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**VARIATION IN THE SEVERITY OF MUMMY BERRY DISEASE
AMONG LOWBUSH BLUEBERRY CLONES**

By

Laura Penman

B. S. Allegheny College, 2000

A THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of
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(in Botany and Plant Pathology)

The Graduate School

The University of Maine

August, 2003

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VARIATION IN THE SEVERITY OF MUMMY BERRY DISEASE AMONG LOWBUSH BLUEBERRY CLONES

By Laura Penman

Thesis Advisor: Dr. Seanna Annis

An Abstract of the Thesis Presented
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Lowbush blueberry production is important for the economy of Maine and the Maritime provinces, and mummy berry disease, caused by *Monilinia vaccinii-corymbosi*, often reduces plant stand vigor and fruit production. The severity of mummy berry disease among clones of lowbush blueberry were measured in four fields over a two year period (2001 and 2002). In each of 37 clones, 10 flowering stems with and without symptoms of blight were randomly selected at flowering to represent "diseased" and "healthy" stem populations, respectively. The severity of leaf and flower blight and fruit mummification was measured for each of these stems, and the relationships between blight and fruit mummification, adjusted fruit set, and berry weight was examined. There were significant differences in the severity of leaf blight among clones within each field, but significant differences in the severity of flower blight and fruit mummification among clones were observed in only half of the fields. The average severity of leaf blight per clone (the average proportion of leaves with symptoms of blight on "diseased" stems) was consistently correlated with the average incidence of blight (the average proportion of blighted stems within a clone). However, there was not a consistent relationship between the average severity of flower blight

and the average incidence of blight. Furthermore, there was no relationship between the severity of leaf blight and the severity of fruit mummification. In some fields, as the severity of leaf blight increased, adjusted fruit set and average berry weight decreased, which may justify current attempts to reduce leaf blight through chemical and cultural controls.

In order to examine the possible relationship between pathogen virulence and the severity of leaf blight, isolates of *M. vaccinii-corymbosi* were obtained from four clones of lowbush blueberry with different amounts of blight, and *in vitro* pectinase activities of these isolates was examined. Significant differences in pectinase production were observed among isolates, but pectinase activity of isolates did not account for differences in blight severity among clones.

To examine whether host factors contribute to the severity of mummy berry blight, ten "phenology" stems were randomly selected in each of 27 clones prior to bud break in 2002. The development of leaf and flower buds on each "phenology" stem was examined weekly until Julian day 145, and the height of each stem was recorded. The average severity of leaf blight per clone decreased as the average height of stems within the clone increased, but the relationship was not significant in all fields. However, the severity of leaf and flower blight on "phenology" stems increased with the developmental stage of their leaf and flower buds, respectively, on Julian days 131 through 139. Clones with less susceptible tissue during ascospore release had less blight than clones with more susceptible tissue, which suggests that avoidance or escape may account for differences in the severity of blight among clones. However, differences in inoculum density and host biochemical resistance may have also been involved.

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CHAPTER ONE: LITERATURE REVIEW

Lowbush blueberry production is important for the economy of Maine, Quebec, and the Maritime provinces and involves the management of naturally-seeded stands of *Vaccinium angustifolium* Ait. and *Vaccinium myrtilloides* Michx. Because seedlings are naturally established and spread laterally by rhizomes, lowbush blueberry fields contain a mixture of phenotypically-diverse clones (DeGomez and Smagula 1990). Researchers have observed that clones of *V. angustifolium* vary in their susceptibility to infection by *Monilinia vaccinii-corymbosi* (Reade) Honey, which is the most important pathogen of lowbush blueberry in Maine (Annis, personal observation; Lambert 1990; Lambert 1995). Factors that may contribute to these observed clonal differences in susceptibility have not been examined. Furthermore, the effects of leaf infection on fruit quality and quantity are not known.

This literature review will describe lowbush blueberry management practices and discuss aspects that may influence lowbush blueberry yield. The biology of *M. vaccinii-corymbosi* will be described, and factors that may affect the severity and incidence of infection by this fungus will be discussed.

Lowbush Blueberry Production

Managed lowbush blueberry fields occupy about 85,800 ha in North America and produce about 25,000-40,000 tons of fruit per year (Hancock 1995; Yarborough 2003b). Lowbush blueberry comprises approximately one third of the United States blueberry crop and is of major economic importance in Maine (26,300 ha), Quebec (16,200 ha), and the Maritime provinces (12,150 ha) (Yarborough 2003b). In comparison to lowbush blueberry, highbush blueberry produces more berries (55,000

tons per year) on less acreage (14,000 ha), and is often considered the most economically important blueberry species in North America (Hancock 1995). Rabbiteye blueberry produces approximately 4,500 tons of fruit per year on 2,400 ha (Hancock 1995). Most research on disease resistance in blueberry has focused on cultivars of highbush and rabbiteye blueberry because of their economic importance.

There are many characteristics that differ between lowbush blueberry and cultivated blueberry production. The most basic of these differences lies in the origin of the plants. Most commercial lowbush blueberry fields are developed by removing overstory trees and shrubs in naturally-occurring populations of lowbush blueberry and applying fertilizers, pesticides, and irrigation to maximize growth and production of these uncultivated plants (Shoemaker 1978; Yarborough 1998). Lowbush blueberry plants originate from seedlings and form clones through the production of new shoots from underground rhizomes (Hall et al. 1979; DeGomez and Smagula 1990). As a result, lowbush blueberry fields contain plants with a variety of genotypes and phenotypes. In contrast, highbush and rabbiteye blueberry fields contain cultivars that were bred to maximize factors such as fruit size, fruit flavor, productivity, length of ripening season, cold-tolerance, and disease resistance (Eck 1988). In order to maintain uniformity and simplify harvesting, most cultivated fields contain rows of one to a few genetically-identical cultivars (Shoemaker 1978; Hancock 1995), which contrasts sharply with the genetic diversity that results from the management of pre-existing lowbush blueberry clones. A heterogeneous host population is expected to exert non-uniform selection pressures on the pathogen population, which may result in greater intraspecific pathogen variability relative to that found in homogenous host crops (Agrios 1988; Zhu et al. 2000).

Lowbush blueberry production also differs from cultivated blueberry production in the location, climate, and species involved. Managed lowbush blueberry fields in Maine are comprised primarily of tetraploid *Vaccinium angustifolium* Aiton and its various subspecies (including *V. nigrum* and *V. lamarckii*) but also contain clones of the diploid *V. myrtilloides* Michx. (Shoemaker 1978; Vander Kloet 1978; Aalders and Hall 1961; Aalders and Hall 1963a). Lowbush blueberry plants require long chill periods in order to produce normal flowers, and the production of lowbush blueberries is most intense in Maine, Quebec, and the Maritime provinces (Hancock 1995). Blueberry production in other regions of the United States and Canada involves appropriate selection and management of cultivars for each region. Blueberry production in British Columbia, Ontario, and states in the Pacific Northwest, the Midwest, and the Mid-Atlantic regions of the United States is dependent on the Northern highbush blueberry (*V. corymbosum* L.) (Eck 1988). In Florida and other southeastern states, the rabbiteye blueberry (*V. ashei* Reade) and the Southern highbush blueberry (*V. australe*: a result of a cross between *V. corymbosum* and *V. darrowi* Camp) are the most important species (Eck 1988; Shoemaker 1978).

The management of established fields also differs between lowbush and cultivated blueberries. In order to control weed populations and increase fruit production, lowbush blueberry fields are typically pruned on a two-year cycle (Ismail and Hanson 1982; Lambert 1990; Shoemaker 1978). After harvest, “bearing” fields are burned or mowed. New, unbranched shoots develop in these “pruned” or “non-bearing” fields during the following growing season and form vegetative and reproductive buds for the next season’s growth. Once these reproductive buds bear flowers and fruit and the fruit is harvested, the plants are ready to be pruned again. In contrast, pruning of highbush blueberry occurs annually and involves removal of some of the lower and upper branches in young plants or the removal of weaker shoots in

older plants to maximize fruit size and quality (Shoemaker 1978). Rabbiteye blueberry plants are typically pruned less frequently and severely than highbush blueberry because rabbiteye cultivars rarely overbear (Shoemaker 1978). Even when highbush and rabbiteye bushes are heavily pruned, enough bearing shoots are left for a successful harvest in the next growing season, which differs from the extreme pruning typical of lowbush blueberry production. In addition to maximizing fruit production, alternate-year pruning of lowbush blueberry may also interrupt pest reproduction cycles. For example, the blight stage of mummy berry disease is rare in "pruned" fields relative to "bearing" fields (Lockhart 1961), presumably because susceptible host tissue is not available when infective spores are present. Furthermore, burning fields biennially destroys a considerable proportion of the overwintering populations of some blueberry pests, including blueberry spanworm and *Monilinia vaccinii-corymbosi*, the fungus responsible for mummy berry disease (Lambert 1990; DeGomez et al. 1990).

Factors Affecting Yield

Pollination

Insect pollination is critical for maximizing yield of *Vaccinium* species. The level of self-fertility varies among clones of lowbush blueberry, and a maximum fruit set of 52% was found for self-pollinated flowers (Aalders and Hall 1961). In contrast, Aalders and Hall (1961) observed up to 90% fruit set in cross-pollinated flowers of *V. angustifolium*. Although the above experiment did not utilize insect pollinators, the results justify attempts to maximize pollinator presence in lowbush blueberry fields through the importation of honeybees or bumblebees (Stubbs et al. 2001) and the maximization of suitable habitats for native pollinators, particularly bumblebees, mason bees, and alfalfa leafcutter bees (Stubbs et al. 2000, Drummond and Stubbs 2003).

Pollinators can only improve yields if they can successfully transfer pollen between compatible flowers. Aalders and Hall (1961) determined that cross-pollination between *V. angustifolium* and *V. myrtilloides* resulted in abortion of the fertilized fruit. Furthermore, they found that pollination of *V. angustifolium* with a 1:1 mixture of pollen from another *V. angustifolium* clone and *V. myrtilloides* significantly reduced fruit set, fruit size, and seed count, and significantly increased maturation time. They suggested that incompatibility of pollen from *V. angustifolium* and *V. myrtilloides* may explain why previous researchers (Bell 1957) observed low seed production in fields with nearly equal amounts of *V. myrtilloides* and *V. angustifolium*, even when pollination rates were maximized (Aalders and Hall 1961). Therefore, both pollination rates and pollen quality need to be improved in order to increase actual yields of lowbush blueberry.

Pollination and fertility are also affected by flower age. Wood and Wood (1963) determined that as flowers of *V. angustifolium* aged, their ability to set fruit decreased, and there was no fruit set if flowers were pollinated 8 days after anthesis. Furthermore, by comparing the decrease in percent fruit set over time between hand-pollinated and open-pollinated stems, they concluded that as the flowers aged, their attractiveness to insect pollinators also decreased (Wood and Wood 1963).

Water Availability and Efficiency

Despite the deep tap root systems characteristic of lowbush blueberry plants, yields are negatively influenced by water stress (Hall et al. 1979; Hall 1957; Hinkleton et al. 2000). Benoit et al. (1984) found that water stress in the “prune” year reduces the number of flowers and fruit produced during the subsequent “bearing” year. Water stress in the “bearing” year has been shown to reduce yields by reducing fruit size and weight (Hinkleton et al. 2000). Furthermore, there is evidence that the degree of

tolerance to water stress varies between genetic individuals (Benoit et al. 1984; Hinkleton et al. 2000), which may contribute to clonal differences in yield and resistance to pest pressure. Although research suggests that yields would be improved by monitoring soil moisture levels and irrigating as needed during both years of the crop cycle (Benoit et al. 1984; Hinkleton et al. 2000), few small growers irrigate during the “bearing” year and “prune” year irrigation is rare (Yarborough 1998). Large growers usually irrigate in both years, but recent water shortages have reduced the frequency of irrigation in the “prune” year (Yarborough, personal communication).

Photosynthate

Carbohydrate production and allocation are two major factors affecting plant growth. When photosynthate is limited, competition between tissues occurs, resulting in stunted growth of tissues relative to their potential growth capabilities. Blueberry plants are deciduous perennials, so initial leaf growth relies on reserve photosynthate stored in underground rhizomes (Smagula and DeGomez 1999). Once the photosynthetic tissue is able to sustain itself without relying on carbohydrate reserves, it can export “current” photosynthate to developing leaves, roots, stems, and reproductive tissue. Therefore, the timing and amount of vegetative tissue relative to reproductive tissue affects the allocation of carbohydrates to developing fruit.

Reproductive growth often competes strongly with vegetative growth for nutrients and photosynthate (Gardner et al. 1985). In species like lowbush blueberry that exhibit determinate growth (Bell 1950), vegetative growth is greatly reduced upon flowering and nearly all resources are subsequently devoted to flowering and reproduction (Gardner et al. 1985). In blueberry crops, several flushes (1-5, depending on blueberry type and cultivar) of vegetative growth occur per season, each

of which results in apical abortion (Eck 1988). Lowbush blueberry plants have only one or two flushes of vegetative growth per season (Hancock 1995).

The timing of floral bud break and fruit set relative to vegetative bud break is an important factor in determining carbohydrate allocation and yield. If reproductive growth occurs before or at the same time as vegetative growth, the amount of stored carbohydrate available for vegetative growth is expected to be relatively small due to competition with reproductive tissues (Darnell and Birkhold 1996; Birkhold et al. 1992). However, if vegetative growth occurs before reproductive growth, a large portion of reserve carbohydrate would be invested in photosynthetic tissue that is capable of producing "current" photosynthate. Darnell and Birkhold (1996) attributed the difference in fruit size between two rabbiteye blueberry cultivars to differences in the timing of vegetative bud break, suggesting that the late-leafing cultivar had smaller fruit due to lower levels of "current" photosynthate available for reproductive growth and development.

Because developing fruit makes rigorous demands on the carbohydrate budget of plants, the ratio of vegetative tissue to reproductive tissue affects both yield and quality of fruit crops. Leaf to fruit ratios were shown to be positively correlated with fruit weight of tomato (Hurd et al. 1979), fruit soluble solids in apple (Ferree and Cahoon 1987) and fruit weight, firmness, and soluble solids in sweet cherries (Facteau et al. 1983). Maust et al. (1999) studied the effects of reducing flower bud density upon vegetative tissue and fruit development in two southern highbush blueberry cultivars with different developmental phenologies. Leaf area: fruit ratios were positively correlated with fruit weight and fruit soluble solids for both cultivars. Furthermore, increases in leaf area to fruit ratios greatly reduced the ripening time of fruits in the late-leafing cultivar.

Nutrition

Nitrogen and phosphorus are limiting nutrients for most agricultural plants (Gardner et al. 1985). Most commercial blueberry fields in Maine are not deficient in nitrogen (Smagula and Dunham 1995). An excess of nitrogen applied in the "pruned" year may reduce yields by causing deficiencies in micronutrients, increasing susceptibility to winter injury, promoting growth of weeds, or stimulating an over-production of flower buds relative to the nutrient budget in the "bearing" year (Smagula and DeGomez 1999; Benoit et al. 1984; Yarborough et al. 1986; Penney and McRae 2000). As a result, the effects of "prune" year nitrogen applications upon yield have been variable, with experiments reporting yield gains (Rayment 1965; Smagula and Hepler 1978; Ismail et al. 1981), yield losses (Yarborough et al. 1986; Penney and McRae 2000), or no effect (Benoit et al. 1984). On the other hand, evaluations of nitrogen fertilizer applications in the spring of the "bearing" year have all demonstrated increased yields (Rayment 1965; Penney and McRae 2000; Percival and Sanderson 2002; Karemangingo and Melanson 2002). Unlike nitrogen, phosphorous is often limiting in Maine lowbush blueberry fields (Yarborough and Smagula 1993), and fertilizer applications of diammonium phosphate (DAP) (Smagula and Dunham 1995; Yarborough and Smagula 1993) or elemental phosphorous (Rayment 1965; Yarborough and Smagula 1993) in the spring of the "prune" year have increased yield. Elemental phosphorous applications in the spring of the "bearing" year have also been shown to increase fruit set and yield (Percival and Sanderson 2002).

Genetic Factors

In addition to variation in morphological characteristics, such as stem, leaf, flower, and fruit color (Barker et al. 1963; Wood and Barker 1963; Aalders and Hall 1963a; Vander Kloet 1978; Hall et al. 1979), stem height (Hall et al. 1979),

developmental phenology (Barker et al. 1963; Hall et al. 1979), drought-tolerance (Benoit et al. 1984; Hinkleton et al. 2000), herbicide-tolerance (Yarborough et al. 1986), and photoperiod effects on growth and development (Hall and Ludwig 1961), lowbush blueberry clones also vary in potential yield. Hall et al. (1966) evaluated percent fruit set in 15 clones over 3 or 4 production cycles and concluded that fruit set varies significantly among clones, but within-clone variation in fruit set is minor between years. Hepler and Yarborough (1991) evaluated yields of 100 blueberry clones grown under intensive management conditions, including high-density pollination, fertilization, irrigation, and weeding. The variability of clone productivity was quite large, with standardized yields ranging from 400 to 17,000 kg/ha. Variation in productivity among clones may be attributed to clonal differences in flower and fruit number, berry size, and berry weight (Barker et al. 1963; Pritts et al. 1985; Smagula et al. 1997) or to variation in the level of female fertility (Wood and Wood 1963; Hall et al. 1966). Smagula et al. (1997) also found that berry firmness, acidity, and sugar content varied among clones.

Pests

The availability of nutrition, water, and photosynthate determines the potential yield of crops within given genetic limitations. Insect pests and pathogens reduce actual yields by causing direct damage to the marketable commodity and/or causing indirect damage through injury to non-marketable plant parts. Blueberry sawfly, blueberry flea beetle, blueberry leaf beetle, blueberry spanworm, blueberry thrips, grasshoppers, and strawberry rootworm are responsible for damage to foliage of blueberry plants, and many of these insects also damage flowers or fruit (Collins et al. 1994; Collins et al. 1995a; Collins et al. 1995b; Collins et al. 1995c; Collins et al. 1995d; Collins et al. 1995e; Collins et al. 1995f). In contrast, parasitism of blueberry

fruit by the blueberry maggot fly frequently causes direct economic damage (Anonymous 1987). As a result, the blueberry maggot fly is considered to be the most important insect pest of lowbush blueberry (Anonymous 1987). Pathogens also vary in the type of yield reductions they cause in lowbush blueberry. Diseases responsible for damage to vegetative tissues include *Septoria* leaf spot and *Gloeosporium* stem canker (Lambert 1995). Red leaf, caused by *Exobasidium vaccinii*, is responsible for stand losses and reduced fruit production in lowbush blueberry (Lambert 1995). Botrytis blossom blight, caused by *Botrytis cinerea*, and blueberry anthracnose, caused by *Colletotrichum gloeosporioides*, both result in flower death and directly reduce yields (Lambert 1995). The most important disease of lowbush blueberry is mummy berry disease, caused by *Monilinia vaccinii-corymbosi* (DeGomez et al. 1990; Lambert 1990). Mummy berry disease causes both direct and indirect damage to blueberry crops. The primary infection stage, also referred to as mummy berry blight, causes death of leaves, flowers, and in extreme cases, entire clones or fields. The secondary infection stage, also referred to as fruit mummification, reduces the number of marketable fruit by replacing tissue of pollinated blueberry ovaries with fungal mycelium.

Mummy Berry Disease

Biology of *Monilinia vaccinii-corymbosi*

Monilinia vaccinii-corymbosi Honey belongs to the Ascomycota, class Discomycetes, order Leotiales, and family Sclerotiniaceae (Hawksworth et al. 1996). *Monilinia* species have apothecia located upon stalks and stroma in the form of either sclerotia or mummified host tissue (Hawksworth et al. 1996). The ascospores of *M. vaccinii-corymbosi* are binucleate and ovoid, with dimensions of 12-15 X 5-7 μm (Milholland 1977). Although some members of the Sclerotiniaceae do not produce

conidia (Hawksworth et al. 1996), *M. vaccinii-corymbosi* produces monilioid macroconidia (~ 27 x 25 µm) in chains on the upper surface of infected tissue (Honey 1936; Batra 1983). *Monilinia vaccinii-corymbosi* also produces microconidia (~2.5 x 2.5 µm) on the surface of ascospores, macroconidia, and mycelium (Batra 1983; Batra 1991). Microconidia have been shown to function as spermatia in other members of the Sclerotiniaceae (Drayton 1937; Groves and Drayton 1939; Byrde and Willetts 1977), but there have been no investigations indicating that these structures have a similar function in *M. vaccinii-corymbosi* (Batra 1991).

Compared to other *Monilinia* species that attack ericaceous hosts, *M. vaccinii-corymbosi* is less species specific, attacking several *Vaccinium* species (Batra 1983). The infection cycle of *M. vaccinii-corymbosi* begins in the spring with the emergence of apothecia from mummified fruit ("mummy berries") (Honey 1936). The apothecia discharge ascospores, which are responsible for blighting of leaf and flower buds. Cox and Scherm (2001b) recently provided evidence that wind is the main factor responsible for dispersing ascospores of *M. vaccinii-corymbosi*. Primary ascospore infection causes general necrosis (or blight) of the infected tissue. Infected flowers are no longer able to produce fruit, infected leaf petioles characteristically droop, and *M. vaccinii-corymbosi* produces chains of conidia on the petioles of infected leaves (Batra 1983). Pollinators are the most important vectors for secondary infection, which results from the transfer of conidia and pollen to the stigmas of flower buds (Cox and Scherm 2001b; Batra and Batra 1985). Once in the ovary, *M. vaccinii-corymbosi* kills blueberry seeds within four weeks (Shinners and Olson 1996) and mycelium occupies the entire ovary within 3-4 months (Milholland 1977). The shriveled, white or gray-colored "mummy berry" that results will drop to the ground, overwinter, and produce new apothecia and ascospores in the spring. Greenhouse inoculations of leaf buds with

ascospores and flowers with pollen and conidia have reproduced primary and secondary infection symptoms, respectively (Batra 1983). However, inoculations of leaves with conidia and flowers with a combination of ascospores and pollen failed to produce infection, suggesting the conidia and ascospores are specialized in function (Batra 1983).

Yield Reductions by *M. vaccinii-corymbosi*

Blight of flower buds or mummification of fruit results in direct yield losses, but it is not known how much leaf blight indirectly reduces fruit yield and quality in lowbush blueberry. Highbush blueberry, rabbiteye blueberry, and lowbush blueberry are susceptible to mummy berry disease, but they are differentially affected by the primary and secondary infection stages. Highbush blueberry yield is greatly reduced by secondary conidial infections (Milholland 1977), with some yield losses of highbush blueberry estimated at 70-85% (Wallace et al. 1976). Yield of lowbush and rabbiteye blueberry, on the other hand, is more affected by ascospore infection of flowers than the secondary conidial infection (Hildebrand and Braun 1991; Milholland 1977; Stretch and Ehlenfeldt 1997). Observations of mummy berry disease on *V. angustifolium* suggest that severe leaf blight may reduce berry size (Hildebrand and Braun 1991). In a survey of fields in Nova Scotia, Prince Edward Island, and New Brunswick, mummy berry blight was designated a serious threat to 40% of fields not sprayed with fungicide and was observed to cause complete crop losses in areas of up to 8 ha (Lockhart et al. 1983).

Resistance to the blight stage of mummy berry disease is significantly different among cultivars of highbush blueberry (Pepin and Toms 1969; Stretch et al. 1995; Ehlenfeldt et al. 1996), and the susceptibility of highbush cultivars to mummy berry blight was found to increase with *V. angustifolium* ancestry (Ehlenfeldt et al. 1996).

Highbush blueberry cultivars also exhibited significant differences in susceptibility to fruit rot, but unlike studies of blight severity between cultivars, many of the most resistant cultivars had large percentages of *V. angustifolium* ancestry (Stretch and Ehlenfeldt 2000). There has not yet been a detailed study of mummy berry blight resistance between clones of *V. angustifolium*, although it has been observed that different clones appear to exhibit varied levels of susceptibility (Annis, personal observation; Lambert 1990; Lambert 1995).

Factors Affecting Disease Severity

Traditionally, the interaction between the environment, host plant, and pathogen has been illustrated by diagramming each of these components as a side of the disease triangle (Agrios 1988). In this way, the disease triangle demonstrates that pathogen virulence and abundance, host susceptibility and distribution, and environmental conditions must all be favorable in order for disease to occur and progress in the host. Therefore, if the environment is constant within a compatible host-pathogen complex, then variation in the amount of disease is due to variation in factors that affect host susceptibility and/or variation in factors that affect pathogen virulence.

Sources of Variation in Virulence of Plant Pathogens

As fungi have coevolved with their plant hosts, they have developed ways to effectively overcome host defenses. Penetration of the host seems to rely on a combination of mechanical force and enzymatic breakdown of host barriers (Agrios 1988). Once host barriers have been breached, necrotrophic fungi, like *M. vaccinii-corymbosi*, are able to live saprophytically off of dead host material and often use

toxins and/or cell wall-degrading enzymes (CWDE) in order to kill host tissue in advance of growing hyphae (Batra 1983; Agrios 1988).

Considerable research has been performed in order to determine whether CWDE production contributes to the virulence of *Sclerotinia* species (a genus that is closely related to *Monilinia*). In studying the production of polygalacturonase, cellulase, and xylanase by two isolates of *S. sclerotiorum*, Marciano et al. (1983) did not find a correlation between enzyme production and virulence. Similarly, Errampalli and Kohn (1995) did not find a relationship between *S. sclerotiorum* aggressiveness and the isoforms of pectin methyl esterase and polygalacturonase produced by field isolates. Morrall et al. (1972) also was unable to find a correlation between virulence and total pectic enzyme production among 38 isolates of *Sclerotinia* spp. isolated from different hosts and geographical locations. However, Lumsden (1976) observed a positive relationship between *in vivo* polygalacturonase production and virulence by 10 isolates of *S. sclerotiorum* on bean, and Chan and Sackston (1970; 1972) found that *in vivo* and *in vitro* production of cellulase and polygalacturonase was correlated with virulence of *S. bataticola* on sunflower. Because of the differences in methodology, species, and isolates used in the aforementioned studies, it is unclear whether CWDE production is correlated with virulence in *Sclerotinia* species and whether this possible correlation may also be observed in closely related species of *Monilinia*.

There has not been an examination of CWDE production by *M. vaccinii-corymbosi*. However, there have been several studies of CWDE production and its relationship to virulence in *Monilinia* species responsible for brown rot of pome and stone fruits. *M. fructigena* produced high amounts of pectinase activity and negligible cellulase activity in apple tissue infected with brown rot (Calonge et al. 1969). Howell (1975) performed correlation and regression analysis on 119 mutagenic isolates obtained from a single spore isolate of *M. fructigena* and found that virulence was

positively correlated with alpha-L-arabinofuranosidase, pectin esterase, and polygalacturonase production. These results suggest that pectinase production may be important for virulence of *Monilinia* spp. and other pathogenic fungi in the family Sclerotiniaceae.

Sources of Variation in Susceptibility of Plant Hosts

Plants have developed several mechanisms to reduce their susceptibility to pathogen attack. Some of these defenses are constitutively present, while others are only expressed in response to encounters with the pathogen (Cowling and Horsfall 1980; Agrios 1988). Host defenses can be further characterized as physical or biochemical in nature. Constitutively-expressed host defenses include physical boundaries at the host plant surface and fortifications around individual cells, as well as fungistatic and fungitoxic compounds located at the host surface (Cowling and Horsfall 1980; Agrios 1988). Wax deposits, like those observed on lowbush blueberry leaves and fruit (Aalders and Hall 1963a), may avert host attack by repelling water necessary for the germination of fungal spores (Agrios 1980). The timing of stomatal development may also affect the ability of many fungal pathogens, including *M. vaccinii-corymbosi* to enter the host via the stomata (Agrios 1980; Hildebrand and Braun 1991). Upon entering the host, preformed tannins and phenolics may inhibit cell wall-degrading enzymes, and plant enzymes may hydrolyze the cell walls of the invading hyphae (Cowling and Horsfall 1980; Agrios 1988). The degree and combination of these factors determine the constitutively-expressed resistance of host plants to a given pathogen. Plants also express a variety of induced physical and chemical defenses in response to pathogen attack, including formation of abscission layers and tyloses and lignification of cell walls (Beckman 1980; Agrios 1988). Induced chemical defenses

include the hypersensitive response, detoxification of fungal toxins, phytoalexin production, and increased production of phenolics (Ono et al. 2001; Patil 1980; Agrios 1988). The resistance to mummy berry blight that has been observed in some highbush blueberry cultivars may be due to constitutive or induced biochemical mechanisms (Ehlenfeldt et al. 1996).

Host Avoidance

The ability of a host to prevent and react to pathogen attack via physical and chemical resistance mechanisms is important in reducing host susceptibility, but the amount of disease may also be affected by temporal factors. Host plants can avoid pathogen attack by timing susceptible stages of development to minimize encounters with the pathogen during these vulnerable periods (Agrios 1980; Agrios 1988). As discussed below, blueberry plants are only susceptible to primary and secondary infection by *M. vaccinii-corymbosi* during certain stages of leaf and flower bud development, respectively (Hildebrand and Braun 1991; Ngugi et al. 2002).

In order for primary leaf infection to occur, timing of ascospore release must coincide with the host's vegetative and reproductive development. In lowbush blueberry fields, ascospore release by *M. vaccinii-corymbosi* and bud development of *Vaccinium* species occurs between late April and early May during daylight hours (Hildebrand and Braun 1991; Ramsdell et al. 1974). Dormant vegetative and reproductive buds exhibited 0-1% infection incidence when inoculated with an ascospore suspension of 10^6 spores/mL (Hildebrand and Braun 1991). Infection incidence increased with bud development stage: infection rates of 85% were observed for vegetative buds with separating leaves and infection rates of 90% were observed for reproductive buds with the corolla extended beyond the calyx (Hildebrand and Braun 1991). The positive relationship between bud development and infection

incidence was attributed to increases in stomatal density associated with maturation of leaf and flower buds (Hildebrand and Braun 1991). Cultivar phenology was shown to be a critical factor in the severity of *M. vaccinii-corymbosi* blight on highbush blueberry; the length of shoots during ascospore release was positively correlated with the severity of blight (Ehlenfeldt et al. 1996). However, Pepin and Toms (1969) did not find a relationship between leaf bud development and the number of leaf and shoot infections in highbush blueberry grown in British Columbia. This discrepancy may be attributed to differences in the methods used to quantify host development and susceptibility. Pepin and Toms (1969) used five semi-continuous classes to categorize leaf bud development, and observed that all of the cultivars they studied had susceptible leaf tissue during periods of ascospore production. In contrast, Ehlenfeldt et al. (1996) used continuous measurements of shoot length as an indication of host development. Furthermore, Ehlenfeldt et al. (1996) compared the percentage of blighted shoots as a measure of susceptibility among cultivars, but Pepin and Toms (1969) placed cultivars into arbitrary classes of susceptibility (susceptible, moderately susceptible, and resistant) based on the number of infections relative to a highly susceptible variety. It is likely that the semi-continuous classification system used by Pepin and Toms (1969) was less sensitive in detecting differences in leaf bud development and susceptibility among cultivars than the continuous system used by Ehlenfeldt et al. (1996).

Several studies have illustrated that the timing of apothecial development and ascospore release correspond to the timing of host bud development (Ramsdell et al. 1975; Batra 1983 ; Lehman and Oudemans 1997; Hildebrand and Braun 1991 ; Ramsdell et al. 1974). Lehman and Oudemans (2000) reasoned that if pathogen inoculum production consistently corresponds with host bud break, then host phenology must exert a strong selective force on the pathogen. In a study of the timing

of apothecial development and ascospore release in several *M. vaccinii-corymbosi* populations on highbush blueberry, Lehman and Oudemans (2000) concluded that the sexual phenology of the fungus is moderately to highly heritable.

Just as susceptible host tissue is necessary for successful primary infection, conidia must contact floral stigmas during vulnerable periods in the host's reproductive development in order for secondary infection to occur. As previously mentioned, pollinators are implicated as being the major vectors of conidia in the secondary infection stage of mummy berry disease. After deposition on the stigma, germ tubes travel down the stylar canal and proceed to infect host ovaries: a process that occurs within seven days under favorable conditions (Milholland 1977; Shinnars and Olson 1996). In a study of two cultivars, hyphal growth rates down the stylar canal were highest when flowers were inoculated with 50 conidia/stigma on the same day as anthesis (Ngugi et al. 2002). Hyphal growth rates decreased linearly with time, so that flowers inoculated 5 days after anthesis exhibited negligible growth of hyphae (Ngugi et al. 2002). Furthermore, incidence of secondary infection decreased exponentially in response to flower age, with infection incidences ranging from 76.4% when flowers were inoculated at anthesis to 15.5% when they were inoculated 4 days after anthesis. Ngugi et al. (2002) also determined that pollination status affects hyphal growth rates and secondary infection incidence. Pollination of stigmas one to two days before inoculation reduced hyphal growth rates and disease incidence relative to flowers that simultaneously received inoculum and pollen (Ngugi et al. 2002). Pollination of stigmas after inoculation with conidia had no significant effect on hyphal growth rates (Ngugi et al. 2002). These results demonstrate that flower age and pollination status are important factors in determining the success of secondary infection by *M. vaccinii-corymbosi*.

Environment

Environmental factors are also important in disease development, as they affect survival, growth, and development of both the host and the pathogen. Oversummer survival of mummy berries in highbush blueberry fields is reduced by prolonged contact with warm, moist soil (Cox and Scherm 2002a), and overwinter survival is highest if pseudosclerotia were in dense weeds beneath highbush bushes (Wallace et al. 1976). Apothecial germination from mummy berries in highbush blueberry fields requires 900-1200 hours of temperatures below 7 C, and apothecial development in both highbush and lowbush blueberry fields is favored by warmer temperatures (15 C) and 16 hour day length (Milholland 1974; Milholland 1977; Hildebrand and Braun 1991). Ramsdell et al. (1975) suggested that daily shifts in relative humidity stimulate daytime ascospore discharge, and that ascospore germination occurs primarily at night in association with increases in relative humidity. In addition to affecting pathogen development, temperature has also been shown to have an effect on host susceptibility. In a greenhouse study, Hildebrand and Braun (1991) determined that leaf and flower buds of lowbush blueberry plants were more susceptible to ascospore infection when exposed to below-freezing temperatures. These studies demonstrate how temperature and humidity influence the amount of mummy berry disease by affecting growth and survival of *M. vaccinii-corymbosi* at important stages of its life cycle.

Thesis Objectives

The overall purpose of this thesis is to determine whether clones of lowbush blueberry differ significantly in infection by *Monilinia vaccinii-corymbosi*, and to investigate host and pathogen factors that may be responsible for any observed differences. Specific host and pathogen characteristics of interest include phenology of leaf and flower bud development in *Vaccinium angustifolium* and *in vitro* pectinase production by field isolates of *M. vaccinii-corymbosi*. Furthermore, I wanted to determine the effect of leaf blight on secondary infection and yield.

