the season. The last disease assessment was made on 15 November 2002 and 8 November 2003, after which necrosis associated with natural leaf senescence made leaf spot counts impossible. Leaf abscission (described below) was monitored for another 3 weeks after the last disease assessment date.

The numbering system for tracking individual leaves enabled us to determine the time of abscission of each leaf based on the absence of that leaf from the node on which it was present on the previous assessment date. However, since assessment dates were 3 to 10 days apart, it could not be determined exactly on which day leaf abscission occurred; instead, it was assumed that the time of abscission for a leaf that dropped between two successive assessment dates was at the mid-point of the two assessment dates. Based on this estimate of time of abscission, time to abscission, *T*, was calculated for each leaf using 23 August 2002 (day of the year 235) or 28 August 2003 (day 240) as starting dates. These dates were chosen because they corresponded to the assessment dates that marked the transition from negligible, sporadic leaf loss to the onset of more sustained levels of defoliation (Fig. 1). Any defoliation that occurred prior to these dates, mainly due to factors other than disease, was not considered in the analysis.

Descriptive analysis. For each year separately, monitored leaves were assigned to four classes based on their *T* values. In 2002, three of the groups corresponded to *T* values of < 50, 50 to 80, and > 80 days after 23 August, while the fourth group was comprised of leaves that had not abscised by the end of the assessment period. In 2003, groups were defined similarly except that the first three groups had *T* values of < 40, 40 to 70, and > 70 days after 28 August.

Different classes were used in the 2 years due to differences in the dynamics of defoliation (Fig. 1). For each class, the distribution of final disease severity values among all the leaves within that class was examined using box-whisker plots generated with the UNIVARIATE procedure in SAS (v. 8.2; SAS Institute, Inc., Cary, NC), whereby final disease severity was defined as the number of spots per leaf on the disease assessment date prior to leaf drop. Separately for the 2 years, differences among the four classes in the distribution of final disease severity values were tested for significance using the Kruskal-Wallis test (Stokes *et al*., 1995), a non-parametric test of the null hypothesis that the distribution of a response is the same in multiple groups.

Survival analysis concepts. Data that describe time to an event (e.g., time to leaf abscission) are generically referred to as survival data or failure time data. Conventional statistical techniques such as linear regression analysis are inappropriate for such data because complete knowledge of survival times may not be available due to censoring, most commonly because the period of observation ends before all individuals experience the event of interest. Furthermore, survival times often do not follow a normal distribution (Lawless, 2003). In this study, since the data set contained censored observations (i.e., leaves that had not abscised by the end of the assessment period), and since time to leaf abscission, the dependent variable, is readily interpreted as a "survival time" (Dungan et al, 2003; Scherm and Ojiambo, 2004; Zens and Peart, 2003), survival analysis techniques were used to describe and model the data. Central to this analysis is the determination of the leaf survival distribution function $S(t) = Pr(T \geq t)$ which gives the probability of observing a survival time T larger than or equal to some value t.

A fundamental assumption of survival analysis is that observations for different individuals are statistically independent. This was a potential concern in the present study where times to abscission of leaves located on the same shoot could have been correlated. To test for

the validity of the independence assumption, we used the ARIMA procedure in SAS to determine whether times to abscission of leaves within shoots were autocorrelated. Only two and five of the 50 shoots included in 2002 and 2003, respectively, showed significant ($P < 0.05$) autocorrelation patterns (*data not shown*), suggesting that observations from different leaves on the same shoot were independent.

Comparison of survival distribution functions among groups of leaves. Binary variables were constructed for initial disease severity at harvest (< 5 or ≥ 5 spots per leaf on 14 June and 20 June in 2002 and 2003 [days 165 and 171], respectively, marking the time when ~50% of the fruit had been harvested), leaf age (older or younger leaves on the shoot), and leaf location in the canopy (≤ 80 or >80 cm, corresponding to above-ground shoot position in lower and upper portions of the canopy, respectively). For each leaf group defined by these binary variables, leaf survival distribution functions were obtained using the Kaplan-Meier estimation within the LIFETEST procedure in SAS (Allison, 1997). Differences between leaf groups were compared using the log-rank test (Harrell, 2001).

Modeling leaf survival. The accelerated failure time (AFT) model (Allison, 1997; Scherm and Ojiambo, 2004) was used to parameterize the effects of initial disease severity at harvest (*DS*, expressed as number of spots per leaf), leaf age (*LA*, a binary variable as defined above), and leaf location in the canopy (*LL*, in cm above-ground) on leaf survival. This modeling approach was selected after initial analyses indicated that the assumptions of the proportional hazards model (Allison, 1997), an alternative model for survival times, were not satisfied for the 2003 data set. Technical details of these two modeling approaches have been reviewed by Scherm and Ojiambo (2004) and are discussed extensively by Allison (1997).

The AFT model was of the form $\log_e T = \beta_0 + \beta_1 DS + \beta_2 LA + \beta_3 LL + \sigma\varepsilon$, where ε is a random error term and β_0, β_1, β_2, β_3, and σ are parameters to be estimated. The model was implemented with the SAS procedure LIFEREG using the Weibull model to describe the underlying survival time distribution. This choice of probability distribution was based on comparing the log-likelihoods for the fitted survival time distribution models (Table 4.1). The Weibull model consistently had the lowest log-likelihoods in the 2 years, and lower absolute values of log-likelihoods correspond to a better model fit (Allison, 1997). Final AFT model parameter estimates were obtained by dropping non-significant terms ($P > 0.05$) and refitting the model.

RESULTS

Disease progress and abscission of individual leaves. In 2002, symptoms of Septoria leaf spot were first observed at the end of April, followed by an exponential increase in disease severity up to late September (Fig. 4.1A). Thereafter, disease severity decreased until the end of the assessment period, presumably because of abscission of severely infected leaves. In 2003, onset of disease occurred nearly 2 months later than in the previous year (Fig. 4.1B), yet the time when the disease progress curve peaked and the average disease severity at that time (about 60 spots per leaf) were similar for the 2 years. Thus, after an initial delay, the disease progressed more rapidly in 2003 than in 2002.

The earlier disease onset in 2002 may have been associated with warmer springtime temperatures (Fig. 4.2). For example, the monthly mean temperature for April was 18.1°C in 2002 but only 15.5°C in 2003. In contrast, the more rapid disease progression during the second

year was likely due to increased precipitation. The total amount of rain recorded between July and September was 482 mm in 2003 compared with 236 mm in 2002.

In 2002, very little defoliation was observed before the end of August (Fig. 4.1A). By the end of the first week of September, defoliation exceeded 10%, and by mid-November, about one-third of all leaves had abscised. This was followed by a steep increase in defoliation until early December, at which time >80% of the leaves had dropped. In 2003, about 15% of the leaves abscised before the end of May, followed by no additional defoliation until late August (Fig. 4.1B). This early leaf loss in the spring was unusual and followed a blossom blight epidemic (caused by *Botrytis cinerea*), whereby dehiscent, infected corollas, upon landing on leaf surfaces, incited large necrotic areas, ultimately leading to abscission of affected leaves. Following a period of absence of defoliation during the summer, leaf abscission increased steadily starting in early September and reached >80% by the end of the assessment period in late November.

Distribution of time to leaf abscission. In both years, final disease severity was highest for leaves that abscised early (i.e., had the shortest *T* values) and lowest for leaves that had not abscised by the end of the assessment period (Fig. 4.3). Leaves with intermediate *T* values also had intermediate levels of disease. Based on the Kruskal-Wallis test, the distributions of final disease severity values among the four defoliation time classes were different as indicated by highly significant ($P < 0.0001$, df = 3) Chi-Square values of 217.9 and 210.7 in 2002 and 2003, respectively.

Comparison of survival distribution functions among groups of leaves. In both years, disease severity at harvest and leaf age had highly significant ($P < 0.0001$) effects on survival of individual leaves, whereas leaf location in the canopy was marginally significant ($P =$

0.0347) in 2002 but not significant ($P = 0.2105$) in 2003 (Table 4.2). For the two disease severity groups, the difference in leaf survival was evident from the estimated survival functions (Fig. 4.4). Each point on the survival function represents the probability that a leaf will survive (i.e., not abscise) until a given point in time. For example, in 2003 the estimated probability for a leaf to survive for 50 days after 28 August was 0.70 and 0.42 for leaves in the low (< 5 spots/leaf) and high (≥ 5 spots/leaf) disease severity groups, respectively (Fig. 4.4B). At the end of the assessment period, survival was zero for leaves with ≥ 5 spots at harvest compared with a survival probability of 0.20 for leaves having < 5 spots. In both years, mean survival times of leaves with low disease at harvest were about 3 weeks longer than those of leaves with a higher level of disease (Table 4.2).

Modeling leaf survival. The AFT model described the data well, with log-likelihood ratios of –282.3 and –455.6 in 2002 and 2003, respectively (Table 4.1). The parameter estimates and associated test statistics (Table 4.3) revealed that the effects of both disease severity at harvest (expressed as the number of spots per leaf) and leaf age on risk of leaf abscission was highly significant ($P < 0.01$) in the 2 years. Parameter estimates for leaf location in the canopy were not included in the final AFT model due to the non-significant ($P > 0.05$) effect of this variable in both years.

Since the dependent variable in the AFT model is $\log_e T$, the parameter estimate for disease severity, β_1, can be used to calculate the relative change in survival time for each additional leaf spot using the formula $100\% \times (e^{\beta_1} - 1)$ (Allison, 1997). Based on this transformation, each additional spot present at harvest accelerated the time to defoliation by 1.9 and 4.5% in 2002 and 2003, respectively. Similarly, relative differences in time to defoliation between the two leaf age groups can be computed based on the estimate for β_2; this calculation

indicated that older leaves defoliated 13.1 and 40.7% earlier than younger leaves in 2002 and 2003, respectively.

DISCUSSION

This study provides the first quantitative description of the dynamics of premature defoliation of blueberry induced by Septoria leaf spot. Leaves with high disease severity and/or older leaves abscised earlier than those having low disease severity and/or younger leaves. The relative decrease in leaf survival due to Septoria leaf spot differed somewhat across the 2 years in that each additional leaf spot present at harvest accelerated time to defoliation by 1.9% in 2002 and by 4.5% in 2003. Assuming an average value of about 3%, the predicted time to abscission for a leaf with n spots at harvest is only $(97/100)^n \times 100\%$ as long as that of a disease-free leaf. Since disease severity can reach very high levels on susceptible cultivars (Scherm *et al*., 2003), the analysis revealed a considerable and highly significant quantitative effect of Septoria leaf spot on the risk of leaf abscission.

The ability of foliar diseases to incite premature defoliation in fruit crops has been documented for a number of pathosystems. In apple, for example, high levels of infection by *Botryosphaeria obtusa*, *Phomopsis* spp., or *Marssonina coronaria* have been shown to result in early leaf loss (Rosenberger *et al*., 1996; Sharma and Bhardwaj, 2003). Severe defoliation of orange trees due to high levels of citrus greasy spot caused by *Mycosphaerella citri* also has been reported (Hidalgo *et al*., 1997). Similar effects of disease can occur in cherry, where infection by the leaf spot pathogen *Cercospora circumscissa* results in early and severe leaf loss (Sztejnberg, 1986). All these reports discussed the yield implications of disease-induced premature defoliation. In deciduous fruits, a large proportion of leaf carbohydrates (Choi *et al*., 2003;

Oliveira and Priestly, 1988) and nitrogenous compounds (Titus and Kang, 1981) moves into the woody parts of the trees during autumnal senescence. These reserves play an important role in early growth of shoots and fruit in the following spring (Layne and Flore, 1993).

