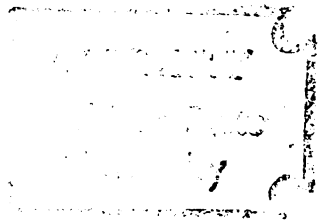

This is to certify that the

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SOURCES, COMPONENTS, AND INHERITANCE OF RESISTANCE TO COCCOMYCES HIEMALIS IN PRUNUS SPECIES

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Thomas Martin Sjulin

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SOURCES, COMPONENTS, AND INHERITANCE OF RESISTANCE TO *COCCOMYCES HIEMALIS* IN *PRUNUS* SPECIES

By

Thomas Martin Sjulin

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ABSTRACT

SOURCES, COMPONENTS, AND INHERITANCE OF RESISTANCE TO *COCCOMYCES HIEMALIS* IN *PRUNUS* SPECIES

By

Thomas Martin Sjulin

Resistance to *Coccomyces hiemalis*, the cause of fungal leaf spot of cherry, was evaluated in field and greenhouse studies with several *Prunus* species. In a field planting of a total of 25 cultivars of *P. avium*, *P. cerasus*, *P. gondouinii*, and *P. fruticosa*, cultivars differed in rate and severity of both infection and defoliation, but none was completely resistant. *P. avium* cultivars were more resistant to defoliation than *P. cerasus* and *P. gondouinii* cultivars. Field resistance to defoliation was negatively correlated with lesion development and sporulation in the greenhouse.

In greenhouse studies, cultivars of *P. avium*, *P. cerasus*, and *P. gondouinii* differed in components of resistance, including numbers of lesions, time and rate of lesion appearance, and lesion size and sporulation. Lesions on *P. avium* appeared later, were smaller, and produced fewer spores than lesions on *P. cerasus* and *P. gondouinii*. Numbers of spores per lesion varied with lesion size, time of lesion appearance, leaf age, and numbers of lesions per unit area of inoculated leaf. Cultivar x isolate interactions

were not significant for cultivars of these three species and six fungal isolates. These isolates differed in all components except time and rate of lesion appearance.

Inheritance of components of resistance was evaluated in families of juvenile seedlings from an incomplete diallel of four P. cerasus and one P. gondouinii cultivars. No discrete classes of resistance were observed and broad-sense heritabilities of all components except lesion size were less than 0.5 on an individual plant basis. Thus, resistance did not appear to be simply inherited. General combining ability differed among cultivars at two of three dates of inoculation, but family x date of inoculation interactions were detected for all components except numbers of spores per lesion.

Seedlings and clones representing 15 Prunus species and interspecific hybrids were inoculated with an isolate from P. cerasus. Members of the Padus subgenus, the Pseudocerasus and Mahaleb sections of Prunus, and interspecific hybrids between these sections and the Eucerasus section of Prunus exhibited complete resistance.

To

Kay and Scott Michael

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INTRODUCTION AND LITERATURE REVIEW

Cherry leaf spot is a serious fungal disease of cultivated cherries in most cherry producing regions of the world (2). The disease was first described in New York in 1878 on native black cherry (*Prunus serotina* Ehrh.), although the pathogen was incorrectly named *Septoria cerasina* (28). The disease was reported in Europe on European bird cherry (*Prunus padus* L.) in 1884 and the pathogen was named *Cylindrosporium padi* (17). American workers subsequently reported *Cylindrosporium* species on several cherry and plum species. Finally, Higgins in 1914 divided the *Cylindrosporium* species occurring on *Prunus* species into three separate species of the Ascomycete genus *Coccomyces* (11). The fungi occurring on sour cherry (*P. cerasus* L.), sweet cherry (*P. avium* L.) and pin cherry (*P. pennsylvanica* L.) were named *Coccomyces hiemalis*; those found on plum species were named *C. prunophorae*; and those found on black cherry, choke cherry (*P. virginiana* L.) and *P. mahaleb* L. were named *C. lutescens*.