CHAPTER TWO: VARIATION IN THE SEVERITY AND INCIDENCE OF MUMMY BERRY DISEASE AMONG LOWBUSH BLUEBERRY CLONES

Introduction

Lowbush blueberry plants are managed for fruit production on 85,800 ha in Maine, Quebec, and the Maritime provinces (Lambert 1990; Lambert 1995; Yarborough 2003b). Mummy berry disease is the most damaging and widespread disease of lowbush blueberry in Maine (Lambert 1990) and is caused by the fungus *Monilinia vaccinii-corymbosi* (Batra 1983). Ascospore infection by *M. vaccinii-corymbosi* blights leaves and flowers of *Vaccinium* species (primary infection), and conidia produced on these blighted tissues subsequently infect ovaries of *Vaccinium* species, resulting in the formation of mummified fruit, or “mummy berries” (secondary infection) (Honey 1936; Batra 1983). Sweet lowbush blueberry (*Vaccinium angustifolium*) and sour-top blueberry (*V. myrtilloides*) are the most frequently observed species in lowbush blueberry fields (Yarborough 1998), and reductions in fruit production by *M. vaccinii-corymbosi* are primarily due to ascospore infection in these species (Hildebrand and Braun 1991). Mummy berry blight has caused complete crop loss of areas up to 8 ha in size in lowbush blueberry fields not treated with fungicides (Lockhart et al. 1983).

Managed lowbush blueberry fields are established through the removal of overstory vegetation to encourage growth of pre-existing lowbush blueberry plants, and fields are typically pruned biennially in order to increase fruit production (Lambert 1990; Penney et al. 1997). Pruned plants produce flower buds at the end of the growing season in the “non-bearing” year and produce flowers and fruit during the following “bearing” year (Yarborough 1998). Mummy berry disease is rare in “pruned”

fields but occurs frequently in “bearing” fields (Lockhart 1961), presumably because leaf tissue is not present in “pruned” fields during periods of ascospore release.

Established seedlings of *V. angustifolium* and *V. myrtilloides* spread asexually through underground rhizomes to produce stands of genetically-uniform stems, which are called clones. These clones vary in a variety of morphological and physiological characteristics, including leaf, stem, and fruit color (Barker et al. 1963; Wood and Barker 1963; Aalders and Hall 1963a; Vander Kloet 1978; Hall et al. 1979), stem height (Hall et al. 1979), developmental phenology (Barker et al. 1963; Hall et al. 1979), tolerance to stress (Benoit et al. 1984; Hinkleton et al. 2000; Yarborough et al. 1986), flower number, fruit number, berry size, and berry weight (Barker et al. 1963; Hall et al. 1966; Pritts et al. 1985; Hepler and Yarborough 1991; Smagula et al. 1997). Observations of mummy berry blight in lowbush blueberry suggest that lowbush blueberry clones also vary in their susceptibility to mummy berry blight (Annis, personal observation; Lambert 1990; Lambert 1995), but these observations have not been subjected to statistical analysis.

The objectives of this chapter were to examine differences in the severity and incidence of mummy berry disease among clones of lowbush blueberry.

Materials and Methods

Selection of Clones

Two bearing commercial fields (Airport I and Junior Grant) were studied in Deblois, ME during the summer of 2001. Ten distinct clones of *V. angustifolium* were selected in Airport I during flowering based on observed qualitative differences in clonal morphology and blight severity. Airport I was in its second year of fruit production after pruning, and missed one of two applications of Orbit (propiconazole), which is a

protectant fungicide that is currently being used to control mummy berry disease in Maine. Four morphologically distinct clones with symptoms of mummy berry blight were also selected in Junior Grant. These clones were located in a region of the field that typically does not receive full pesticide applications due to its proximity to an irrigation pond. Airport I and Junior Grant were both irrigated during the bearing year.

Three bearing commercial fields (Airport II, Columbia, and Sam Hill) were evaluated in the spring and summer of 2002 in Deblois, Maine. Airport II was adjacent to Airport I, but Airport II contained different clones and was bearing fruit a year later than Airport I. Most of the eight clones in Airport II, twelve clones in Columbia, and seven clones in Sam Hill were chosen in July of 1998 by running two diagonal transects across the field and selecting distinct clones that had symptoms of secondary fruit infection (mummy berries). Clones were initially marked by pounding plastic stakes into the ground, and in 2000 these clones were marked using GPS (Geographic Positioning System) way points. In some cases, these previously selected clones could not be clearly identified in March, 2002, so morphologically distinct clones within the proximity of the original GPS position were selected prior to occurrence of disease symptoms. In Airport II, three previously unsurveyed clones were chosen in order to increase sample size. Airport II and Columbia were irrigated during the bearing year, but Sam Hill did not receive irrigation. None of the fields studied in 2002 received fungicide applications. Airport II and Columbia were in their first year of production since pruning. Based on the amount of branching on stems and records of fruit production in previous years, Sam Hill appears to have been in its third year of fruit production since pruning.

Disease Severity and Incidence

Selection of Stems

Blight severity was investigated by randomly selecting ten flowering stems with symptoms of blight within each clone by tagging the diseased stem closest to a thrown object. Some effort was used to sample throughout the clone by selecting stems near the center and perimeter of the clone.

In order to examine differences among clones in the absence of mummy berry blight, ten flowering stems without symptoms of primary infection were also randomly selected within each clone. These "healthy" stems were not the same stems as those used to study bud development, stem height, or blight severity, and care was taken to avoid clumped distributions of "healthy" stems. Because many clones in Sam Hill had few flowering stems, "healthy" stems in Sam Hill were selected by identifying the first flowering stems encountered without symptoms of primary infection.

Table 1. Evaluation schedule of "healthy" and "diseased" stems

(Dates expressed in Julian days)

Field	1° infection (During flowering)	1° infection/ fruit set (Green fruit)	2° infection (Pre-harvest)
Airport 1	148-158 (2001)	179-180 (2001)	211 (2001)
Junior Grant	156-158 (2001)	184 (2001)	207 (2001)
Airport 2	161 (2002)	181 (2002)	220 (2002)
Columbia	155 (2002)	180 (2002)	220 (2002)
Sam Hill	152 (2002)	177 (2002)	215 (2002)

The total numbers of leaves, flowers, and fruit on each “healthy” or “diseased” stem were counted at flowering, during the green fruit stage, and shortly before harvest (Table 1). The number of leaves, flowers, and fruit with symptoms of mummy berry disease were also counted at each time period (Table 1). With the use of Proc GENMOD (SAS Institute, Cary, North Carolina), the count data was fitted to a negative binomial distribution, and Type 3 analysis was selected due to the complex and unbalanced “treatment” structure. Model statements were used to compare the total number of leaves, flowers, and fruit among fields, between “healthy” and “diseased” populations, among clones, and among observation dates. All three observation periods were used in the model for vegetative units (leaves), but the model for reproductive units (flowers and fruit) only included counts obtained during flowering and pre-harvest. Furthermore, separate models for flowering and pre-harvest were needed to compare the blight and mummification of reproductive units, respectively, among model components. Junior Grant was not included in the models because of its comparatively small sample size (4 clones).

Measurements of Disease Severity

Leaf blight severity was quantified as the proportion of infected leaves on each “diseased” stem during flowering, the green fruit stage, and just prior to harvest (Table 1). Kruskal-Wallis ANOVA ($\alpha = 0.05$) (Proc NPAR1WAY in SAS) was used to determine if the severity of leaf blight differed among clones within each field. Dunn's multiple comparison test with a correction for tied values was used to compare leaf blight severity among clones within a field (Neave and Worthington 1988).

The severity of flower blight was calculated as the proportion of blighted flowers on each "diseased" stem. (This data was missing for Jr. Grant). Flower blight severity was measured at flowering and during the green fruit stage (Table 1). Because many stems had no flowers with symptoms of mummy berry blight, permutation analysis was used to determine whether observed differences among clones in each field were significantly different from 1000 random assortments of the same observed values (Sokal and Rohlf 1995). If there were significant differences, Dunn's multiple comparison test ($\alpha = 0.05$) was used to compare flower infection severity among clones within each field.

The severity of fruit mummification was determined by calculating the proportion of healthy flowers (during flowering) that produced mummy berries. The number of mummies on each "healthy" and "diseased" stem was counted shortly before harvest (Table 1). Because there were no records of the amount of flower blight in Jr. Grant, the severity of fruit mummification in that field was calculated as the proportion of total flowers on each stem that produced mummy berries. Permutation analysis was performed on 1000 permutations of the observed data in order to determine whether clone and health status of stems in each field influenced the distribution of observed values, and whether there was a significant interaction between clone and stem health.

Measurements of Disease Incidence

Incidence of blight was measured in all fields in 2002 (Columbia, Sam Hill, and Airport II) during flowering. In order to sample throughout the clone, each clone was visually divided into quadrants and a 10 cm square was tossed into each quadrant. The incidence of blight was measured as the proportion of stems within the square that had

symptoms of primary infection. Kruskal-Wallis ANOVA ($\alpha = 0.05$) was used to determine whether clones within the same field differed in their incidence of blight.

Spearman's correlation procedure was used to determine whether there was a significant relationship between the average severity and average incidence of blight within a clone.

Results

Quantitative Differences Among Fields, Clones, and Observation Dates

Vegetative Tissue

The GENMOD model for vegetative tissue sufficiently fit the data set (Pearson Chi-square goodness of fit value of 1.0732). The Type 3 ANOVA table generated from this model is located in Appendix A. Across all fields, "diseased" stems generally had more leaves than "healthy" stems (Figs. 1, 2, 3, and 4), and this difference made a significant contribution to the model ($p < 0.0001$). During flowering, there was a greater range in average leaf number per stem among clones within the "diseased" population (28.5 to 127.8 leaves per stem) than within the "healthy" stem population (16.8 to 82.0 leaves per stem). The difference in the number of leaves between "healthy" and "diseased" populations across all fields was significantly influenced by growth stage (ie. flowering, green fruit, pre-harvest) ($p < 0.0001$) (Figs. 1, 2, 3, and 4). In Airport I and Columbia, "diseased" stems in clones that had more leaf blight (located to the right of the graph) had declines in the number of leaves between flowering and the green fruit stage and between flowering and pre-harvest. Clones with less severe leaf blight (located to the left of the graph) in these fields gained leaves during the observation period.

Although the number of leaves on sampled stems differed among fields (Figs. 1, 2, 3, and 4), field was not an important factor in the model ($p < 0.9913$). However, the interaction between field and growth stage did make a significant contribution to the model ($p < 0.0001$). Within the “healthy” stem population, there was an increase in average leaf number in both Airport I and Sam Hill after flowering, but this trend was not as pronounced in Columbia and was not observed in Airport II (Figs. 1, 2, 3, and 4). There were significant differences in leaf number among clones within each field (Kruskal-Wallis $p < 0.0001$ in all fields for both “healthy” and “diseased” populations), and these differences in leaf number contributed significantly to the model ($p < 0.0001$). Differences in the number of leaves among clones were influenced by health status of the stem, and this interaction was a significant factor in the model ($p < 0.0001$). However, the growth stage during which observations were made did not affect differences in leaf number among clones within a field ($p = 0.9815$).

Reproductive Tissue

The GENMOD model generated for reproductive tissue had Pearson Chi-square goodness of fit value of 1.3304. The Type 3 ANOVA table generated from this model is located in Appendix B. “Diseased” stems had significantly more reproductive units than “healthy” stems ($p < 0.0001$), which was probably due to differences in the number of flowers between “healthy” and “diseased” stems at flowering, which is when stems were selected. At flowering, the average flower number per clone was higher for “diseased” stems than for “healthy” stems (the range for “diseased” stems was 4.6 to 37.6 flowers per stem compared to a range of 3.9 to 28.7 flowers per “healthy” stem).

Airport I

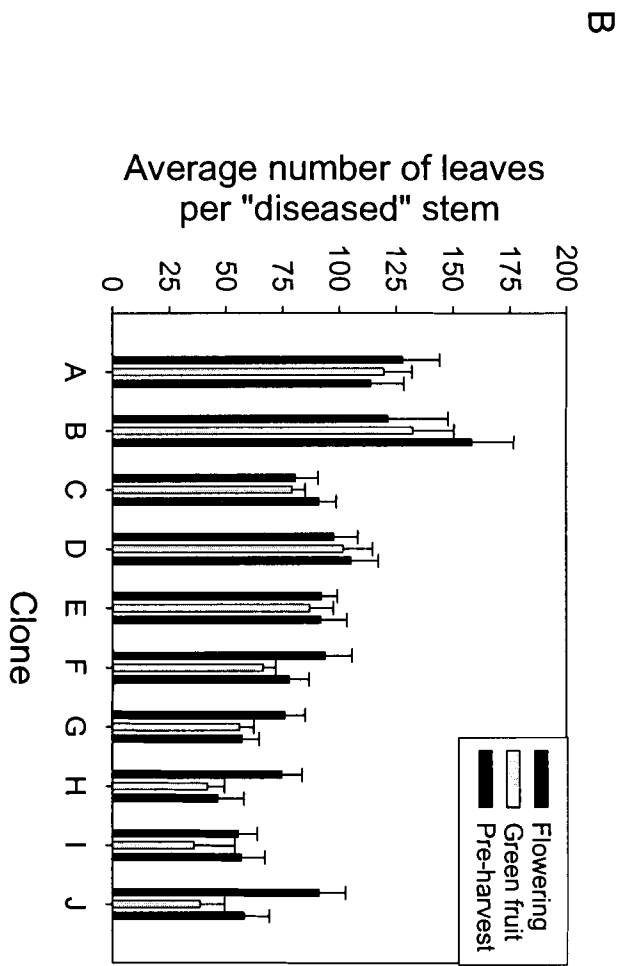
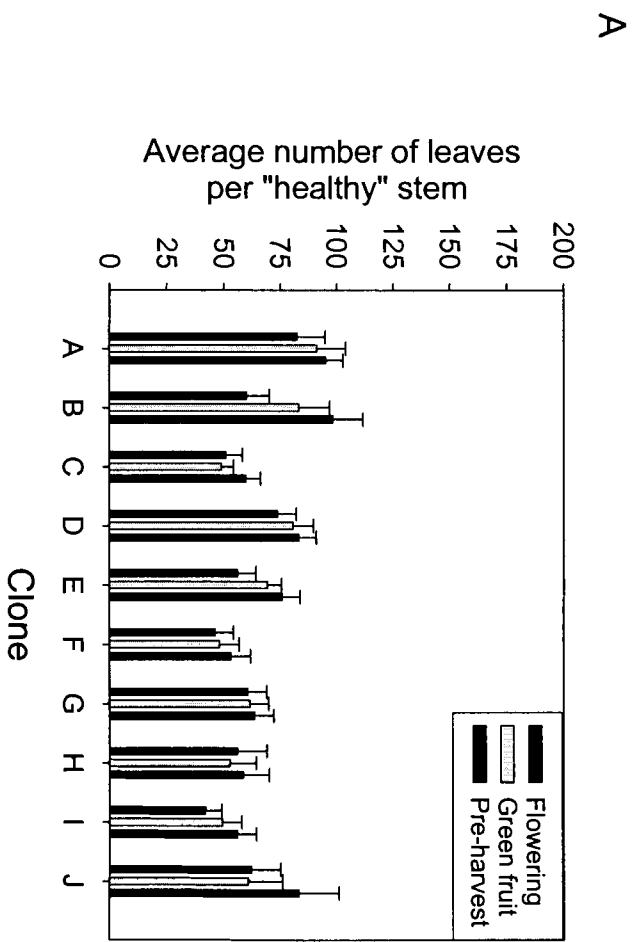


Figure 1. Average number of leaves for "healthy" (A) and "diseased" (B) stems in Airport I, within each clone at different growth stages (Table 1). Clones are arranged in order of increasing severity of leaf blight. Bars show the standard error of the means.

Airport II

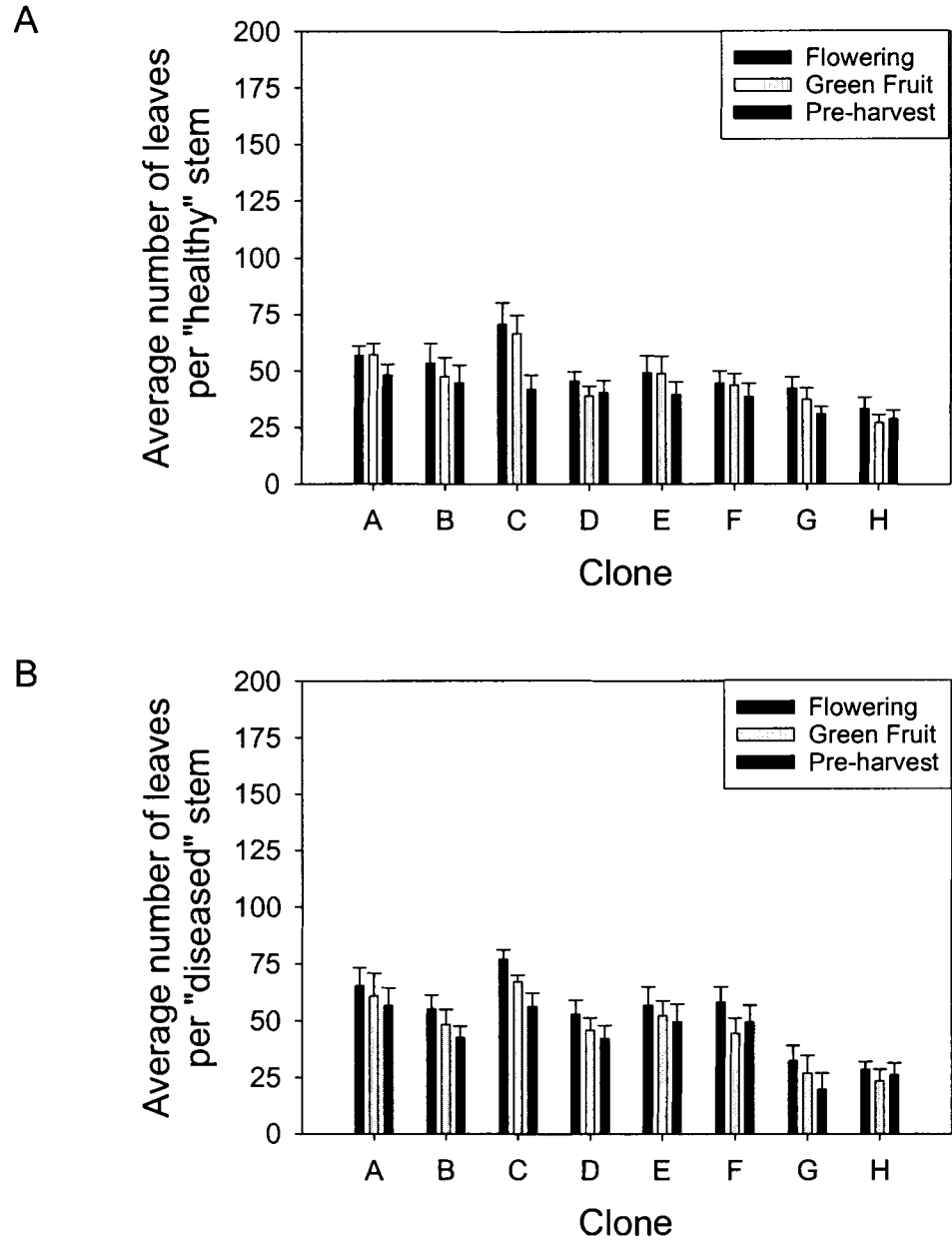


Figure 2. Average number of leaves for "healthy" (A) and "diseased" (B) stems in Airport II, within each clone at different growth stages (Table 1). Clones are arranged in order of increasing severity of leaf blight. Bars show the standard error of the means.

Columbia

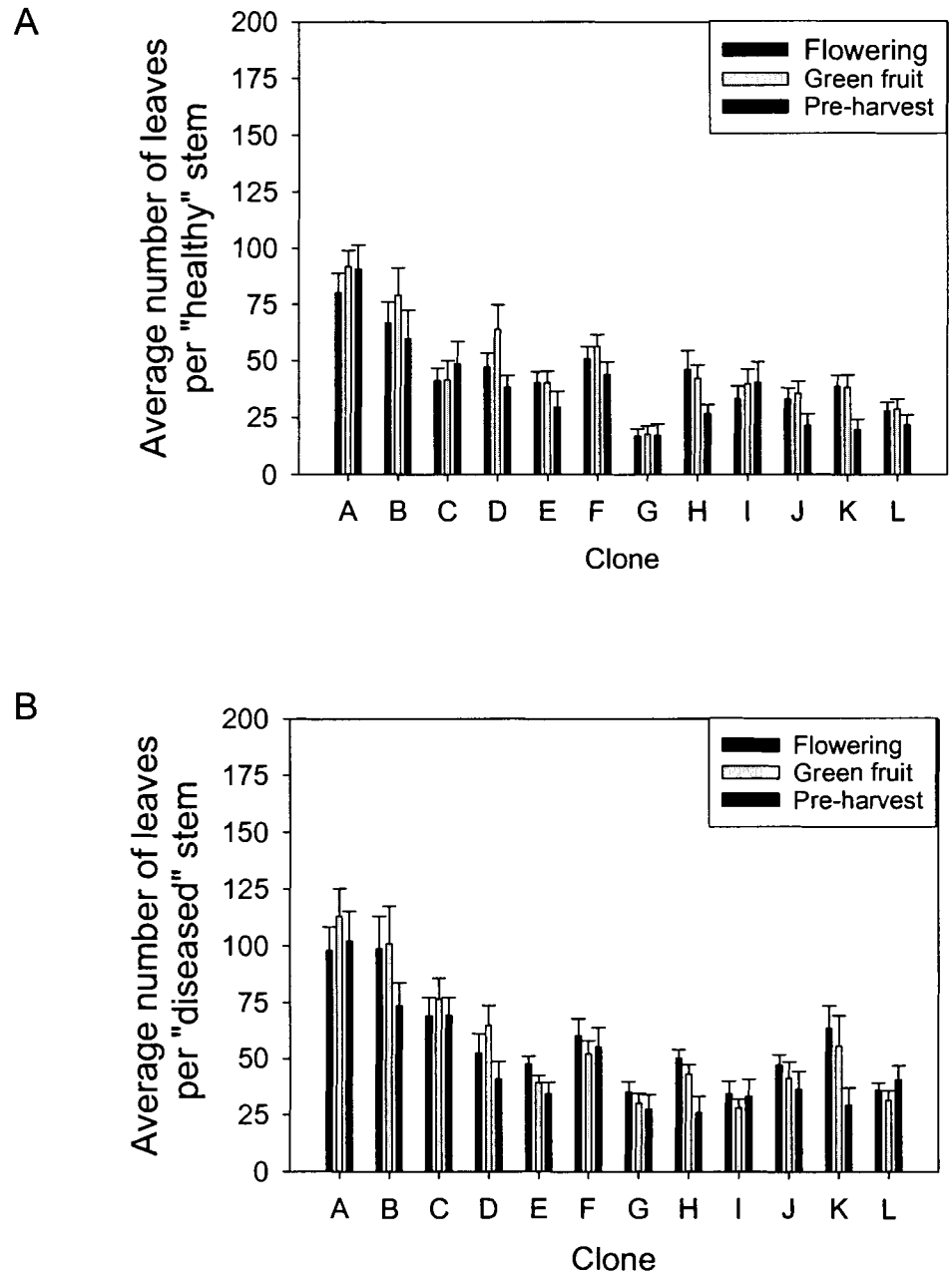


Figure 3. Average number of leaves for "healthy" (A) and "diseased" (B) stems in Columbia, within each clone at different growth stages (Table 1). Clones are arranged in order of increasing severity of leaf blight. Bars show the standard error of the means.

Sam Hill

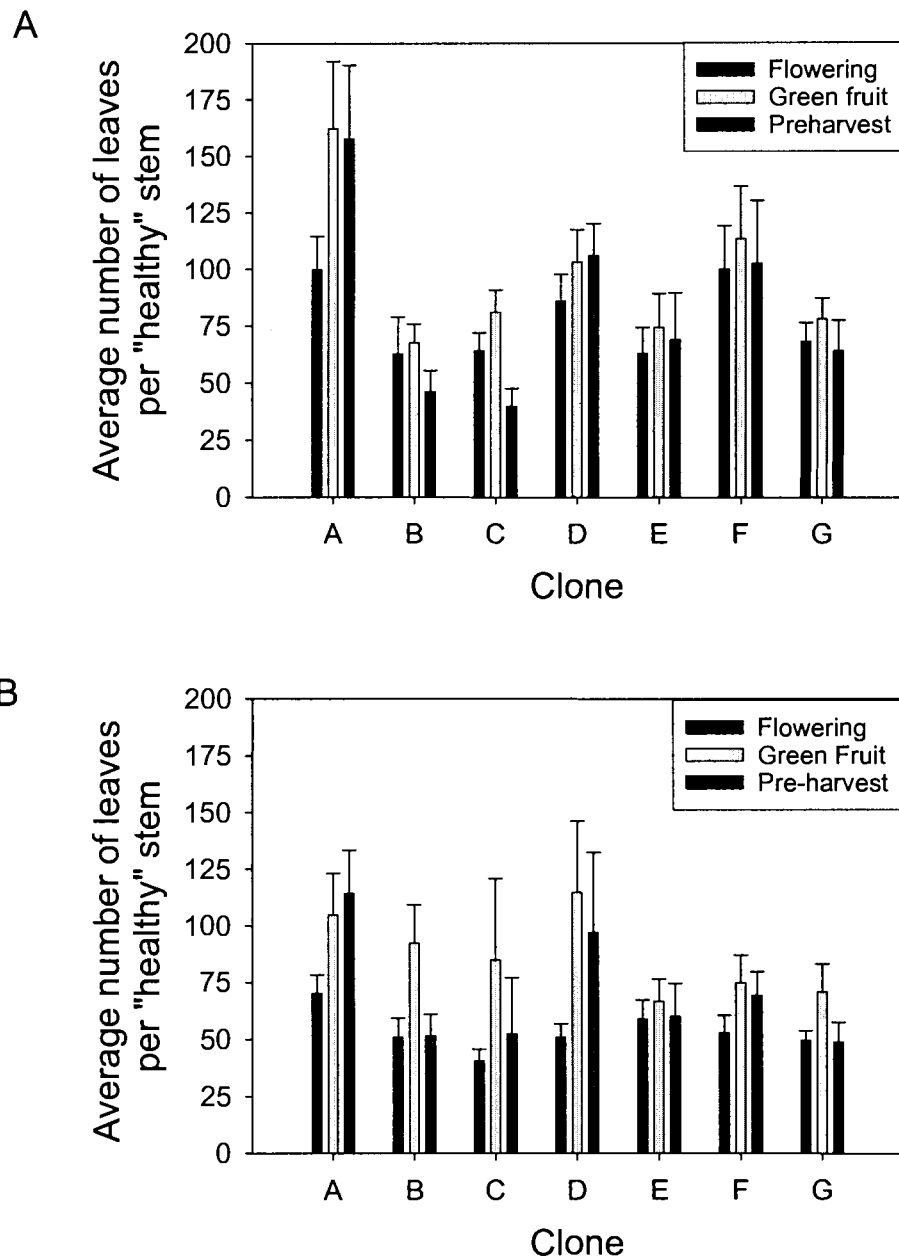


Figure 4. Average number of leaves for "healthy" (A) and "diseased" (B) stems in Sam Hill, within each clone at different growth stages (Table 1). Clones are arranged in order of increasing severity of leaf blight. Bars show the standard error of the means.

Prior to harvest, “diseased” stems still had more viable reproductive units per clone than “healthy” stems, but the magnitude of the difference between “healthy” and “diseased” stems was smaller than it was at flowering (the range for “diseased” stems prior to harvest was 2.0 to 26.9 fruit per stem, while the range for “healthy” stems was 1.1 to 21.2 fruit per stem). The difference in the number of reproductive units between “healthy” and “diseased” stems and differences across combinations of stem health and growth stage both made significant contributions to the model ($p < 0.0001$ for both factors).

The number of reproductive units per stem also varied among fields ($p < 0.0001$), and the average number of reproductive units per stem in a field was influenced by both the health status of the stem ($p = 0.0014$) and the growth stage of the plants ($p < 0.0001$) (Figs. 5, 6, 7 and 8). Within the “healthy” stem population, stems in Airport I and Sam Hill had more flowers on average than stems in Airport II and Columbia. Just prior to harvest, “healthy” and “diseased” stems in Airport I had more fruit on average than the other fields (Figs. 5, 6, 7, and 8).

The number of reproductive units differed among clones within a field and made a significant contribution to the model ($p < 0.0001$). Furthermore, the growth stage of the clone and health status of stems both affected differences in the number of reproductive units among clones ($p < 0.0001$ and $p = 0.0011$, respectively) (Figs. 5, 6, 7, and 8).

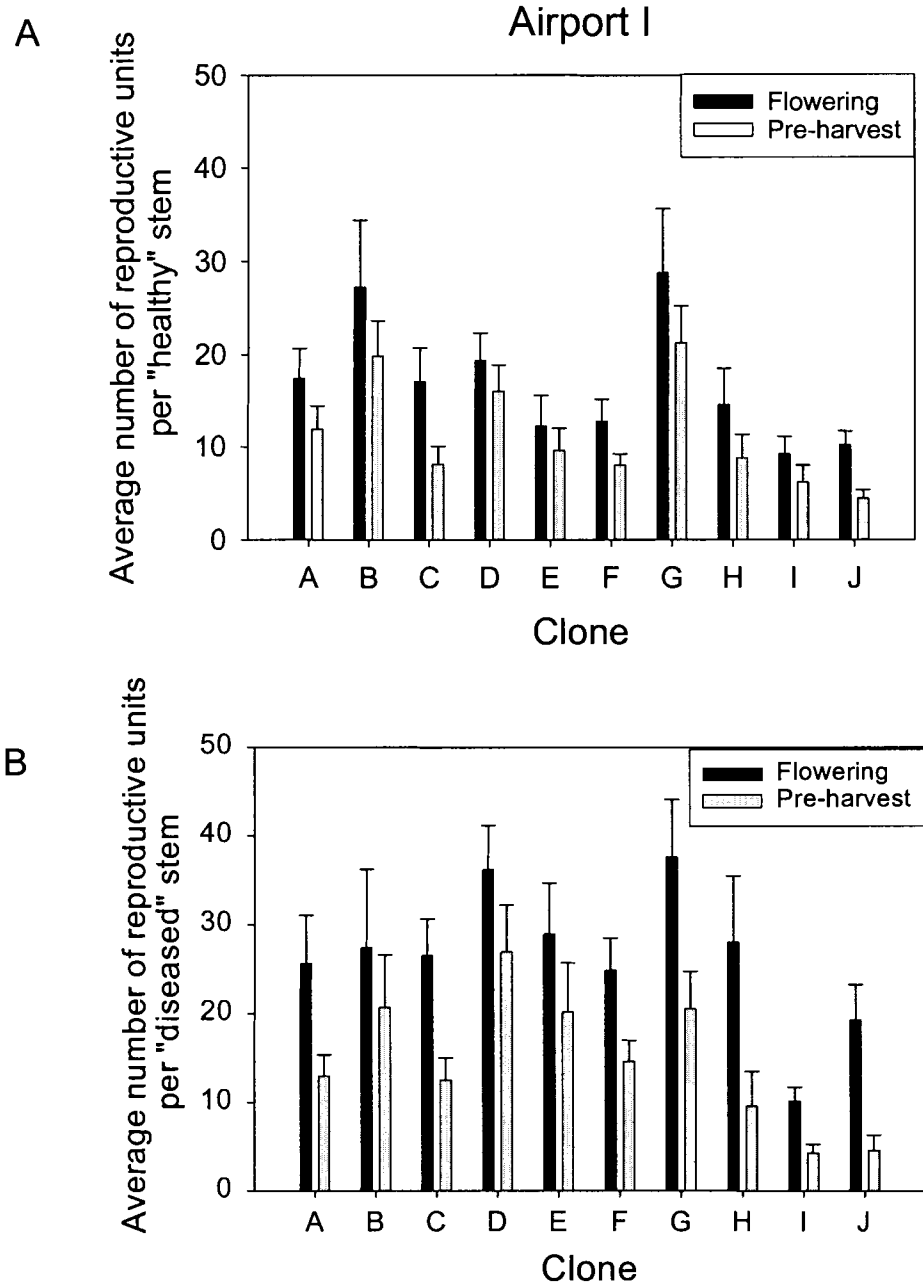


Figure 5. Average number of reproductive units for "healthy" (A) and "diseased" (B) stems in Airport I, within each clone at different growth stages (Table 1). Clones are arranged in order of increasing severity of leaf blight. Bars show the standard error of the means.

Airport II

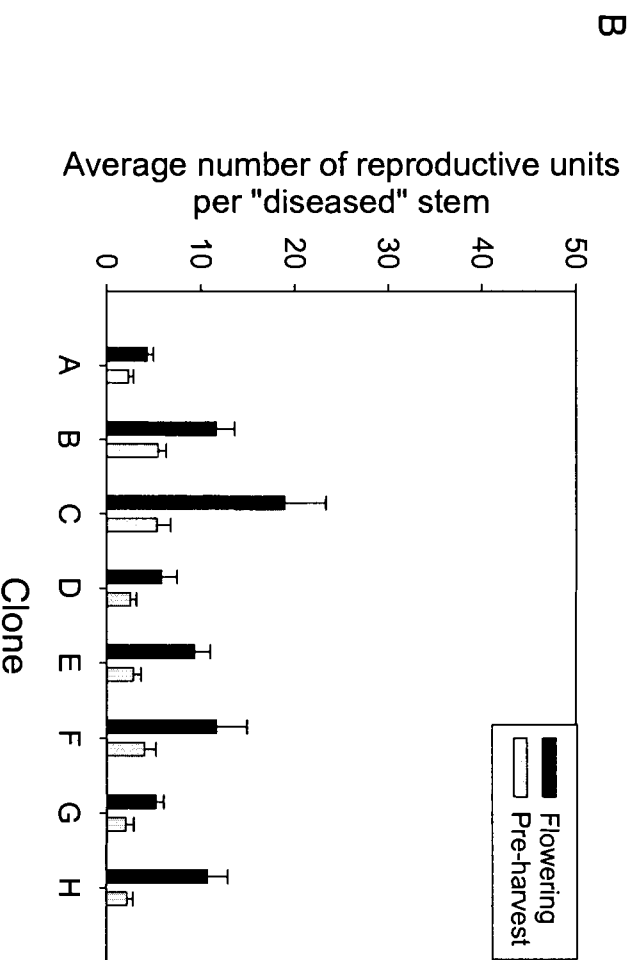
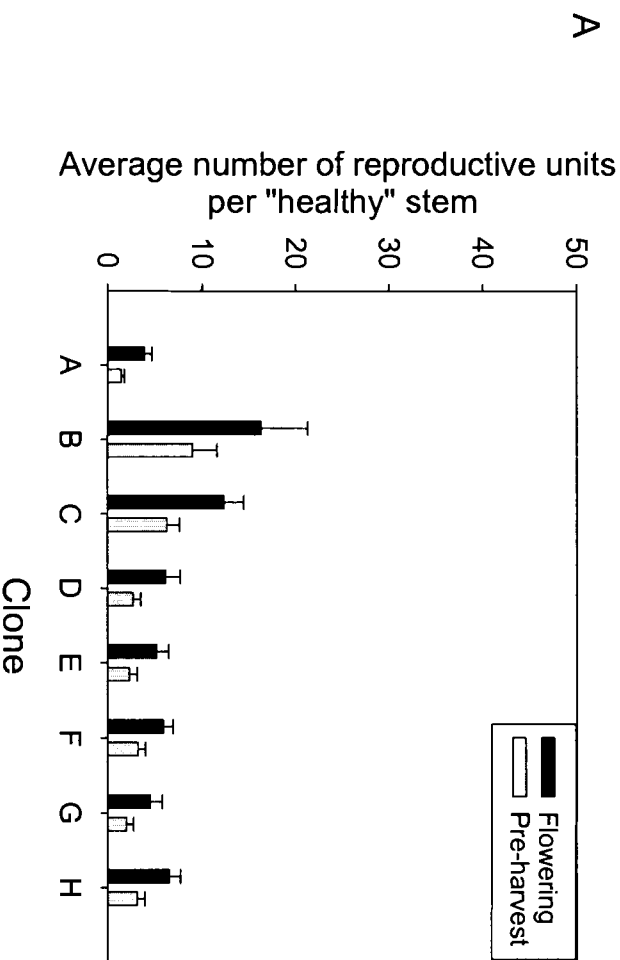


Figure 6. Average number of reproductive units for "healthy" (A) and "diseased" (B) stems in Airport II, within each clone at different growth stages (Table 1). Clones are arranged in order of increasing severity of leaf blight. Bars show the standard error of the means.