In blueberry, retention of leaves throughout the fall enhances the ability of the plants to form flower buds (Darnell, 1991). Based on mechanical defoliation experiments, Lyrene (1992) determined that early fall defoliation of *V. ashei* reduced flower bud set; for example, only 0.8% of the nodes on defoliated shoots produced flower buds compared with 42.6% of the nodes on non-defoliated shoots. In experiments in which leaves were removed from alternating nodes on each shoot, flower buds failed to form at the defoliated nodes, indicating that defoliation removes a nearby source of carbohydrates and thus reduces the potential of an axillary bud to be transformed into a flower bud (Lyrene, 1992). It was also suggested that premature defoliation in blueberry may reduce flower bud initiation by removing receptors of the short-day stimulus. In similar mechanical defoliation experiments on southern highbush blueberry, partial or complete premature defoliation inhibited flower bud set and resulted in lower return yields (Williamson and Miller, 2002). The effects of disease-induced defoliation on flower bud induction and subsequent yield may be even more pronounced than those documented for mechanical defoliation, given the negative physiological effects of disease on infected leaves prior to leaf abscission. Indeed, in other pathosystems, leaf spots have been shown to lower yields by reducing the leaf area available for photosynthesis as well as the photosynthetic capacity of the remaining leaf area (Jesus Junior *et al*., 2003; Lopes and Berger, 2001). In a study to determine the effect of Septoria leaf spot on photosynthesis of blueberry leaves, Roloff *et al*. (2004), using both rabbiteye and southern highbush blueberries, documented a reduction in photosynthesis in infected leaves, whereby net assimilation rate was lowered by approximately one-half at 20%

disease severity and values approached zero for leaves with >50% necrotic leaf area. Interestingly, the leaf area in which photosynthesis was impaired was about three times as large as the area covered by necrosis (Roloff *et al*., 2004). Collectively, these studies illustrate the importance of maintaining disease-free foliage to maximize yields in the following growing season.

Our analysis also showed that older leaves are more likely to abscise prematurely than younger leaves. In addition to undergoing earlier natural senescence due to their advanced physiological age, older leaves are also likely to accumulate higher levels of disease, given their presence on the plant for a longer period of time. These increased disease levels, as discussed above, further increase the risk of premature leaf abscission. Differences in cumulative disease between older and younger leaves also have been reported for Septoria leaf spot on *Eucalyptus nitens*, caused by *Septoria pulcherrima* (Hood *et al*., 2002). Although differences in leaf susceptibility, inoculum availability, and environmental conditions during the season may also influence disease severity on leaves of different ages, no studies have been conducted to examine this relationship in detail.

Survival analysis proved to be a powerful tool for assessing the effects of disease severity and leaf age on time to defoliation. This method of analysis is dynamic in that it does not merely provide a 'snapshot' at one particular point in time, but rather shows how the risk of defoliation changes over time with respect to a set of covariates. For example, unlike logistic regression, which requires that each observation be categorized as, say, low or high disease at a specified time, the dependent variable in survival analysis, T, is continuous, allowing for a more complete use of the information in the data. Similar conclusions were reached by Dungan *et al*. (2003) who used growth equations and survival analysis to estimate the effect of date of emergence on

the leaf life-span of a winter-deciduous compared with an annual plant species. In that study, although growth equations precisely described leaf emergence of the two species, they obscured key differences between their leaf life-spans that were identified with survival analysis. In a study to describe the distribution of the time-dependent developmental trait 'time to flowering' and compare differences in various maize genotypes, Vermerris and McIntyre (1999) also reported that their conclusions were enhanced by using survival analysis compared with conventional statistical procedures. In plant pathology, previous applications of survival analysis, reviewed by Scherm and Ojiambo (2004), have been limited (Dallot *et al*., 2004; Jules *et al*., 2002; Madden and Nault, 1983; Westra *et al*., 1994).

Although this study documented a strong and significant effect of Septoria leaf spot on premature defoliation and previous work established a link between premature defoliation and reduced return yields (Lyrene, 1992; Williamson and Miller, 2002), further research is needed to determine the physiological and quantitative effects of the disease on the processes involved in yield formation in blueberry. In addition, a more detailed examination of the temporal progress of the disease prior to defoliation is needed to identify host, pathogen, and environmental factors that determine epidemic development. Such information on disease progress, when integrated with data on the dynamics of disease-induced defoliation and associated yield losses, could lead to development of treatment thresholds, using an approach similar to that used for Septoria diseases of wheat (Verreet *et al*., 2000). Ultimately, this would have to incorporate other foliar diseases such as rust, anthracnose, and Gloeosporium leaf spot, which can affect certain blueberry cultivars in the southeastern United States (Scherm *et al*., 2003). Potential interactions among these diseases and their relative effects on defoliation and yield need to be investigated.

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Table 4.1. Comparison of four models to describe the survival distribution for estimating differences in time to defoliation of individual leaves of 'Premier' rabbiteye blueberry in a field study carried out in Georgia in 2002 and 2003

	Log-likelihood			
Year	Exponential	Log-logistic	Log-normal	Weibull
2002	–394.3	–148.9	–168.6	–145.2
2003	–510.5	–403.5	–433.5	–356.2

Table 4.2. Effects of Septoria leaf spot severity, leaf age, and leaf location in the canopy on the time to abscission (*T*) of individual leaves of 'Premier' rabbiteye blueberry in a field study in Georgia in 2002 and 2003

Variable	2002			2003		
	n	*T* (days) mean ± s.e.	Pr > Chi-Square	*n*	*T* (days) mean ± s.e.	Pr > Chi-Square
Disease severity at harvest [a]						
< 5 spots/leaf	173	87.9 ± 1.61		423	64.3 ± 1.32	
≥ 5 spots/leaf	210	68.4 ± 2.01	< 0.0001	36	42.6 ± 4.12	< 0.0001
Leaf age [b]						
Younger leaves	174	84.5 ± 1.64		263	69.8 ± 1.57	
Older leaves	209	68.6 ± 2.23	< 0.0001	196	52.9 ± 1.97	< 0.0001
Leaf location [c]						
≤ 80 cm	122	68.7 ± 2.88		130	62.6 ± 2.17	
> 80 cm	261	81.3 ± 1.51	0.0347	329	62.6 ± 2.38	0.2105

[a] Disease severity in mid-June when ~50% of the fruit had been harvested. Sample size (*n*) at that time was lower than the number of leaves tagged in the spring as some leaf loss had occurred due to factors other than Septoria leaf spot.

[b] Leaves on the lower (older leaves) or upper (younger leaves) halves of the assessed shoots.

[c] Height above the ground of the assessed shoots.

Table 4.3. Parameter estimates and test statistics for accelerated failure time models describing the time to abscission of individual leaves of 'Premier' rabbiteye blueberry in a field study in Georgia in 2002 and 2003

Parameter	df	Estimate	Standard error	Chi-Square	Pr > Chi-Square
2002					
Intercept	1	4.583	0.0446	10545.8	<0.0001
Disease severity [a]	1	–0.020	0.0454	40.8	<0.0001
Leaf age [b]	1	0.123	0.0032	7.4	0.0064
Weibull scale parameter	1	0.364	0.0184		
Weibull shape parameter	1	2.746	0.1391		
2003					
Intercept	1	4.196	0.0399	11051.4	<0.0001
Disease severity [a]	1	–0.046	0.0528	37.7	<0.0001
Leaf age [b]	1	0.341	0.0076	41.9	<0.0001
Weibull scale parameter	1	0.496	0.0228		
Weibull shape parameter	1	2.016	0.0926		

[a] Number of spots per leaf due to *Septoria albopunctata* in mid-June when ~50% of the fruit had been harvested.

[b] Coded as 0 and 1 for leaves on the lower (older leaves) or upper (younger leaves) halves of the assessed shoots, respectively.

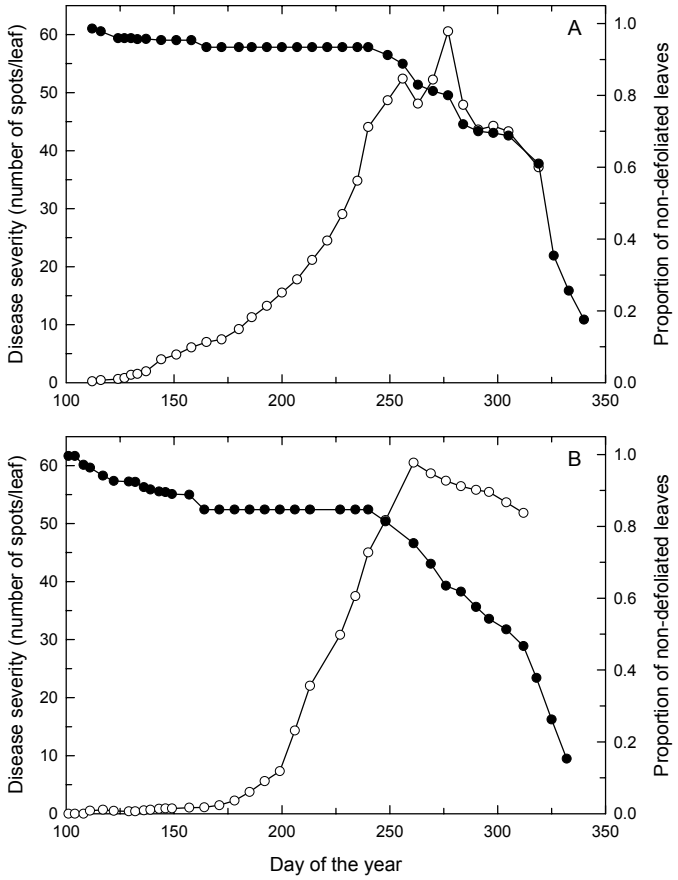

Fig. 4.1. Temporal progress of Septoria leaf spot severity (○) and defoliation (●) of individual leaves of 'Premier' rabbiteye blueberry in a field study carried out in Georgia in 2002 (n = 410; **A**) and 2003 (n = 542; **B**).

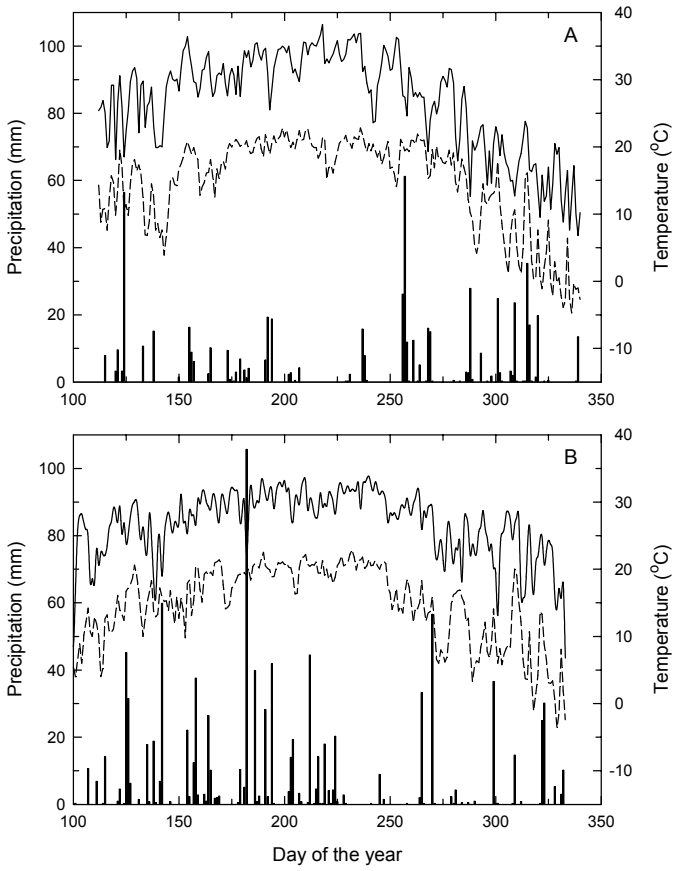

Fig. 4.2. Daily maximum and minimum air temperatures and precipitation totals at the experimental site in 2002 (**A**) and 2003 (**B**). The vertical bars represent daily precipitation and the solid and dashed lines represent maximum and minimum temperatures, respectively.