Higgin's classification continues to be accepted by most American workers. However, European workers in recent years have accepted von Arx's reclassification of the three *Coccomyces* species into a single species named *Blumeriella jaapii* (4). Higgin's classification will be used for the remainder of this dissertation.

The disease on cultivated cherries (P. avium, P. cerasus and P. gondouinii Rehd.) is characterized by small necrotic lesions on leaves, petioles and occasionally on fruit pedicels (34). Primary infection of expanding leaves in the spring is from ascospores discharged from apothecia produced over winter in fallen leaves. Ascospores are discharged during rainy periods from the time of first leaf emergence until about 6 to 7 weeks after petal fall (1). Secondary infections are from conidia splashed from acervuli produced on the primary infections and subsequent secondary infections. Secondary infections can occur throughout the growing season as long as susceptible host tissue is present and conditions are favorable for infection. Conditions favorable for both primary and secondary infection are determined mainly by temperature and leaf wetness (8). Thus, the increase of disease during the growing season is not continuous, but instead, occurs in discrete stages called infection periods (19).

Severely infected leaves usually become chlorotic and subsequently abscise. If defoliation is severe before harvest, fruit may fail to ripen properly (10). More commonly, premature defoliation reduces both the vigor and hardiness of the tree in subsequent seasons. Reduction in vigor in sour cherry is in turn related to reduction in yield and fruit quality (20). Dutton and Wells (7) observed reduced bud survival, fruit set and fruit size the year following severe defoliation from leaf spot. In addition, fruiting spur development, flower bud survival and fruit set were reduced the second year following defoliation. Howell and Stackhouse (12) also observed reduced

bud survival and fruit set for two seasons following premature defoliation by C. hiemalis. Furthermore, they observed delayed acclimation in the fall and more rapid deacclimation in the spring of both vegetative and flower buds of prematurely defoliated trees.

Fungicidal sprays are currently the principal means for preventing leaf spot infection and subsequent defoliation. In Michigan, the initial fungicide application is made at petal fall, followed by four additional sprays at 10 to 14-day intervals until harvest. A final application is made soon after harvest (13).

Cherry cultivars with increased resistance to C. hiemalis would be useful in several ways. First, use of a cultivar immune to C. hiemalis could eliminate several costly fungicide sprays. Second, an increased (but not complete) level of resistance could reduce the number of fungicide sprays. Finally, an increased level of resistance could allow use of less effective fungicides if the preferred fungicides became unavailable due to resistant pathogen strains or loss of their use for economic or environmental reasons. Strains of C. hiemalis resistant to benzimidazole fungicides have been found in Michigan (15).

Resistance in Cherry to Coccomyces Hiemalis

Little is known about the relative resistance of either sour cherry or sweet cherry cultivars to C. hiemalis. Sweet cherry cultivars are, in general, more resistant than sour cherry cultivars (32), but little within species variation in resistance was thought to exist (1).

Recent reports from Eastern Europe indicate that within species variability for resistance does exist (3,6,16,21,22,29,31, 35,36,40). Although few, if any, cultivars in these studies were found to be completely resistant to C. hiemalis, there does appear to be potential for selecting for increased resistance in both P. avium and P. cerasus. Enikeyev (9) found that certain cultivars of both P. cerasus and P. avium produced a higher percentage of resistant seedlings in their progenies than did other cultivars. Also, crosses involving the European ground cherry (P. fruticosa Pall.) were generally much more susceptible than crosses not involving this species. P. cerasus is thought to have arisen as an allotetraploid of P. fruticosa and P. avium (25).

Several species of cherry in the Pseudocerasus, Lobopetalum and Mahaleb sections of the subgenus Cerasus, and in the subgenus Padus (30) appear to have much greater resistance to C. hiemalis than cultivated cherries. The Pseudocerasus and Lobopetalum sections contain the Japanese flowering cherries. Many of these species appear to remain free of cherry leaf spot infection in ornamental plantings. In addition, a number of these species have been reported to form hybrids with P. avium (5). However, no systematic evaluation of the resistance of these species to C. hiemalis has been made to date.