Columbia

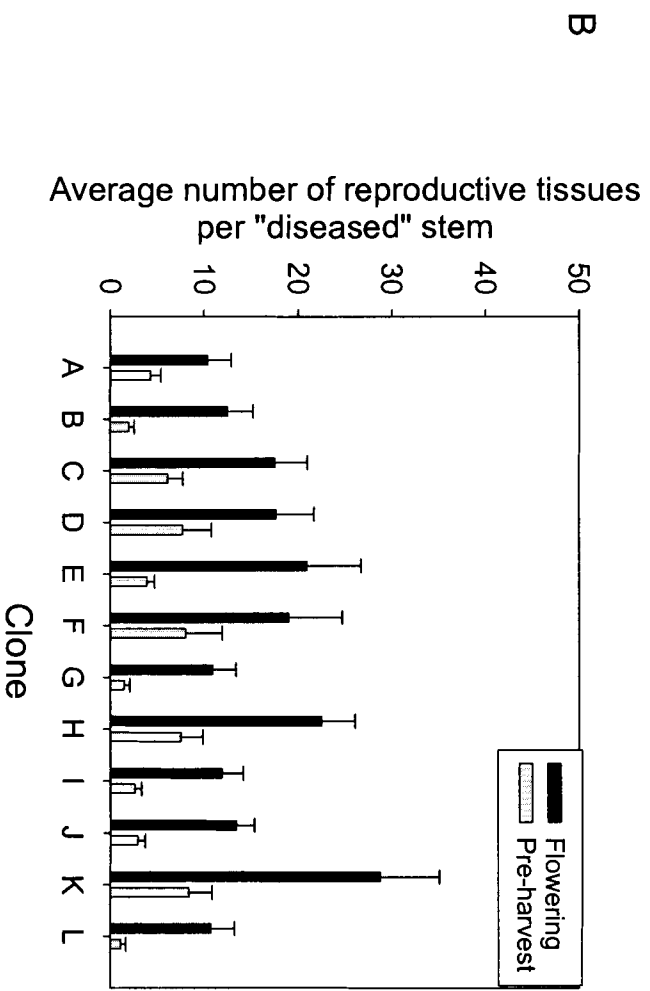
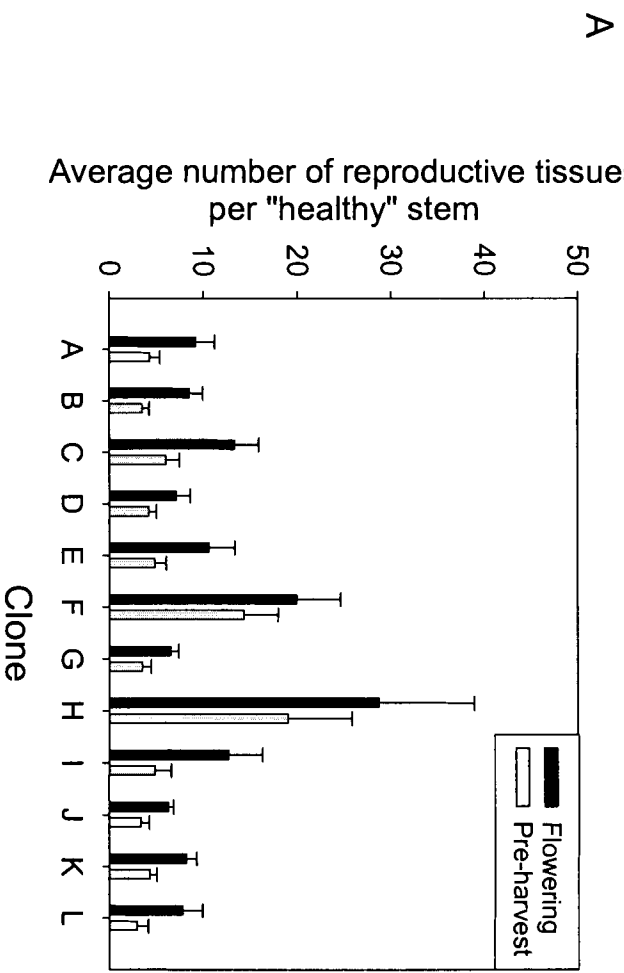


Figure 7. Average number of reproductive units for "healthy" (A) and "diseased" (B) stems in Columbia, within each clone at different growth stages (Table 1). Clones are arranged in order of increasing severity of leaf blight. Bars show the standard error of the means.

Sam Hill

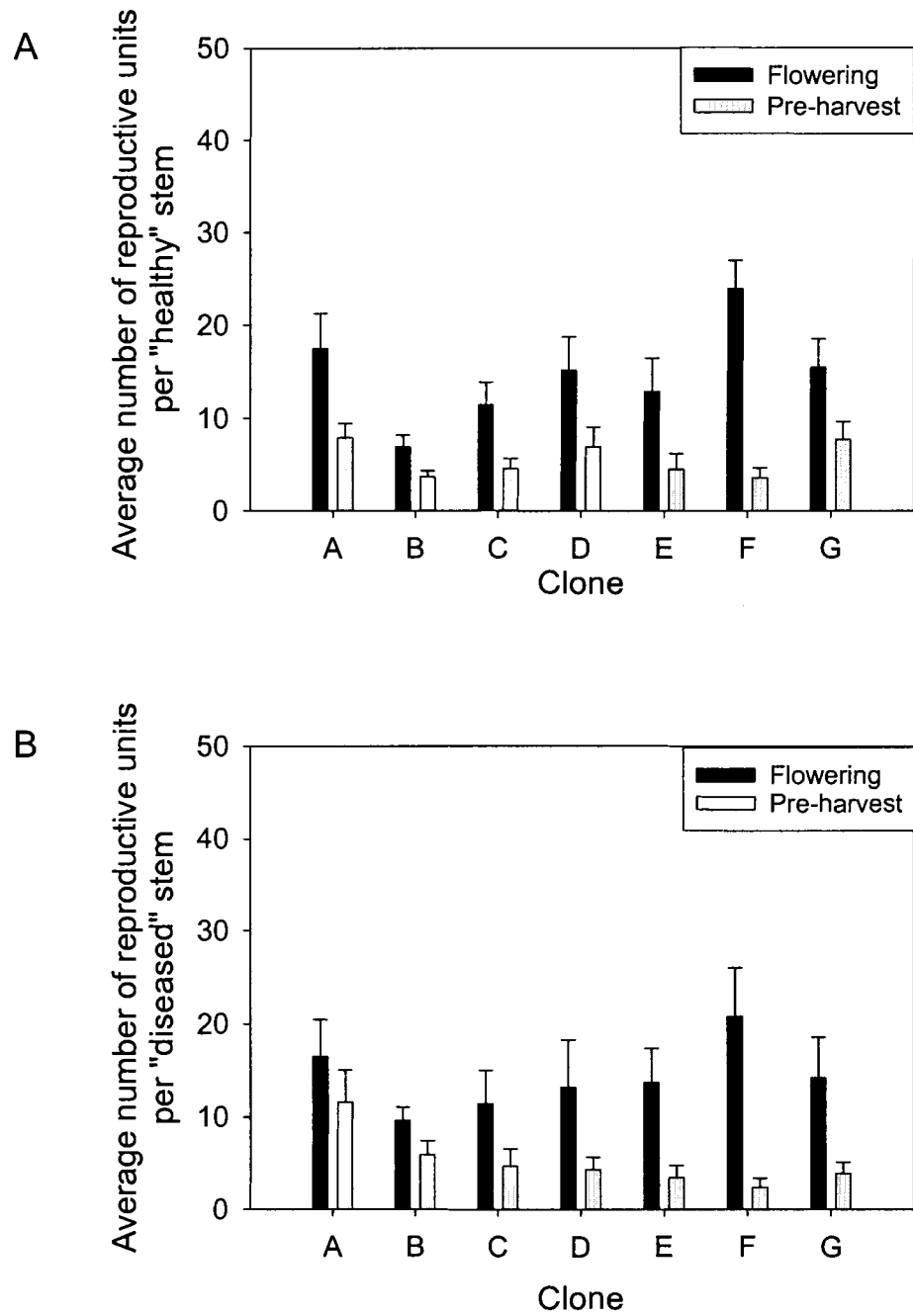


Figure 8. Average number of reproductive units for "healthy" (A) and "diseased" (B) stems in Sam Hill, within each clone at different growth stages (Table 1). Clones are arranged in order of increasing severity of leaf blight. Bars show the standard error of the means.

Proportion of Blighted Vegetative Tissue

Within the “diseased” stem population, there were generally more healthy leaves than diseased leaves, as the average proportion of leaves with symptoms of blight was usually under 0.5 (Figs. 9a, 10a, 11a, 12a, and 13a). Furthermore, the proportion of leaves with blight symptoms decreased throughout the growing season. For example, “diseased” stems in Airport II had average leaf blight severities per clone ranging from 0.09 to 0.43 during flowering. However, during the green fruit stage, the average severity of blight per clone ranged from 0.03 to 0.21, and no blighted leaves were observed on “diseased” stems just prior to harvest.

On “diseased” stems, the proportion of leaves with blight varied significantly among clones in Airport I, Airport II, Columbia, and Sam Hill ($p=0.0001$, $p=0.0001$, $p=0.0001$, and $p=0.0026$, respectively) (Figs. 9a, 11a, 12a, and 13a). Clones in Airport I had more severe leaf blight (ranging from 0.07 to 0.63) than clones in Airport II, Columbia, and Sam Hill (which collectively ranged from 0.05 to 0.43). There was not a significant difference in the severity of leaf blight among clones in Junior Grant, which had average leaf blight severity ranging from 0.03 to 0.05 (Fig. 10a).

Proportion of Blighted Flowers

The GENMOD model generated for flower blight had Pearson Chi-square goodness of fit value of 0.9557. The Type 3 ANOVA table generated from this model is located in Appendix C. There were generally more healthy flowers than blighted flowers on stems within the “diseased” population, as the average proportion of flowers with symptoms of blight was consistently below 0.50 (Figs. 9b, 11b, 12b, and 13b). The proportion of flowers with symptoms of blight varied significantly among fields ($p<0.0001$) and among clones within a field ($p<0.0001$). “Diseased” stems in second-

year bearing fields (Airport I and Sam Hill) had less flower blight than “diseased” stems in first-year fields (Airport II and Columbia) (Figs. 9b, 11b, 12b, and 13b). The severity of flower blight within clones in Airport I and Sam Hill were similar during flowering (ranging from 0.03 to 0.20 and from 0.01 to 0.11, respectively), but the severity of flower blight for clones in Airport II (from 0.02 to 0.46) and Columbia (from 0.00 to 0.34) were approximately 2 X greater.

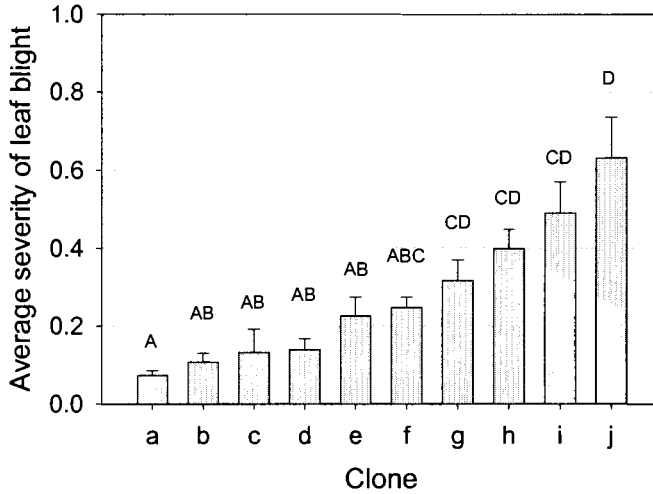
The severity of flower blight on “diseased” stems was significantly different among clones in Airport I ($p=0.001$ as determined by Kruskal-Wallis ANOVA) and Airport II ($p=0.017$ as determined by Kruskal-Wallis ANOVA) (Figs. 9b and 11b), but differences among clones were not significant in Columbia or Sam Hill (Figs. 12b and 13b). Although significant differences in the severity of flower blight were not detected among clones in Columbia, Clone A had less flower blight than other clones in the same field (Fig. 12b). The proportion of flowers with symptoms of blight was not measured in Junior Grant.

Severity of Fruit Mummification

The Pearson Chi-square goodness of fit value of GENMOD model generated for fruit mummification was 1.2787, and the Type 3 ANOVA table generated from this model is located in Appendix D. The severity of fruit mummification, measured as the proportion of healthy flowers that produced mummy berries, varied significantly among fields ($p<0.0001$).

Airport I

A



B

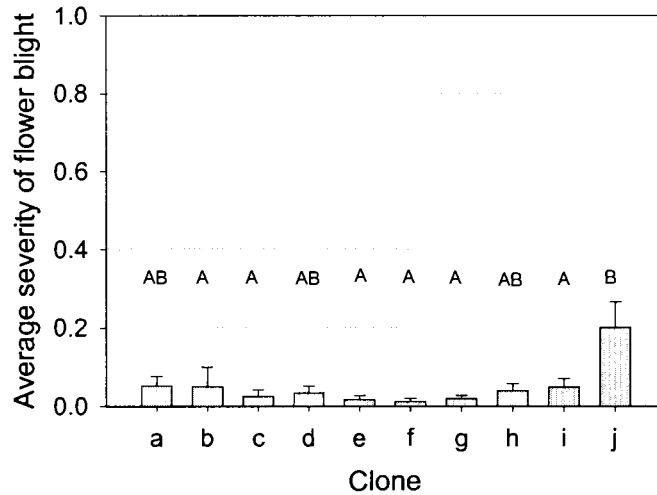


Figure 9. Average severity of leaf and flower blight among clones in Airport I during flowering. Average severity values of “diseased” stems are presented with standard error bars. Clones in both graphs are arranged in order of increasing severity of leaf blight. Clones with different letters are significantly different at $\alpha=0.05$. A) Blight severity of leaves was calculated as the proportion of leaves with symptoms of primary infection. B) Blight severity of flowers was calculated as the proportion of flowers with symptoms of primary infection.

Junior Grant

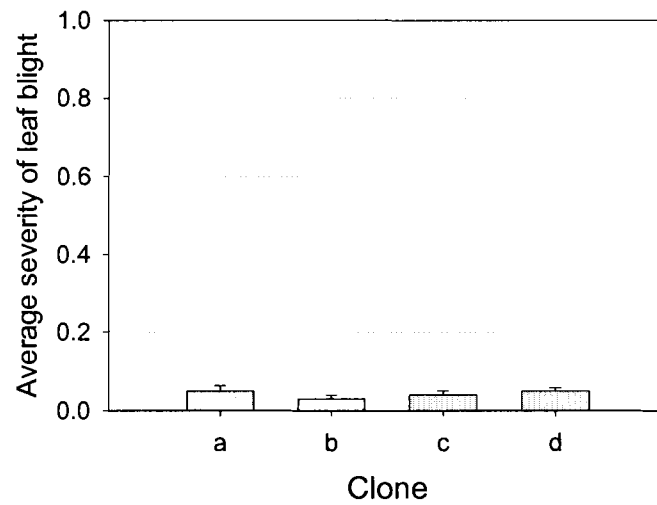


Figure 10. Average severity of leaf blight among clones in Junior Grant during flowering. Average severity values of “diseased” stems are presented with standard error bars. Blight severity of leaves was calculated as the proportion of leaves with symptoms of primary infection.

Airport II

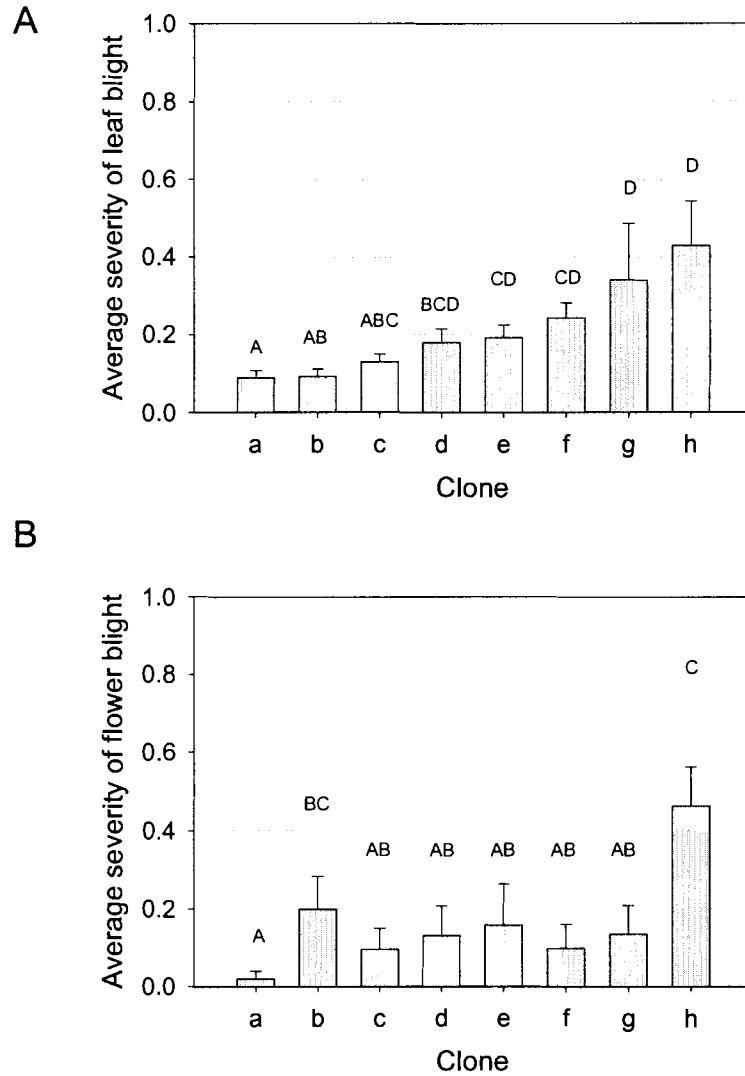


Figure 11. Average severity of leaf and flower blight among clones in Airport II during flowering. Average severity values of “diseased” stems are presented with standard error bars. Clones in both graphs are arranged in order of increasing severity of leaf blight. Clones with different letters are significantly different at $\alpha=0.05$. A) Blight severity of leaves was calculated as the proportion of leaves with symptoms of primary infection. B) Blight severity of flowers was calculated as the proportion of flowers with symptoms of primary infection.

Columbia

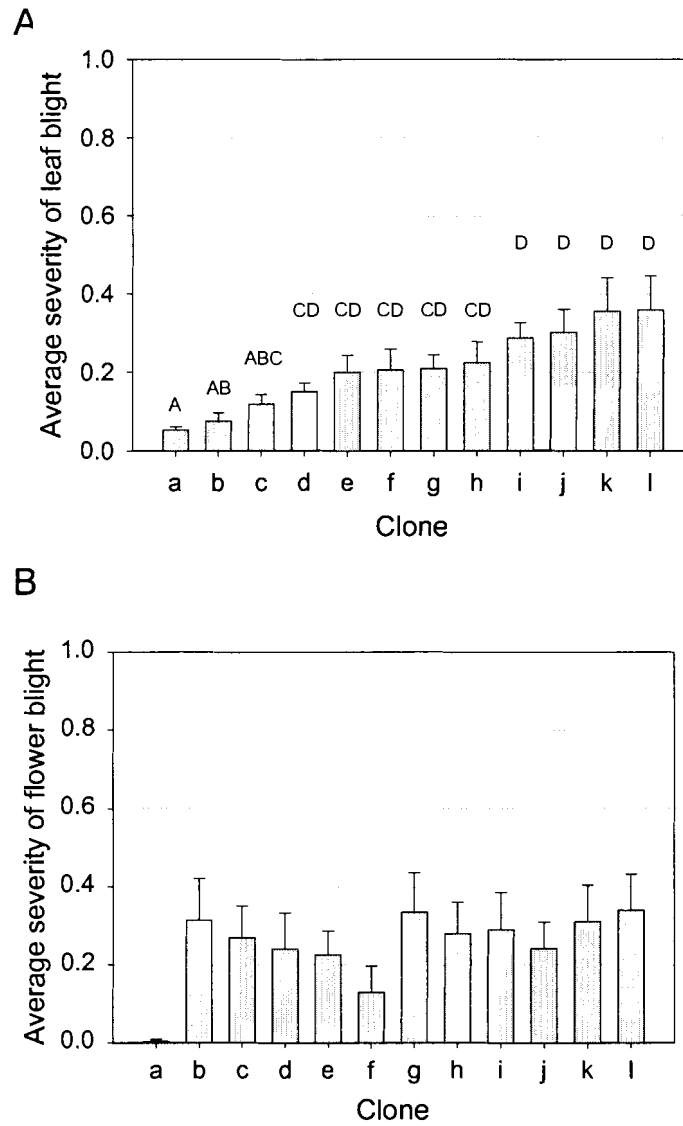
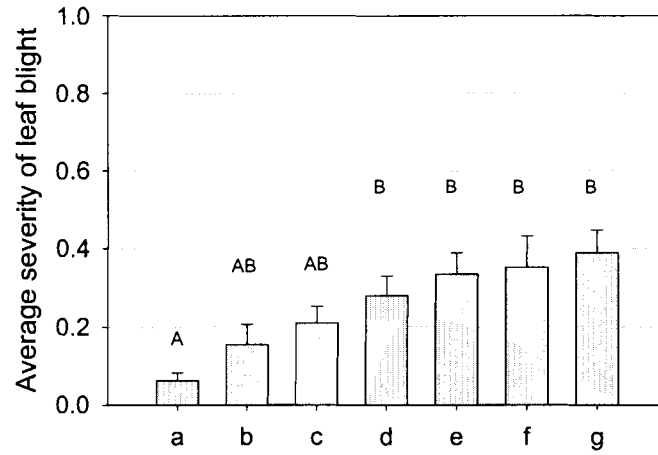


Figure 12. Average severity of leaf and flower blight among clones in Columbia during flowering. Average severity values of “diseased” stems are presented with standard error bars. Clones in both graphs are arranged in order of increasing severity of leaf blight. Clones with different letters are significantly different at $\alpha=0.05$. A) Blight severity of leaves was calculated as the proportion of leaves with symptoms of primary infection. (B) Blight severity of flowers was calculated as the proportion of flowers with symptoms of primary infection.

Sam Hill

A



B

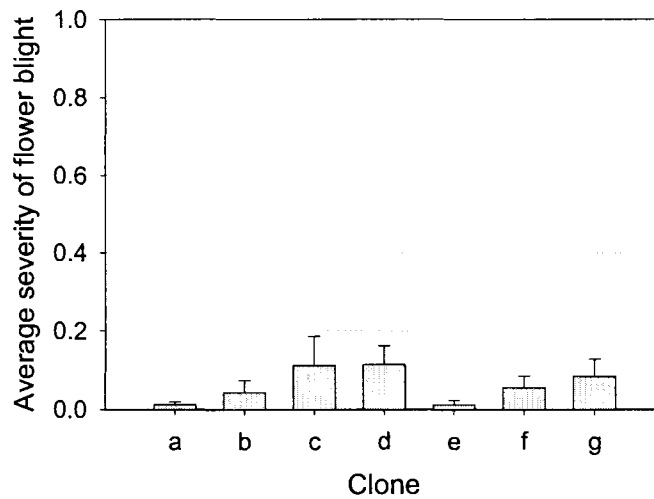


Figure 13. Average severity of leaf and flower blight among clones in Sam Hill during flowering. Average severity values of “diseased” stems are presented with standard error bars. Clones in both graphs are arranged in order of increasing severity of leaf blight. Clones with different letters are significantly different at $\alpha=0.05$. A) Blight severity of leaves was calculated as the proportion of leaves with symptoms of primary infection. B) Blight severity of flowers was calculated as the proportion of flowers with symptoms of primary infection.

The severity of fruit mummification for each field varied by whether it was influenced by the health status of the stem or by the clone from which the stem originated. In Airport I and Sam Hill, the severity of fruit infection was significantly different among clones (Kruskal-Wallis $p < 0.001$ and $p = 0.008$, respectively), but significant differences were not observed among clones in Junior Grant, Airport II, or Columbia (Fig. 14 and Fig. 15). In Airport I, the severity of fruit infection was significantly higher for “healthy” stems than for “diseased” stems (Kruskal-Wallis $p = 0.006$), but differences in the severity of fruit infection between “healthy” and “diseased” populations were not observed in Junior Grant, Airport II, Columbia, or Sam Hill (Fig. 14 and Fig. 15). In all of the fields studied, the interaction between clone and stem health was not significant, indicating that stem health did not significantly influence differences in the severity of fruit infection among clones.

Relationship Between the Severity of Leaf Blight and the Severity of Flower Blight and Fruit Mummification

There was not a consistent relationship between the severity of leaf blight and the severity of flower blight on “diseased” stems. The severity of leaf blight for individual “diseased” stems was positively and significantly correlated with the average severity of flower blight among clones in Columbia, although the correlation was not strong (Fig. 17). The severity of leaf blight for “diseased” stems was not significantly correlated with the severity of flower blight in Airport I, Airport II, or Sam Hill (Figs. 16 and 17). On individual “diseased” stems, the severity of fruit infection was not correlated with the severity of leaf infection for any of the fields.

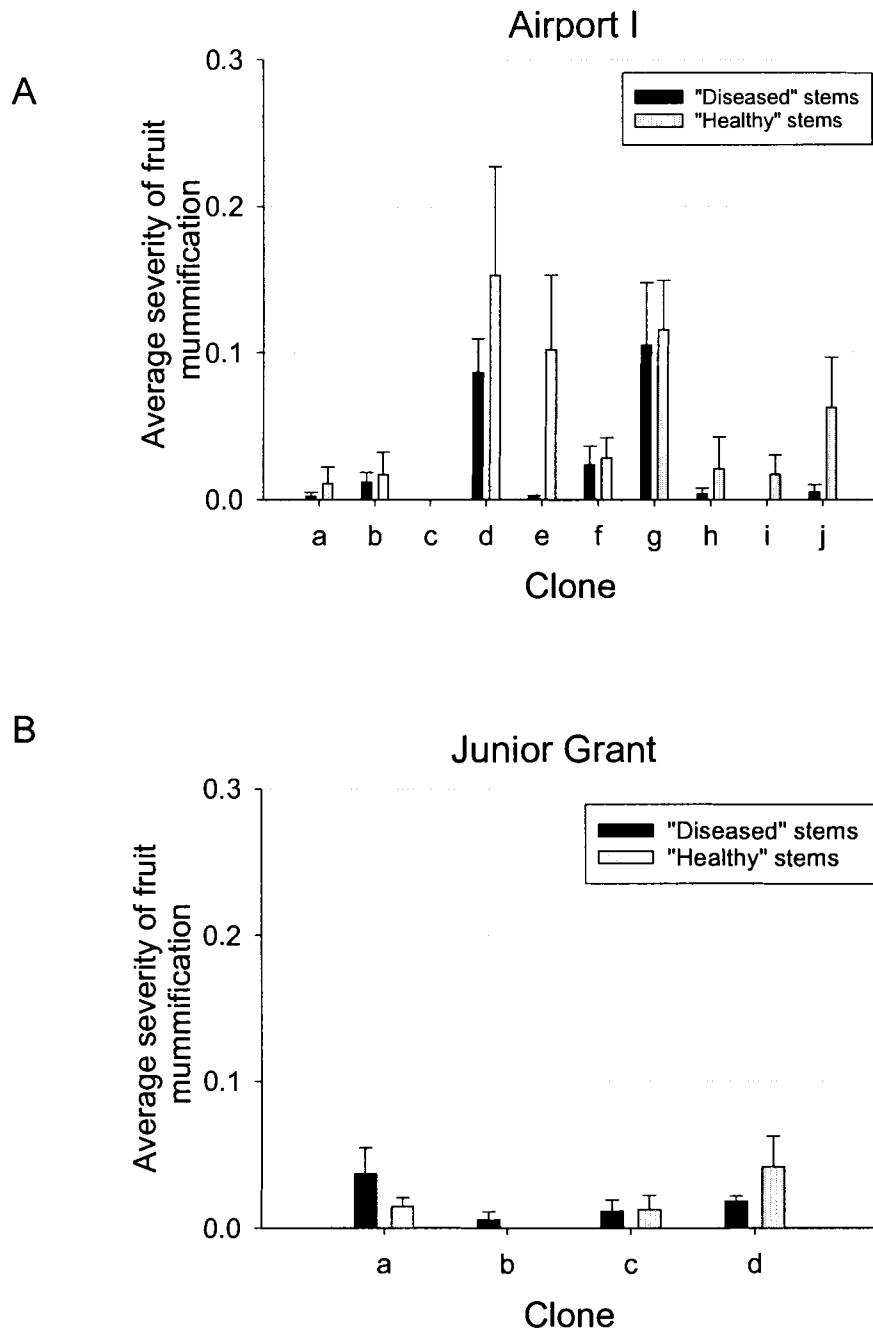


Figure 14. Average severity of fruit mummification of stems separated by clone and health status in Airport I (A) and Junior Grant (B). Severity of fruit infection was measured as the proportion of healthy flowers on each stem that produced mummy berries. Clones were arranged in order of increasing severity of leaf blight. Clones without bars did not produce mummy berries on sampled stems. Bars show the standard errors of the means.

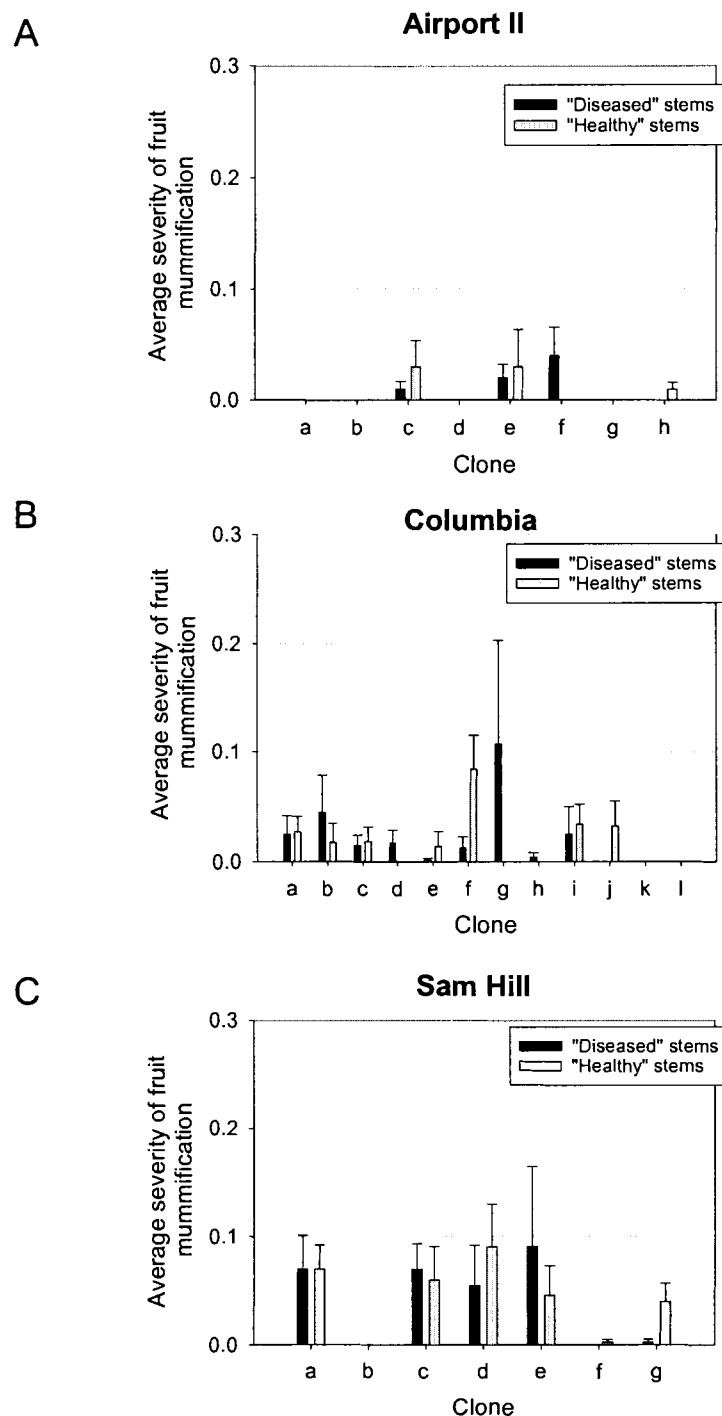


Figure 15. Average severity of fruit mummification of stems separated by clone and health status in Airport II (A), Columbia (B), and Sam Hill (C). Severity of fruit infection was measured as the proportion of healthy flowers that produced mummy berries. Clones were arranged in order of increasing severity of leaf blight. Clones without bars did not produce mummy berries on sampled stems. Standard error bars are provided.