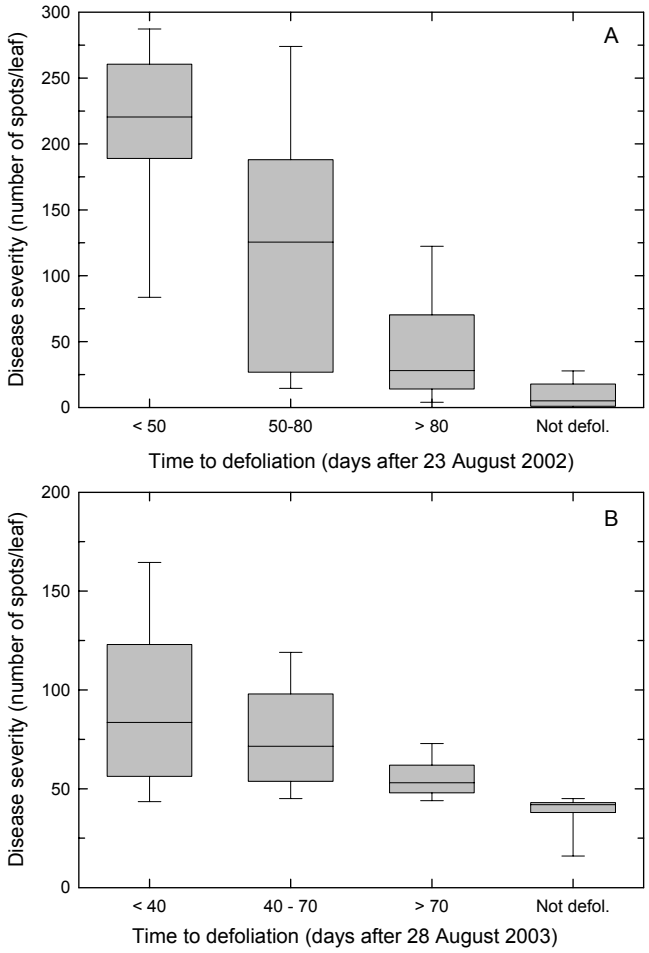

Fig. 4.3. Box-whisker plots showing the distribution of Septoria leaf spot severity values for individual leaves of 'Premier' rabbiteye blueberry that defoliated at different times in a field study carried out in Georgia in 2002 (*n* = 389; **A**) and 2003 (n = 459; **B**). The boxes represent the interquartile range, the whiskers indicate the 5- and 95-percentiles and the lines within the boxes represent the median disease severity. Not defol. = leaves that had not defoliated by the end of the assessment period in late November.

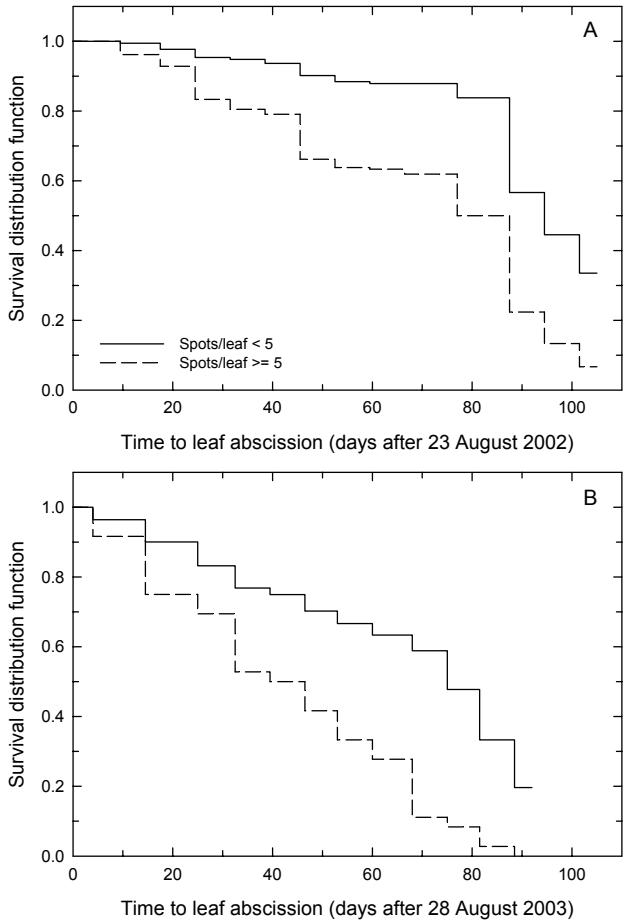

Fig. 4.4. Kaplan-Meier estimates of survival functions describing time to abscission of individual leaves of 'Premier' rabbiteye blueberry in a field study in Georgia in 2002 (*n* = 383; **A**) and 2003 (*n* = 459; **B**). Leaves were classified according to severity of Septoria leaf spot in mid-June when ~50% of the fruit had been harvested. The starting date for time to defoliation was in late August which marked the transition from a period of negligible, sporadic leaf loss to the onset of more sustained levels of leaf loss.

CHAPTER 5

SEPTORIA LEAF SPOT REDUCES FLOWER BUD SET AND YIELD OF RABBITEYE AND SOUTHERN HIGHBUSH BLUEBERRIES[1]

[1]Ojiambo, P.S., Scherm, H., and Brannen, P.M. 2005. Submitted to *Plant Disease*.

Septoria Leaf Spot Reduces Flower Bud Set and Yield of Rabbiteye and Southern Highbush Blueberries

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ABSTRACT

In field trials on 'Premier' rabbiteye blueberry, individual shoots were selected and tagged in the fall of 2001, 2002, and 2003 to quantify the effects of Septoria leaf spot severity and disease-induced premature defoliation on flower bud set and return yield. Experiments were similarly carried out on 'Bluecrisp' southern highbush blueberry using shoots tagged after fruit harvest in the summer of 2002 and 2003. Leaves on the distal 20-cm segments of these shoots were monitored for disease severity (number of spots per leaf) through the remainder of the fall; at the same time, defoliation (expressed as the proportion of nodes with missing leaves) was recorded for each of the shoot segments. Flower bud set was assessed subsequently in winter or early spring, and berries were harvested as they matured the following summer to determine return yield. In both cultivars, there was no statistical relationship between flower bud numbers or return yield per shoot and final disease severity, area under the disease progress curve, or area under the defoliation progress curve measured previously on the same shoots. Specifically, while shoots with high disease levels always had low flower bud set and yields, those having low disease levels had highly variable bud numbers and yields. Nonetheless, the data revealed a clear pattern for flower bud set potential (i.e., the maximum number of buds on shoots having a given

disease severity level) to decrease linearly as disease severity increased ($P < 0.0005$). Flower bud set decreased by one bud per shoot as disease severity in the previous fall increased by 18 and 12 spots per leaf for 'Premier' and 'Bluecrisp', respectively. Relationships between yield and disease variables were similar to those of flower bud numbers and disease, except that the decrease in yield potential (i.e., the maximum yield for a given disease severity level) was less gradual than for flower bud set potential. On 'Premier', there was evidence for a threshold effect whereby yield potential dropped markedly as final disease severity exceeded about 50 to 60 spots per leaf on average. Evidence for such a threshold effect was weaker on 'Bluecrisp', possibly because of the lower number of data points for this cultivar combined with lower yields due to poor pollination.

INTRODUCTION

In Georgia, blueberry is the second most important fruit crop and is currently produced on more than 3,200 ha statewide (Boatright and McKissick, 2003). Among the different species of blueberry grown in the state, cultivars of the native rabbiteye blueberry (*Vaccinium ashei*) constitute about 90% of the production acreage, with the remaining 10% planted to the recently introduced, earlier-maturing southern highbush blueberry (*V. corymbosum* interspecific hybrids) (Scherm and Krewer, 2002). According to a recent disease survey (Scherm *et al*., 2003), both species can be affected by leaf spot diseases (Brannen, 2001), of which Septoria leaf spot, caused by *Septoria albopunctata*, is the most prevalent in Georgia (Scherm *et al*., 2003) and other southeastern states (Cline, 2002).

The disease is characterized by small circular leaf lesions with white to tan centers and purple margins (Milholland, 1995). Ostiolate pycnidia, usually one but occasionally up to five

per spot, occur on the upper leaf surface. Stem lesions are typically sunken, 5 to 6 mm in diameter, with tan or gray centers and reddish-brown margins. Most of the southern highbush and several rabbiteye blueberry cultivars are highly susceptible to Septoria leaf spot (Scherm *et al*., 2003).

In Georgia, foliar symptoms of Septoria leaf spot appear first by early May and disease severity increases rapidly after fruit harvest between June and September (Chapter 3; Ojiambo and Scherm, 2004). High levels of disease during this period can lead to pronounced reductions in photosynthesis of affected leaves; indeed, in field experiments carried out by Roloff *et al*. (2004), net assimilation rate of leaves was reduced by approximately one-half at 20% disease severity, and values approached zero for leaves with >50% necrotic leaf area. In addition, the disease can trigger premature defoliation during summer and early fall (Chapter 4; Brannen *et al*., 2002, 2003; Cline, 2002; Ojiambo and Scherm, 2004). In mechanical leaf removal experiments, premature defoliation of blueberry bushes resulted in lower yields in the subsequent growing season (Williamson and Miller, 2002), presumably because defoliation reduced flower bud set during the fall by eliminating photoreceptors and/or lowering carbohydrate reserves during critical periods (Darnell, 1991; Lyrene, 1992).

Based on these considerations, it seems likely that epidemics of Septoria leaf spot will reduce flower bud set and lower return yields by reducing photosynthesis of diseased leaves (Roloff et al., 2004) and/or by causing premature defoliation (Chapter 4; Ojiambo and Scherm, 2004). However, no studies have been conducted to determine the relationships among disease severity, defoliation, and reproductive development of blueberry. Therefore, the objective of this study was to quantify the effects of disease and disease-induced premature defoliation on flower bud set and return yield in field conditions.

MATERIALS AND METHODS

Field site and data collection. The study was carried out in an experimental rabbiteye blueberry planting at the University of Georgia Horticulture Farm near Athens (northeastern Georgia) from 2001/2002 to 2003/2004 (3 years), and in a commercial southern highbush blueberry planting near Homerville (southern Georgia) in 2002/2003 and 2003/2004 (2 years). The Athens planting consisted of alternating rows of cultivars 'Premier' and 'Climax' to which no fungicides were applied throughout the study period. The Homerville planting was part of a fungicide evaluation test (Brannen et al., 2003) and was comprised of alternating rows of cultivars 'Bluecrisp' and 'Star'. Plants at both sites were mature, and maintenance of the plantings, including fertilization, pruning, and weed control, followed commercially recommended practices (Austin, 1994). Supplemental overhead irrigation was applied as needed.