P. mahaleb has been observed to be more resistant to C. hiemalis than either P. avium or P. cerasus (37). A number of P. avium x P. mahaleb clones selected as potential cherry rootstocks (38) may be possible sources of increased resistance to C. hiemalis in P. avium.

Three cherry species in the subgenus Padus (P. padus, P. serotina and P. virginiana) were completely resistant to infection by isolates of C. hiemalis from P. avium or P. cerasus (11,18). However, all three species are susceptible to strains of Coccomyces that Higgins considered to be a separate species, C. lutescens (11). The use of any of these cherry species as a source of complete resistance to C. hiemalis would have to be done guarding against introduction of susceptibility to C. lutescens. Furthermore, no reports have been found to date of interspecific hybrids between these species and the cultivated cherries. A fourth member of this subgenus, P. maackii Rupr., has been successfully crossed with cultivated cherries (24). It is not yet known if this species is resistant to C. hiemalis.

There appear to be two basic approaches to breeding cherries resistant to Coccomyces leaf spot. One is to select for increased levels of resistance within the cultivated species (P. avium, P. cerasus and P. gondouinii). Most studies indicate that cultivars differ quantitatively in terms of the incidence or severity of disease in the field. This type of resistance, called partial resistance, is a type of incomplete resistance that reduces the rate of pathogen mutiplication even though the host is susceptible to infection (26,33). Breeding for partial resistance is sometimes enhanced or simplified by selecting for one or more component of resistance contributing to partial resistance (26).

The alternate approach is to identify sources of complete resistance in other cherry species, and incorporate the resistance

into cultivated cherries, if complete resistance is not found within cultivated cherries to begin with. This approach has been used successfully in Malus to breed scab resistant apple cultivars (39).

Which approach is chosen depends upon several factors, including (1) the level of resistance needed; (2) the horticultural characteristics of the resistance sources; (3) the number and nature of the genes controlling resistance; and (4) the genetic variability of the pathogen to the resistance. At present our understanding of these factors is too poor to wisely choose the best approach.

Variation in Coccomyces Hiemalis

Higgins (11) first examined pathogenic variation among Coccomyces strains isolated from Prunus species. The results of cross-inoculation studies indicated pathogenic specialization by Coccomyces among several species of Prunus. Isolates from hosts in the subgenus, Padus would not infect hosts in the subgenera, Cerasus and Prunophora, with the exception of P. mahaleb in Cerasus. Isolates from hosts in the subgenera Cerasus and Prunophora would not infect hosts outside of their respective subgenus. Higgins assigned these three groupings of isolates to separate species of Coccomyces, because there were morphological differences between the groups of isolates when grown in culture.

Keitt (18) examined in greater detail the ability of Coccomyces isolates to cross-infect other Prunus species. His results generally supported Higgins' conclusions. However, the grouping of isolates was not as distinct as that found by Higgins. Isolates from

all seven hosts tested in three subgenera of Prunus (Prunophora, Cerasus and Padus) readily infected P. mahaleb. P. munsoniana Wight & Hedr. (subgenus Prunophora) was readily infected by isolates from P. cerasus (subgenus Cerasus), but not by isolates from P. domestica L. (subgenus Prunophora). Isolates from P. virginiana (subgenus Padus) readily infected members of both Padus and Prunophora, as well as P. mahaleb. Isolates from P. serotina (subgenus Padus) did not infect other members of Padus. Keitt's work does not support Higgin's decision to assign three species designations within Coccomyces. The use of formae speciales designations for isolates from different hosts would also be confusing, as several hosts are readily infected by more than one group of isolates. It is probably best to designate these groups of isolates as different pathogenic races until the genetic relationships among groups can be determined.