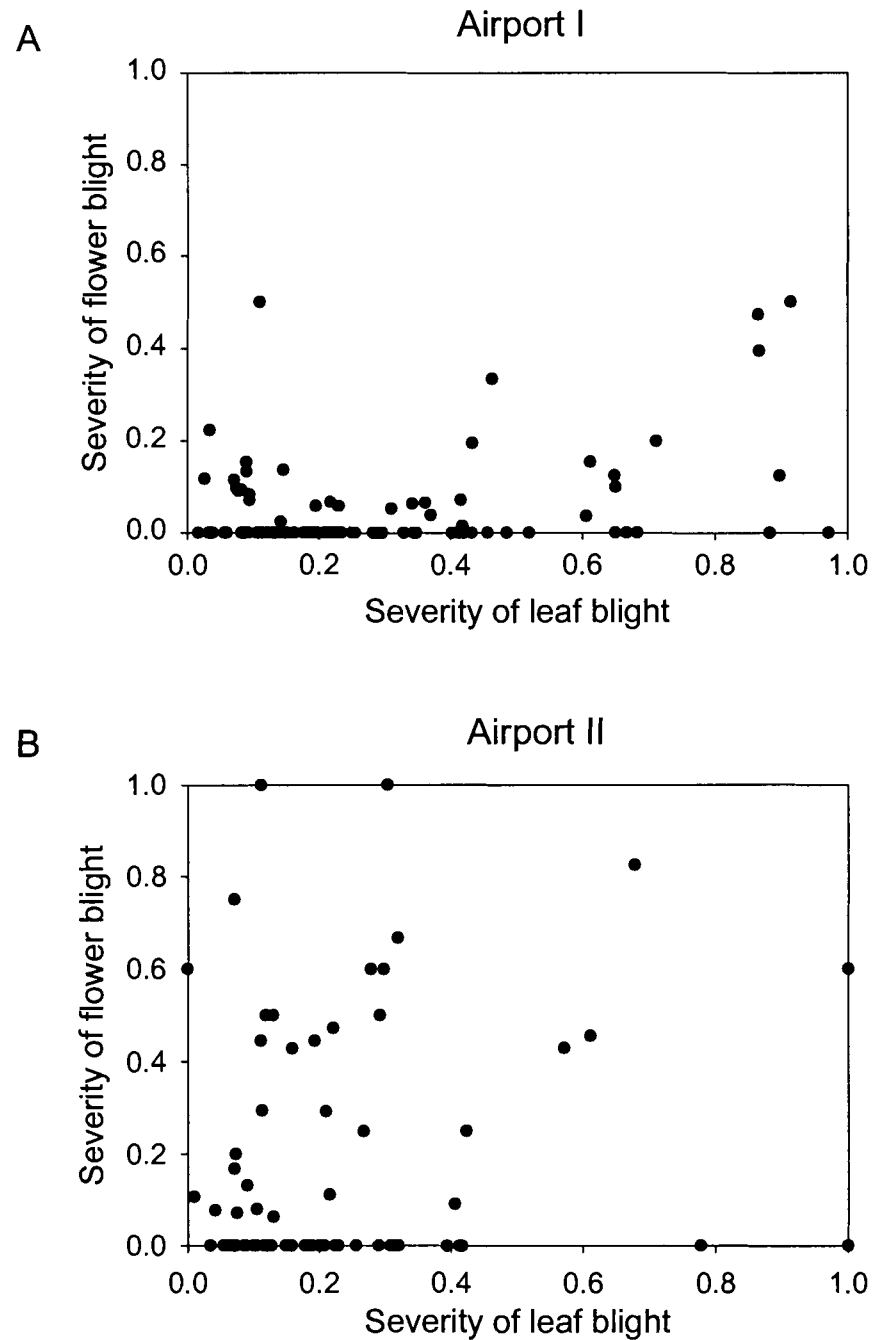


Figure 16. The relationship between the severity of leaf blight and the severity of flower blight per “diseased” stem in (A) Airport I ($r_s = +0.153$, $p=0.128$) and (B) Airport II ($r_s = +0.169$, $p=0.135$).

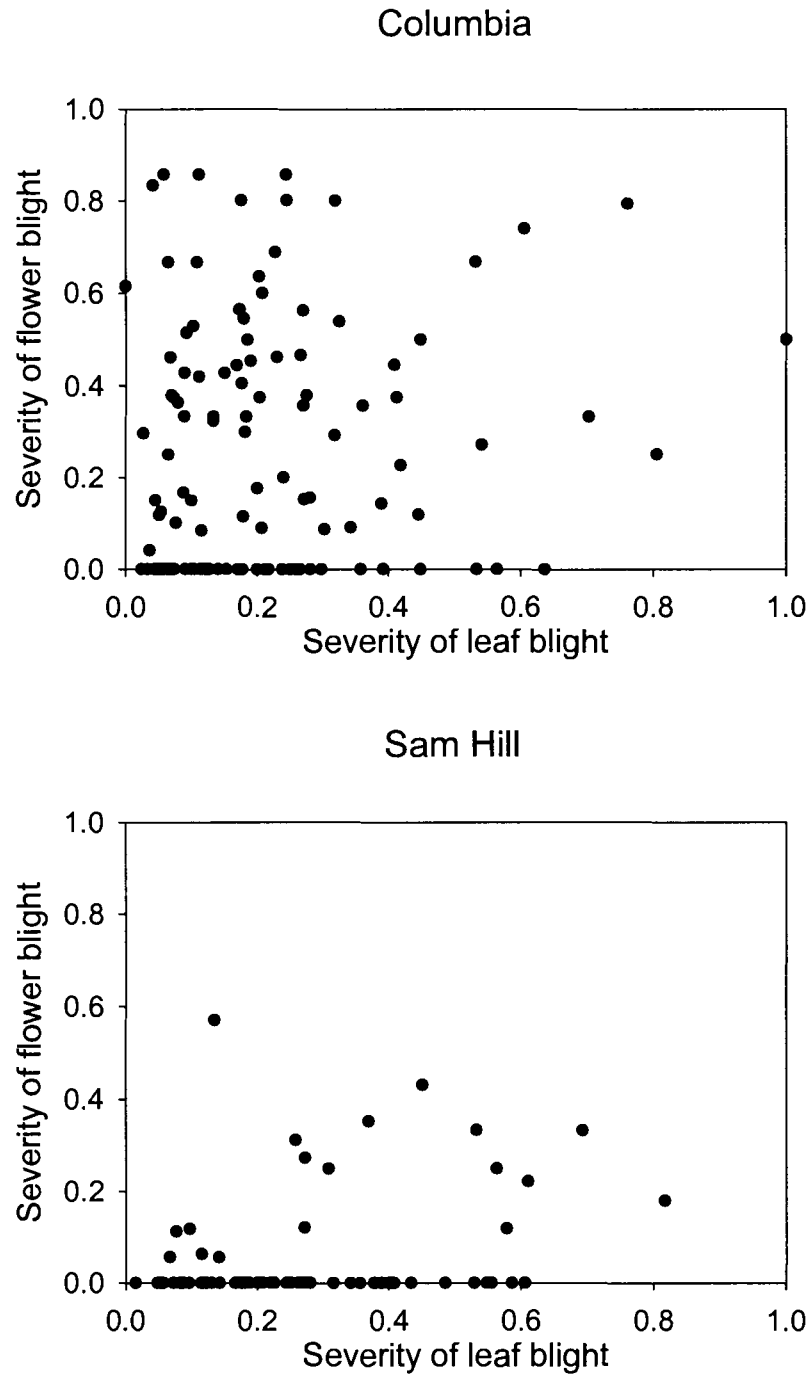


Figure 17. The relationship between the severity of leaf blight and the severity of flower blight per “diseased” stem in (A) Columbia ($r_s = +0.191$, $p=0.037$) and (B) Sam Hill ($r_s = +0.222$, $p=0.068$).

Relationship Between Disease Severity and Incidence

In each field surveyed in 2002, the average severity of leaf blight per clone (the proportion of leaves with symptoms of primary infection on a sampled stem) was significantly correlated with the average incidence of leaf blight per clone (the proportion of stems with symptoms of either leaf or flower blight within a 100 cm² area) (Fig. 18). The average incidence of blight was significantly correlated with the severity of flower blight (the proportion of leaves with symptoms of primary infection on a sampled stem) in Airport II, but significant correlations were not observed in either Columbia or Sam Hill (Fig. 18).

Relationship Between the Number of Leaves or Flowers and the Severity of Leaf or Flower Blight

The severity of leaf blight decreased as the number of leaves on “diseased” stems increased. This correlation was significant in Airport I, Airport II, and Columbia, but was not significant in Sam Hill at the $\alpha=0.05$ significance level (Figs. 19 and 20). In contrast, the severity of flower blight on “diseased” stems increased as the number of flowers on each stem increased (Figs. 21 and 22). Although the correlation between flower number and flower blight severity was not strong, it was significant in Airport II, Columbia, and Sam Hill, but not in Airport I.

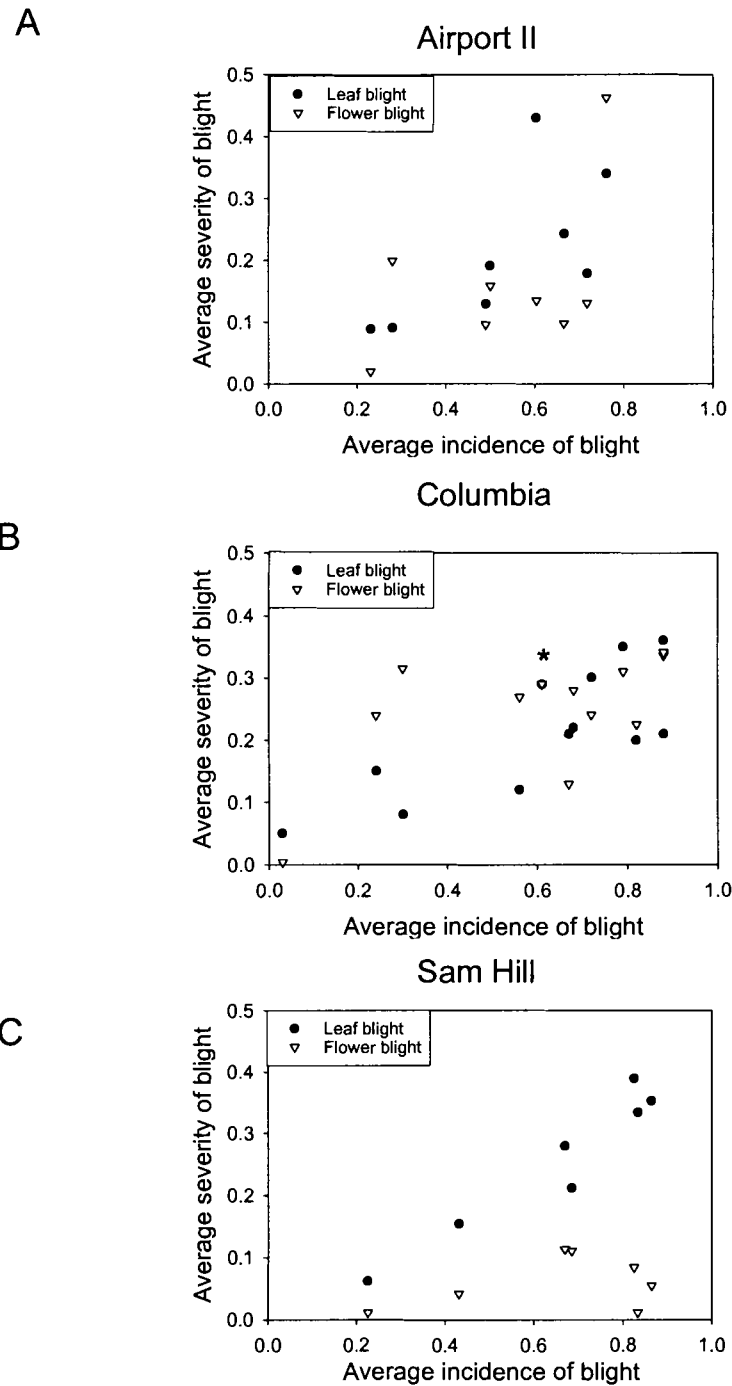


Figure 18. The relationship between the incidence of blight and the severity of leaf and flower blight of clones in A) Airport II ($r_s=+0.755$, $p=0.031$ and $r_s=+0.731$, $p=0.040$ for leaf and flower blight, respectively) B) Columbia ($r_s=+0.680$, $p=0.015$ and $r_s=+0.372$, $p=0.234$ for leaf and flower blight, respectively) and Sam Hill ($r_s=+0.786$, $p=0.036$ and $r_s=-0.342$, $p=0.452$ for leaf and flower blight, respectively). Symbols marked with an “*” are overlapping.

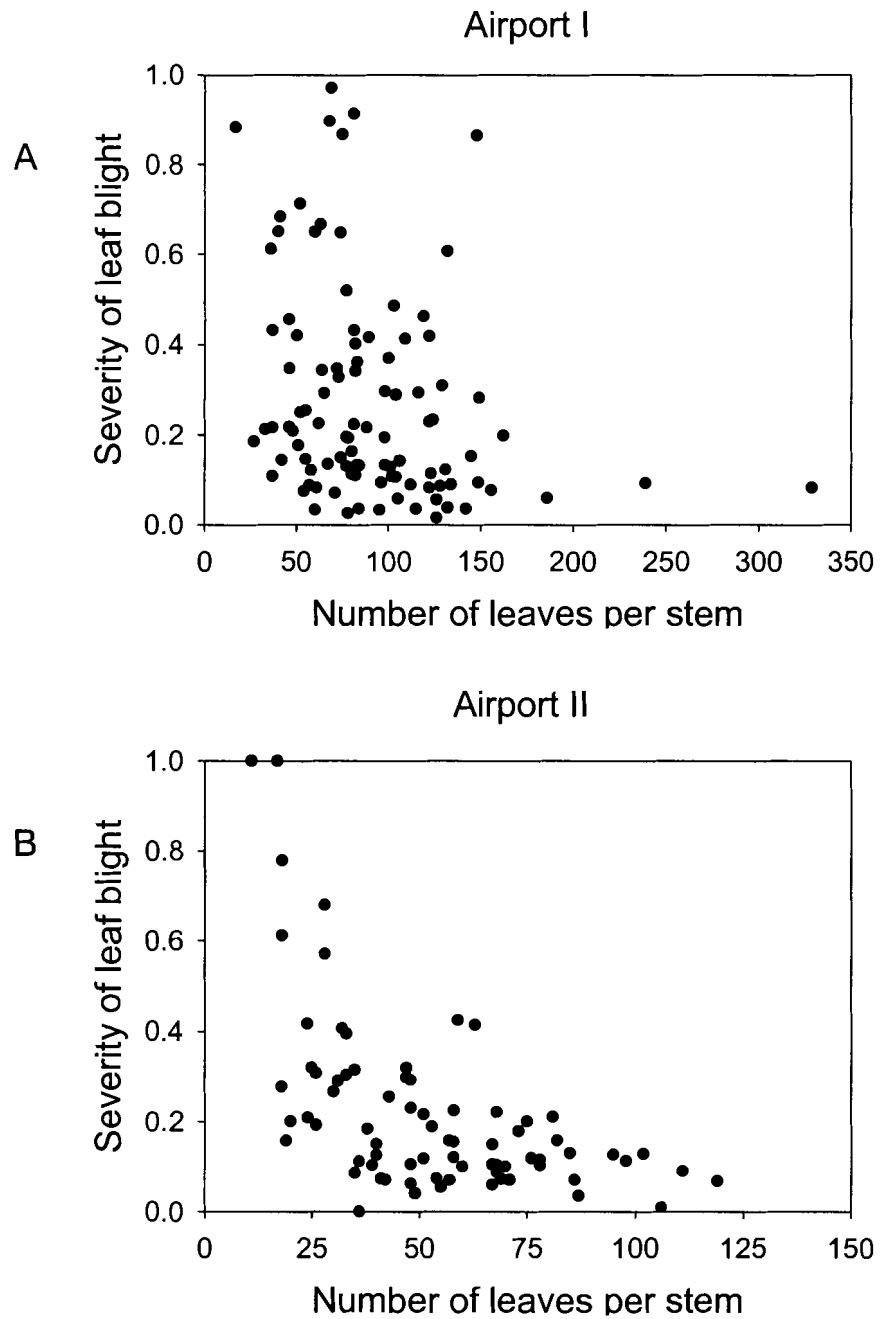


Figure 19. The relationship between leaf number and the severity of leaf blight for “diseased” stems in (A) Airport I ($r_s = -0.334$, $p = 0.001$) and (B) Airport II ($r_s = -0.527$, $p < 0.001$).

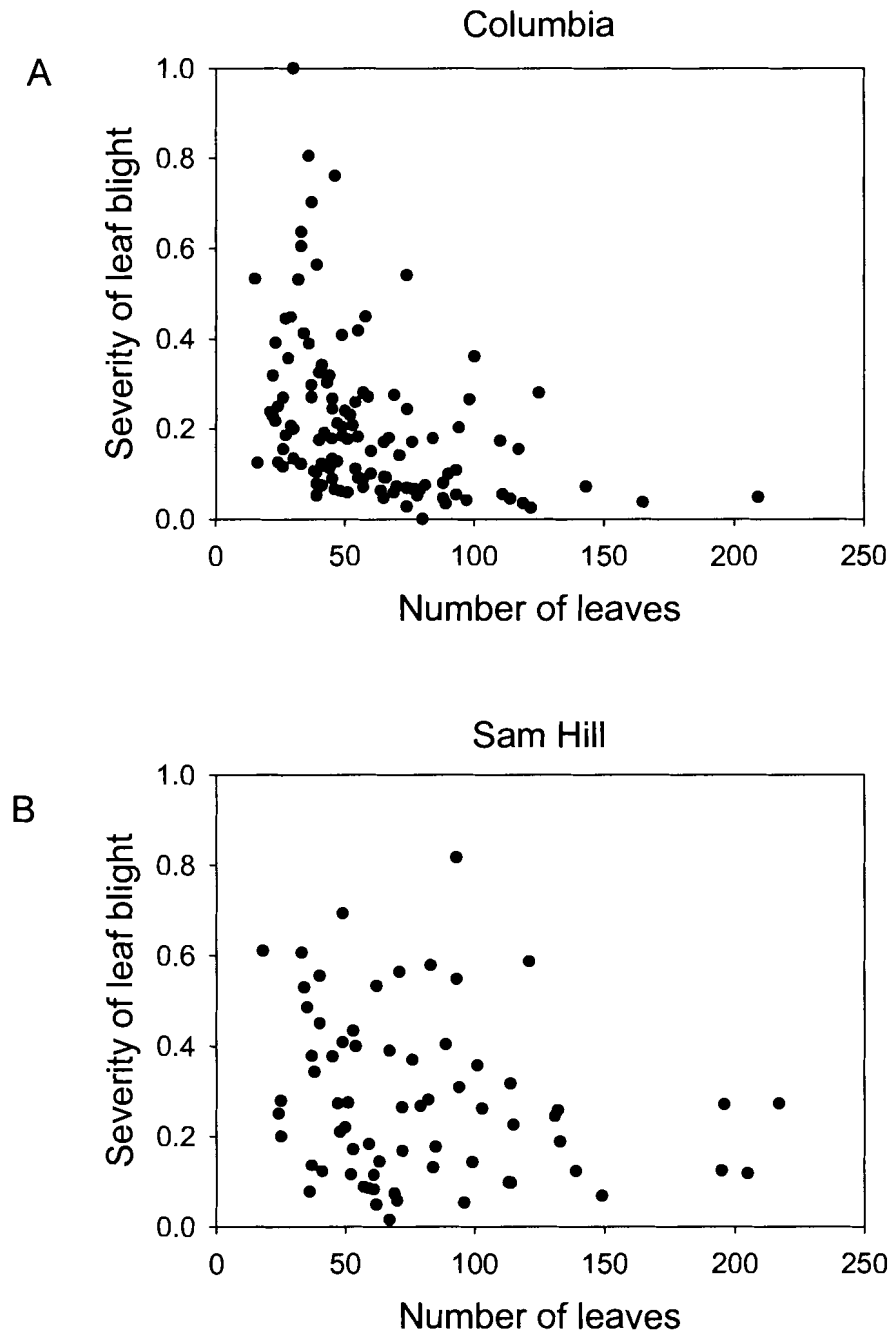


Figure 20. The relationship between leaf number and the severity of leaf blight for “diseased” stems in (A) Columbia ($r_s = -0.525$, $p < 0.001$) and (B) Sam Hill ($r_s = -0.219$, $p = 0.072$).

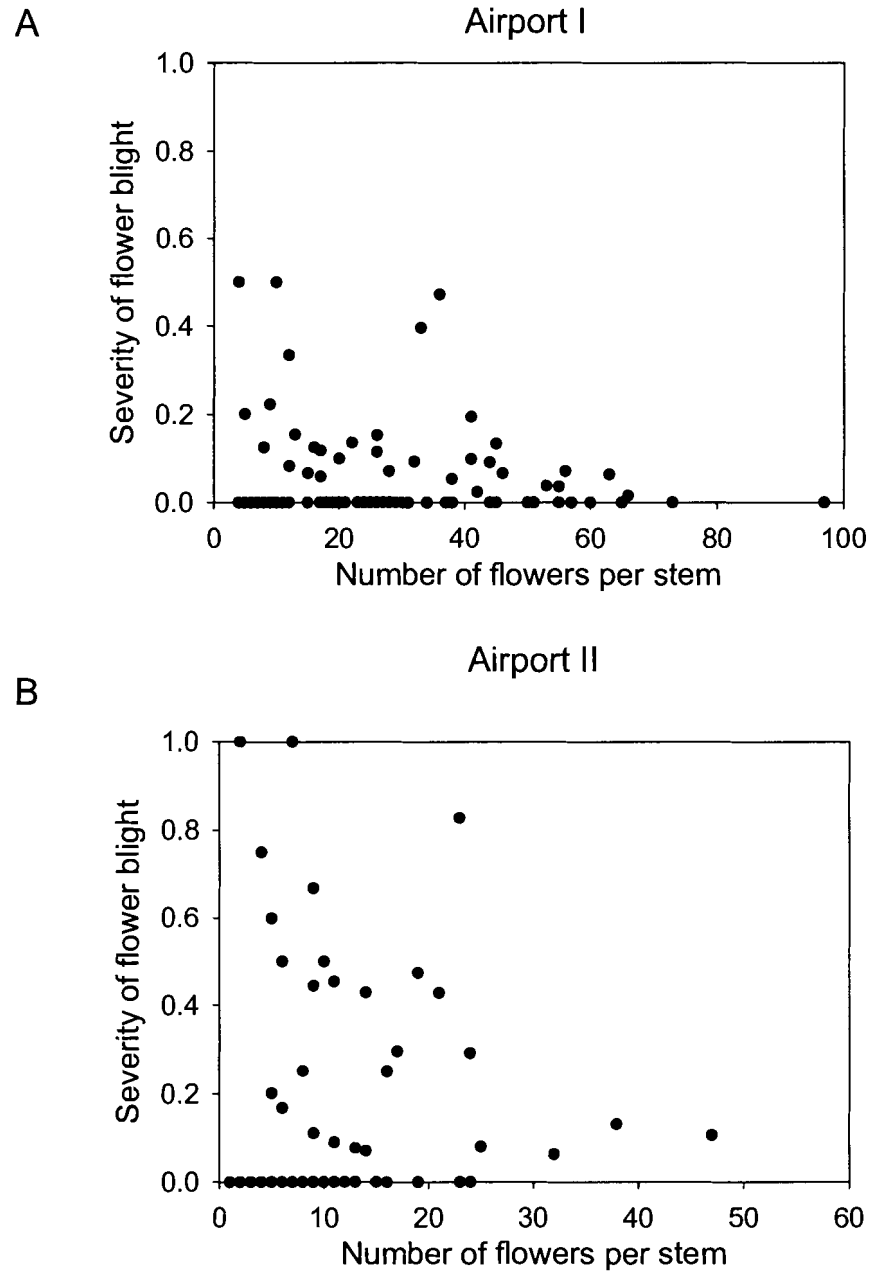


Figure 21. The relationship between flower number and the severity of flower blight for “diseased” stems in (A) Airport I ($r_s = -0.343$, $p = 0.527$), and (B) Airport II ($r_s = -0.316$, $p = 0.004$).

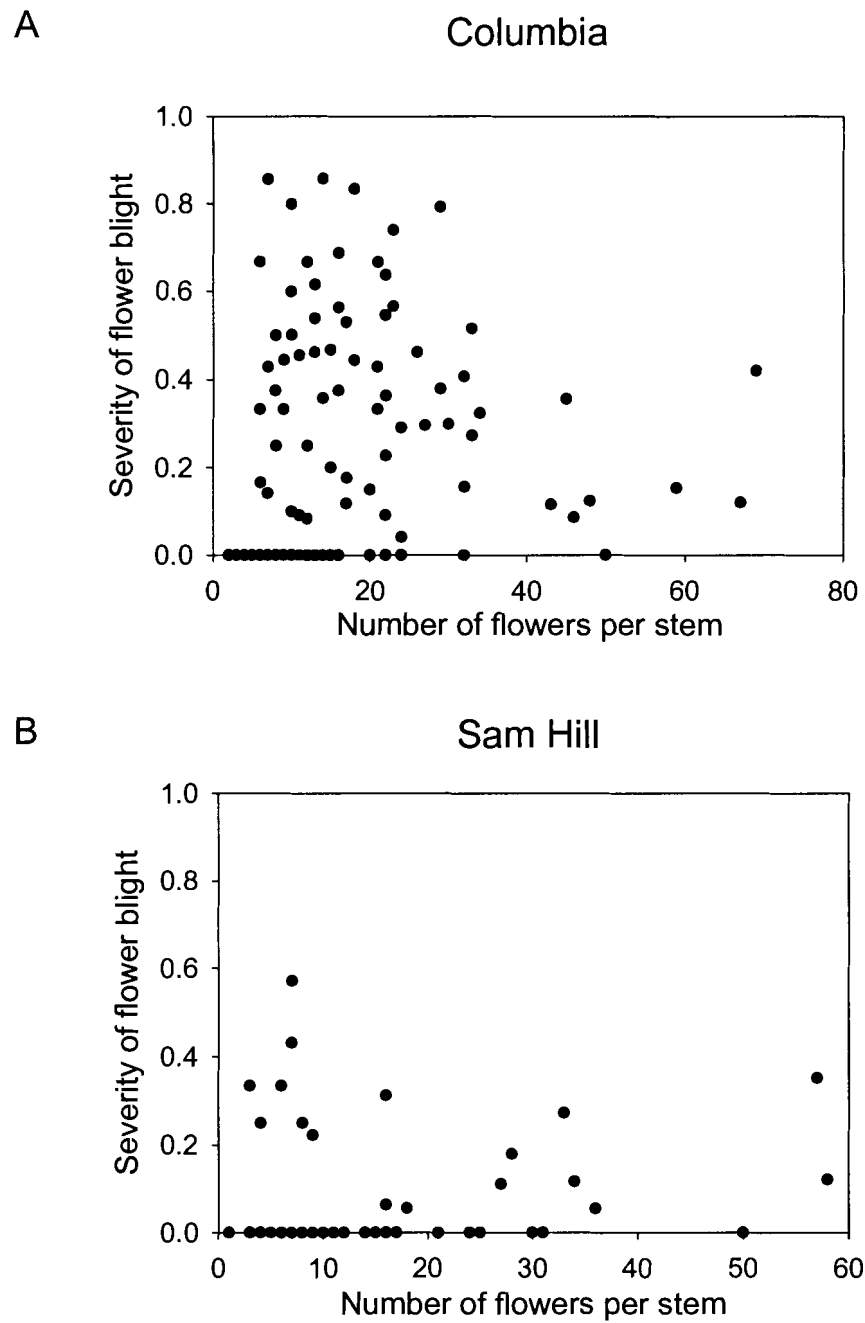


Figure 22. The relationship between flower number and the severity of flower blight for “diseased” stems in (A) Columbia ($r_s = -0.343$, $p < 0.001$), and (B) Sam Hill ($r_s = -0.254$, $p = 0.036$).

Discussion

The fields used in this study varied in many factors, including the number of years in production since pruning, the intensity of irrigation, and their exposure to pest pressures. Differences in these conditions among fields may have resulted in the observed differences in leaf, flower, and fruit number. Although lowbush blueberry fields are typically pruned after each bearing year, Airport I and Sam Hill had more than one consecutive year of fruit production. Because branch formation on stems increases each year in the absence of pruning (DeGomez 1988), differences in the number of years since pruning may account for why stems in Airport I and Sam Hill had more leaves than stems in either Airport II or Columbia. Furthermore, because leaf buds on individual stems developed at different times (data not shown), many of the leaf buds in Airport I and Sam Hill may not have expanded until after flowering. This may explain why “healthy” stems in Airport I and Sam Hill exhibited increases in leaf number after flowering.

Differences in leaf retention among fields can also be attributed to differences in field management. Water stress may have caused the average number of leaves in Sam Hill to decline between the green fruit stage and the blue fruit stage (pre-harvest). Fields that were irrigated during the bearing year (Airport I, Airport II, and Columbia) should have been less susceptible to water stress and therefore may have retained their leaves longer than non-irrigated fields (Sam Hill). Furthermore, stems in Sam Hill showed signs of insect damage, which may have contributed to premature leaf drop.

Airport I (surveyed in 2001) had considerably more reproductive units (flowers or fruit) per stem than fields surveyed in 2002 (Airport II, Columbia, and Sam Hill). Relatively low yields were observed across Maine in the summer of 2002, a

phenomenon that has been attributed to a combination of warm, dry weather during flower bud formation and cold, rainy weather during flowering, which reduced pollination (Yarborough 2003a). Furthermore, the average number of flowers was generally higher on stems with two or three years of vegetative growth since pruning (Airport I and Sam Hill) in comparison to fields with only one year of vegetative growth since pruning (Airport II and Columbia). Although biennial pruning is practiced in order to increase fruit production (Yarborough 1998), clones that have had more than one year of vegetative growth may have more carbohydrate reserves available for flower production than clones that must devote a large portion of carbohydrate reserves to initial stem growth and elongation (Smagula and DeGomez 1999). Furthermore, because the number of branches increases with the number of years since pruning (DeGomez 1988), plants that have had more than one year of vegetative growth may also have more side branches available for flower production.

One major assumption of this research is that all of the clones were exposed to uniform inoculum. The genetic and phenotypic diversity of *M. vaccinii-corymbosi* within and among fields of lowbush blueberry is not known, so it is possible that stems within some fields or within some clones may have been infected with more virulent strains of *M. vaccinii-corymbosi* than other stems. Because ascospores of *M. vaccinii-corymbosi* can travel 30 m from the point of discharge (Cox and Scherm 2001b), it is likely that clones contain strains of *M. vaccinii-corymbosi* originating from many different apothecia. It is also possible that stems in the proximity of apothecia may have more severe blight than stems located some distance away from the source of ascospores, which would result in a clumped distribution of blighted stems. In order to account for the potential of nonuniform ascospore densities, stems were randomly selected throughout the clone.

Airport I had a greater range of leaf blight relative to other fields, which is probably an artifact of the clone-selection process. Whereas clones in 2002 were selected based on previous years' data, clones in 2001 (Airport I) were selected at flowering in order to represent a wide range of infection severity. Furthermore, clones in Airport I were damaged by frost during ascospore release, and frost injury has been shown to increase the susceptibility of *V. angustifolium* buds to mummy berry blight (Hildebrand and Braun 1991).

We found unexpected differences in the severity of flower blight among fields. "Diseased" stems in second- or third-year bearing fields (Airport I and Sam Hill) had significantly less flower blight than "diseased" stems in first-year fields (Airport II and Columbia). This observation may be due to delayed development of flower buds, as was observed in Sam Hill (see Chapter 3). More field surveys should be conducted in order to determine whether there are consistent differences in the severity of flower blight between first year and second year production fields, and to examine possible differences in flower development between first and second- or third- year fields.

Despite differences in management and in overall leaf, flower, and fruit number among fields, the severity of leaf blight varied significantly among clones within each field (excluding Junior Grant), and the severity of flower blight was significantly different among clones in half of the fields studied. These results are consistent with observations reported by Lambert (1990 and 1995), but this report contains the first verification of significant differences in the severity of blight among clones of lowbush blueberry. Differences in the severity of leaf blight were observed in Airport I (n=10 clones), Airport II (n=8 clones), Columbia (n=12 clones), and Sam Hill (n=7 clones), but were probably not observed in Junior Grant due to the combination of the uniformly low infection severities and a small sample size (n=4 clones) of clones with symptoms of blight. Significant differences in the severity of flower blight among clones were

observed in Airport I and Airport II, but were not observed in the other two fields.

Although Clone A in Columbia had less flower blight than other clones in the same field, significant differences were probably not detected because of the high variability in the severity of blight for other clones in this field. In contrast, the severity of flower blight was uniformly low in Sam Hill, which may be related to delayed flower development of clones in that field (See chapter 3).

“Diseased” stems had significantly more leaves and flowers than “healthy” stems. However, this observation does not necessarily indicate that stems with fewer leaves and flowers have less blight. On the contrary, as the number of leaves on “diseased” stems increased, the severity of infection decreased. These observations may at first seem contradictory, but they can be explained by the stem selection process and the method of measuring blight severity. “Healthy” stems often had fewer leaves and flowers than “diseased” stems because of the difficulty in finding flowering stems without symptoms of primary infection in clones with severe amounts of blight. Often, the random selection process in heavily-blighted clones selected “healthy” stems with few flowers, a relatively small number of leaves, and no branches. Assuming that the inoculum density is constant, a stem with few leaves, flowers, and branches is less likely to intercept infective spores than a neighboring stem with many leaves, flowers, and branches. Because blight severity was measured as the proportion of leaves with symptoms of blight, if two stems with different numbers of leaves have the same number of blighted leaves, the severity of infection would be greater on the stem with fewer leaves. This illustrates why “diseased” stems with more leaves have less severe blighting than “diseased” stems with few leaves.

In contrast, there were weak positive correlations between the number of flowers and the severity of flower blight on “diseased” stems. This relationship was observed in Airport I, Columbia, and Sam Hill, but was probably not observed in Airport

I because flowers were more common in Airport I than in the other fields. Assuming inoculum levels to be constant, if a stem has many flowers then there is a greater probability that at least one of the flowers will be blighted relative to a stem with few flowers. Because most stems have no flower blight, “diseased” stems with even one blighted flower and many healthy flowers will have more severe blight than a “diseased” stem with only a few “healthy” flowers.

Positive correlations between the average severity of leaf blight and the average incidence of blight of clones were consistently significant within all fields. However, Airport II was the only field that had a significant relationship between the average severity of flower blight and the average incidence of primary infection. Because leaf infection is more common than flower infection, it is not surprising that the average proportion of stems with primary infection would be more closely related to the proportion of blighted leaves than with the proportion of blighted flowers.

In most fields, the severity of leaf blight for “diseased” stems was not positively correlated with the severity of flower blight. The lack of a relationship may also be due to the fact that nearly all “diseased” stems had blighted leaves, but many “diseased” stems did not have any blighted flowers. Other potential factors might include the timing of flower bud development or the position of flowers on the stem.