On 'Premier', which is highly susceptible to Septoria leaf spot (Scherm *et al*., 2003), 50 spring shoots were selected arbitrarily from >10 bushes on 28 September in 2001 and on 22 August in 2002 and 2003. Previous field studies (Chapter 4; Ojiambo and Scherm, 2005) documented minimal defoliation prior to late August, followed by sustained increases in disease and defoliation thereafter. On 'Bluecrisp', which is also highly susceptible to the disease (Scherm *et al*., 2003), 45 and 35 spring shoots were selected (one shoot per bush) about 1 month after fruit harvest on 11 and 13 June in 2002 and 2003, respectively. Each shoot was tagged 20 cm from its tip, and its height above the ground was measured to provide an indication of shoot position within the canopy. Leaves on the distal 20-cm of these shoots were assessed individually at weekly to biweekly intervals for severity of Septoria leaf spot (expressed as number of spots per leaf), and a mean disease severity value was calculated for each shoot. On average, 10 to 13 leaves were present on each 20-cm shoot segment. At the same time,

defoliation (expressed as the proportion of nodes with missing leaves) was recorded for each of the shoot segments. On both cultivars, the last disease assessments were made in early to mid-November, after which leaf spots became difficult to count due to necrosis associated with natural leaf senescence. Shoots were monitored for defoliation for another 2 weeks after the last disease assessment date. Throughout the experimental period, Septoria leaf spot was the only noticeable foliar disease on the two cultivars used in the study.

Following each growing season, flower bud set and return yield were recorded in winter and spring to early summer, respectively. Flower bud set was determined by counting the number of flower buds on each of the tagged shoot segments in early February for 'Premier' and in December or January for 'Bluecrisp'. On 'Premier', fruit were harvested as they matured for a period of 2 to 4 weeks starting in early June. Due to poor pollination that resulted in low fruit set of 'Bluecrisp' in the 2002/2003 trial, fruit were only harvested in the second year in early May 2004. Fruit numbers and total fresh weights were determined for each tagged shoot segment, and yield was expressed as total fruit weight per shoot.

Data analysis. Disease severity and defoliation data for each shoot segment were used to calculate areas under the disease progress curve (AUDPC, expressed as spot-days) and areas under the defoliation progress curve (AUDefPC, expressed as proportion-days), respectively (Campbell and Madden, 1990). An estimate of final disease severity for each shoot was obtained by averaging disease severity on the last assessment date prior to leaf abscission for all leaves on that shoot. Final disease severity, AUDPC, and AUDefPC were examined graphically to explore relationships with flower bud numbers and return yield. The distribution of final disease severity values for shoots with different numbers of flower buds was examined using box-whisker plots generated using PROC UNIVARIATE in SAS (v. 8.2; SAS Institute Inc., Cary, NC). The

correlation between fruit weight and number of fruits per shoot was examined using PROC CORR in SAS, separately for the two cultivars, using the pooled data from all the years.

RESULTS

Disease severity and defoliation. No more than two tagged shoots were lost in any of the trials during the study period. Final disease severity in early to mid-November was generally higher on 'Premier' than on 'Bluecrisp' (Table 5.1). On 'Premier', disease was most severe in fall of 2002 (78.4 spots per leaf on average) and least severe in fall of 2003 (40.0 spots per leaf). In contrast, disease severity on 'Bluecrisp' was similar in 2002 and 2003. Trends in AUDPC and AUDefPC across years and cultivars followed those of disease severity, except that the lowest value of AUDefPC on 'Premier' was observed in 2001 (Table 5.1).

Relationships between flower bud set and disease. The number of flower buds formed on each 20-cm shoot segment ranged from 0 to 14 for 'Premier' and 0 to 12 for 'Bluecrisp' (Fig. 5.1). For both cultivars, plots of flower bud numbers versus final disease severity (Fig. 5.1 A and B) revealed considerable scatter in the data. Specifically, while shoots with high disease levels always had low flower bud set, those having low disease levels had highly variable bud numbers. While this indicates that disease severity was a poor predictor of *actual* flower bud set, flower bud set *potential* (defined here as the maximum number of buds on shoots with a given disease severity level) appeared to decrease linearly as disease severity increased (illustrated by the diagonal boundary lines in Fig. 5.1A and B). This trend was confirmed by histograms showing the maximum number of flower buds per shoot for different disease severity classes for the two cultivars (Fig. 5.2). When linear regression analysis was used to determine the relationship between maximum flower bud number per shoot (y) and the midpoints of the disease severity

classes (x) in Fig. 5.2, significant negative relationships were found for 'Premier' ($y = 13.8 - 0.058\ x$; $r^2 = 0.954$, $P = 0.0002$, $n = 156$), and 'Bluecrisp' ($y = 11.5 - 0.087\ x$; $r^2 = 0.926$, $P = 0.0005$, $n = 80$). Based on the slopes of the regression equations, flower bud set potential decreased by one bud per shoot as final disease severity increased by 18 and 12 spots per leaf for 'Premier' and 'Bluecrisp', respectively. A negative relationship between flower bud set and disease was also evident from the box-whisker plots showing the distribution of final disease severity values for shoots having different numbers of flower bud (Fig. 5.3).

Considerable scatter was also apparent in plots illustrating the relationship between numbers of flower buds per shoot segment and AUDPC or AUDefPC (Fig. 5.1C-F). However, as was the case with final disease severity, a negative trend was apparent between flower bud set potential and the two variables in both cultivars.

Relationships between return yield and disease. Fruit weight and number of fruits per shoot were strongly correlated in 'Premier' ($r = 0.969$, $P<0.0001$, $n = 46$) and 'Bluecrisp' ($r = 0.931$, $P<0.0001$, $n = 33$) and hence only fruit weight data is presented here. Fruit yields of 'Premier' ranged from 0 to 72.4 g per 20-cm shoot segment and were highest in the 2001/2002 trial (Figs. 5.4A, C, E). In 'Bluecrisp', fruit yields were measured only in the 2003/2004 trial and were compromised by low fruit set, the highest yield being only 9.3 g per shoot (Figs. 5.4B, D, F). As was the case with the relationship between flower bud numbers and disease, considerable scatter was observed in plots of yield versus final disease severity, AUDPC, or AUDefPC (Fig. 5.4). Nonetheless, the highest yields were observed at low disease or defoliation levels, while higher levels of the latter variables were associated consistently with shoots having the lowest yields. On 'Premier', for example, yield potential (i.e., the maximum yield at a given disease level) remained relatively unaffected by disease up to about 50 to 60 spots per leaf and then

dropped markedly thereafter (Fig. 5.4A). Plots of yield versus AUDPC and especially AUDefPC showed similar patterns (Figs. 5.4C and E). Due to the low number of data points, the evidence for a disease or defoliation threshold associated with a rapid drop in yield potential was weaker in 'Bluecrisp' (Fig. 5.4B, D, F).

DISCUSSION

This is the first study to provide quantitative information about the effects of Septoria leaf spot severity and disease-induced defoliation on reproductive development in blueberry. Specifically, we document here the negative effect of epidemics in the summer and fall on subsequent flower bud set and return yield potential in rabbiteye and southern highbush blueberry. Previously, Cline (2002) reported reductions in flower bud set and return yield of highbush blueberry affected by a complex of foliar diseases including Gloeosporium and Septoria leaf spots in North Carolina, but the relative contribution of the individual diseases could not be quantified.

The negative effects of Septoria leaf spot on flower bud set and return yield potential in the present study were likely due to a reduction of the photosynthetic capacity of affected leaves; directly by reducing the leaf area available for photosynthesis and the photosynthetic capacity of the remaining green leaf area (Roloff *et al*., 2004), and indirectly by inducing premature defoliation (Chapter 4; Ojiambo and Scherm, 2004). Similar effects of foliar diseases on return yield have been reported in other fruit crop pathosystems (Hidalgo *et al*., 1997; Rosenberger *et al*., 1996; Sharma and Bhardwaj, 2003; Sztejnberg, 1986). All these reports attribute reductions in return yield to reduced storage of carbohydrates (Choi *et al*., 2003; Oliveira and Preistly, 1988) or nitrogenous compounds (Petrie *et al*., 2003; Titus and Kang, 1982) in woody plant

organs during autumnal senescence. These reserves play an important role in early growth of shoots and fruit during the following spring (Layne and Flore, 1993). In blueberry, defoliation in early fall also reduces the potential for transformation of axillary buds into flower buds by removing receptors of the short-day stimulus (Lyrene, 1992).

In both cultivars, plots of flower bud numbers or yield versus final disease severity showed considerable scatter in the data with some shoots having low flower bud numbers or yield despite having had leaves with low disease severity. This variability was not reduced when integrated disease variables such as AUDPC or AUDefPC were used. The lack of a simple statistical relationship should not be surprising, given that numerous biological and environmental factors (Brown *et al*., 1995; Darnell, 1991) in addition to disease can affect flower bud set and return yield. Even in annual crops, relationships between disease and yield are often weak due to the complex interactions between these factors (Teng, 1987; Waggoner and Berger, 1987). In perennial fruit crop pathosystems such as citrus-greasy spot (McGovern *et al*., 2003), peach-rusty spot (Furman *et al*., 2003), apple-*Marssonina coronaria* (Rosenberger *et al*., 1996), and coffee-leaf rust (Brown *et al*., 1995), factors such as nutritional status and biennial bearing pattern have been documented to complicate the relation between disease and yield even further. In blueberry, reduced flower bud set and low yields on shoots with low levels of disease could be due to other shoot-related factors that affect carbohydrate supply, e.g., shoot position in the canopy, shoot orientation, or shoot diameter (Gough, 1994; Darnell, 1991). While shoot orientation and diameter were not measured in the present study, we recorded heights of shoots above the ground as a surrogate for shoot position within the canopy. However, no significant correlation ($P > 0.05$) was observed between shoot height and flower bud numbers or yield (*data*

not shown). Thus, differences in canopy position among shoots were not large enough to be a confounding factor in the present study.

Despite the variability in the relationships between flower bud numbers or yield and disease, some useful patterns could be discerned. For flower bud set, there was a striking pattern for the maximum number of buds on shoots having a given disease severity level (interpreted as flower bud set potential) to decrease linearly as disease levels increased. This relationship (represented by the boundary lines in Fig. 5.1 and the histograms in Fig. 5.2) shows that the gap between flower bud set potential in the absence of Septoria leaf spot and bud set potential in the presence of the disease becomes progressively wider as disease severity increases. This gap may be interpreted as representing the contribution of Septoria leaf spot to reductions in flower bud number, while the scatter below the boundary lines in Fig. 5.1 may indicate reductions due to other causes. For each additional leaf spot, reductions in flower bud set potential were greater for 'Bluecrisp' than for 'Premier'. This can be explained by the larger diameters of leaf spots on 'Bluecrisp' compared with those on 'Premier' (Scherm *et al*., 2003). Thus, reductions in flower bud numbers are more likely to occur at lower leaf spot numbers for 'Bluecrisp' than for 'Premier'.

Relationships between yield and disease variables were similar to those between flower bud numbers and disease, except that the decrease in yield potential was less gradual than the decrease in flower bud set potential. On 'Premier', for example, there was evidence for a threshold effect, whereby yield potential dropped markedly as final disease severity, AUDPC, and AUDefPC exceeded about 50 to 60 spots per leaf, 2000 spot-days, and 20 proportion-days, respectively (Fig. 5.3). Evidence for such a threshold effect was weaker on 'Bluecrisp', possibly due to the lower number of data points for this cultivar combined with the lower yields.