Magie (23) studied the variability of C. hiemalis isolates collected from a single host species, P. cerasus. Isolates differed considerably in growth habit, growth rate and spore production on artificial media. Isolates also differed in the type and number of infections produced on leaves of both sweet cherry and sour cherry. No evidence of pathogenic races was found. However, only one sweet cherry cultivar and one sour cherry cultivar were used, and the conditions of the experiments varied considerably. Parlevliet and Zadoks (27) have demonstrated that biologically significant cultivar by isolate interactions may contribute only a small part to the total experimental variance. It is important that experiments of this kind be well controlled to minimize residual error variance.

The demonstrated variability in Coccomyces at the species level of the host is of immediate concern if attempts are to be made to introduce complete resistance into cultivated cherries by interspecific hybridization. Care must be taken that incorporation of resistance to one group of Coccomyces isolates does not introduce susceptibility to another. In addition, utilizing sources of partial resistance apparently existing within cultivated cherries does not preclude consideration of the variability of the pathogen to this type of resistance. Partial resistance in several host-pathogen systems have been found to be race-specific in nature (14).

Research Objectives

This research presents the first attempts to systematically identify, characterize and utilize resistance in Prunus species to Coccomyces hiemalis. The specific objectives were (1) identify sources of complete or partial resistance in cultivated cherries; (2) identify components of resistance contributing to the resistance identified in the first objective; (3) obtain preliminary estimates of the heritable components of resistance; (4) evaluate the variability of the pathogen population to the resistance identified in the first objective; and (5) identify sources of complete resistance to C. hiemalis in related cherry species.

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CHAPTER I

FIELD RESISTANCE OF CHERRY CULTIVARS AND SELECTIONS TO *COCCOMYCES HIEMALIS*

CHAPTER I

FIELD RESISTANCE OF CHERRY CULTIVARS AND SELECTIONS TO *COCCOMYCES HIEMALIS*

Abstract

Resistance to infection and defoliation by *Coccomyces hiemalis* Higg. was measured in a field planting of 25 cultivars and selections representing four *Prunus* species (*P. avium* L., *P. cerasus* L., *P. fruticosa* Pall. and *P. gondouinii* Rehd.). No cultivar was completely resistant to either infection or defoliation. The rates and severity of infection and defoliation, and the estimated dates of 50% infection and defoliation by *C. hiemalis* differed among cultivars. Correlations between defoliation severity and measures of infection were poor. Rates of defoliation and dates of 50% defoliation were highly correlated with defoliation severity. Comparisons between species indicated that *P. avium* cultivars were more resistant than *P. cerasus* or *P. gondouinii* cultivars in terms of infection rate, defoliation rate, date of 50% defoliation and defoliation severity. Defoliation severity was less in *P. avium* cultivars than in *P. fruticosa* cultivars. *P. fruticosa* cultivars were

more resistant than P. cerasus cultivars in terms of infection severity, defoliation rate, date of 50% defoliation, and defoliation severity.

Introduction

Cherry leaf spot, caused by Coccomyces hiemalis, is a serious disease of cultivated cherries throughout the world (2). At present, little information exists on the genetic variation for resistance within sour cherry (Prunus cerasus) or other cultivated cherry species. An evaluation of the relative resistance of available cultivars would aid selection of parent material for use in a cherry breeding program.

Sweet cherries (P. avium) are considered more resistant to leaf spot than sour cherries (15). The relative resistance of duke cherries (P. gondouinii), which are hybrids between sweet and sour cherries, is uncertain. The European ground cherry (P. fruticosa) was reported to be less resistant than either P. avium or P. cerasus (8).

Reports from Eastern Europe indicate that genetic variation for resistance also exists within sweet, sour, and duke cherries (3,6,13,19,21). The purpose of this research was to evaluate the relative resistance to infection and defoliation by C. hiemalis of cultivars and selections within these four species of cherry. In addition, planned statistical comparisons were made between these species on the assumption that the cultivars and selections evaluated were representative of their respective species.