The severity of fruit mummification also varied among clones within a field, but this variation was only significant in Airport I and Sam Hill. Differences may not have been observed in Airport II due to uniformly low production of mummy berries on sampled stems, while in Columbia, the proportion of flowers that produced mummy berries was highly variable. The severity of fruit infection was generally greater on “healthy” stems than on “diseased” stems, but because of low and highly variable infection severities, this difference was not significant. Pollinator preference for flowers on “healthy” stems or differences in receptivity of floral stigmas between “healthy” and

“diseased” stems may have caused “healthy” stems to have more mummified fruit than stems with leaf blight. It is also possible that “diseased” stems prevented the formation of mummy berries through induced abortion of fruit or through induced biochemical resistance. Another possible explanation is that “diseased” stems were stressed and, as a result, aborted more fruit (healthy or infected) than “healthy” stems. If stress due to leaf blight is the cause of reduced mummy berry production in “diseased” stems, one would expect that stems with severe blight symptoms would produce fewer mummy berries than stems with mild blight symptoms. However, there was no relationship between the severity of primary leaf infection and the severity of secondary fruit infection in all of the fields studied. The frequency of fruit infection may have been too low to detect possible correlations between the severity of leaf blight and fruit infection. However, Stretch and Ehlenfeldt (2000) found “no significant correlation between blighting resistance and fruit infection resistance” of highbush blueberry cultivars, which suggests that the severity of leaf blight and the severity of fruit mummification are unrelated. The dispersal mechanisms for ascospores and conidia of *M. vaccinii-corymbosi* differ. Whereas ascospores are dispersed primarily by wind, conidia of *M. vaccinii-corymbosi* rely on transmission by bees in order to infect ovaries of its *Vaccinium* host (Batra and Batra 1985; Cox and Scherm 2001b). Unlike other pollinators that tend to visit each flower on a stem, honeybees tend to forage by visiting only one or two flowers before moving to a neighboring stem (Drummond, personal communication). Therefore, in fields pollinated by honeybees, conidia produced on the leaf of a stem may not infect flowers on the same stem or even within the same clone. Fruit mummification is further complicated by the finding that pollinated flowers are much less receptive to conidia of *M. vaccinii-corymbosi* than unpollinated flowers, and that receptivity also declines with the number of days since anthesis (Ngugi et al. 2002).

CHAPTER THREE: CLONAL CHARACTERISTICS RELATED TO THE SEVERITY AND INCIDENCE OF MUMMY BERRY DISEASE

Introduction

Mummy berry disease is caused by the fungus *Monilinia vaccinii-corymbosi* and is an important disease of blueberry (*Vaccinium*) species. Ascospores of *M. vaccinii-corymbosi* infect blueberry hosts during flowering, resulting in the blight stage of mummy berry disease, and conidia produced on the surface of blighted tissues infect the ovaries of pollinated flowers, resulting in mummification of the fruit.

In Maine and the Maritime provinces, lowbush blueberry fields contain naturally-seeded stands of *V. angustifolium* and *V. myrtilloides* that are often managed for blueberry production through irrigation, fertilization, and pesticide applications. Lowbush blueberry fields are usually also pruned biennially in order to increase fruit production. Plants established from seedlings of *V. angustifolium* and *V. myrtilloides* spread laterally through underground rhizomes to form stands of genetically-identical plants, which are called clones. As a result of rhizomal growth of seedlings, lowbush blueberry fields contain many clones that differ in several phenotypic characteristics, including leaf color, berry color, and height. As reported in the previous chapter and by Lambert (1990 and 1995), clones of lowbush blueberry also differ in their severity and incidence of mummy berry disease.

Cultivars of highbush blueberry (*V. corymbosum*) and rabbiteye blueberry (*V. ashei*) also vary significantly in their susceptibility to both stages of mummy berry disease (Pepin and Toms 1969; Stretch et al. 1995; Ehlenfeldt and Stretch 2000; Stretch and Ehlenfeldt 2000). Resistance of highbush cultivars to mummy berry blight has been attributed primarily to disease avoidance, as cultivars with relatively early

shoot growth generally had higher percentages of blighted shoots relative to later-developing cultivars (Ehlenfeldt et al. 1996). However, some highbush blueberry cultivars with early shoot growth were resistant to mummy berry blight (Ehlenfeldt et al. 1996), and when cultivars at the same stage of leaf bud development were inoculated with ascospores, they developed different severities of mummy berry blight (Ehlenfeldt et al. 1997). These results suggest that biochemical mechanisms may also be involved in resistance. It is likely that the timing of vegetative development is also related to the resistance of *V. angustifolium* clones to mummy berry blight, but this relationship has not been investigated. There have also been no attempts to associate other clonal traits with resistance to mummy berry disease.

The objectives of this chapter are to relate the phenology of leaf and flower development to the severity and incidence of mummy berry disease. Relationships between height of lowbush blueberry clones and the severity and incidence of infection were also investigated.

Materials and Methods

Characterization of Clones: Morphology and Bud Development

Three fields (Airport II, Columbia, and Sam Hill) were studied in 2002. 8 clones were selected in Airport II, 12 clones were selected in Columbia, and 7 clones were selected in Sam Hill prior to leaf and flower bud expansion, as described in Chapter 2. Airport II and Columbia had one year of vegetative growth since pruning, whereas the highly-branched stems in Airport I and Sam Hill indicated that those fields had more than one year of vegetative growth since pruning.

Clonal morphology and bud development were observed in order to determine whether differences in these traits corresponded with variations in the level of mummy berry disease among clones. Before budbreak, 10 stems with flower buds were randomly selected within each clone in Airport II, Columbia, and Sam Hill, and the heights of these “phenology” stems were recorded in Airport II and Columbia.

For each “phenology” stem in Airport II, Columbia, and Sam Hill, the maximum and most frequently observed reproductive and vegetative bud stages were estimated at ~ 7 day intervals (beginning Julian date 114-123) by examining these stems using the scale described by Hildebrand and Braun (1991) (Table 2). If a stem had approximately equal numbers of buds in different stages, the average of the values was used as the most frequently observed development stage. Observation of clonal leaf and flower bud development ceased in mid May (Julian date 143-149) when the most mature vegetative and reproductive buds on each stem had attained the highest ranking on the scale.

Table 2. Stages of Leaf and Flower Bud Development
as described by Hildebrand and Braun (1991)

Stage	Leaf development	Stage	Flower development
V0	Tightly closed dormant bud	T0	Tightly closed dormant bud
V1	< 2 mm green tissue	T1	Swollen bud
V2	2-5 mm green tissue	T2	Separation of bud scales
V3	>5 mm green tissue, leaves not separated	T3	Flower buds visible, covered with sepals
V4	Leaves separating	T4	Corolla visible
		T5	Corolla growth past calyx

Monitoring Ascospore Production

Mummy berries that were producing apothecia were collected from Airport II (Julian day 131), Columbia (Julian day 131), and Sam Hill (Julian days 107 and 136) and taken back to the laboratory for observation. Apothecia were placed in 250 ml beakers on top of moistened sand so that the distance between the apothecial cup and the top of the beaker was less than 3 cm. The beakers were covered loosely with petri dish lids, and a glass coverslip was attached to the inside of the petri dish lid to capture discharged ascospores. The containers of apothecia were kept in a incubator with a 16h photoperiod and at temperatures of 8° C/night and 16° C/day, as described by Hildebrand and Braun (1991). Coverslips were washed with deionized water and monitored daily for ascospore release from Julian day 135 to 148.

Measurements of Disease Severity and Incidence

At flowering, 10 flowering stems with symptoms of blight and 10 flowering stems without symptoms of blight were randomly selected and represented the "diseased" and "healthy" population, respectively, for each clone. As described in the previous chapter, the severity of leaf and flower blight for "diseased" stems was measured as the proportion of leaves and flowers, respectively, with symptoms of blight during flowering. The severity of fruit mummification was measured as the proportion of healthy flowers on both "healthy" and "diseased" stems that produced mummy berries. The average severities of leaf blight, flower blight, and fruit infection for each clone were reported in Chapter 2.

In order to relate height and the phenology of leaf and flower buds to the severity of infection on individual stems, estimates of leaf and flower blight were recorded for "phenology" stems shortly before anthesis. As with "diseased" stems, the

severity of flower blight was measured as the proportion of flowers with symptoms of blight. In contrast, the severity of leaf blight on "phenology" stems was measured as the proportion of leaf clusters with symptoms of blight. The incidence of blight was measured as the proportion of blighted stems within a 10 cm² frame, as described in the previous chapter. Estimates of the severity of leaf and flower blight were not obtained for "phenology" stems in Sam Hill.

Clonal Factors Affecting Disease Severity and Incidence

Spearman's correlation procedure (Systat, Richmond, California) (a non-parametric correlation procedure that ranks data to determine relationships) was used to determine whether the severity of leaf and flower blight of clones in Airport II and Columbia were correlated with the height of their stems. Spearman's correlation procedure was also used to determine whether the severity of leaf and flower blight of clones was correlated with their maximum and most frequently observed leaf and flower development stages, respectively, on Julian dates 128 through 139. Trendlines for the relationship between blight severity and the most frequently observed bud development stage of individual "phenology" stems were obtained using Sigmaplot (SPSS, Chicago, Illinois), but Spearman's correlation coefficients for these "phenology" stems do not reflect the strength of the relationship between data points and the trendline. Spearman's correlation analyses were also performed using clonal averages of height ("phenology" stems), bud development ("phenology" stems), and disease severity ("diseased" stems).

Spearman's correlation procedure was also used to determine the strength and significance of the relationship between the average incidence of blight and the average stem height, leaf development stage, and flower development stage within a clone.

Results

Relationship Between Leaf and Flower Development

In all fields, the most frequently observed flower development stage of individual "phenology" stems was correlated with their most frequently observed leaf development stage on the same date. This relationship was significant for all correlates; Airport II (Julian day 131: $r_s = +0.426$, $p=0.000$ and Julian day 139: $r_s = +0.437$, $p=0.000$), Columbia (Julian day 131: $r_s = +0.487$, $p=0.000$ and Julian day 139: $r_s = +0.536$, $p=0.000$) and Sam Hill (Julian day 128: $r_s = +0.314$, $p=0.011$ and Julian day 136: $r_s = +0.408$, $p=0.001$). However, clonal averages of the most frequently observed flower development stage on "phenology" stems were not consistently correlated with the average most frequently observed leaf development stage. Only in Columbia were the averages of the most frequently observed leaf and flower development stages of clones significantly correlated on Julian days 131 ($r_s = +0.820$, $p=0.001$) and 139 ($r_s = +0.883$, $p=0.000$).

Ascospore Production

Under controlled light and temperature conditions in the laboratory, the apothecia collected from Airport II, Columbia, and Sam Hill fields on Julian dates 131-136 released ascospores on Julian day 136 through Julian day 148, indicating that inoculum was probably present in these fields during this time period.

Clonal Factors Contributing to the Severity of Primary Leaf Infection

Height

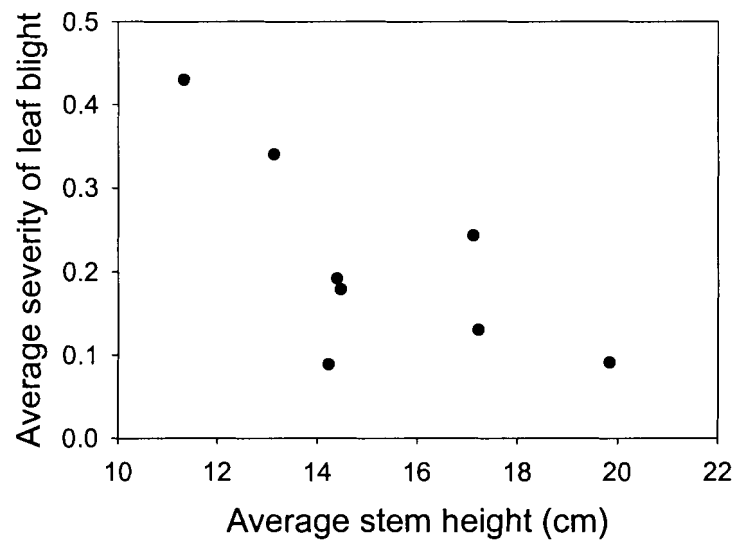
The severity of leaf blight tended to decrease as stem height increased, but the strength and significance of this relationship varied between fields as well as between analysis of individual stems or clonal averages. There was no relationship between the height of individual “phenology” stems and the severity of leaf blight on those stems. In contrast, when the average stem height of “phenology” stems was plotted against the average disease severity of “diseased” stems, there was a significant negative correlation among clones in Columbia ($r_s = -0.758$) (Fig. 23b). A similar trend was observed in Airport II, but the relationship was not significant ($r_s = -0.571$) (Fig. 23a). Stem heights were not recorded in Sam Hill.

Phenology

The average maximum and most frequently observed leaf development stages of “phenology” stems were measured to examine the potential relationship between leaf bud phenology on Julian days 128 -139 and the eventual severity of leaf blight on “diseased” stems. The phenology of leaf and flower bud development was measured on Julian days 131 and 139 in Airport II, on Julian days 131 and 139 in Columbia, and on Julian days 128 and 136 in Sam Hill. Clonal averages of the maximum and most frequently observed leaf development stages were significantly and positively correlated among clones within each field for at least one of the observation dates (Julian days 128-139). The correlation was significant in Airport II on Julian day 139 ($r_s = + 0.903$, $p=0.002$), in Columbia on Julian days 131 and 139 ($r_s = + 0.900$, $p<0.001$

A

Airport II



B

Columbia

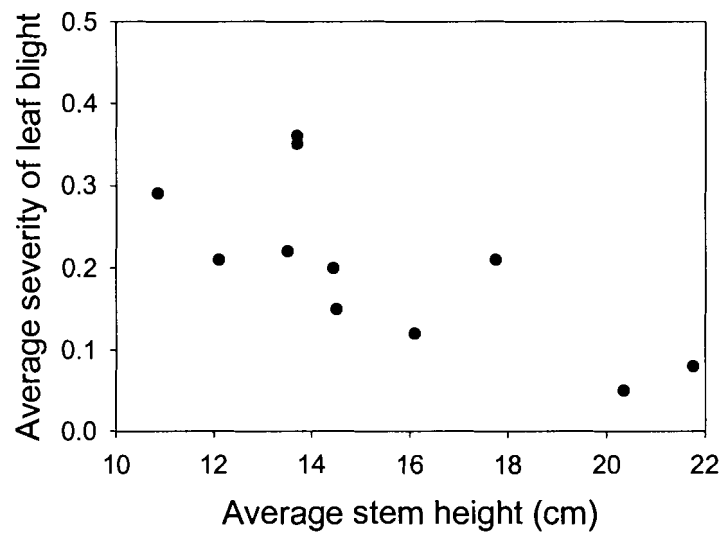


Figure 23. Relationship between average stem height of “phenology” stems and average severity of leaf blight of “diseased” stems for clones within A) Airport II ($r_s = -0.571$, $p = 0.139$) and B) Columbia ($r_s = -0.758$, $p = 0.007$).

and $r_s = + 0.979$, $p < 0.001$, respectively) and in Sam Hill on Julian day 128 ($r_s = + 0.893$, $p = 0.007$). The correlation between the maximum and most frequently observed leaf development stage was not significant in Airport II on Julian day 131 or in Sam Hill on Julian day 136. Estimates of the most frequently observed leaf development stage were more representative of the majority of leaf tissue on individual stems and therefore were used in phenology analyses.

When the relationship between leaf bud phenology and the severity of leaf blight was examined using individual “phenology” stems, the most frequently observed leaf development stage on Julian dates 131 and 139 was positively correlated with the severity of leaf blight in both Airport II and Columbia (Figs. 24 and 25). Severity estimates were not recorded for individual “phenology” stems in Sam Hill.

The average severity of leaf blight of “diseased” stems measured during flowering (Julian days 148-161) was greater in clones with higher leaf development stages during Julian days 128 –139. However, this relationship was not consistently significant for different fields or observation dates. There were significant positive correlations between the average of the most frequently-observed leaf development stage and the average severity of leaf blight for clones in Sam Hill on Julian day 136 and for clones in Columbia on Julian days 131 and 139 (Fig. 26b and c). For clones in Sam Hill, there was no significant correlation between the average severity of leaf blight and the most frequently observed leaf development stage on Julian day 128 (Fig. 26c), and no significant relationships were observed for clones in Airport II on either Julian date 131 or 139 (Fig. 26a).

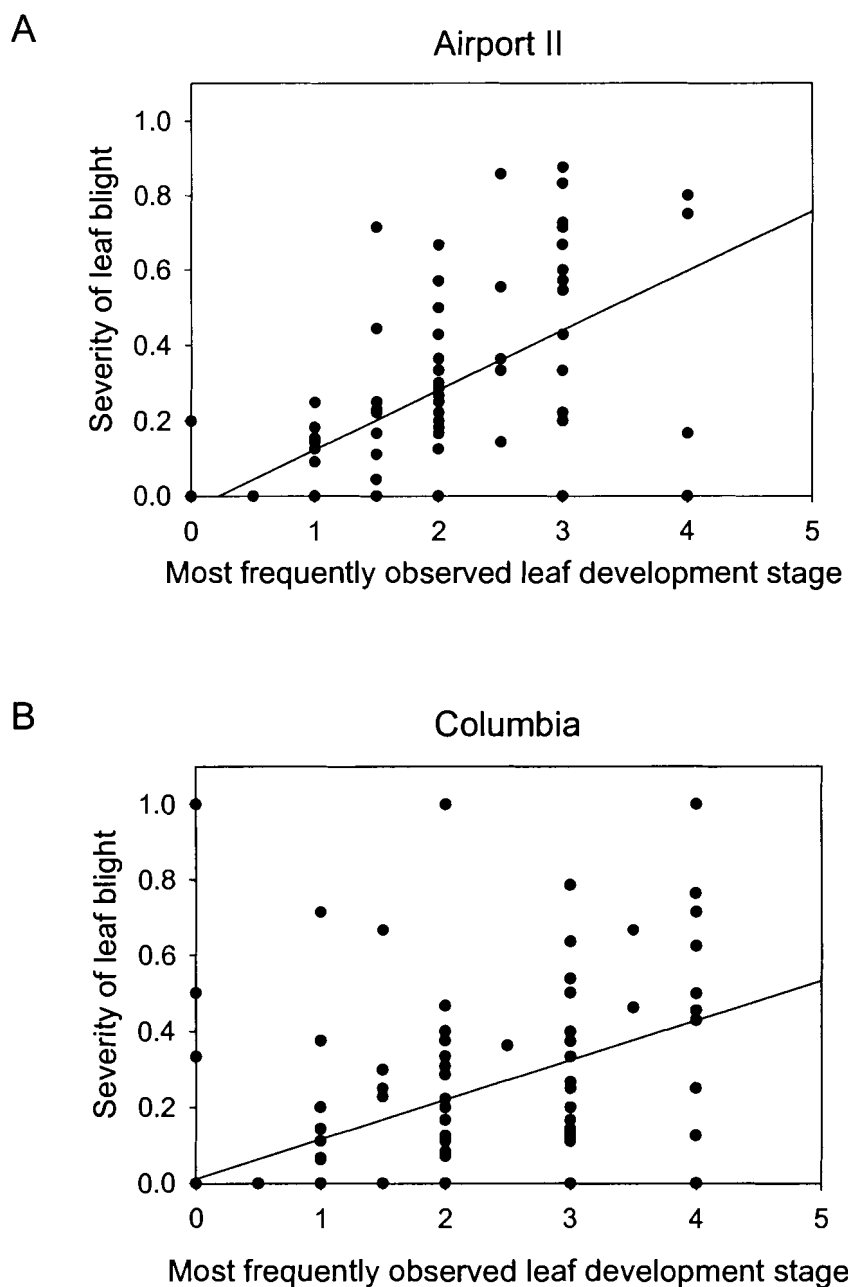


Figure 24. Relationship between the most frequently observed leaf development stage on Julian day 131 and the severity of leaf blight during flowering for individual “phenology” stems in two fields. A) Airport II ($r_s = +0.570$, $p < 0.001$) and B) Columbia ($r_s = +0.583$, $p < 0.001$)

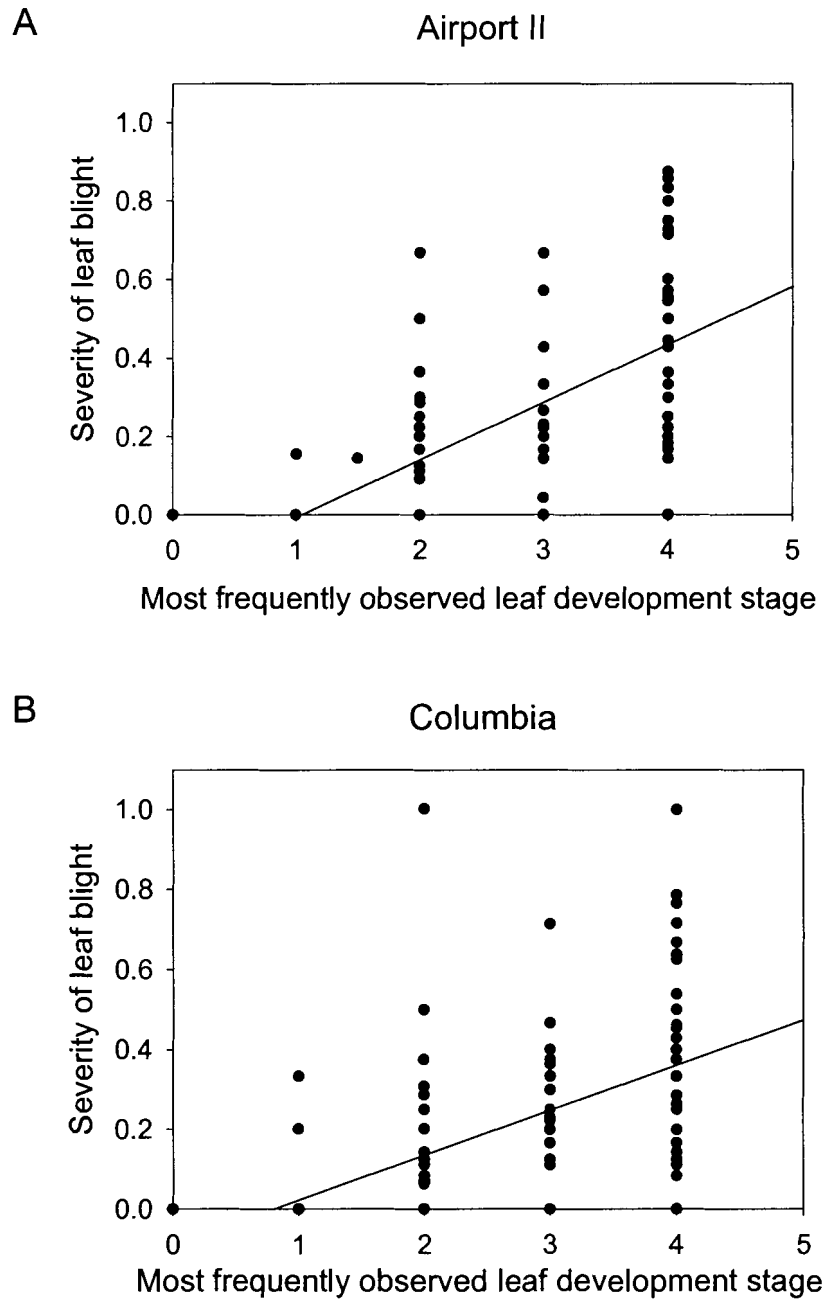


Figure 25. Relationship between the most frequently observed leaf development stage on Julian day 139 and the severity of leaf blight during flowering for individual “phenology” stems in two fields. A) Airport II ($r_s = +0.585$, $p < 0.001$) and B) Columbia ($r_s = +0.650$, $p < 0.001$)

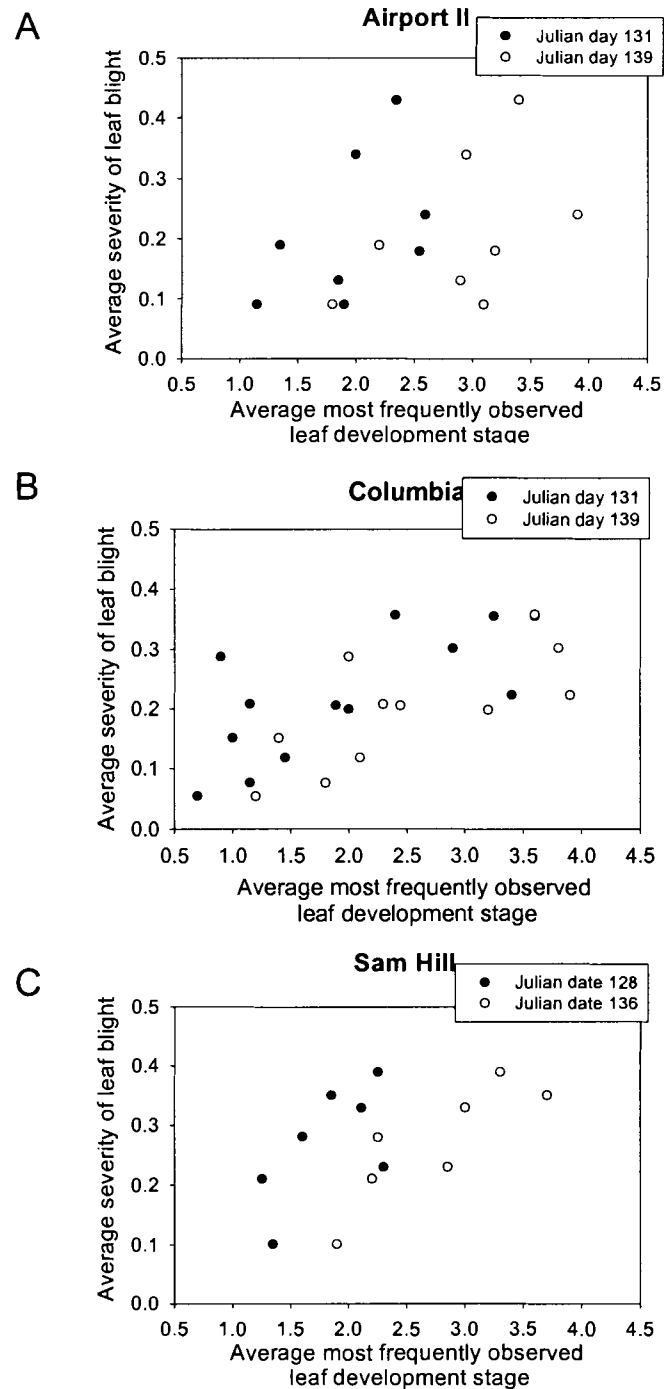


Figure 26. The relationship between the average most frequently observed leaf development stage during leaf bud expansion and the average severity of leaf blight during flowering for clones in three fields. A) Airport II (Julian day 131: $r_s = +0.563$, $p = 0.146$ and Julian day 139: $r_s = +0.527$, $p = 0.180$), B) Columbia (Julian day 131: $r_s = +0.629$, $p = 0.029$ and Julian day 139: $r_s = +0.739$, $P = 0.006$), and C) Sam Hill (Julian day 128: $r_s = +0.571$, $p = 0.180$ and Julian day 136: $r_s = +0.929$, $P = 0.003$).

In contrast, when the average maximum leaf development stage of clones was used in the analyses, significant positive correlations were found only for clones in Columbia (Julian day 131: $r_s = +0.752$, $p = 0.005$; Julian day 139: $r_s = +0.720$, $p = 0.008$). Correlations between these variables were not significant in Sam Hill (Julian day 128: $r_s = +0.571$; Julian day 136: $r_s = +0.595$) and were not observed in Airport II (Julian day 131: $r_s = 0.217$; Julian day 139: $r_s = 0.430$).

Clonal Factors Contributing to the Severity of Primary Flower Infection

Height

There was no significant correlation between the height of “phenology” stems and their severity of flower blight in either Airport II or Columbia. Furthermore, there was no significant correlation between the average height of “phenology” stems per clone and the average severity of flower blight for “diseased” stems per clone in either Airport II or Columbia (Fig 27). With the exception of one clone, the average severity of flower blight was lower for clones in Columbia with smaller average stem heights (Fig. 27b). Height data was not collected in Sam Hill.

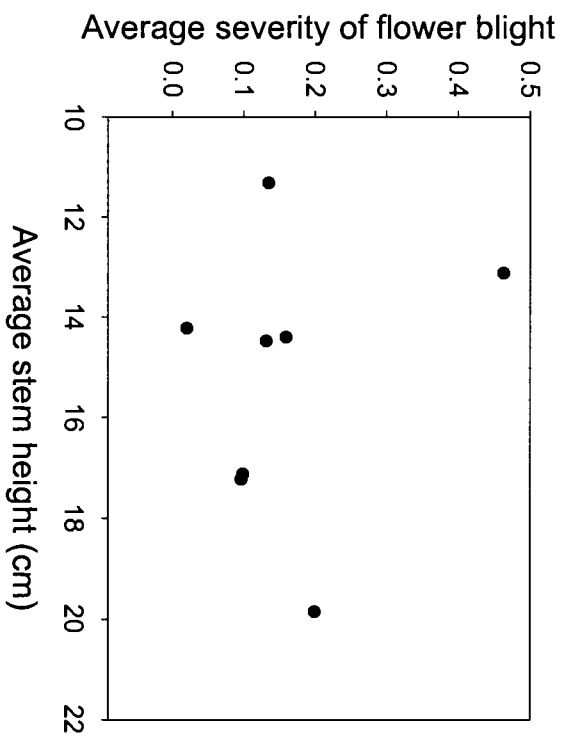
Phenology

In Airport II and Columbia, the severity of flower blight for individual “phenology” stems measured at flowering was positively correlated with their most frequently observed flower development stage on Julian days 131 and 139. (Figs. 28 and 29). The severity of flower blight was not determined for “phenology” stems in Sam Hill.

Although the average severity of flower blight on “diseased” stems generally was greater for clones with higher average flower development stages on Julian days 128-139, the correlations between these factors were not significant in Airport II,

A

Airport II



B

Columbia

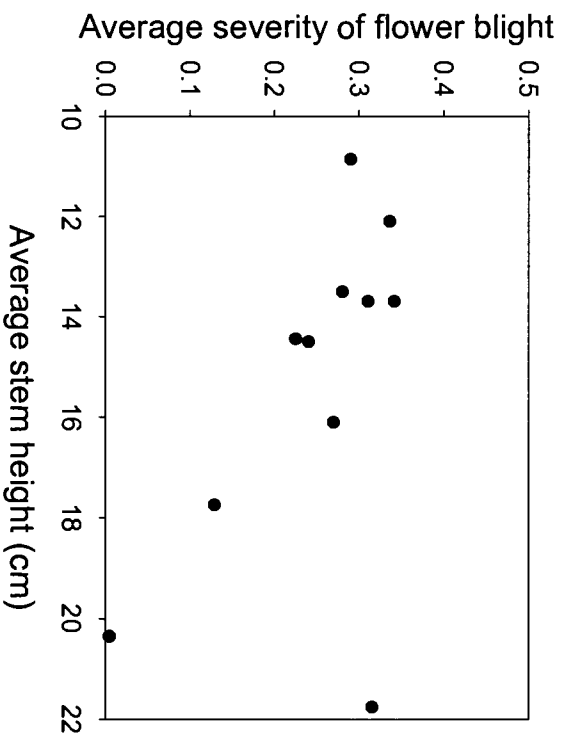


Figure 27. Relationship between average stem height and average severity of flower blight of clones within A) Airport II ($r_s = -0.143$, $p = 0.736$) and B) Columbia ($r_s = -0.478$, $p = 0.137$).

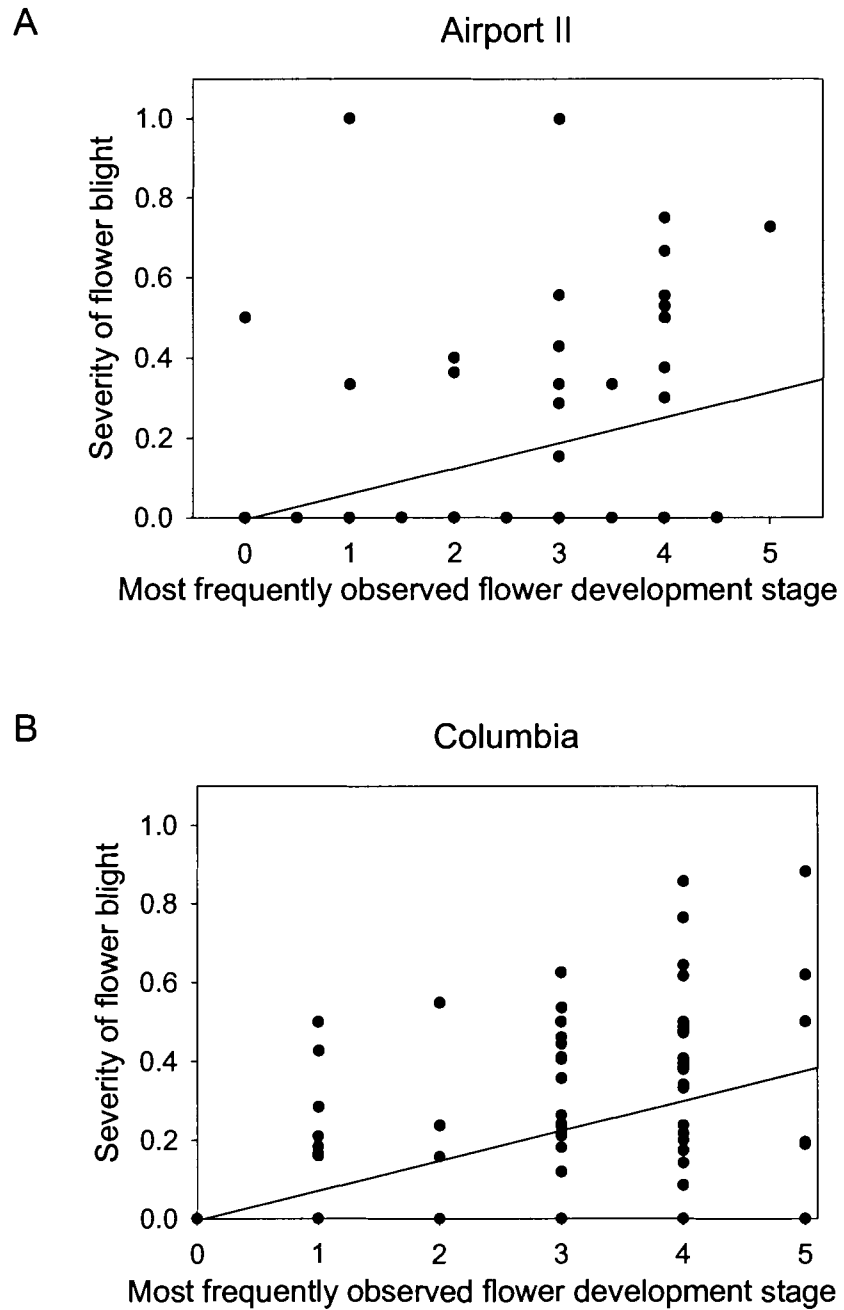
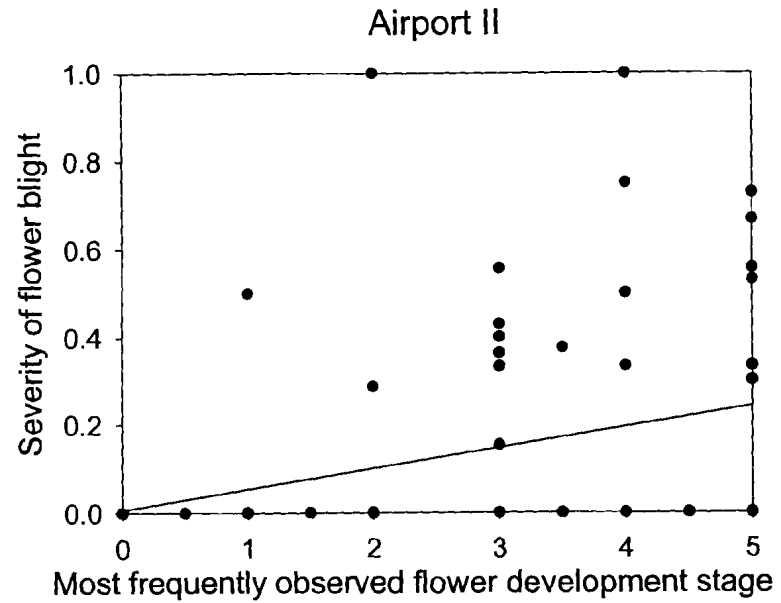


Figure 28. Relationship between the most frequently observed flower development stage on Julian day 131 and the severity of flower blight during flowering for individual “phenology” stems in two fields. A) Airport II ($r_s = +0.455$, $p < 0.001$) and B) Columbia ($r_s = +0.439$, $p < 0.001$).