Our arguments concerning relationships among disease variables, flower bud set, and yield are primarily based on a visual assessment of the patterns apparent in scatter plots and histograms of these variables. In general, patterns are defined as discontinuities in data exhibiting some measure of repetition (Roberts, 1999). Recognition of such patterns can help in interpretation of data and development of new ideas (such as the concepts of flower bud set potential and yield potential per shoot in the present study) as well as in designing of follow-up experiments (Roberts, 1999). In another plant science-related example, visual pattern recognition has been instrumental in resolving apparently complex effects of photoperiod and the basic temperature response in soybean (Roberts *et al*., 1996; Upadhyay *et al*., 1994). Although curve-fitting using standard statistical approaches plays an important role in recognition of the form of processes, the approach provides little benefit when relationships are very complex or where coefficients describing the relationships have no biological meaning. Use of boundary lines to delineate patterns has been used successfully to develop soil nutrient norms for soybean production (Evanylo and Sumner, 1987).

Further research is needed to explore the usefulness of the patterns observed in this study for making disease management decisions. First, one needs to remember that these patterns were observed for *potential* flower bud set and yield; relationships for *actual* flower bud numbers and yield were much more variable. Second, the disease variables used in this study were based on season-long or end-of-season assessments, while management decisions need to be made earlier in the season. In many cases, however, yield loss assessments utilizing single-point disease measurements early in the season have failed to adequately explain the relationships between yield and disease severity in most pathosystems, including even annual crops where disease development and yield formation occur in the same season (Danielsen and Munk, 2004; Teng,

1987; Waggoner and Berger, 1987). We are currently investigating the season-long temporal dynamics of Septoria leaf spot epidemics in blueberry (Chapter 3), and information generated from that study could be used to predict early in the season whether the disease or defoliation thresholds indicated above will be exceeded.

Waggoner and Berger (1987) proposed yield loss models based on a healthy leaf area index, which considered growth of the host, disease severity, and defoliation throughout the season. Although green leaf area was not measured in our study, future studies in blueberry should consider utilizing this approach. Healthy leaf area index-based models (Bryson, 1997; Waggoner and Berger, 1987) have been developed and tested for pathosystems where both disease and yield effects occur in the same growing season; it will be interesting to determine whether such models better describe the relationship between return yield and disease severity in the previous season, which is relevant not only for Septoria leaf spot of blueberry but also for other pathosystems involving perennial crops.

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Table 5.1. Summary statistics of Septoria leaf spot-related variables on individual shoots of 'Premier' rabbiteye and 'Bluecrisp' southern highbush blueberry in field trials in Georgia from 2001/2002 to 2003/2004

Cultivar/year	n [a]	Final disease severity (number of spots per leaf) [b]		Area under the disease progress curve (spot-days)		Area under the defoliation progress curve (proportion-days)	
		Mean	Range	Mean	Range	Mean	Range
'Premier'							
2001/2002	50	66.6	3.6 – 215.8	1655	66.3 – 4411	8.9	0 – 27.4
2002/2003	48	78.4	0.0 – 242.5	2578	0.0 – 8917	31.4	0 – 105.1
2003/2004	48	40.0	3.8 – 117.0	1918	226.5 – 5301	20.3	0 – 59.6
'Bluecrisp'							
2002/2003	43	20.4	1.5 – 117.5	1144	71.3 – 4322	16.5	0 – 70.1
2003/2004	35	26.9	2.8 – 158.5	1125	220.1 – 4443	26.7	0 – 83.4

[a] Number of 20-cm shoot segments used for assessment of disease variables, flower bud set, and return yield. Each shoot segment had 10 to 13 leaves on average.

[b] Mean number of spots per leaf on the last assessment date prior to leaf abscission, averaged across all leaves per shoot segment.

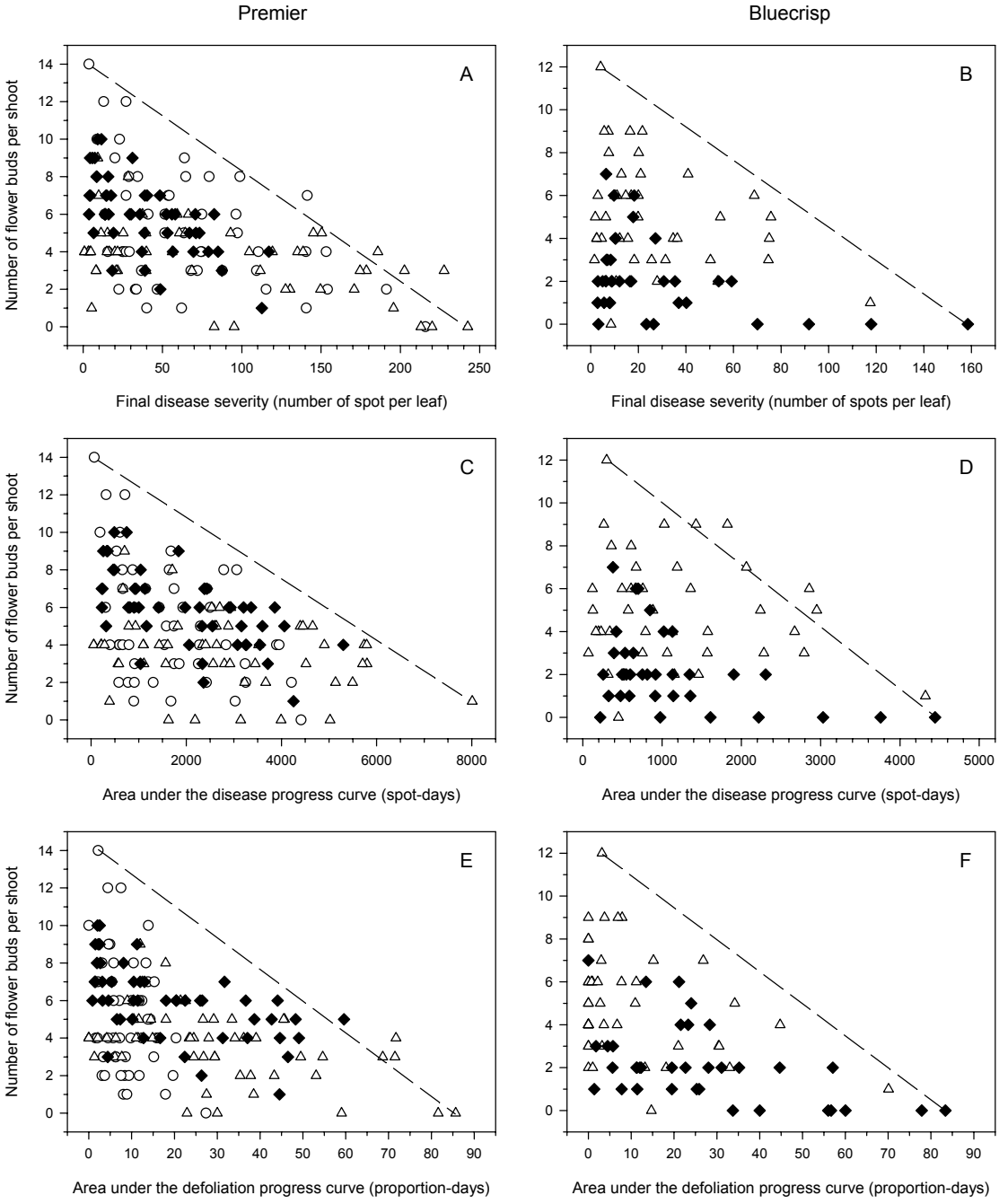

Fig. 5.1. Relationships between flower bud set and Septoria leaf spot-related variables on individual shoots of 'Premier' rabbiteye and 'Bluecrisp' southern highbush blueberry in field trials in Georgia in 2001/2002 (○) , 2002/2003 (△), and 2003/2004 (◆). **A** and **B**, final disease severity (mean number of spots per leaf on the last assessment date prior to leaf abscission, averaged across all leaves per 20-cm shoot segment); **C** and **D**, area under the disease progress curve; **E** and **F**, area under the defoliation progress curve. The diagonal boundary lines, constructed by joining the points with the highest number of buds and the highest disease or defoliation level, represents the decrease in flower bud set potential due to Septoria leaf spot.

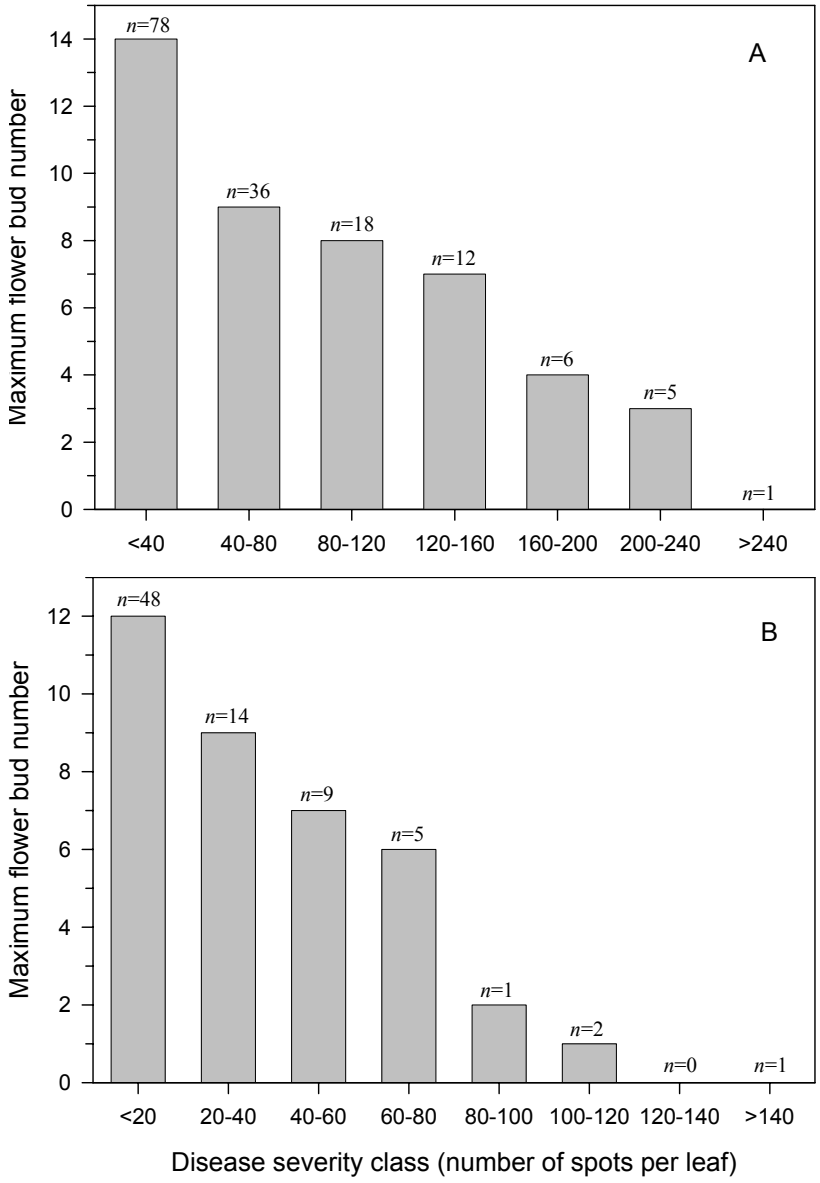

Fig. 5.2. Histograms showing the maximum number of flower buds per 20-cm shoot segments of (**A**) 'Premier' rabbiteye and (**B**) 'Bluecrisp' southern highbush blueberry for different classes of final Septoria leaf spot severity in field trials in Georgia from 2001/2002 to 2003/2004 (**A**) or 2002/2003 to 2003/2004 (**B**). Final disease severity is defined as the mean number of spots per leaf on the last assessment date prior to leaf abscission, averaged across all leaves per 20-cm shoot segment. The sample size, *n*, represents the number of shoots within each disease severity class.