Materials and Methods

Plant material. Twenty-five cultivars and selections representing four species of cultivated cherries (P. avium, P. cerasus, P. gondouinii and P. fruticosa) were chip budded in September, 1979, onto seedling rootstocks of P. mahaleb L. All budwood except that of P. avium 'Governor Wood', P. avium 'Yellow Glass', P. cerasus 'SHT-2' and P. gondouinii 'SHT-3' was obtained as virus-free budwood from the U.S.D.A. Interregional Project No. 2 repository in Prosser, WA 99350. Budwood of 'Governor Wood' and 'Yellow Glass' was obtained from Interstate Nurseries, Inc., Hamburg, IA 51640. Budwood of 'SHT-3' and 'SHT-2' was obtained from the original seedling trees at the South Haven Experiment Station, South Haven, MI. 'SHT-2' is an open-pollinated seedling of an unknown morello sour cherry. 'SHT-3' is an apparent interspecific hybrid between P. cerasus 'North Star' and an unknown culitvar of P. avium. Both 'SHT-2' and 'SHT-3' were previously selected at the South Haven Experiment Station for their resistance to C. hiemalis (R. L. Andersen, unpublished). The remaining cultivars were selected to represent a genetically diverse sampling of cultivars within each species.

The budded trees were dug in November, 1979, and stored at 1-4 C until planting in April, 1980. Trees were planted at 1.5 m by 1.8 m spacing in Locke sandy loam in a randomized complete block with five replications. Trees were irrigated daily throughout the growing season with a biwall drip irrigation system buried 2- to 5-cm deep adjacent to each tree. Seedling tops were removed from each tree

within 10 days after planting to force growth from the propagated bud. A single vegetative shoot was forced from each tree and maintained as a single shoot until terminal bud formation. Each tree received approximately 30 g of 12% N-12% P_2O_5-12% K_2O fertilizer in a single application 2 weeks after budbreak and a supplementary application of 33 g of urea one month later. Trees were sprayed as needed throughout the growing season to control insects and mites. Pyrazophos (Afugan 30 EC, 0.5%, v/v) was applied twice within 4 weeks after inoculation to control powdery mildew (14).

Inoculation with Coccomyces hiemalis. Each tree in the experiment was inoculated with C. hiemalis on July 7, 1980. Conidia for inoculation were washed from naturally infected leaves of P. cerasus 'Montmorency' from trees adjacent to the experimental plot. A 2 cm^2 area on the lower surface of the second unfolded leaf below the shoot apex was inoculated by spraying a suspension of 10^5 conidia per ml with a mist bottle. The inoculated leaf was enclosed over night in a polyethylene bag containing a wet paper napkin for approximately 12 hours. Subsequent spread of inoculum and infection occurred throughout the growing season during periods of natural leaf wetness.

Evaluation of resistance. Trees were evaluated starting July 7, 1980, and at 1- to 2-week intervals through October 2, 1980. Percent of total leaves that were infected or defoliated were determined at each evaluation. The total number of leaves for an individual tree was the number of unfolded leaves present on August 6,

1980, when the terminal leaf had unfolded on the first trees to cease terminal growth.

Preliminary analysis of angular-transformed data as a split-plot in time (18) indicated that there was a highly significant cultivar by time of evaluation interaction for both percent infection and percent defoliation. In addition, the angular transformation did not adequately linearize the data, as a highly significant non-linear residual term remained after fitting the linear term.

Gompertz and logistic equations were fit to the sigmoidal percent infection and percent defoliation curves to determine if at least part of the interaction was due to differences in slope and/or position of the curves. Examination of the residuals and correlation coefficients for individual trees indicated a better fit with the Gompertz transformation ($-\ln(-\ln(y))$). Linear regression of Gompertz-transformed values against time of rating was used to estimate rates (k) of infection and defoliation for each tree (4). In addition, the time in days of the year to 50% infection and 50% defoliation were estimated from the regression equations.