A



B

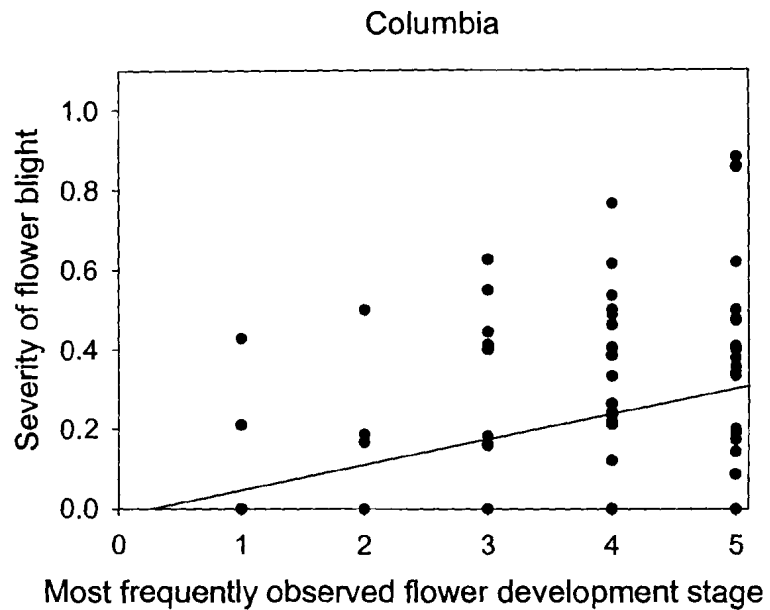


Figure 29. Relationship between the most frequently observed flower development stage on Julian day 139 and the severity of flower blight during flowering for individual “phenology” stems in two fields. A) Airport II ($r_s = +0.427$, $p < 0.001$) and B) Columbia ($r_s = +0.404$, $p < 0.001$)

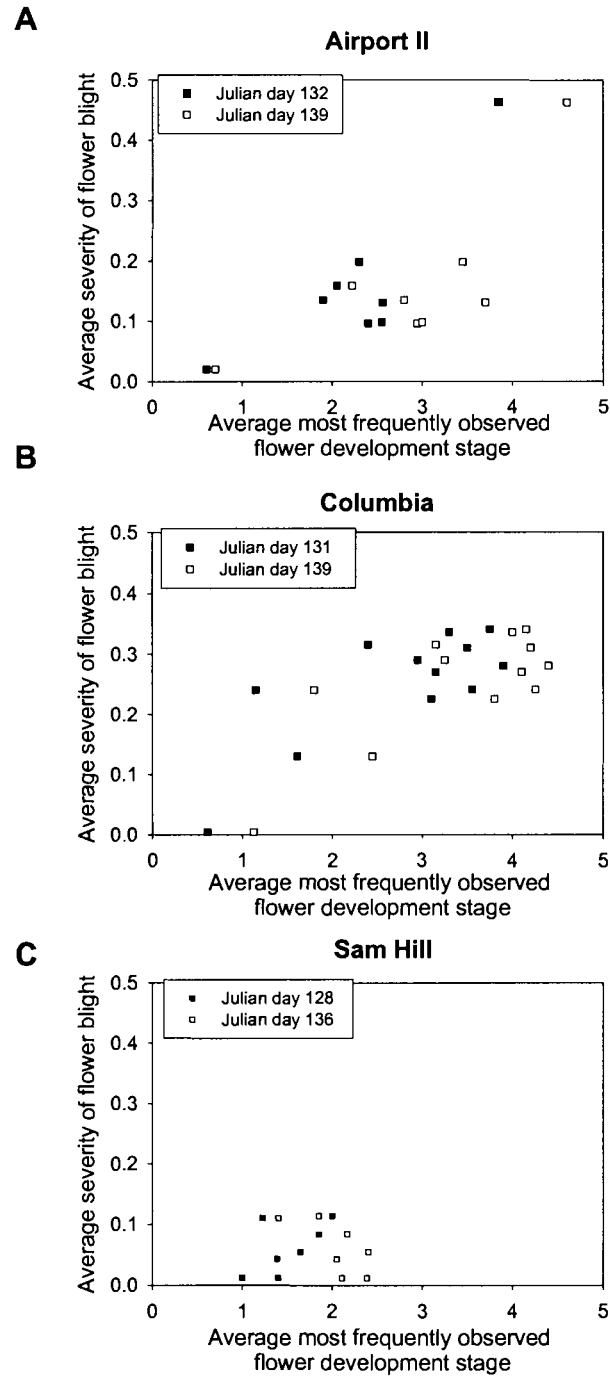


Figure 30. The relationship between the average most frequently observed flower development stage during bud expansion and the average severity of flower blight of clones during flowering for clones in three fields. A) Airport II (Julian day 131: $r_s = +0.347$, $p = 0.399$ and Julian day 139: $r_s = +0.204$, $p = 0.629$) B) Columbia (Julian day 131: $r_s = +0.375$, $p = 0.230$ and Julian day 139: $r_s = 0.253$, $p = 0.429$) and C) Sam Hill (Julian day 128: $r_s = 0.559$ and $p = 0.192$ and Julian day 136: $r_s = -0.391$ and $p = 0.386$).

Columbia, or Sam Hill (Fig. 30). The clones observed in Sam Hill (Fig. 30c) had slower flower development and a smaller proportion of blighted flowers than many of the clones in Airport II and Columbia (Fig. 23a and b).

Clonal Factors Contributing to the Incidence of Blight

Height

Clones with higher average stem heights had lower average incidences of blight (measured as the proportion of stems within a clone that had symptoms of either leaf or flower blight). However, the negative correlation between stem height and blight incidence was only significant in Columbia, (Fig. 31b). While this trend was seen in Airport II, there was no significant correlation between these two variables among clones (Fig. 31a). Stem height data was not collected in Sam Hill.

Phenology

The average incidence of blight (observed during flowering) tended to be greater in clones with higher average common development stage of their leaves (observed between Julian days 128 and 136). However, this relationship was not consistently significant. There was a positive and significant correlation between blight incidence and leaf bud development in Columbia on Julian days 131 and 139 ($r_s=+0.616$, $p= 0.033$ and $r_s=+0.735$, $p= 0.006$, respectively) (Fig. 33a and b), and in Sam Hill on Julian day 136 ($r_s=+0.964$, $p<0.001$) (Fig. 34b). However, the relationship between blight incidence and leaf development was not significant in Airport II on Julian day 131 or 139 ($r_s=+0.524$, $p= 0.183$ and $r_s=+0.452$, $p= 0.260$, respectively) (Fig. 32a and b), or in Sam Hill on Julian day 128 ($r_s= +0.607$, $p= 0.148$) (Fig 34a).

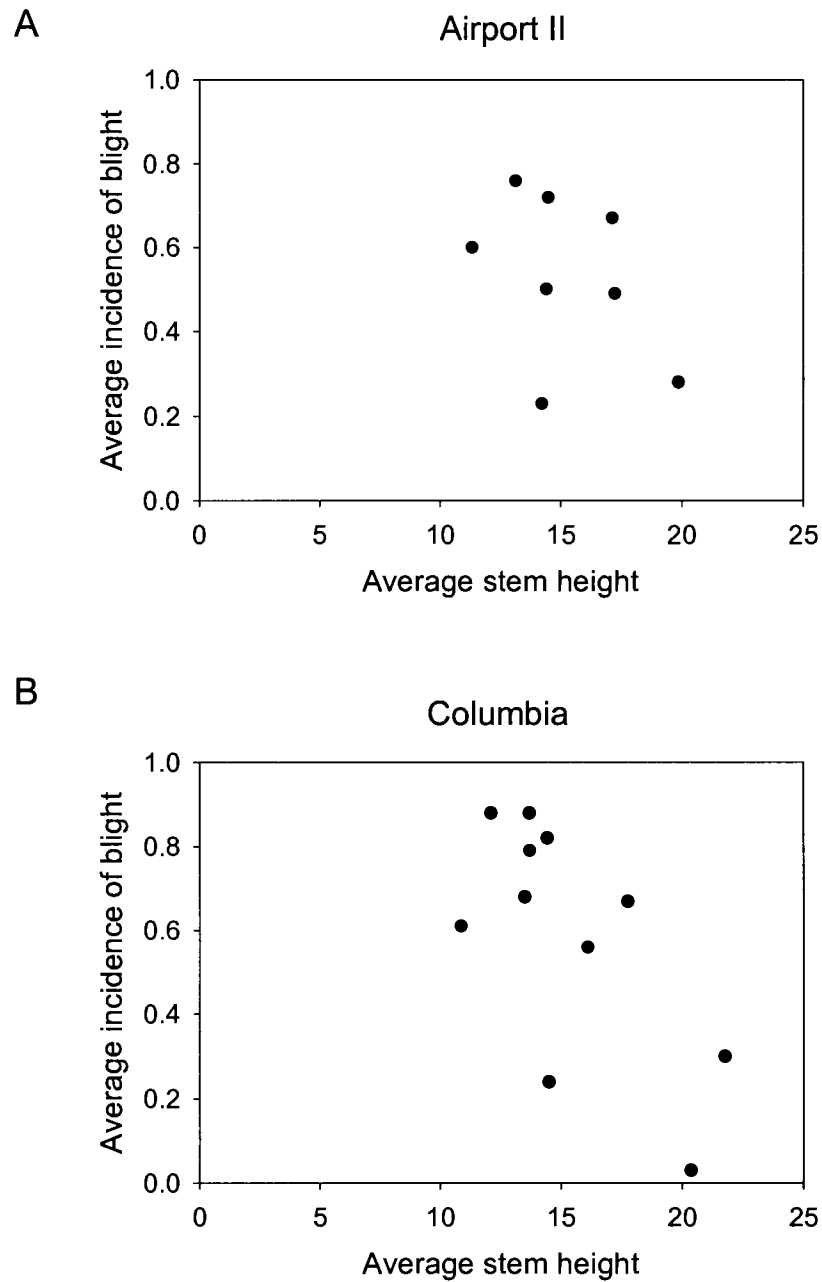


Figure 31. The relationship between the average stem height and average incidence of blight (measured as the proportion of stems with symptoms of blight during flowering) of clones in A) Airport II ($r_s = -0.333$, $p = 0.420$) and B) Columbia ($r_s = -0.612$, $p = 0.046$).

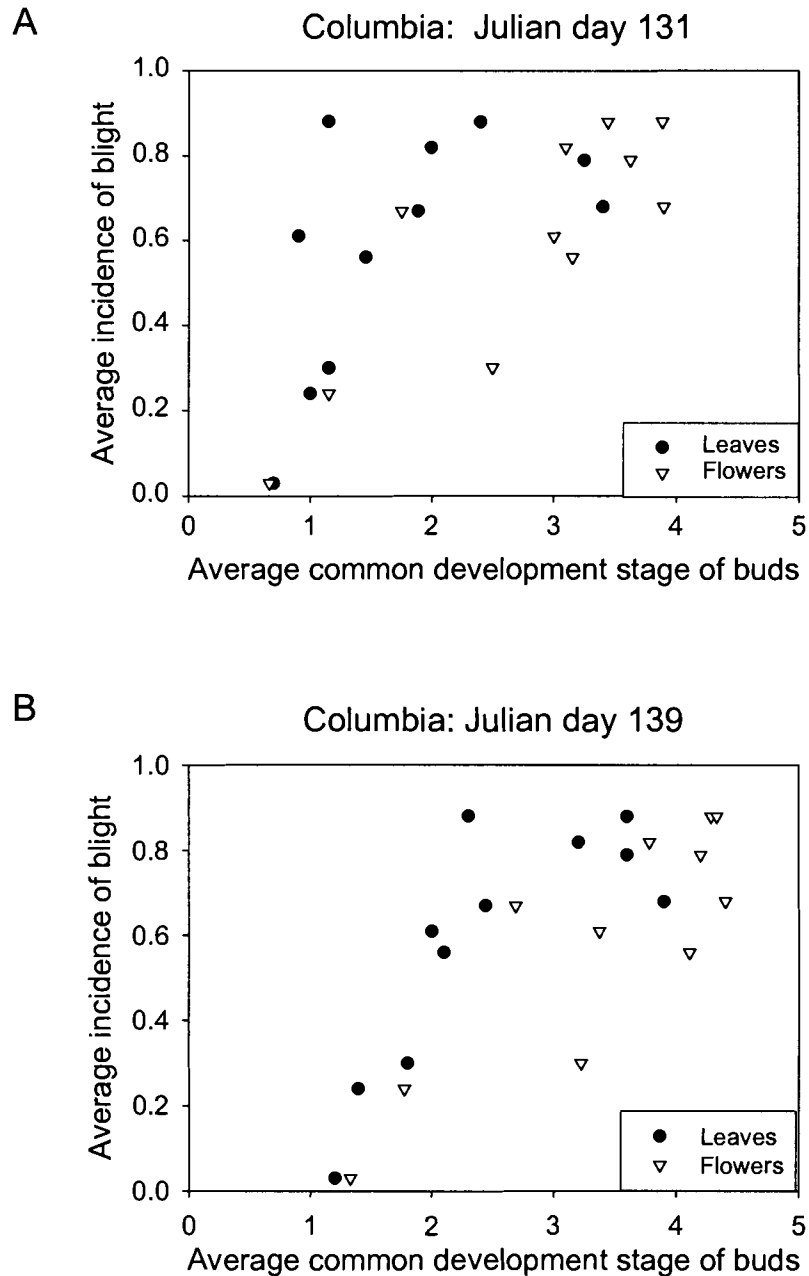


Figure 33. The relationship between the average incidence of blight per clone in Columbia (measured on Julian day 155) and the average of the most frequently observed leaf and flower development stages of the clones measured on A) Julian day 131 and B) Julian day 139.

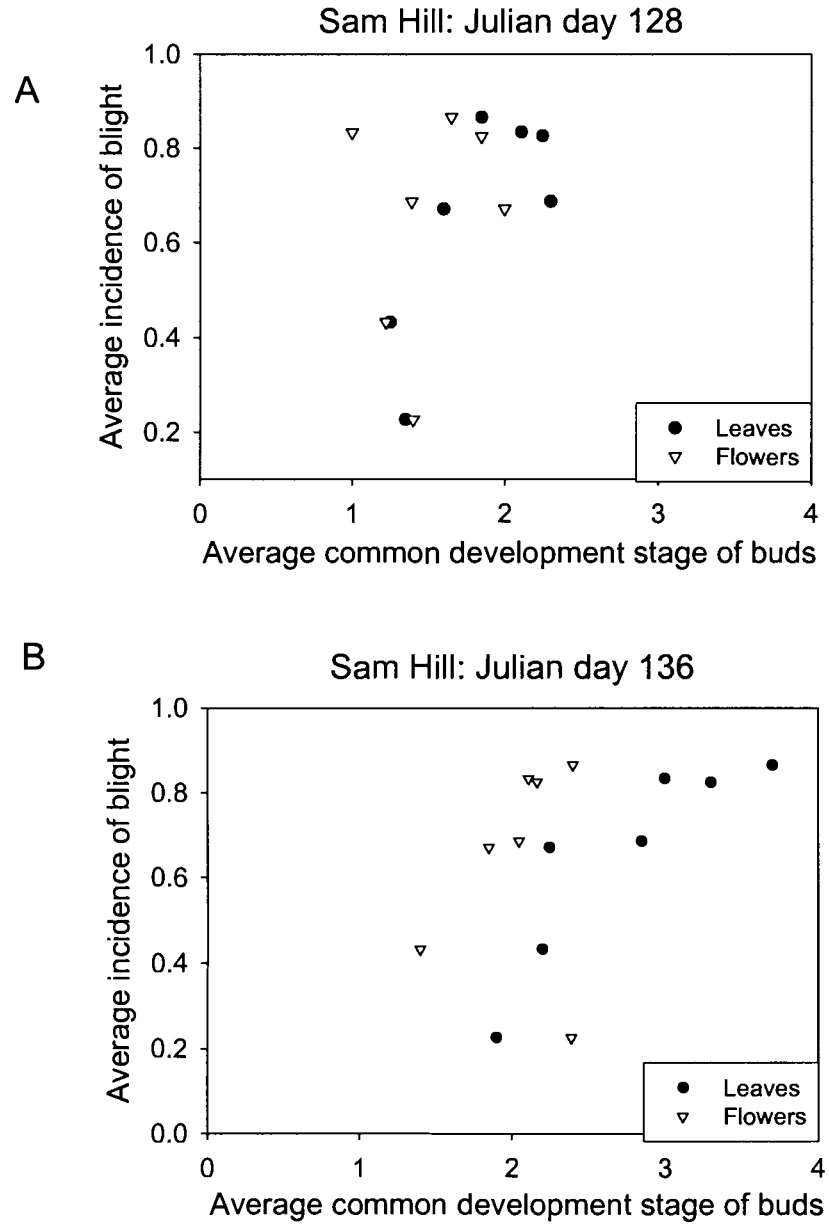


Figure 34. The relationship between the average incidence of blight per clone in Sam Hill (measured on Julian day 152) and the average of the most frequently observed leaf and flower development stages of the clones measured on A) Julian day 128 and B) Julian day 136.

As the average common flower development stage on Julian days 131-139 increased, the average incidence of blight tended to increase. However, this positive correlation was significant only in Columbia ($r_s=+0.739$, $p=0.006$ and $r_s=+0.774$, $p=0.003$) (Fig. 33). The relationship between blight severity and flower bud development was not significant for Julian days 131 and 139 in Airport II ($r_s=+0.571$, $p=0.139$ and $r_s=+0.595$, $p=0.120$, respectively) (Fig. 32) or for Julian days 128 and 136 in Sam Hill ($r_s=0.000$, $p=1.000$ and $r_s=+0.306$, $p=0.504$, respectively) (Fig. 33)

Relationship Between Phenology and the Severity of Fruit Mummification

The average common flower development stage of clones was not significantly correlated with their eventual severity of fruit mummification. In Columbia, the average severity of fruit mummification decreased as the average common flower development stage on Julian dates 131 and 139 increased ($r_s= -0.507$ and $r_s= -0.420$, for Julian days 131 and 139, respectively), but the correlations were not significant. In Airport II and Sam Hill, there was no correlation between the average severity of fruit mummification and the average common flower development stage measured from Julian day 128 to 139.

Discussion

Two different populations of stems were used to examine the height, bud phenology, and disease severity of clones. The height and phenology of stems in the “phenology” stem population were compared to the severity of mummy berry disease in “diseased” and “phenology” stem populations. “Phenology” stems were chosen prior to budbreak, and therefore were randomly selected from the population of stems with

flower buds within each clone. In contrast, “diseased” stems were selected from the population of flowering stems with symptoms of blight. Therefore, all “diseased” stems have some blighted tissue, whereas “phenology” stems theoretically represent the severity and incidence of disease in a small random sample of stems within a clone, which may or may not have disease. The severity of disease on “phenology” stems was related to their height and their leaf and flower bud phenology in order to determine direct relationships between these factors. The average height and phenology of clones were also related to their average severity and incidence of blight reported in Chapter 2 in order to determine if height and phenology are clonal characteristics related to the severity and incidence of blight within a clone.

The phenology of leaf bud development is an important factor affecting differences in the severity and incidence of leaf blight among lowbush blueberry clones. When individual stems were used in the analysis, the severity of leaf blight was positively correlated with leaf bud development observed on Julian days 128-139. The average severity of leaf blight was also correlated with the average common leaf development stage for clones in two of three fields on at least one observation date. Furthermore, the average incidence of blight for clones was related to their severity of leaf blight and increased with the average leaf and flower bud development of “phenology” stems, but the incidence of blight was not consistently correlated with the average common development stage of leaf buds. Other researchers have also reported significant positive relationships between vegetative phenology and the severity and incidence of mummy berry blight on blueberry cultivars, but this is the first study of this relationship among clones in commercial lowbush blueberry fields. Hildebrand and Braun (1991) inoculated leaf buds of *V. angustifolium* with ascospores of *M. vaccinii-corymbosi*, and found that the incidence of leaf blight increased with the stage of leaf bud development, particularly if the developing leaves were frost-

damaged. When resistance to *Monilinia* leaf blight was compared among cultivars of highbush and rabbiteye blueberry, the percentage of blighted shoots was significantly greater in cultivars which exhibited earlier shoot growth relative to other cultivars (Ehlenfeldt et al. 1996; Stretch and Ehlenfeldt 1997; Ehlenfeldt and Stretch 2000). It is likely that avoidance is an important resistance mechanism to mummy berry disease in both lowbush and highbush blueberry, and that delayed leaf development may decrease the amount of susceptible leaf tissue during ascospore release (Agrios 1980; Agrios 1988; Ehlenfeldt et al. 1996; Stretch and Ehlenfeldt 1997).

As was seen with leaf blight, the severity of flower blight also varied among clones within a field, and the severity of flower blight on individual “phenology” stems was positively correlated with their most frequently observed stage of flower bud development. A positive correlation between flower blight severity and flower bud development was also found by Hildebrand and Braun (1991) using frost-damaged *V. angustifolium* plants. However, when the averages of the flower development stages for clones were compared to the average severities of flower blight for “diseased” stems, positive, but non-significant, relationships were observed. The failure to detect a significant correlation may be due to the difficulty determining all of the developmental variation within flower clusters (Hildebrand et al. 2001) as well as the variation in development of individual flower buds on a stem. Furthermore, Hildebrand and Braun (1991) reported that the incidence of flower infection peaked with stage 3 flower buds in plants that were not exposed to frost, which further complicates the relationship between flower phenology and blight severity.

There is evidence that susceptible host tissue is important for the reproductive success of *M. vaccinii-corymbosi*. The release of ascospores coincides with budbreak of highbush, lowbush, and rabbiteye blueberry hosts (Ramsdell et al. 1975; Batra 1983 ; Hildebrand and Braun 1991 ; Ramsdell et al. 1974), and the phenology of apothecia

production differs between early- and late-season cultivars grown in fields within the same geographical region (Lehman and Oudemans 1997). Lehman and Oudemans (2000) inoculated highbush floral stigmas with conidia produced by “early”- and “late”-emerging populations of apothecia in order to evaluate the phenology of the resulting offspring. After determining the amount of phenological variation that was attributed to genetic vs. environmental factors, Lehman and Oudemans (2000) concluded that the phenology of apothecia production is moderately to highly heritable. These results suggest that the phenology of populations of *M. vaccinii-corymbosi* is controlled by both genetic and environmental factors and that the fitness of apothecia is influenced by the timing of host bud development relative to ascospore discharge.

Clones with higher stem heights had lower severities of leaf blight than clones with shorter stems, but the height of individual stems was not related to the severity of leaf blight on those stems. Height of a stem by itself does not influence its severity of leaf blight, but it is a clonal characteristic that may indicate the severity of leaf infection. Taller clones may have slower development relative to shorter clones, or, if height is controlled by genetic factors, there may be a biochemical resistance mechanism common to most “tall” clones. Furthermore, taller stems are expected to have more leaves than shorter stems, and the severity of leaf blight on stems with many leaves tends to be lower than leaf blight severity on stems with few leaves (as reported in Chapter 2). Although stem height does not seem to be directly related to blight resistance, it may be a useful indicator of relatively resistant clones. Stem height remains fairly constant over a period of months, unlike leaf bud development, which is much more temporal. Although the severity of leaf blight decreased with stem height, there was not a strong relationship between stem height and the severity of flower

blight. As was previously mentioned, flower blight was a relatively rare event, which may have prevented the detection of a relationship between flower blight and stem height.

There was no relationship between the average common stage of flower development on Julian days 128-139 and the average severity of fruit mummification. Other researchers have reported that flower age is an important factor affecting the successful infection of floral stigmas by conidia of *M. vaccinii-corymbosi* (Ngugi et al. 2002). Relationships may not have been found in this study because of dates used to evaluate flower bud development. Ascospores were present on Julian days 128-139, but only conidia are able to cause fruit infection and conidia are not produced on host tissue until ~10 days after ascospore germination (Lockhart et al. 1983; Hildebrand and Braun 1991). It is likely that flower bud development measured on Julian days 128-139 does not reflect the development of flower buds during conidia production. Honeybee activity is strongly influenced by weather (Drummond 2002), which further complicates the possible relationship between flower development and the severity of fruit mummification. Furthermore, the scale of flower bud development that was used in this study had a visible corolla as the maximum flower development stage, and so it did not differentiate between closed and open flowers.

This study only examined the phenology of leaf and flower development for one growing season, so it is not known whether clones with relatively late bud development will continue to have relatively low amounts of *Monilina* blight. Lyrene (1985) found that the phenology of leaf and flower buds for clones of rabbiteye blueberry (relative to other clones) was consistent on a year to year basis. Assuming that the relative phenology of lowbush blueberry clones is also consistent over years, it is likely that clones of *V. angustifolium* that have delayed leaf and flower bud development will continuously avoid blight by *M. vaccinii-corymbosi*. However, it is also possible that

apothecial production by *M. vaccinii-corymbosi* is dependent on slightly different environmental cues than bud development of *V. angustifolium*. By continuing to examine the relationship between the phenology of bud development and the severity of leaf blight for the clones used in this study, the consistency of blight severity and bud phenology of clones of *V. angustifolium* can be determined.

CHAPTER FOUR: EFFECTS OF MUMMY BERRY BLIGHT UPON THE QUALITY AND QUANTITY OF LOWBUSH BLUEBERRY FRUIT

Introduction

Blueberries are an important crop in Maine and the Maritime provinces, and many of the berries produced in these regions are from naturally-seeded stands of the lowbush blueberry species *V. angustifolium* and *V. myrtilloides* (Yarborough 1998). Mummy berry disease is one of the most important diseases of lowbush blueberry and is caused by the fungus *Monilinia vaccinii-corymbosi* (Batra 1983). Mummy berry blight of leaves and flowers is caused by ascospore infection during bud expansion. When conidia produced on blighted tissues are transported to pollinated floral stigmas, the fruit becomes mummified, drops to the ground, and is an overwintering structure for the fungus (Batra 1983). Unlike highbush blueberry cultivars, yields of lowbush blueberry plants are reduced more by the blight stage than by the fruit infection stage of mummy berry disease (Hildebrand and Braun 1991; Stretch and Ehlenfeldt 1997; Stretch et al. 2001).

Mummy berry blight causes direct economic damage through injury to reproductive tissue, but the effects of leaf blight on berry number and berry quality are not well understood. Hildebrand and Braun (1991) observed that severe leaf blight of *V. angustifolium* in greenhouse studies was associated with reductions in berry size, but this relationship has not been examined under field conditions. Severe leaf blight may reduce fruit quality by reducing the production and allocation of photosynthate to developing fruit. Reductions of leaf to fruit ratios have been associated with reductions in weight and soluble solids in southern highbush blueberry (Maust et al. 1999) and other fruits (Hurd et al. 1979; Fecteau et al. 1983), but these reductions in leaf to fruit

ratios were artificially created through the removal of vegetative or reproductive tissues rather than through pathogen-induced death of photosynthetic tissue.

The purpose of study is to determine the effects of mummy berry blight on fruit quantity and berry weight, and to investigate the possible role of leaf to fruit ratios in yield reductions.

Materials and Methods

The effects of blight on fruit quality and quantity was studied in one field in 2001 (Airport I) and in three fields in 2002 (Airport II, Columbia, and Sam Hill). Airport I was an irrigated field in its second year of production since pruning and had missed one application of fungicide in the spring of 2001. Airport II and Columbia were irrigated fields that were in their first year of production since pruning, and Sam Hill was a non-irrigated field that appeared to be in its third year of production since pruning. Airport II, Columbia, and Sam Hill did not receive any applications of fungicide in 2002. Ten clones were selected in Airport I, eight clones were selected in Airport II, twelve clones were selected in Columbia, and seven clones were selected in Sam Hill, as described in Chapter 2.

As previously described, 10 “diseased” and 10 “healthy” stems with flowers were randomly selected in each clone at flowering (Chapter 2). The number of healthy and blighted leaves and flowers were counted for each stem at flowering, during the green fruit stage, and just prior to harvest (See chapter 2 for a list of dates). The severity of leaf blight was calculated for each stem as the proportion of leaves with symptoms of blight. The number of set fruit was counted at the green fruit stage, and

the number of ripe fruit, unripe fruit, and mummy berries were counted just prior to harvest. The adjusted fruit set of individual stems was the proportion of healthy flowers in May that produced healthy fruit.

The data for the adjusted fruit set of individual stems was ranked, and the ranked data was analyzed using Proc GLM Analysis of Variance (SAS Institute) to determine whether adjusted fruit set differed among clones within a field and between “healthy” and “diseased” stem populations. A model statement was also included to test whether there was a significant interaction between clone and stem health. Spearman’s correlation procedure was used to determine whether there was a relationship between adjusted fruit set and the severity of blight on individual “diseased” stems.

The average berry weight per stem was also measured for each “healthy” and “diseased” stem in 2001 and 2002. Whereas all of the set fruit on a stem were used to determine the adjusted fruit set, only blue fruit were measured for average berry weight. Spearman’s correlation procedure was used to determine whether there was a significant relationship between average berry weight and the severity of blight of individual “diseased” stems. Spearman’s correlation procedure was also used to determine whether the ratios of leaf number to fruit number at the green fruit stage were related to the eventual average berry weight of “healthy” and “diseased” stems within each field. Trendlines for these relationships were obtained using Sigmaplot (SPSS, Chicago, Illinois), but Spearman’s correlation coefficients do not reflect how closely the data points are related to the trendline.

Results

Changes in the Number of Reproductive Units Over Time

The majority of flowers were counted during “flowering”, but some stems produced flowers during the green fruit stage, which resulted in more reproductive units (green fruit and flowers) at this stage than the number of reproductive units present during flowering (Figs. 35, 36, 37, and 38). Furthermore, the potential fruit production observed during the green fruit stage was usually larger than the number of ripe and unripe fruit observed at harvest (Figs. 35, 36, 37, and 38). The effects of disease on fruit production were seen when the number of reproductive units per flower produced at the green fruit stage were compared to the number of fruit per flower prior to harvest (Figs. 35, 36, 37, and 38). In Airport II and Columbia, differences in slopes between the green fruit stage and prior to harvest were greater for “diseased” stems than for “healthy” stems. However, in Airport I and Sam Hill, “healthy” and “diseased” stems did not differ in their reductions in fruit number between the green fruit stage and pre-harvest. “Healthy” and “diseased” stems in Airport I produced more fruit than stems in Airport II, Columbia, or Sam Hill. In all fields, the production of mummy berries was uniformly low and did not differ between “healthy” and “diseased” stems.

Variation in Adjusted Fruit Set Among Clones

The adjusted fruit set of stems varied among clones within most fields. Adjusted fruit set was significantly different among clones within Airport I ($p < 0.0001$), Columbia ($p = 0.0016$), and Sam Hill ($p < 0.0001$), but was not significantly different among clones in Airport II (Figs. 39 and 40). Flowers from “healthy” stems produced significantly more blueberries than flowers from “diseased” stems in Airport I ($p = 0.0019$) and Columbia ($p < 0.0001$) (Figs. 39a and 40a), but there was not a

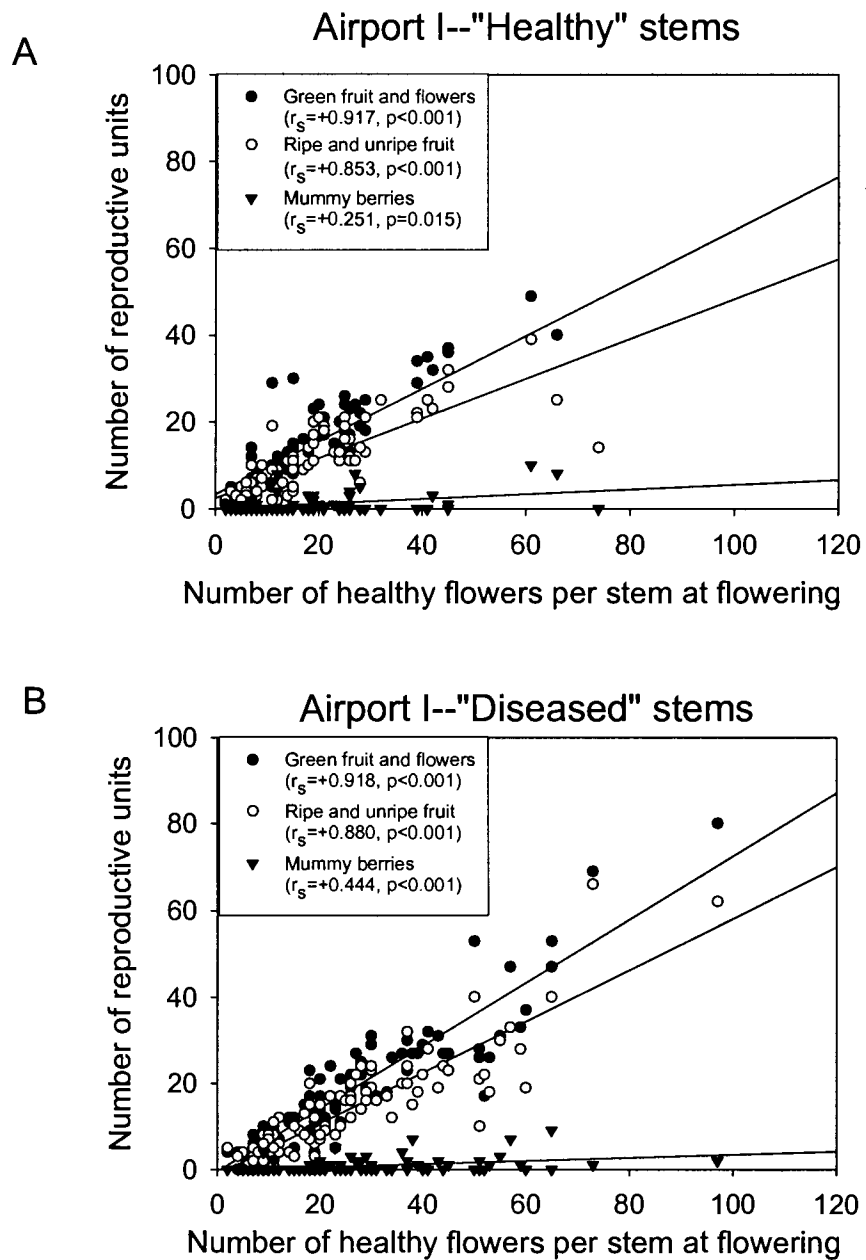


Figure 35. Changes in the number of reproductive units per stem over time on A) "healthy" and B) "diseased" stems in Airport I. Healthy flowers were counted on Julian days 148 -158. Green fruit and flowers were counted on Julian days 179-180. Ripe and unripe fruit and mummy berries were counted on Julian day 211.