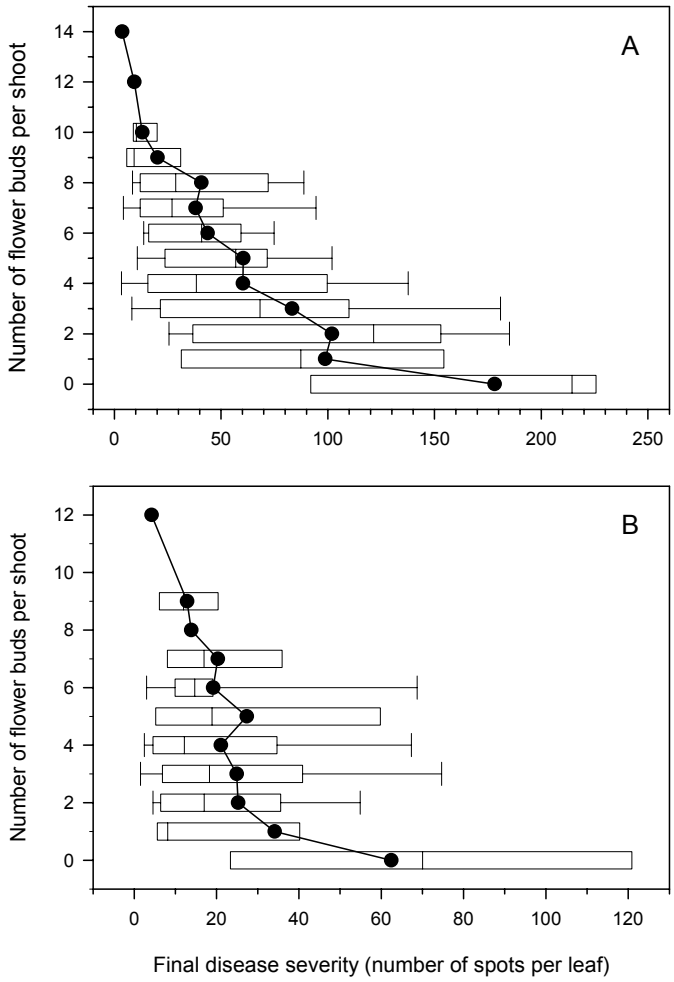

Fig. 5.3. Box-whisker plots showing the distribution of final Septoria leaf spot severity values on individual shoots of (**A**) 'Premier' rabbiteye and (**B**) 'Bluecrisp' southern highbush blueberry with different numbers of flower buds in field trials in Georgia from 2001/2002 to 2003/2004 (**A**) or 2002/2003 to 2003/2004 (**B**). Final disease severity is defined as the mean number of spots per leaf on the last assessment date prior to leaf abscission, averaged across all leaves per 20-cm shoot segment. The boxes represent the interquartile range, with the whiskers indicating the 5- and 95-percentiles. Lines and circles within the boxes represent median and mean disease severity values, respectively.

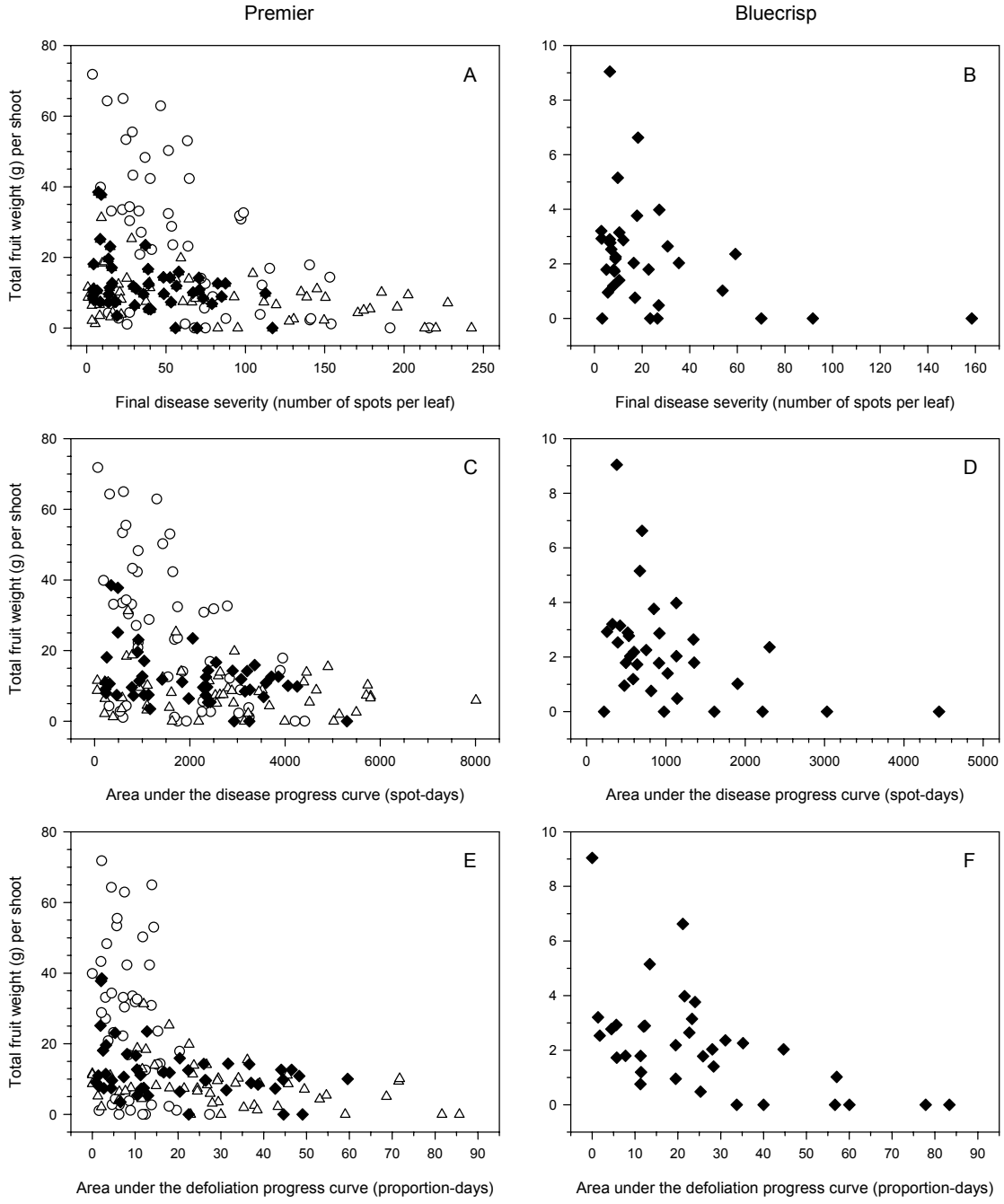

Fig. 5.4. Relationships between return yield and Septoria leaf spot-related variables on individual shoots of 'Premier' rabbiteye and 'Bluecrisp' southern highbush blueberry in field trials in Georgia in 2001/2002 (○), 2002/2003 (Δ), and 2003/2004 (♦). **A** and **B**, final disease severity (mean number of spots per leaf on the last assessment date prior to leaf abscission, averaged across all leaves per 20-cm shoot segment); **C** and **D**, area under the disease progress curve; **E** and **F**, area under the defoliation progress curve.

CHAPTER 6

OPTIMUM SAMPLE SIZE FOR DETERMINING DISEASE SEVERITY AND DEFOLIATION ASSOCIATED WITH SEPTORIA LEAF SPOT OF BLUEBERRY[1]

[1]Ojiambo, P.S., and Scherm H. 2004. To be submitted to *Plant Disease*.

Optimum Sample Size for Determining Disease Severity and Defoliation Associated with Septoria Leaf Spot of Blueberry

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ABSTRACT

In a 3-year field study, 'Premier' rabbiteye blueberry plants were sampled at three hierarchical levels (leaf, shoot, and bush) to assess severity of Septoria leaf spot (caused by *Septoria albopunctata*) and disease-associated defoliation. A positive linear relationship (R^2 = 0.977, $P < 0.0001$, $n = 2127$) was observed between two measures of disease severity, viz. the number of spots per leaf and percent necrotic leaf area, both assessed on individual leaves in mid- to late October. For data summarized at the shoot level, percent defoliation increased nonlinearly ($R^2 = 0.729$, $P < 0.0001$, $n = 224$) with disease severity, and a plateau of about 90% defoliation was reached at ~60 spots per leaf. Variance components were calculated for disease severity to partition total variation into variation among leaves per shoot, shoots per bush, and bushes within the field. In all cases, leaves and shoots accounted for about 90% of the total variation. On the basis of estimates of variance components and linear cost functions (which considered the time required to assess each leaf and select new shoots and bushes for assessment), the mean optimum number of leaves per shoot was 0.7 and 1.5 for assessing disease severity as number of spots per leaf and percent necrotic leaf area, respectively. The mean optimum number of shoots per bush and bushes within a field for assessing the two disease

severity variables were similar with a mean of 2.2 shoots and 22.9 bushes. A sample of 2.6 shoots per bush and 7.9 bushes within a field was the optimum sample size for evaluating defoliation across the 3 years.

INTRODUCTION

Among the foliar diseases that affect cultivated blueberries (*Vaccinium* spp.), Septoria leaf spot, caused by *Septoria albopunctata*, is the most prevalent in Georgia and other southeastern states (Cline, 2002; Scherm *et al*., 2003). Foliar disease symptoms include small circular lesions with white to tan centers and purple margins (Milholland, 1995). In Georgia, these symptoms appear first by early May and then increase rapidly between June and September (Chapter 3). When left uncontrolled, the disease can result in premature defoliation in late summer or early fall (Chapter 4; Brannen *et al.*, 2002, 2003; Cline, 2002; Ojiambo and Scherm, 2005), and this has the potential to reduce flower bud set in late fall and yield the following spring (Chapter 5; Lyrene, 1992; Ojiambo *et al*., 2005; Williamson and Miller, 2002).

With the increasing acreage and intensity of production, Septoria leaf spot is developing into an important production problem (Scherm and Krewer, 2003). Thus, research is needed to gain a better understanding of the epidemiology of the disease. Efficient disease assessment methods and sampling procedures are critical for epidemiological studies, crop loss assessment, and for evaluating disease management practices (Danielsen and Munk, 2004; Seem, 1984). Although methods for disease assessment and related sampling schemes have been developed for various crops (Cooke, 1998), no such procedures are available for Septoria leaf spot of blueberry. In the latter pathosystem, disease severity can be assessed either as number of spots per leaf or percent necrotic leaf area. While counting of spots on leaves is more time-consuming,

it can result in a more objective measure of disease severity. However, no studies have been carried out to determine the relationship between these two measures of disease severity, the time needed for making the respective assessment, and how it affects the sample size needed for disease assessment. In addition, although Septoria leaf spot can lead to premature defoliation (Chapter 4; Ojiambo and Scherm, 2005), no information is available on the sample size required to assess this disease-associated variable.

When disease severity is assessed at the leaf level, there are almost infinite possibilities by which leaves can be selected for assessment among the leaves on a given shoot, among shoots within a bush, and among bushes within the field. Thus, there is a need to determine the most efficient sampling plan for disease assessment through a sampling optimization procedure (Thal and Campbell, 1987). Although optimum sample sizes for assessing foliar disease have been established for various pathosystems (Analytis and Kranz, 1972; Aubertot et al., 2004; Duthie *et al*., 1991; Filajdić and Sutton, 1994; Thal and Campbell, 1987), no such protocol is available for Septoria leaf spot of blueberry. Based on these considerations, the objectives of this study were to 1) determine the relationship between Septoria leaf spot severity assessments obtained by counting the number of spots per leaf and estimating percent necrotic leaf area; 2) determine sources of variation (leaves per shoot, shoots per bush, and bushes within the field) for disease severity and defoliation assessments to decide how to allocate sampling resources; and 3) determine the optimum number of leaves per shoot, shoots per bush, and bushes within the field necessary to assess disease-associated variables based on the time required to evaluate these plant structures.