Estimates of disease severity were made by calculating the areas under the percent infection and percent defoliation curves. Areas for each tree were calculated by the following equation:

$$\text{Area} = \sum_{i=1}^{n} ((R_i + R_{i+1})/2)(t_{i+1} - t_i)$$

where t_i = day of the year at evaluation "i," R_i = percent infection or percent defoliation at evaluation "i," and i = 1 to 9.

Results

Inoculation of a single leaf per tree resulted in the nearly uniform establishment of infection throughout the experimental plot. At the time of inoculation, 57% of the trees showed no visible symptoms of infection on any leaves. All but one of the 125 trees showed visible symptoms of infection on the inoculated leaf 14 days later.

The level of infection increased rapidly in the plot. The mean percent of leaves infected was less than 5% on the day of inoculation (Figure 1). The mean percent of leaves infected reached 70% at 4 weeks after inoculation (day 219), and increased to greater than 98% at eight weeks after inoculation (day 248).

Variation in infection and defoliation existed within and between the four species of cherry (Tables 1-4). No cultivar was free of infection or defoliation, and significant quantitative differences in infection and defoliation between cultivars were detected.

Rates of infection varied over a 2-fold range among the 25 cultivars from a low of 0.062 for P. avium 'Hedelfingen' to a high of 0.126 for P. cerasus 'North Star' (Table 1). Estimated date of 50% infection varied by less than 10 days among the 25 cultivars. Infection severity (area under infection curve) ranged from a low of 5754 for P. avium 'Yellow Glass' to a high of 6679 for P. avium 'Schmidt'.

Figure 1. Mean percent infection curves for cultivars of four cherry species inoculated with Coccomyces hiemalis on day 189 (inoc). Curves are the mean of 9, 6, 6, and 4 cultivars for Prunus avium, P. cerasus, P. gondouinii and P. fruticosa, respectively.