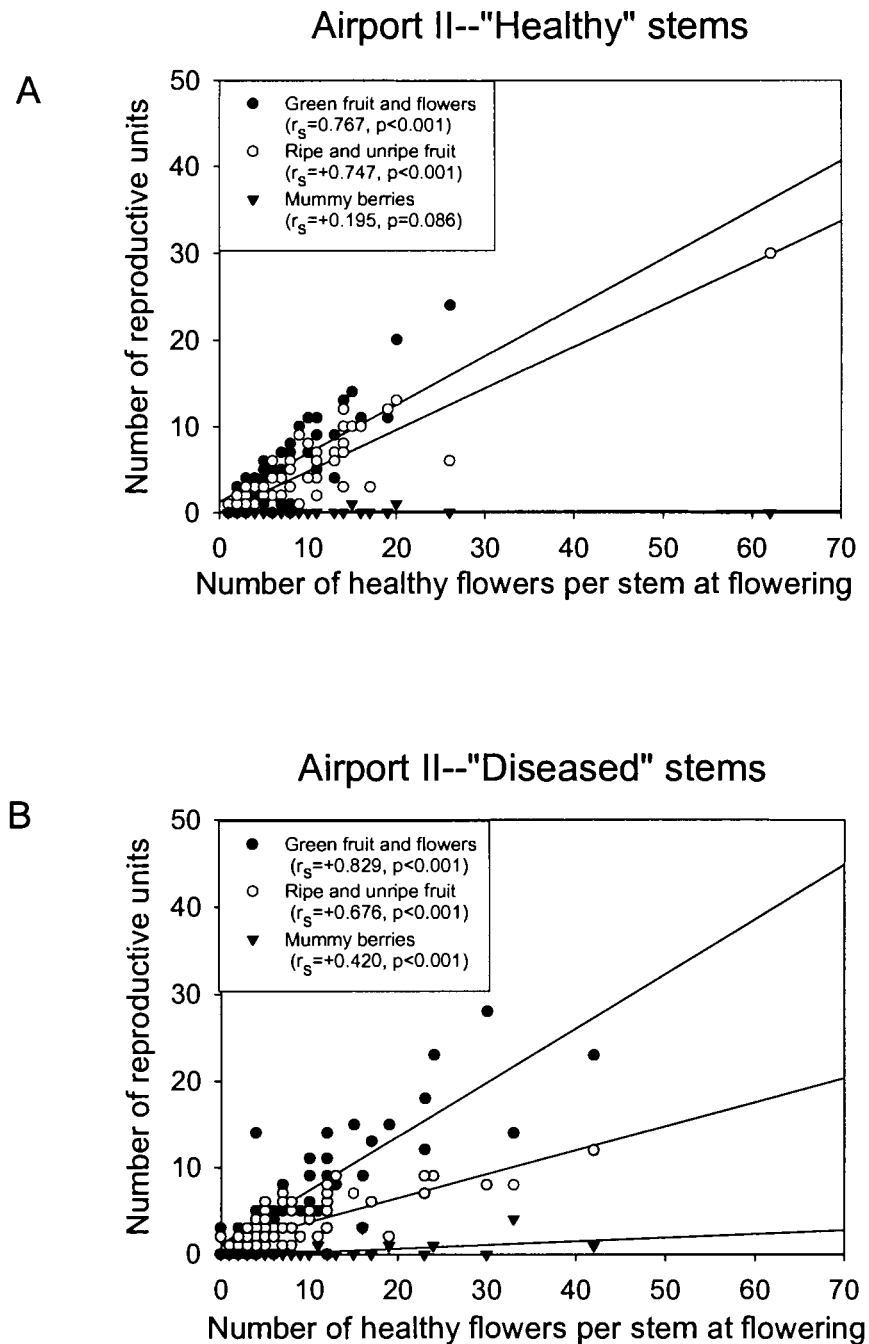


Figure 36. Changes in the number of reproductive units per stem over time on A) "healthy" and B) "diseased" stems in Airport II. Healthy flowers were counted on Julian day 161. Green fruit and flowers were counted on Julian day 181. Ripe and unripe fruit and mummy berries were counted on Julian day 220.

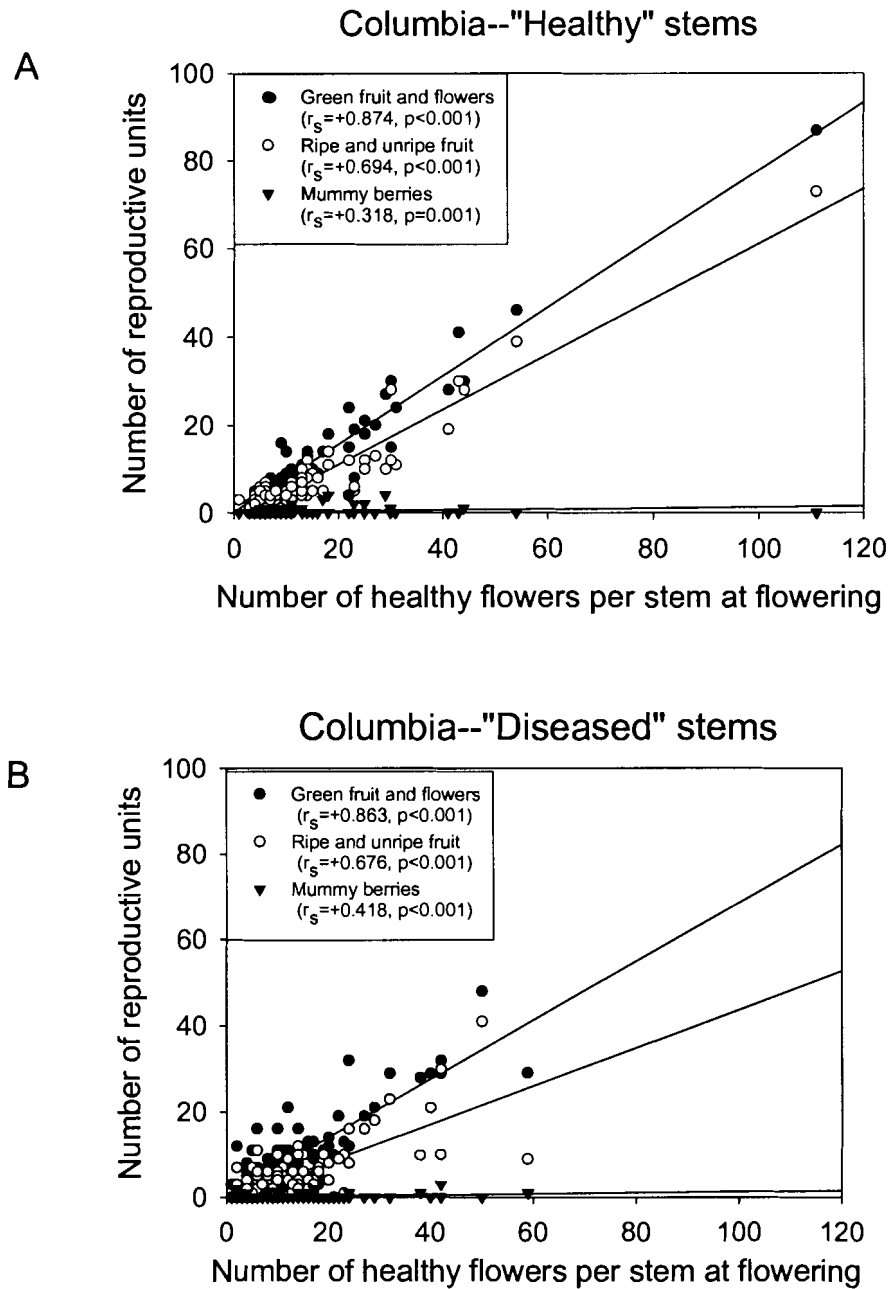


Figure 37. Changes in the number of reproductive units per stem over time on A) "healthy" and B) "diseased" stems in Columbia. Healthy flowers were counted on Julian day 152. Green fruit and flowers were counted on Julian day 177. Ripe and unripe fruit and mummy berries were counted on Julian day 215.

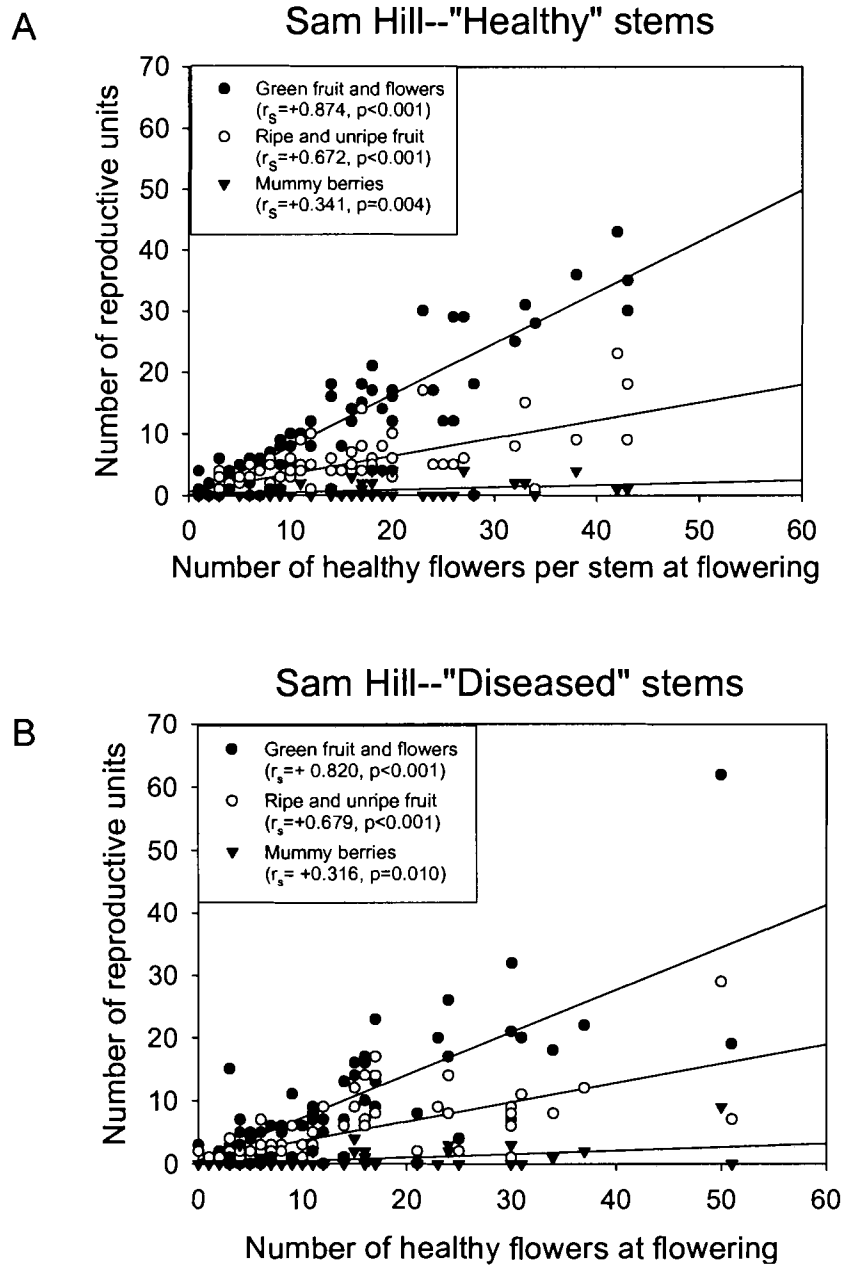


Figure 38. Changes in the number of reproductive units per stem over time on A) "healthy" and B) "diseased" stems in Sam Hill. Healthy flowers were counted on Julian day 152. Green fruit and flowers were counted on Julian day 177. Ripe and unripe fruit and mummy berries were counted on Julian day 215.

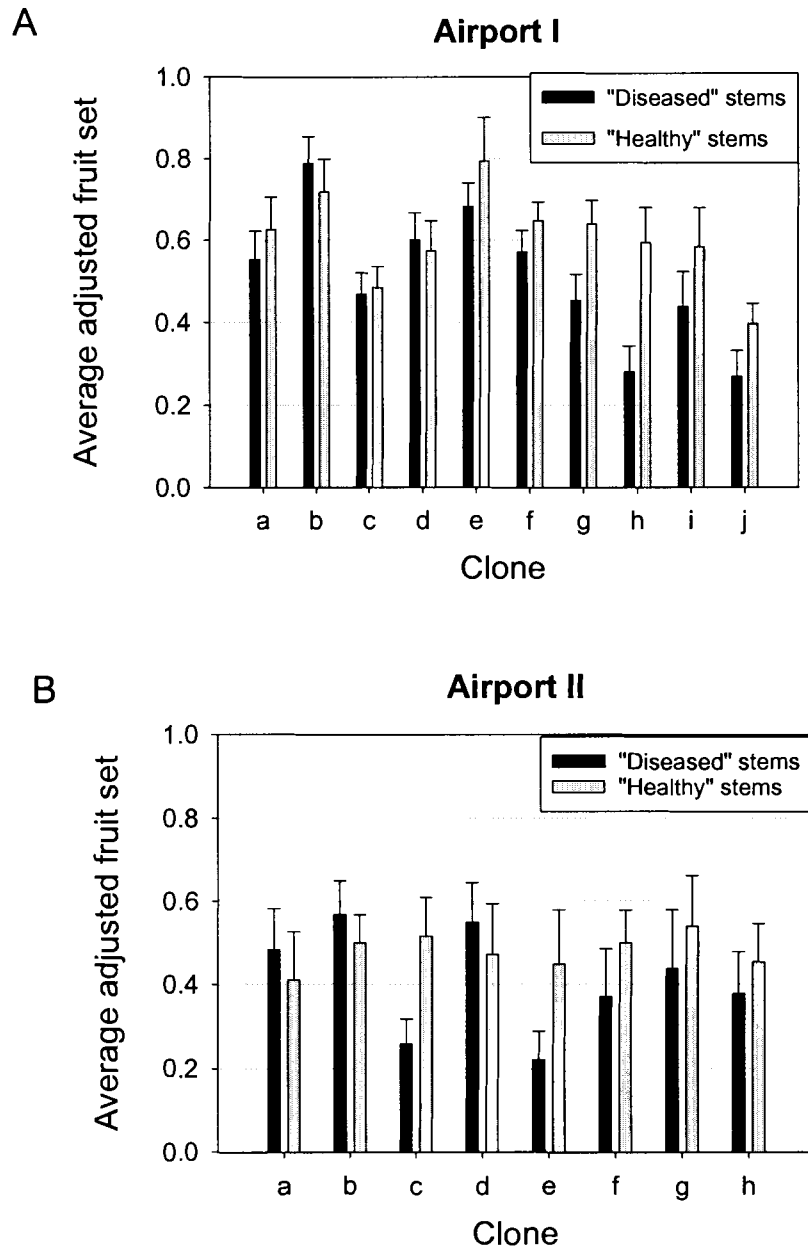


Figure 39. Average adjusted fruit set among clones and “healthy” and “diseased” stems in A) Airport I and B) Airport II. Adjusted fruit set is the proportion of healthy flowers at flowering that produced healthy fruit just prior to harvest. Bars represent the standard error of the means. Clones are arranged in order of increasing severity of leaf blight.

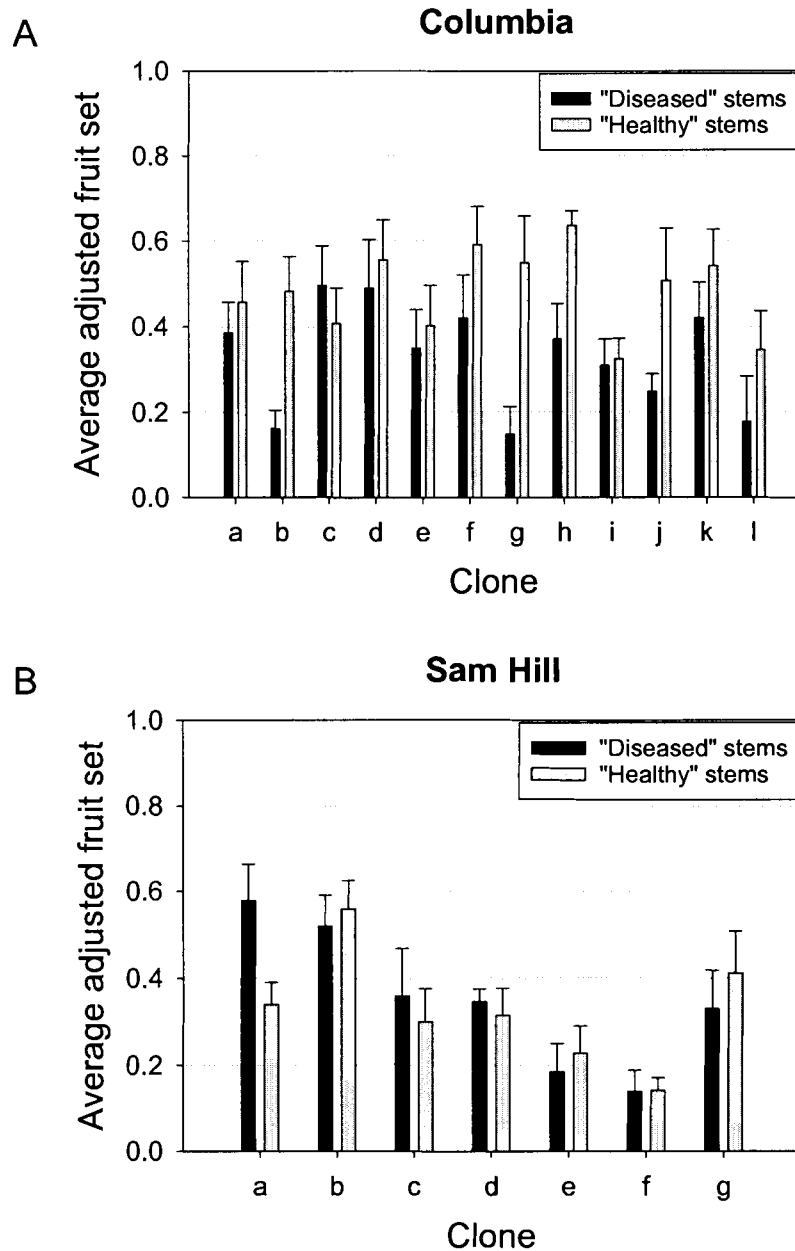


Figure 40. Average adjusted fruit set among clones and “healthy” and “diseased” stems in A) Columbia and B) Sam Hill. Adjusted fruit set is the proportion of healthy flowers at flowering that produced healthy fruit just prior to harvest. Bars represent the standard error of the means. Clones are arranged in order of increasing severity of leaf blight.

significant interaction between clone and stem health status in either field ($p=0.4146$ and $p=0.3307$, respectively). In Airport II and Sam Hill, the differences in adjusted fruit set were not significant between “healthy” and “diseased” stems ($p=0.1924$ and $p=0.7026$, respectively) (Figs. 39b and 40b).

The Effects of Leaf Blight Upon Adjusted Fruit Set

On “diseased” stems, adjusted fruit set decreased as the severity of leaf blight increased. This relationship was only significant for stems in Airport I at $\alpha=0.05$ ($r_s=-0.451$, $p<0.001$) (Fig. 41a), but was also observed for stems in Airport II ($r_s=-0.144$, $p=0.205$), Columbia ($r_s=-0.170$, $p=0.067$), or Sam Hill ($r_s=-0.211$, $p=0.086$) (Figure 41b and 42).

The Effects of Leaf Blight on Berry Weight

In Airport I and Columbia, the average weight of blue (ripe) berries on individual “diseased” stems decreased as the severity of leaf blight increased ($r_s=-0.312$, $p=0.003$) and $r_s=-0.454$, $p<0.001$, respectively). (Fig. 43a and 45a). There was no significant correlation between the severity of leaf blight and average berry weight in Airport II ($r_s=-0.064$, $p=0.637$) or Sam Hill ($r_s=-0.024$, $p=0.859$) (Figs. 44a and 46a).

Relationships Between Leaf to Fruit Ratios and Berry Weight

The average ripe berry weight on “diseased” stems increased with the ratio of healthy leaves to healthy reproductive units (observed during the green fruit stage) in Airport I ($r_s=+0.329$, $p=0.001$) (Fig. 43b), Airport II ($r_s=+0.402$, $p=0.002$) (Fig. 44b), and Columbia ($r_s=+0.415$, $p<0.001$) (Fig. 45b). There was no relationship between average berry weight and leaf to fruit ratios for “diseased” stems in Sam Hill ($r_s=+0.032$, $p=0.814$) (Fig. 46b). “Healthy” stems in Columbia also had significant

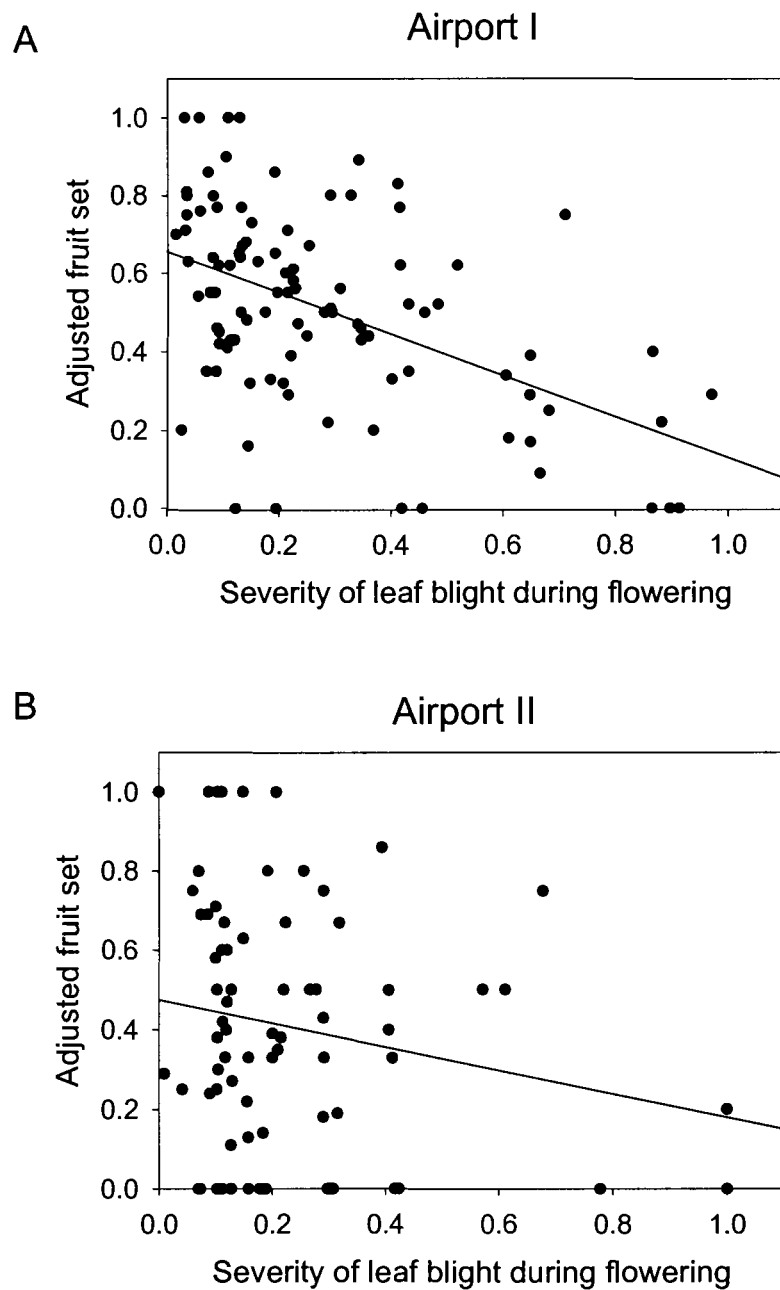


Figure 41. The relationship between the severity of leaf blight during flowering and the adjusted fruit set prior to harvest for “diseased” stems in A) Airport I ($r_s = -0.451$, $p < 0.001$) and B) Airport II ($r_s = -0.144$, $p = 0.205$).

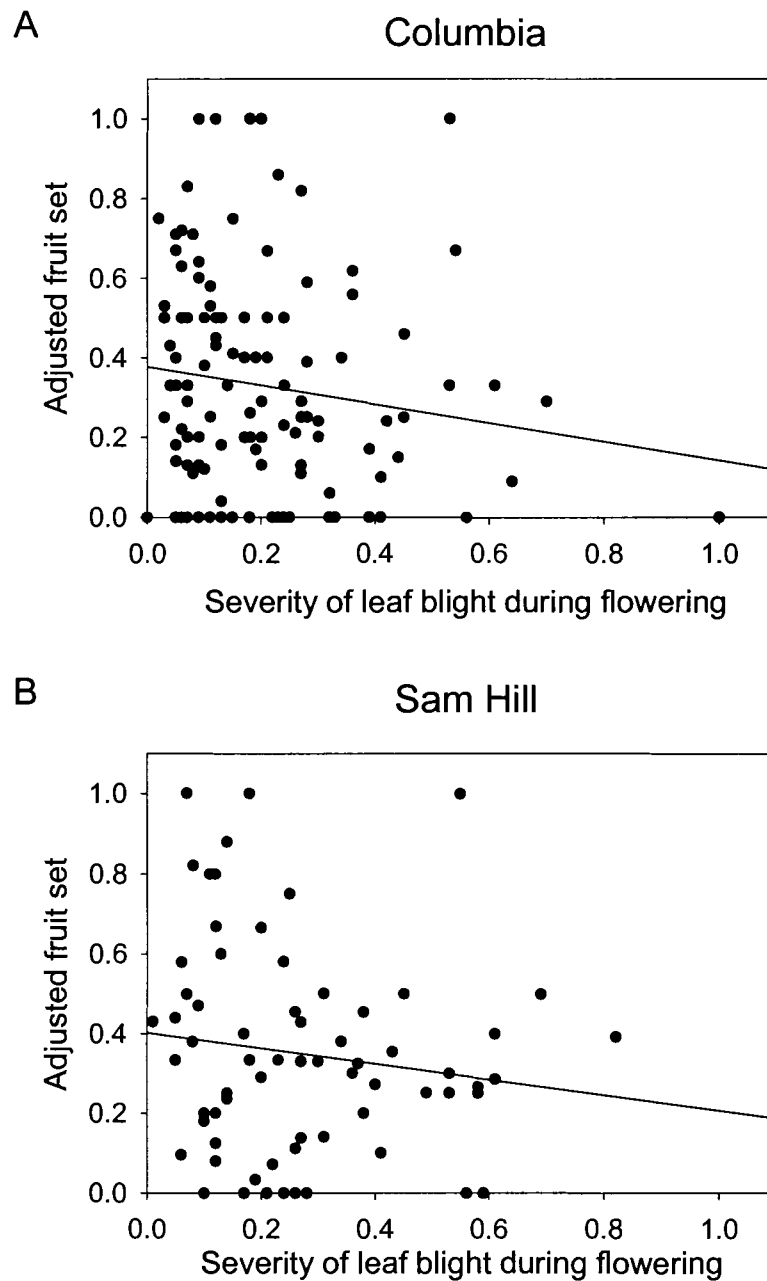


Figure 42. The relationship between the severity of leaf blight during flowering and the adjusted fruit set prior to harvest for “diseased” stems in A) Columbia ($r_s = -0.170$, $p = 0.067$) and B) Sam Hill ($r_s = -0.211$, $p = 0.086$).

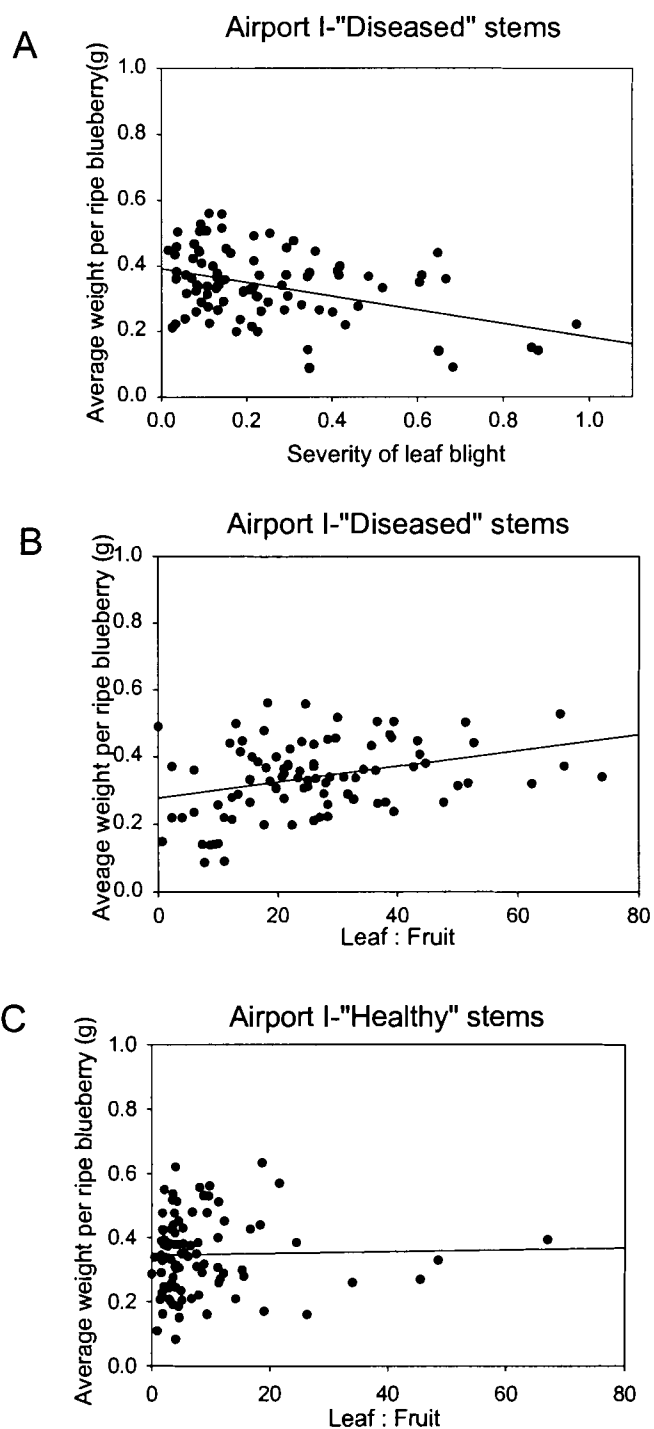


Figure 43. The relationship between average ripe blueberry weight and A) the severity of leaf blight on "diseased" stems during flowering, B) the leaf to fruit ratio on "diseased" stems during the green fruit stage, and C) the leaf to fruit ratio on "healthy" stems during the green fruit stage in Airport I. Leaf to fruit ratios were calculated as the number of healthy leaves divided by the number of healthy fruit.

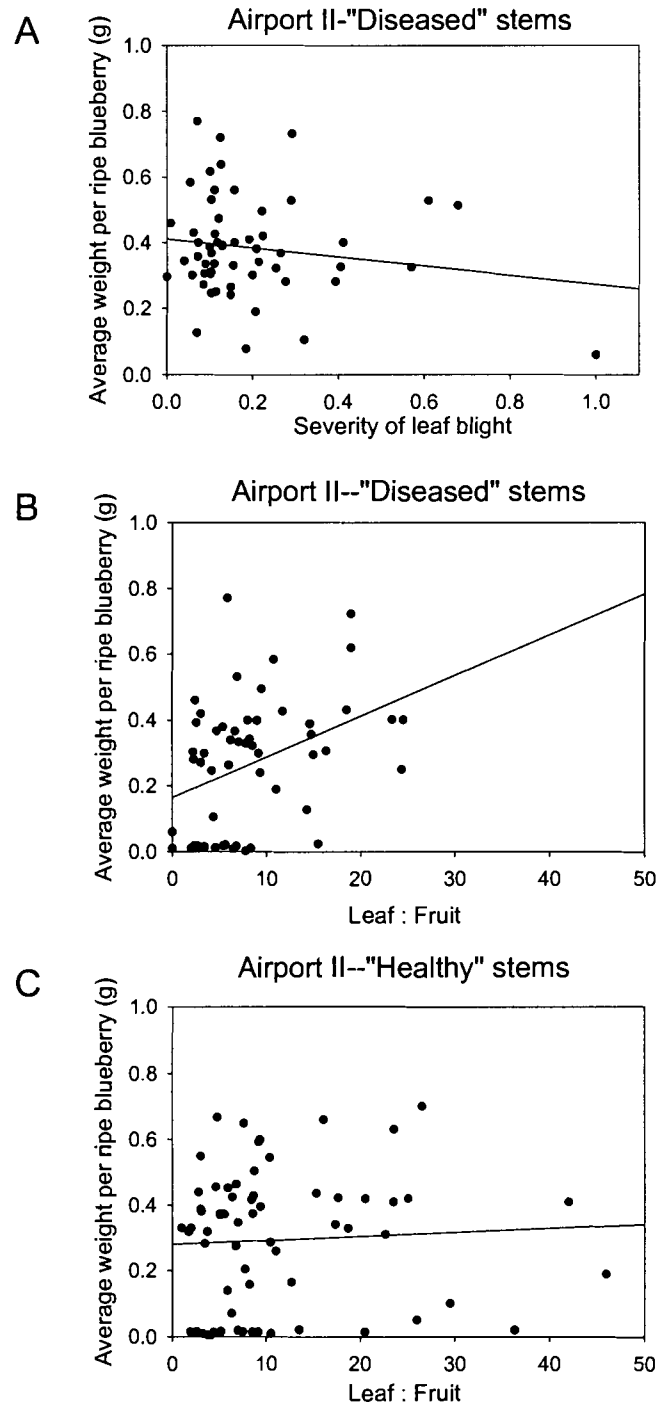


Figure 44. The relationship between average ripe blueberry weight and A) the severity of leaf blight on "diseased" stems during flowering, B) the leaf to fruit ratio on "diseased" stems during the green fruit stage, and C) the leaf to fruit ratio on "healthy" stems during the green fruit stage in Airport II. Leaf to fruit ratios were calculated as the number of healthy leaves divided by the number of healthy fruit.