MATERIALS AND METHODS

Field site and data collection. The study was carried out in an experimental blueberry planting at the University of Georgia Horticulture Farm near Athens as part of a larger study on the epidemiology of Septoria leaf spot (Chapters 3 through 5; Ojiambo and Scherm, 2005; Ojiambo et al., 2005). The planting, 0.15 ha in area, was established in 1988 and consisted of alternating rows of rabbiteye blueberry (*Vaccinium ashei*) cultivars 'Premier' and 'Climax'. Maintenance of the planting, including fertilization, pruning, and weed control, followed generally recommended practices (Austin, 1994). Supplemental overhead irrigation was applied as needed, primarily during the fruit maturation phase in the dry 2002 growing season. No fungicides were applied to the plants throughout the 3-year study period.

On 'Premier', which is highly susceptible to Septoria leaf spot and resistant to other foliar diseases (Scherm et al., 2003), 10, 8, and 10 bushes were selected arbitrarily on 20 September 2001, 16 October 2002, and 29 September 2003, respectively. On each bush, 8 spring shoots were selected arbitrarily and tagged 20 cm from their tips. In mid- to late October, all leaves on these 20-cm shoot segments (each typically with 10 to 13 leaves) were assessed individually for disease severity both as number of spots per leaf and percent necrotic leaf area, whereby percent necrotic leaf area was estimated following training of the assessor with DiseasePro (Nutter, 1997), a computerized disease assessment training program. The same evaluator evaluated all leaves (844, 541, and 742 in 2001, 2002, and 2003, respectively) in all 3 years. Defoliation was assessed on the tagged shoots in mid-November by counting the number of nodes on which leaves had abscised and expressing it as a percentage of the total number of nodes per shoot.

The relative cost (C) associated with assessing disease severity and defoliation was estimated based on the time required to complete these assessments in the field. All other

expenditures of time such as travel to the field and data entry were not considered (Duthie *et al*., 1991). For disease severity, the times (in seconds) required to arbitrarily select a new a bush for assessment within the field (C_B), to arbitrarily select and move to a new shoot (C_S), and to select and assess disease severity on a single leaf (C_L) were recorded with a stopwatch for each of 20 bushes, shoots, and leaves. When defoliation was assessed, the times to select the next bush for assessment (C_{BD}) and to select and assess a single shoot for defoliation (C_{SD}) were determined similarly. Mean values for C_L, C_S, C_B needed to assess the number of spots per leaf were 18.3, 9.1 and 4.4 seconds, respectively, while the corresponding mean values for assessing percent necrotic leaf area were 4.2, 8.3 and 4.1 seconds, respectively. Based on these results, cost ratios of 4:2:1 and 1:2:1 were used for C_L:C_S:C_B to calculate optimum sample sizes (described below) required for assessing disease severity as number of spots per leaf and percent necrotic leaf area, respectively. Similarly, mean values for C_{SD} and C_{BD} when defoliation was assessed were 4.4 and 4.1 seconds, respectively, and the corresponding cost ratio of 1:1 (C_{SD}:C_{BD}) was used to derive optimum sample sizes for assessing defoliation.

Statistical analysis. The relationship between the number of spots per leaf and percent necrotic leaf area was analyzed using simple linear regression for combined data from the 3 years using the PROC REG procedure in SAS (v. 8.2; SAS Institute, Inc., Cary, NC). To determine the relationship between defoliation and disease severity expressed as number of spots per leaf (averaged across all leaves on a given shoot), an exponential equation of the form $y = a(1 - e^{-bx})$ was fitted to the data using SigmaPlot (v. 8.02; SPSS, Inc., Chicago, IL), where y = defoliation (%), x = number of spots per leaf, and a and b are regression coefficients.

To determine the optimum number of leaves, shoots, and bushes for assessment of disease severity, data were analyzed in a three-stage sampling design (leaves within shoots,

shoots within bushes, and bushes within the field) using the SAS procedure VARCOMP (Littell *et al*., 1996). From the analysis, the estimates of variances and variance components associated with sampling leaves, shoots, and bushes, were used to derive the respective optimum sample sizes using the equations given by Campbell and Madden (1990), adopted from Analytis and Kranz (1972):

$$n_L(opt) = [(MS_L \times n_L)/(MS_S - MS_L)]^{1/2} \times (C_S / C_L)^{1/2},$$

$$n_S(opt) = [((MS_S - MS_L) \times n_S)/(MS_B - MS_S)]^{1/2} \times (C_B / C_S)^{1/2}, \text{ and}$$

$$n_B(opt) = [\sigma^2_B + \sigma^2_S / (n_S(opt)) + \sigma^2_L / (n_S(opt)\, n_L(opt))] \times (1/V(x)).$$

In these equations, $n_L(opt)$ = optimum number of leaves per shoot; MS_L = mean square associated with variation among leaves on the same shoot; n_L = actual number of leaves per shoot sampled; MS_S = mean square associated with variation among shoots on the same bush; $n_S(opt)$ = optimum number of shoots per bush; n_S = actual number of shoots sampled per bush; MS_B = mean square associated with variation among bushes within the field; $n_B(opt)$ = optimum number of bushes; σ^2_L, σ^2_S, and σ^2_B = variances associated with leaves, shoots, and bushes, respectively; $V(x)$ = variance around the mean; and C_L, C_S, and C_B as defined above. The variance around the mean was calculated using the equation $V(x)$ = (0.2 × mean disease severity), where the factor of 0.2 allows for a 20% variability around the mean (Analytis and Kranz, 1972).

When assessing defoliation, only the numbers of shoots per bush and bushes in the field need to be optimized because there is only one value of percent defoliation per shoot; thus, data were analyzed in a two-stage sampling design (shoots within bushes and bushes within the field). Based on the estimates of variances and variance components associated with shoots and bushes obtained from the analysis, optimum sample sizes for this variable were determined using the following equations (Analytis and Kranz, 1972; Campbell and Madden, 1990):

$$n_{SD}(opt) = (\sigma_{SD} / \sigma_{BD}) \times (C_{BD} / C_{SD})^{1/2}, \text{ and}$$

$$n_{BD}(opt) = [\sigma^2_{BD} + \sigma^2_{SD} / (n_{SD}(opt))] \times (1/V(x)),$$

where $n_{SD}(opt)$ and $n_{BD}(opt)$ = optimum number of shoots per bush and optimum number of bushes, respectively; σ^2_{SD} and σ^2_{BD} = variances associated with shoots and bushes, respectively; and C_{SD} and C_{BD} as defined above. The variance around the mean ($V(x)$) was calculated as described for disease severity except that the allowed variability around the mean was 10%.

RESULTS AND DISCUSSION

There was a positive, linear relationship ($R^2 = 0.977$, $P < 0.0001$, $n = 2127$) between the number of spots per leaf and percent necrotic leaf area (Fig. 6.1), indicating that the latter disease variable is a reliable predictor of spot number on individual leaves. Based on the combined data from the 3 years, the predictive model to estimate number of spots per leaf (y) using percent necrotic leaf area (x) was $y = 2.33 + 6.07x$. The slope of the regression indicates that, for this particular cultivar, each 1% increment in necrotic leaf area corresponded to approximately 6 leaf spots. Given the epidemiological importance of the number of spots per leaf, Septoria leaf spot severity has been routinely evaluated using this disease variable (Chapters 3 through 5; Brannen *et al*., 2002; 2003; Cline, 2002; Ojiambo and Scherm, 2005; Ojiambo *et al*., 2005). Assessment of disease severity as number of spots per leaf has also been used for Septoria leaf spot of tomato (Ferrandino and Elmer, 1996). The present study shows that with adequate training, visual estimates of percent necrotic leaf area can substitute for the time-consuming task of counting the number of spots per leaf. Based on our measurements of the times required for assessing disease severity, it took about four times longer to count spots on individual leaves than to make visual estimates of percent necrotic area on the same leaves.

At the shoot level, the relationship between defoliation and the number of spots per leaf was characterized by a rapid rise to an upper limit with little change in defoliation above 60 spots per leaf (Fig. 6.2). Based on the combined data from the 3 years, the predictive model to estimate defoliation (y) using number of spots per leaf (x) was $y = 91.2\,(1-e^{-0.047x})$ ($R^2 = 0.729$, $P < 0.0001$, $n = 224$). In blueberry, return yield is strongly affected by the timing and magnitude of defoliation (Lyrene, 1992; Williamson and Miller, 2002), and it may thus be possible to develop action thresholds against Septoria leaf spot based on the anticipated level of disease-induced defoliation. We are currently investigating the relationships among Septoria leaf spot severity, defoliation, flower bud set, and return yield in more detail (Chapter 5; Ojiambo *et al*., 2005) to integrate this information with season-long disease dynamics in an effort to develop thresholds for disease management using an approach similar to that used for Septoria diseases of wheat (Verreet *et al*., 2000).

Mean disease severity levels were very similar across the 3 years, with 30.9, 35.7 and 31.7 spots per leaf and 4.6, 5.6, and 4.8% necrotic leaf area in 2001, 2002, and 2003, respectively (Table 6.1). The greatest sources of variation were variability among leaves on shoots and among shoots within bushes, regardless of whether disease was assessed as number of spots per leaf or percent necrotic leaf area. The variance component associated with leaves accounted for 34.5 to 51.9% of the total variation, while variance among shoots accounted for 38.5 to 62.2% of the total (Table 6.1). The variability associated with bushes was consistently the lowest, ranging from 3.6 to 7.9%. The relative variance component values (expressed as percentages of the total) were similar on average for the two measures of disease severity. When defoliation was assessed, the variance component for shoots accounted for >80% of the total variability, with the

remainder due to variation among bushes. Mean defoliation at the respective assessment dates was very similar across the 3 years (Table 6.1).

The optimum number of shoots per bush and bushes per field needed to estimate disease severity as number of spots per leaf or leaf area infected were similar (Table 6.2). However, the optimum number of leaves per shoot was higher for necrotic leaf area than for number of spots per leaf. The mean optimum number of leaves per shoot, shoots per bush, and bushes to assess disease as number of spots per leaf was 0.7, 2.2, and 23.7, respectively. When disease severity was assessed as percent necrotic leaf area, the corresponding mean optimum sample size was 1.5, 2.2, and 22.1 for leaves, shoots, and bushes, respectively. The mean optimum number of shoots per bush and bushes in the field for assessing defoliation was 2.6 and 7.9, respectively.

This study used formal statistical approaches to sampling for assessing Septoria leaf spot of blueberry and provides the first general sampling protocol for assessing severity of this disease. The relative variation among bushes, shoots within a bush, and leaves within a shoot was investigated to determine optimum sample sizes. The major constraint in most sample surveys, time, was also taken into account. Leaves within shoots and shoots within bushes accounted for the majority of the variation observed. Similar high levels of variability associated with leaves and shoots (i.e., stems or terminals) have been reported for alfalfa leaf spot diseases (Thal and Campbell, 1987) and for Alternaria blotch of apple (Filajdić and Sutton, 1994). Methods of reducing the error variance due to variation from leaf to leaf and shoot to shoot need to be investigated further. It needs to be remembered, however, that assessments in the present study were carried out in a planting untreated with fungicide and consequently, disease severity values on individual leaves varied widely (ranging from 0 to 297 spots per leaf). In experimental plots subjected to specific treatments such as fungicide applications, this range may be smaller,

leading to lower variation among leaves and shoots and correspondingly higher sample sizes for leaves and shoots on a smaller number of bushes. In a sense, therefore, the optimum sampling sizes reported here represent the special situation in which disease is assessed in untreated plots. Of course, the number of bushes sampled would also be reduced relative to those of shoots and leaves when large plantings with bushes located farther apart from one another are sampled, which results in an increase of C_b relative to C_s and C_l.

In summary, in locations where a wide range of Septoria leaf spot severity values is to be expected, we suggest that a sample of 2 leaves per shoot, 3 shoots per bush and 24 bushes in the field will provide a satisfactory assessment of disease severity. For a satisfactory assessment of defoliation, a sample size of 3 shoots per bush and 8 bushes is sufficient. More conservative sample sizes may be needed at the beginning of the season when disease severity is low and relative variability among leaves is high.

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Table 6.1. Estimated variance components for assessing variables associated with Septoria leaf spot, caused by *Septoria albopunctata*, on 'Premier' rabbiteye blueberry

		Sampling level					
		Mean Squares			Percent of total		
Variable [a]/year	Mean	Bush	Shoot	Leaf	Bush	Shoot	Leaf
Spots per leaf							
2001	30.9	110.1	951.3	852.8	5.7	49.7	44.6
2002	35.7	97.3	1656.6	908.1	3.7	62.2	34.1
2003	31.7	83.2	444.4	568.4	7.5	40.6	51.9
Necrotic leaf area (%)							
2001	4.6	2.8	24.1	24.6	5.5	46.7	47.8
2002	5.6	2.7	45.9	25.7	3.6	61.8	34.5
2003	4.8	2.5	11.8	16.5	7.9	38.5	53.6
Defoliation (%)							
2001	60.4	102.4	469.4	…[b]	17.9	82.9	…
2002	63.8	77.5	569.2	…	11.9	88.1	…
2003	65.1	96.1	650.9	…	12.9	87.1	…

[a] Disease severity and defoliation were assessed in mid- to late October and in mid-November, respectively.

[b] Defoliation was assessed on a per shoot basis; thus, there is no variation at the leaf-level.

Table 6.2. Optimum sample size for assessing variables associated with Septoria leaf spot, caused by *Septoria albopunctata*, on 'Premier' rabbiteye blueberry

Variable[a] /year	Optimum number of bushes	Optimum number of shoots per bush	Optimum number of leaves per shoot
Spots per leaf			
2001	29.3	2.0	0.8
2002	24.3	2.9	0.6
2003	17.4	1.8	0.9
Mean	23.7	2.2	0.7
Necrotic leaf area (%)			
2001	27.8	2.0	1.6
2002	21.0	2.9	1.1
2003	17.3	1.6	1.8
Mean	22.1	2.2	1.5
Defoliation (%)			
2001	8.6	2.2	…[b]
2002	7.1	2.7	…
2003	8.1	2.7	…
Mean	7.9	2.6	…

[a] Optimum sample sizes were derived using the variance components presented in Table 1 and their corresponding costs ratios (see text). The allowable variance around the mean used in these calculations was 20 and 10% for disease severity and defoliation, respectively.

[b] Defoliation was assessed on a per shoot basis; thus, there is no sampling at the leaf-level.

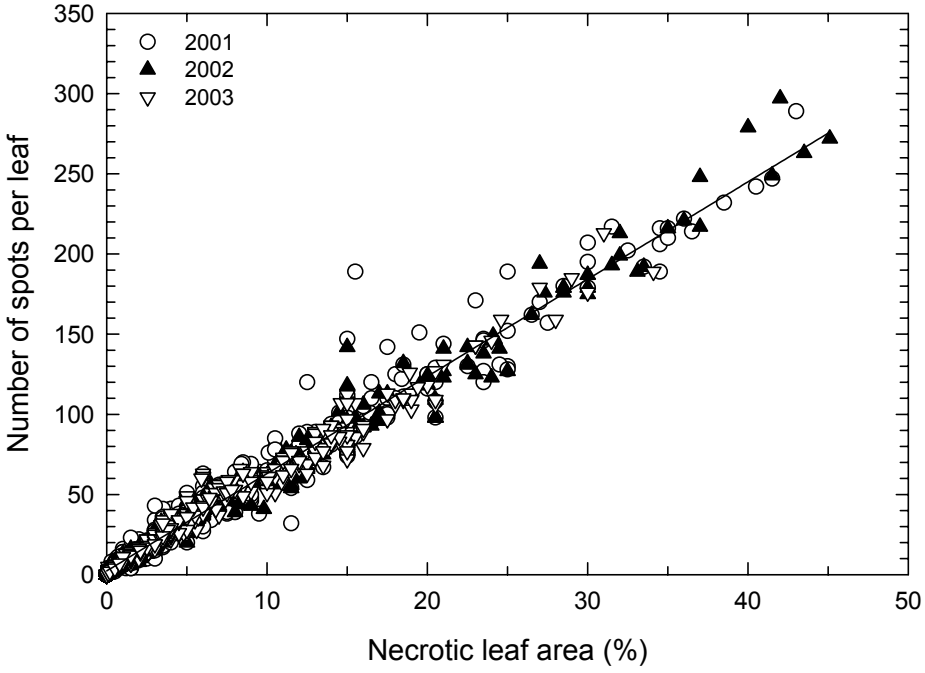

Fig. 6.1. Relationship between number of spots per leaf (y) and percent necrotic leaf area (x) on 'Premier' rabbiteye blueberry affected by Septoria leaf spot. Both variables were assessed in mid- to late October. The regression equation is $y = 2.33 + 6.07\ x$ ($R^2 = 0.977$, $P < 0.0001$, $n = 2127$).

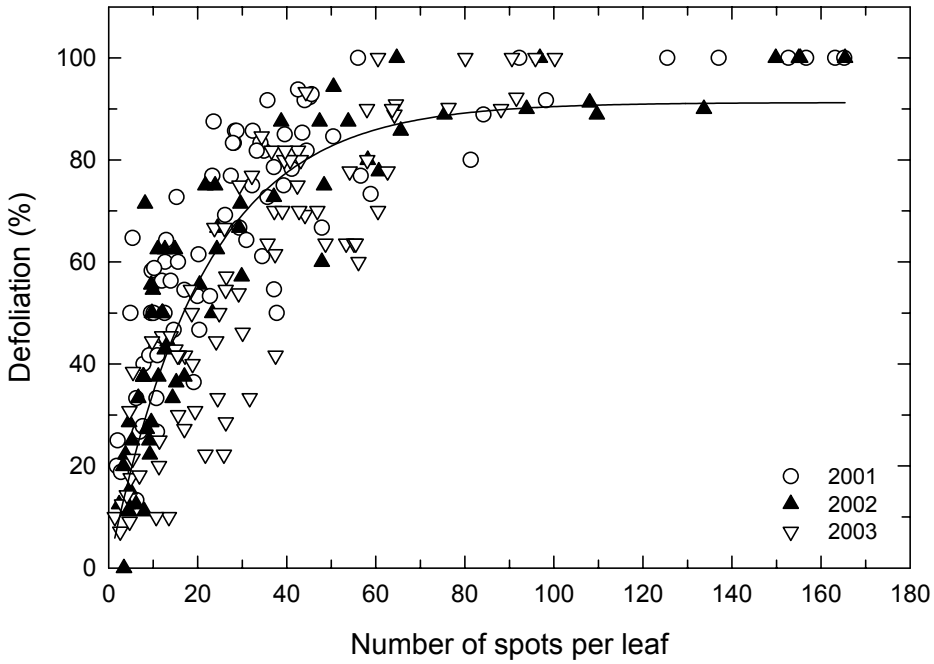

Fig. 6.2. Relationship between percent defoliation (y) and average number spots per leaf (x) on 'Premier' rabbiteye blueberry affected by Septoria leaf spot. Disease severity and defoliation were assessed on per shoot basis in mid- to late October and in mid-November, respectively. The regression equation is $y = 91.2\,(1-e^{-0.047x})$ ($R^2 = 0.729$, $P < 0.0001$, $n = 224$).

CHAPTER 7

CONCLUSIONS

The research carried out as part of this dissertation addressed selected aspects of the Septoria-blueberry pathosystem in an effort to fill critical gaps in knowledge on disease ecology and epidemiology. Difficulties in controlling Septoria leaf spot have been due in part to the limited information available about seasonal disease onset and progression, inoculum dynamics, and the effects of disease on premature defoliation, flower bud set, and return yield. Although the studies presented here did not develop or test specific management guidelines, they provide the basic information needed to critically evaluate the currently used, empirically-derived practice of controlling Septoria leaf spot solely with calendar-based fungicide applications after harvest.

Temporal progress of Septoria leaf spot was typical of polycylic epidemics in which several secondary cycles of the pathogen are produced resulting in continuous infection of the leaves. Pycnidiospore inoculum was present throughout the season, and leaves were infected by *S. albopunctata* season-long. Thus, it is clear that multiple fungicide applications are needed for effective disease suppression. Further, given the onset of the disease in the spring and the high levels of disease on early-emerging leaves, fungicide sprays may have to be initiated earlier than the current practice of initiating applications after fruit harvest in the summer. In this context, it would be important to determine potentially beneficial side-effects against Septoria leaf spot of early-season fungicide applications made to control mummy berry, blossom blight, and fruit rots. Moreover, since disease severity was higher on leaves located on shoots closer to the ground, improved control could also be achieved with more targeted application toward the lower part of the canopy.

Disease severity, defoliation, flower bud set, and return yield were found to be interrelated in the Septoria-blueberry pathosystem. Leaves with high disease severity at harvest subsequently abscised earlier in the fall than leaves with low disease severity, and shoots with severely diseased leaves and/or high levels of defoliation had a reduced potential to set flower buds. Furthermore, such shoots consistently had low return yields the following year. Relative to disease management, these findings emphasize the need for effective disease control before the actual onset of disease-associated defoliation in order to avoid negative effects on yield formation. With further research, it may be possible to identify specific disease severity or defoliation levels that can be tolerated during specific periods of crop development without negatively impacting flower bud set and return yield. For 'Premier', the results reported in this dissertation may be sufficient to develop such a system. Return yield data for this cultivar indicated that yield potential dropped markedly as final disease severity the previous fall exceeded about 50 to 60 spots per leaf (Fig. 5.3). Based on the curve describing disease progress in 2003 (Fig. 3.1B), we anticipate that a final disease severity of ~60 spots per leaf corresponds to a disease level of about 5 to 10 spots per leaf at harvest in late July. Thus, field experiments could be designed to test whether treatments that maintain disease below this level at harvest are indeed able to prevent losses in return yield. Such experiments will have to be carried out at multiple sites, in different years, and for cultivars differing in susceptibility in order to capture a wide range of biologically relevant conditions.

The data base used to develop disease assessment and sampling for Septoria leaf spot was collected during the first 3 years of this research. As such, it was not possible to utilize the guidelines developed for disease assessment and sampling in the epidemiological and yield loss studies carried out concurrently. However, the knowledge that a simple visual estimate of

percent necrotic leaf area is closely correlated with the number of spots per leaf (an unbiased and highly reproducible, but time-consuming measure of disease severity) should greatly facilitate future studies on the epidemiology and management of the disease. Similarly, the hierarchical sampling plans developed here for assessing disease severity and defoliation will be beneficial for obtaining reliable estimates of the two variables with the least expenditure of time.