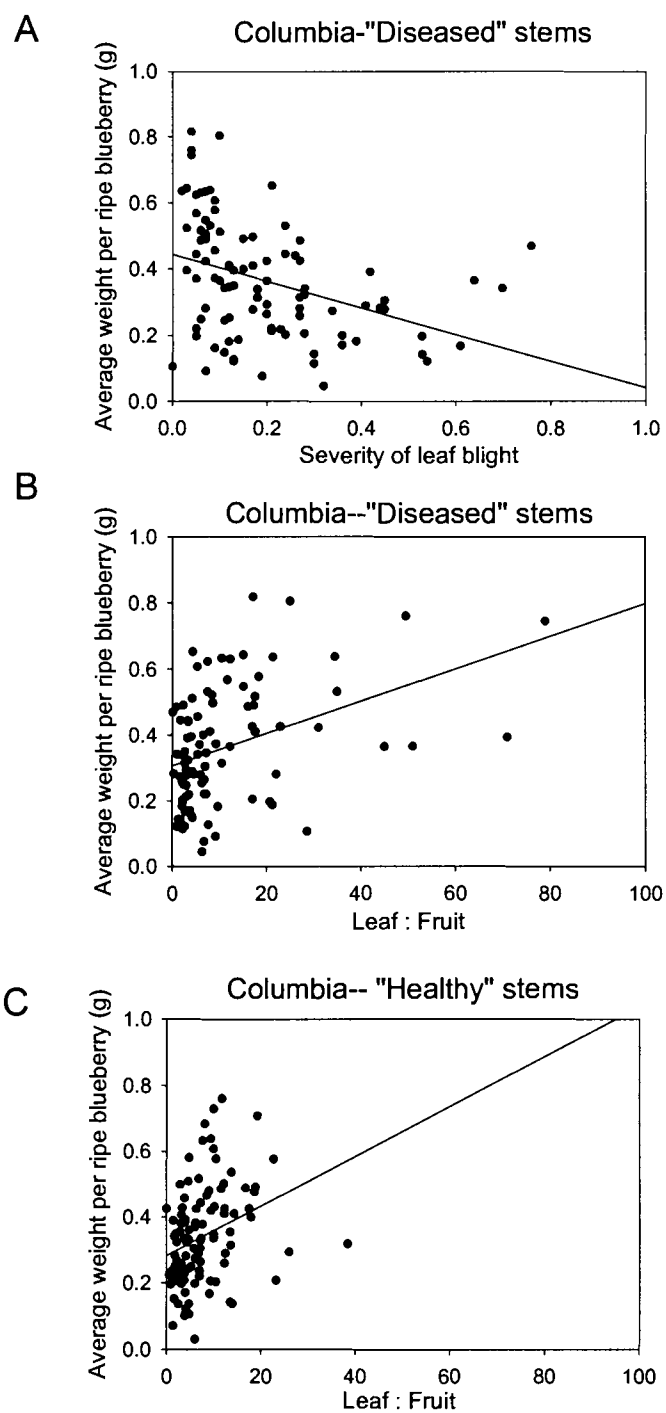


Figure 45. The relationship between average ripe blueberry weight and A) the severity of leaf blight on "diseased" stems during flowering, B) the leaf to fruit ratio on "diseased" stems during the green fruit stage, and C) the leaf to fruit ratio on "healthy" stems during the green fruit stage in Columbia. Leaf to fruit ratios were calculated as the number of healthy leaves divided by the number of healthy fruit.

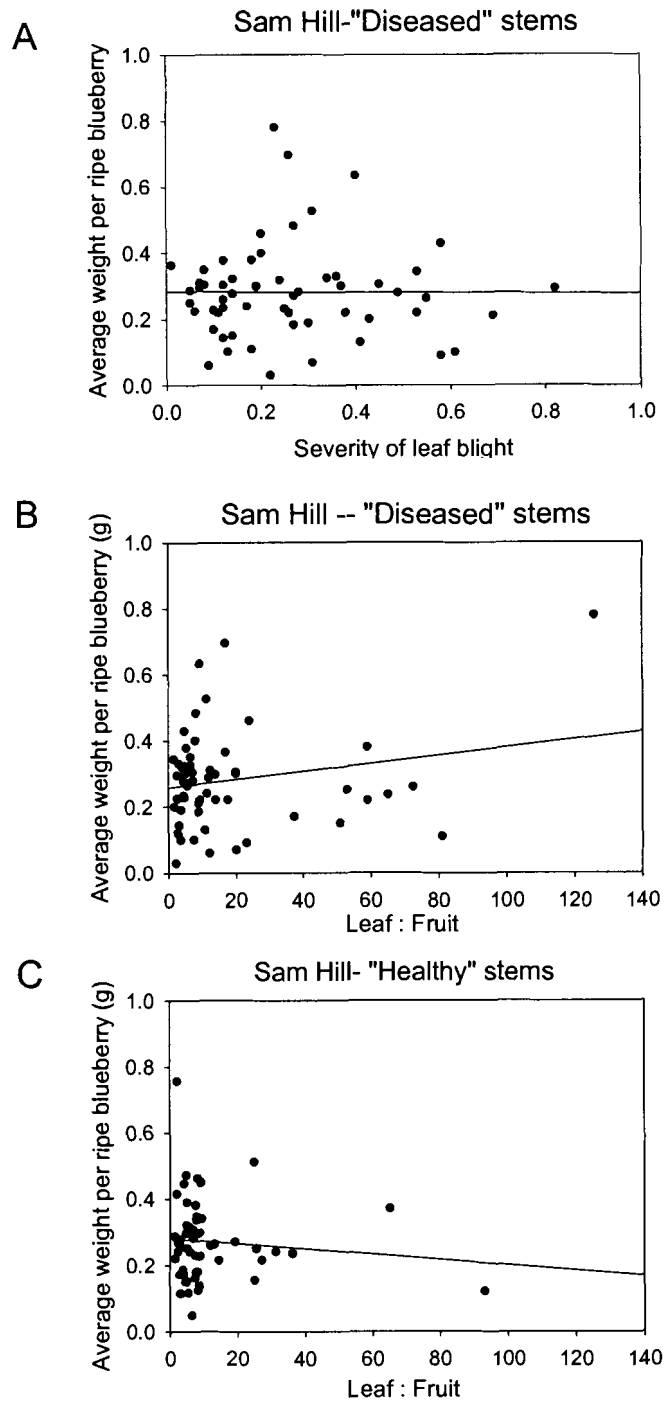


Figure 46. The relationship between average ripe blueberry weight and A) the severity of leaf blight on "diseased" stems during flowering, B) the leaf to fruit ratio on "diseased" stems during the green fruit stage, and C) the leaf to fruit ratio on "healthy" stems during the green fruit stage in Sam Hill. Leaf to fruit ratios were calculated as the number of healthy leaves divided by the number of healthy fruit.

increases in average berry weight as leaf to fruit ratios increased ($r_s = +0.396$, $p < 0.001$) (Fig. 45c), but this relationship was not significant in any other field (Fig. 43c, 44c, and 46c).

Discussion

In this study, the proportion of set fruit was adjusted by eliminating blighted flowers from the population of viable flowers. Even with this adjustment, stems without blight generally set a larger proportion of fruit than stems with blight. “Diseased” stems in Airport II and Columbia appeared to have greater reductions in fruit set between the green fruit stage and the blue fruit stage than “healthy” stems (Figs. 36 and 37). However, significant differences in adjusted fruit set (the proportion of healthy flowers at flowering that produced healthy fruit just prior to harvest) were only found between “healthy” and “diseased” stems in Columbia and in Airport I, which did not have the differences in fruit set that were observed in Columbia (Fig. 35). Adjusted fruit set also varied across clones in Airport I, Columbia, and Sam Hill, but variation among clones did not significantly influence differences in adjusted fruit set between “healthy” and “diseased” stems. By using stems within the same clone, the relationships between the severity of leaf blight, leaf to fruit ratios, and berry weight could be examined without the confounding effect of clonal variation. Furthermore, as the severity of infection increased among “diseased” stems, the adjusted fruit set tended to decrease. It is likely that pollination rates were equal on healthy flowers, regardless of stem health status, unless bees are more attracted to conidia than to flowers. Stress due to infection by *M. vaccinii-corymbosi* may have caused fruit abortion prior to or after the green fruit stage.

Reductions in fruit set after the green fruit stage in both “healthy” and “diseased” populations (Figs. 35, 36, 37, and 38) suggest that some fruit are aborted between the green fruit stage and harvest regardless of the health status of stems. This is the first report of this phenomenon in lowbush blueberry.

In addition to reducing fruit production, *Monilinia* blight also reduces berry weight. Hildebrand and Braun (1991) observed a reduction in fruit size on severely blighted lowbush blueberry plants grown in greenhouse containers, but did not analyze the significance of this relationship. In this study, the average weight per blueberry on “diseased” stems declined as blight severity increased, but this relationship was only significant in Airport I and Columbia fields, which had a sample size of 100 or more stems.

The observed reduction in fruit weight with increasing blight severity may be due to a combination of stress and comparatively low leaf to fruit ratios. The weight of tomato, cherry, and southern highbush blueberry fruit decreased with artificial reductions in leaf to fruit ratios (Hurd et al. 1979; Facteau et al. 1983; Maust et al. 1999). In this study, average berry weight at harvest increased as leaf to fruit ratios increased on “diseased” stems in Airport I, Airport II, and Columbia. In Sam Hill, only 57 “diseased” stems had fruit at harvest, which may have prevented the detection of a relationship between leaf to fruit ratios and berry weight in this field. In contrast, only Columbia had a significant relationship between average berry weight and leaf to fruit ratios for “healthy” stems. The number of samples in other fields may not have been large enough to detect effects within the “healthy” stem population, but physiological mechanisms may also explain the lack of a significant relationship between berry weight and leaf to fruit ratios for “healthy” stems. “Healthy” stems with low leaf to fruit ratios often produced berries with similar average weights as “diseased” stems that had comparatively high leaf to fruit ratios. It is possible that stems with high leaf to fruit

ratios use “surplus” photosynthate for physiological purposes (ie. leaf and flower bud production) which do not contribute to fruit quality. Another possibility is that “diseased” stems with low leaf to fruit ratios may direct some photosynthate and nutrients away from fruit development in order to produce new leaves. Stress due to leaf blight may also account for the significant relationships between leaf to fruit ratios and berry weight, and may explain why similar relationships were not found in most “healthy” populations. It is assumed that plants with greater leaf to fruit ratios have more carbohydrates available for physiological processes, so infected plants with high leaf to fruit ratios may be able to maintain developing fruit despite damage due to *Monilinia* blight. Darnell and Birkhold (1996) used ^{14}C to track carbon allocation in rabbiteye blueberry, and concluded that the development of fruit on healthy plants prevents starch accumulation in stems or roots until after harvest. If similar methods were utilized to study carbon allocation in blighted blueberry plants, the mechanism by which leaf blight reduces berry weight could be better understood.

CHAPTER FIVE: PECTINASE PRODUCTION BY *MONILINIA VACCINII-CORYMBOSI*

Introduction

Monilinia vaccinii-corymbosi causes mummy berry disease and is an important and widespread pathogen of blueberry (*Vaccinium*) species (Batra 1983). Ascospore infection by *M. vaccinii-corymbosi* causes mummy berry blight, which kills both leaves and flowers as they emerge from buds early in the growing season. Conidia produced on the surface of blighted tissues are transported to floral stigmas, and fruits become mummified as mycelium replaces ovary tissue. The “mummy berries” that result from fruit infection serve as overwintering structures for *M. vaccinii-corymbosi* and can be viable for at least 2 years (Lockhart 1961; Batra 1983).

Lowbush blueberry production is important for the economy of Maine and the Maritime provinces of Canada (Yarborough 1998), and mummy berry disease causes large yield reductions relative to other diseases of lowbush blueberry (Lambert 1990). Because lowbush blueberry fields contain many different genotypes of *V. angustifolium* and *V. myrtilloides* (Vander Kloet 1978; Yarborough 1998), they provide an interesting system for studying pathogen diversity among and within host species. Natural pathosystems have heterogeneous host and pathogen populations, each of which has developed varied strategies for resistance and virulence, respectively (Ye et al. 2003). Avoidance appears to be the primary mechanism for resistance of highbush blueberry cultivars to mummy berry disease, but other host resistance mechanisms seem to be involved as well (Ehlenfeldt et al. 1996).

The production of cell wall-degrading enzymes (CWDEs) is associated with the infection processes of many pathogenic fungi (Keon et al. 1987). Individual isolates of fungi grown *in vitro* produce many isoforms of CWDEs that vary in their substrates,

mechanisms, sizes, and isoelectric points (pI) (Keon et al. 1987; Mendgen et al. 1996; Tonukari et al. 2000), and variation in these isoforms has been documented within species of many pathogenic fungi (Errampalli and Kohn 1995; Annis and Goodwin 1996; Annis and Goodwin 1997). The potential relationship between the production of CWDEs and virulence has been studied in *Sclerotinia* species, which are in the same family (Sclerotiniaceae) as *M. vaccinii-corymbosi*. Marciano et al. (1983) did not find a correlation between virulence and *in vivo* production of polygalacturonase, cellulase, and xylanase in two isolates of *S. sclerotiorum* on sunflower. Similarly, Morrall et al. (1972) and Errampalli and Kohn (1995) did not find a relationship between the virulence of *Sclerotinia* isolates and their production of CWDEs *in vitro*. However, several researchers have reported a positive relationship between virulence and *in vivo* and *in vitro* CWDE production in *Sclerotinia* species (Chan and Sackston 1970; Chan and Sackston 1972; Lumsden 1976). The discrepancy regarding the importance of CWDE production to the virulence of *Sclerotinia* species may be due to differences in methodology or sample size, as well as the species, isolates, and specific enzymes evaluated.

There has not been an examination of CWDE production by *M. vaccinii-corymbosi*, but the relationship between pectinase production and virulence has been studied in *M. fructigena*. Apple tissue infected by *M. fructigena* contains high amounts of pectinase activity (Calonge et al. 1968), and Howell (1975) found that virulence was positively correlated with the activity of pectin-degrading enzymes produced *in vitro* by 119 mutants of *M. fructigena*. These results suggest that pectin-degrading enzymes may also be involved in the infection of blueberry tissue by *M. vaccinii-corymbosi*.

In a previous chapter, I demonstrated that leaf and flower bud phenology is variable among clones of lowbush blueberry, and that much of the variation in disease severity and incidence can be attributed to these differences in phenology. In this chapter, I will examine variation in the production of pectinases by isolates of *M. vaccinii-corymbosi* obtained from host plants with different levels of leaf blight.

Materials and Methods

Isolation of *M. vaccinii-corymbosi* Isolates and Production of CWDE in Liquid Medium

During the 2001 field season, 10 diseased stems from each blueberry clone in Airport I were randomly selected for the isolation of *M. vaccinii-corymbosi*. Infected leaves from each stem were surface sterilized by treatment with 70% ethanol for 1-2 minutes, 10% bleach for 1-2 minutes, and 2 rinses in sterile deionized water. Leaves were plated on malt yeast extract agar (MYA) (10g/L malt extract, 3 g/L yeast extract, 15 g/L agar) supplemented with 0.01 % penicillin and 0.01% streptomycin. Isolates were transferred onto new plates until pure cultures of *M. vaccinii-corymbosi* were obtained. Isolates from lowbush blueberry clones a, e, g, and j were selected for enzyme studies in order to represent isolates from plant clones showing a range of disease severity. Three isolates each from different stems were selected from each of the four clones and transferred to MYA (without antibiotics). An isolate from clone e was omitted from analysis once it was verified that it did not produce the macroconidia characteristic of *M. vaccinii-corymbosi*. As a result, clone e is represented by only two isolates. A cork borer (7 mm in diameter) was used to cut plugs of mycelium and agar medium around the growing edge of 2 to 3 week-old colonies. Three mycelial plugs were divided in half and placed in 250 ml flasks containing 25 ml of blueberry cell wall medium using a modification of the medium described by Mankarios and Friend (1980)

(recipe in Appendix E) and grown for 9 days at 20°C with daily agitation. Growth of the isolates of *M. vaccinii-corymbosi* in blueberry cell wall media was minimal, possibly due to large amounts of phenolics that may have been released upon autoclaving of the liquid media. Consequently, the isolates were grown in 250 ml flasks containing 25 ml of synthetic media adapted from Lee et al. (1986) of a 50 mM potassium phosphate (pH 5.8) solution containing 0.67% (w/v) yeast nitrogen base without amino acids (Difco 291940), 0.2% (w/v) DL-asparagine (Sigma A-8256), and supplemented with either 0.1% carboxymethylcellulose (CMC) (Sigma C-5013) or 0.1% citrus pectin (Sigma P-9135). Cultures were grown at 20° C for 9 days with daily agitation.

The contents of each flask were filtered through Whatman #1 filter paper. The filter paper and the adherent mycelium were oven dried and weighed. The culture filtrate was placed in a -20°C freezer and freeze-dried in a lyophilizer. The freeze-dried material was resuspended in 1 ml of 0.05mM Tris-HCl buffer (pH 7.5) and dialyzed against the same buffer, resulting in a culture filtrate 25 times more concentrated than the original. Each isolate was grown three times in liquid media as described above, and the culture filtrates from each repetition were used for the enzyme assays described below.

Verification of Isolate Identity

Samples of liquid cultures were checked under the microscope for the production of microconidia. In order to verify the production of macroconidia, isolates were transferred onto cellulose acetate membranes that were placed on the surface of V-8 agar media (Stretch et al. 2001). If chains of macroconidia were observed, the isolates were designated as the anamorph *Monilia*. Culture filtrates were checked under a compound light microscope for the presence of bacterial contamination.

Determination of Enzyme Activity

Activities of cell wall-degrading enzymes produced by the isolates of *M. vaccinii-corymbosi* were evaluated by measuring the release of reducing sugars from CMC (for cellulase) and pectin (for pectinase) using the spectrophotometric method described by Nelson (1944) and modified by Somogyi (1951). The ratio of culture filtrate, substrate, and reagents was adjusted as described below. 100 μ L of culture filtrate was added to 400 μ L of a 0.1% solution of substrate (CMC or citrus pectin) in 50 mM sodium acetate buffer (pH 5.2) and incubated at 20°C for 48 hours. After this incubation period, 500 μ L of reagent A (described in Appendix F) was added to each tube and the tubes were boiled in a water bath for 20 minutes. Once the solutions cooled to room temperature, 500 μ L of reagent B (described in Appendix F) and 9 mL of dH₂O was added to each tube, and the absorbance of the resulting solution was measured at 510 nm using a GENESYS model 2 spectrophotometer (Spectronic Unicam, Rochester, NY) against a blank of 500 mL of 0.1% substrate in buffer subjected to the same conditions and reagents. Initial screenings revealed that *M. vaccinii-corymbosi* exhibited low cellulase production and low growth rates in CMC medium, but produced higher levels of pectin-degrading enzymes in citrus pectin medium. Consequently, the evaluation of cell wall-degrading enzymes was restricted to pectinases. All culture filtrates were assayed in duplicate.

Statistical Analysis

PROC GLM (SAS Institute Cary, NC) was used to determine whether there were differences in enzymatic activity among isolates obtained from different clones and among isolates obtained within the same clones.

Results and Discussion

All isolates used in these experiments were obtained from blighted leaves of *V. angustifolium* and produced at least one of the conidial types characteristic of *M. vaccinii-corymbosi* (Table 3). Microconidia are produced by many members of the family Sclerotiniaceae and are a useful taxonomic tool for the genus *Monilinia* (Byrde and Willetts 1977; Batra 1991). The production of macroconidia in linear or branched chains (anamorph *Monilia*) is characteristic of the genus *Monilinia* and few other filamentous fungi (Batra 1991). However, because the production of macroconidia *in vitro* is highly variable among and within isolates of *M. vaccinii-corymbosi* (Stretch et al. 2001), many isolates that did not produce macroconidia may still belong to the genus *Monilinia*. Furthermore, although several species of *Monilinia* are pathogenic to *Vaccinium* species, only *M. vaccinii-corymbosi* is pathogenic to blueberry. Therefore, there is adequate evidence that all of the isolates used in these studies are isolates of *M. vaccinii-corymbosi*.

Table 3. *In vitro* production of microconidia and macroconidia by isolates of *M. vaccinii-corymbosi*.

Isolate	Microconidia	Macroconidia	Isolate	Microconidia	Macroconidia
A1	Yes	Yes	G1	Yes	Yes
A2	Yes	Yes	G2	Yes	Yes
A3	Yes	Yes	G3	Yes	Yes
E1	Yes	Yes	J1	Yes	N.A.
E2	Yes	Yes	J2	Yes	Yes
			J3	Yes	Yes

Cellulase Activity

Isolates of *M. vaccinii-corymbosi* exhibited poor growth in media containing carboxymethylcellulose (CMC), and preliminary measures of activity indicated that culture filtrates from *M. vaccinii-corymbosi* had negligible cellulase activity ranging from 7×10^{-5} to 1.3×10^{-5} μmol sugar released/ ml of culture filtrate/ min. Similar results were found for other species of *Monilinia*. Extracts of fruits infected by *M. fructigena* have little or no activity against CMC (Cole and Wood 1961; Calonge et al. 1969), and *M. fruticola* did not grow well in medium containing CMC (Byrde and Willetts 1977). Although cellulase activity is associated with infection by several *Sclerotinia* species (Calonge et al. 1969; Chan and Sackston 1970; Chan and Sackston 1972; Marciano et al. 1983), cellulase activity does not appear to be important in the saprophytic growth of *M. vaccinii-corymbosi* or other *Monilinia* species. Because *in vitro* CWDE production does not necessarily reflect *in vivo* CWDE production (Mendgen et al. 1996), it is not clear whether differences in cellulase activity observed in this study are related to cellulase activity *in vivo*.

Pectinase Activity

Culture filtrates from *M. vaccinii-corymbosi* grown in citrus pectin medium had a range of pectinase activity from 5.00×10^{-3} to 1.56×10^{-2} μmol sugar released/ ml of culture filtrate/ min. These values are more than an order of magnitude lower than was observed for culture filtrates of *Leptosphaeria maculans* at the same pH (Annis and Goodwin 1996). High pectinase activities have been found in fruit infected by *M. fructigena* and *M. fruticola* relative to uninfected fruit (Cole and Wood 1961; Calonge et al. 1969). Furthermore, protoplast injury, depletion of methylated pectin from cell walls, and loss of cell wall structure were observed when pectin lyase, purified from *M.*

fructigena, was applied to cultured apple cells (Hislop et al. 1979; Keon 1985).

Therefore, pectinase production is probably involved in the saprophytic growth of *M. vaccinii-corymbosi*, and may aid necrotrophic growth in the host.

In this experiment, isolates of *M. vaccinii-corymbosi* were selected in order to represent pathogen populations from clones with a wide range of mummy berry blight severity. Because ascospores are dispersed by wind, isolates obtained from stems in the same clone may not have originated from the same apothecium, and as a result, may differ in their virulence. Ascospores have been shown to infect highbush blueberry plants up to 30 m from the site of discharge when average wind speeds were ~1.55 m/s (Cox and Scherm 2001b). Although wind speeds were not recorded in this experiment, ascospores in lowbush blueberry fields may travel even farther due to the comparatively short height of the lowbush blueberry plants. It is also important to note that the disease severity of the clones used in this study was measured as the proportion of infected leaves, which is an indication of the proportion of successful infections but does not necessarily indicate the virulence of individual isolates.

Although the severity of blight differed among clones (severity values of clones a, e, g, and j were reported in the previous chapter), pectinase activity of isolates did not vary among the clones of *V. angustifolium* from which they were obtained (Fig. 47). Because clones of *V. angustifolium* are genetically uniform, it is presumed that stems within each clone have uniform resistance and escape mechanisms. Therefore, isolates of *M. vaccinii-corymbosi* obtained from stems within the same clone had to overcome similar host defenses in order to successfully infect their host. In contrast, isolates obtained from stems in different clones may have had to overcome different types and amounts of host defenses. Because pectinase production was present in all isolates, it is likely that pectinase production is important for the survival of *M. vaccinii-corymbosi* during some portion of its life cycle.

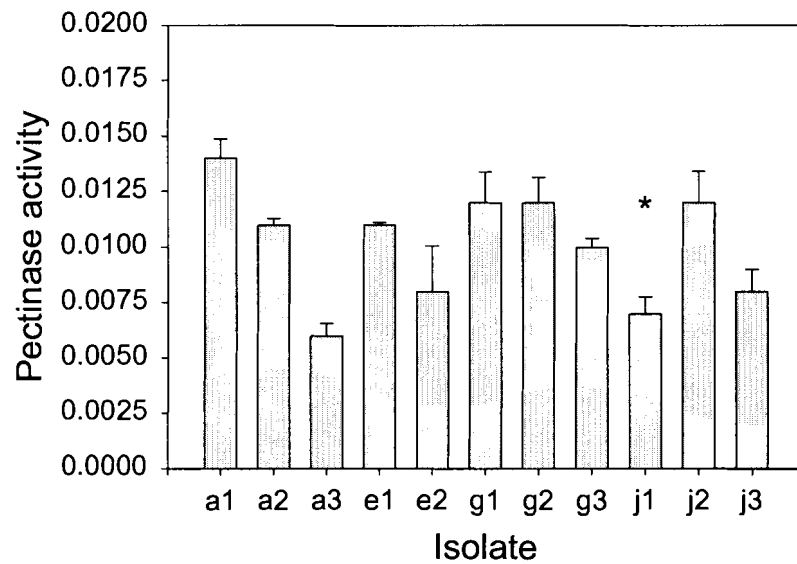


Figure 47. Pectinase activity of *M. vaccinii-corymbosi* isolates obtained from lowbush blueberry stems with symptoms of blight. Pectinase activity was measured in μmol of reducing sugar released per ml of filtrate per minute. Isolates from the same letter were isolated from stems in the same clone. Numbers represent isolates obtained from a different stem within each clone. Bars show the standard error of the means. The isolate marked by an "*" had only two replications.

Differences in disease severity among clones is probably due more to differences in leaf bud phenology during ascospore release than to differences in biochemical resistance or pathogen virulence. In order for a pathogen to successfully infect a host, susceptible host tissue must be present at the same time and in the same space as the inoculum of the pathogen. Once this first condition is met, differences in host resistance or pathogen virulence can influence the severity of infection. Plants can avoid infection by having little or no susceptible tissue present when inoculum densities are high, as has been found for blight of highbush blueberry cultivars (Ehlenfeldt et al.

1996), and lowbush blueberry clones (a previous chapter). If leaf buds were inoculated at uniform development stages in a controlled environment, relationships between virulence factors and disease severity could be examined in the absence of avoidance.

Although significant differences in pectinase production were not found among the clones from which the isolates were obtained, significant differences in pectinase activity were observed among isolates within clones ($p=0.0004$) (Fig. 48). Based on these results, it seems reasonable to suggest that the population of *M. vaccinii-corymbosi* within a field varies in its pectinase production. However, a sample size of 11 isolates obtained from one field is not large enough to make conclusions about differences in populations of *M. vaccinii-corymbosi*. Nevertheless, the variation of *in vitro* pectinase production suggests that populations of *M. vaccinii-corymbosi* in lowbush blueberry fields have phenotypic and genotypic diversity characteristic of natural pathosystems. Genetic analysis of these isolates would be useful in determining whether the observed differences in pectinase activity were genetically-based, and genetic analysis of a large number of isolates would aid in determining the actual genetic variation of *M. vaccinii-corymbosi* within and among clones of *V. angustifolium*.

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Appendix A. Analysis of variance table from Proc GENMOD analysis of vegetative units measured during flowering and shortly before harvest

SOURCE	DF	CHISQ	PR> CHISQ
Site	3	0.10	0.9913
Stem health	1	2310.52	<0.0001
Leaf status	1	6272.20	<0.0001
Clone	33	178.2	<0.0001
Time	2	268.49	<0.0001
Site * Time	6	125.09	<0.0001
Clone * Leaf status	33	205.83	<0.0001
Clone * Stem health	33	163.74	<0.0001
Stem Health*Leaf Status	1	1700.76	<0.0001
Clone*Leaf status* Stem Health	36	173.40	<0.0001
Stem health * Time	2	275.69	<0.0001
Clone * Time	66	44.32	0.9815
Site * Stem health	3	0.00	1.0000
Site * Leaf status	3	0.03	0.9989

Table A.1. Significance values for the number of vegetative units and their health status measured at flowering and shortly before harvest and analyzed using Proc GENMOD.

Appendix B. Analysis of variance table from Proc GENMOD analysis of reproductive units measured during flowering and shortly before harvest

SOURCE	DF	CHISQ	PR> CHISQ
Site	3	64.12	<0.0001
Stem health	1	336.88	<0.0001
Flower health	1	2512.55	<0.0001
Clone	33	110.64	<0.0001
Time	1	332.03	<0.0001
Site* Time	3	66.98	<0.0001
Clone * Flower health	33	139.35	<0.0001
Clone * Stem health	33	72.88	<0.0011
Stem health * Flower health	1	192.71	<0.0001
Stem health * Time	1	125.15	<0.0001
Clone * Time	33	112.01	<0.0001
Site * Stem health	3	15.61	0.0014
Site * Flower health	3	79.78	<0.0001

Table B.1. Significance values for the number of reproductive units and their health status measured at flowering and shortly before harvest and analyzed using Proc GENMOD. Data was fitted to a negative binomial distribution and analyzed with Type III ANOVA.

**Appendix C. Analysis of variance table from Proc GENMOD analysis of
flower number and flower blight**

SOURCE	DF	CHISQ	PR<CHISQ
Field	3	5.17	0.1596
Clone	33	173.23	<0.0001
Stem Health	1	510.65	<0.0001
Health Status	1	1784.40	<0.0001
Field*Flower health	3	7.11	0.0686
Stem health* Flower health	1	453.81	<0.0001
Clone*Flower health	33	154.49	<0.0001
Clone*Stem health	33	34.49	0.3963
Field* Stem health	3	1.03	0.7935
Field*Stem health* Flower health	3	0.99	0.8040

Table C.1. Significance values for flower number and flower blight measured shortly before harvest and analyzed using Proc GENMOD. Data was fitted to a negative binomial distribution and analyzed with Type III ANOVA.

Appendix D. Analysis of variance table from Proc GENMOD analysis of fruit number and fruit mummification

SOURCE	DF	CHISQ	PR<CHISQ
Field	3	177.05	<0.0001
Clone	33	273.37	<0.0001
Stem health	1	2.80	0.0943
Fruit health	1	1006.24	<0.0001
Field*Fruit health	3	33.90	<0.0001
Stem health*Fruit health	1	0.99	0.3185

Table D.1. Significance values for fruit number and fruit mummification measured shortly before harvest and analyzed using Proc GENMOD. Data was fitted to a negative binomial distribution and analyzed with Type III ANOVA.

Appendix E. Preparation of Blueberry Cell Wall Medium

(adapted from Mankarios and Friend (1980))

100 g of blueberry leaves were collected in late September and were homogenized in a blender with 300 ml of 1.0% chilled sodium deoxycholate (3 ml of 1.0% sodium deoxycholate per g of leaves). The mixture was rinsed through a 120 mesh brass sieve with 500 ml of chilled water (5ml cold water per g of leaves).

The leaf mixture was filtered through #1 Whatman filter paper under vacuum in order to remove water. The tissue was rinsed under vacuum with ~100 ml chloroform:methanol (1:1) repeatedly in order to remove lipids (~1000 ml for 100 g of leaf tissue), and allowed to dry. The tissue was then rinsed with 500 ml of cold acetone, or until the green color disappeared from the filtrate.

The cell walls were removed from the filter paper and transferred to a beaker. The solvents were allowed to evaporate in a fume hood for 48 hours.

Blueberry cell wall medium contained 1g/L KH_2PO_4 , 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 g/L DL -asparagine (Sigma A-8256), 10mg/L FeSO_4 , and 1.5% (w/v) blueberry cell walls.

Appendix F. Preparation of Reagents A and B for determination of pectinase activity (Adopted from Nelson 1944).

Reagent A: To prepare 1 L of Reagent A, 180 grams of Na_2SO_4 (anhydrous) were dissolved in 600 ml of hot deionized water. In a separate container, 24 g of Na_2CO_3 (anhydrous), 12 g of sodium potassium tartate ($\text{C}_4\text{H}_4\text{O}_6\text{NaK}\cdot 4\text{H}_2\text{O}$), and 16 g of NaHCO_3 were dissolved in 180 ml of deionized water. Copper sulfate ($\text{CuSO}_4\cdot \text{H}_2\text{O}$) was dissolved in another beaker containing 40 ml of deionized water and then mixed with the solution containing sodium potassium tartate and the other ingredients. Once this new solution was mixed well, it was added to the sodium sulphate solution and the volume was increased to 1 L. The solution was stored at room temperature in a dark bottle.

Reagent B: Because preparation of Reagent B contained sodium arsenate, Reagent B was prepared in a fume hood. 50 g of ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$) was dissolved in 800 ml of deionized water. 42 ml of sulphuric acid was added to this solution and the solution turned pale yellow. In a separate beaker, 6 g of sodium arsenate was dissolved in 100 ml of deionized water, and then added to the ammonium molybdate and sulfuric acid solution. The volume was brought up to 1000 ml and then transferred to a dark bottle. The solution was stirred slowly for 48 hours at 37°C and then stored at room temperature.

BIOGRAPHY OF THE AUTHOR

Laura Penman was born in Mercer County, Pennsylvania on October 15, 1978, where the hospital staff denied Laura's mother the option of spelling Laura's middle name (Nichole) with an "H". Laura's mother was understandably weary and did not argue much with the hospital staff. Several months later, little Laura and her family moved to sunny San Jose, California, where Laura developed a childhood fascination of banana slugs and other slimy things. When Laura was eight years old, the family moved to Hudson, Ohio, where Laura spent most of her formative years. One day, young Laura came home from school and handed her mother a form on which Laura had scrawled her middle name, with an "H". Laura's mother was delighted because she had forgotten the argument with the hospital staff, and had never told her that she originally wanted Laura's middle name to contain an "H".

Laura graduated from Hudson High School in 1996, and went to Allegheny College to pursue her higher education. Professors in the biology department at Allegheny College initiated Laura's interest in mycology, and encouraged her to consider a graduate degree in Plant Pathology. Laura graduated from Allegheny College in 2000 with a double major in Biology and Studio Art, and started her graduate degree in Botany and Plant Pathology at the University of Maine in the fall of 2000. Laura is a member of the American Phytopathological Society and the Mycological Society of America.

After receiving her degree, Laura will continue to spell her middle name with an "H". She is a candidate for the Master of Science degree in Botany and Plant Pathology from The University of Maine in August, 2003.