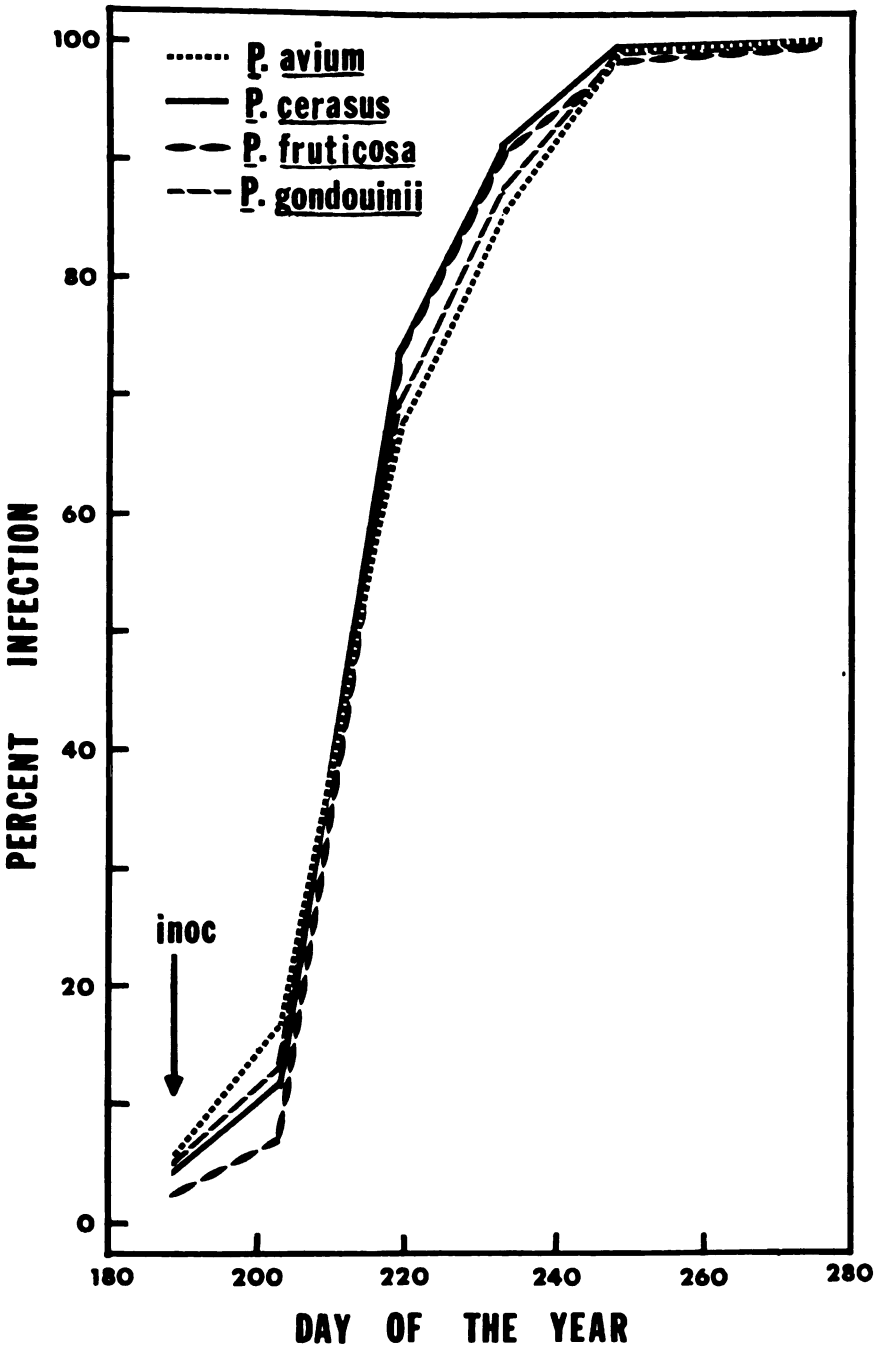

P. avium
P. cerasus
P. fruticosa
P. gondouinii
PERCENT INFECTION
100
80
60
40
20
0
inoc
180
200
220
240
260
280
DAY OF THE YEAR

TABLE 1.--Resistance of cherry cultivars and selections to infection by *Coccomyces hiemalis*[v]

Cultivar	Infection rate[w]	Date of 50% infection (day of the year)[x]	Infection severity (area under inf curve)[y]
Prunus avium			
Black Tartarian	.086 cdefg[z]	211.9 bcdefgh	6251 abcdef
Emporer Francis	.089 bcdefg	212.2 bcdefgh	6191 bcdef
Governor Wood	.085 cdefg	214.8 defgh	5981 defg
Hedelfingen	.062 g	210.7 abcde	6451 abc
Lambert	.071 fg	212.5 bcdefgh	6202 bcdef
Napoleon	.073 efg	211.5 abcdefg	6269 abcde
Schmidt	.079 cdefg	208.9 abc	6679 a
Windsor	.075 defg	213.9 cdefgh	6038 cdefg
Yellow Glass	.083 cdefg	216.6 h	5754 g
P. cerasus			
Early Richmond	.085 cdefg	210.3 abcd	6424 abc
English Morello	.078 cdefg	210.2 abcd	6312 abcd
Meteor	.108 abcd	210.5 abcde	6327 abcd
Montmörency	.107 abcde	208.6 ab	6560 ab
North Star	.126 a	215.0 defgh	6011 cdefg
SHT-2	.112 abc	215.4 efgh	5924 defg
P. gondouinii			
Brassington Duke	.082 cdefg	216.2 gh	5848 efg
Kansas Sweet	.091 bcdefg	216.0 fgh	5905 defg
Krassa Severa	.104 abcdef	206.9 a	6655 ab
May Duke	.082 cedfg	208.9 abc	6421 abc
SHT-3	.085 cdefg	216.2 gh	5818 fg
Wczesna Z Prin	.100 abcdef	212.0 bcdefgh	6219 bcdef
P. fruticosa			
Dwarf Rich	.109 abcd	213.2 bcdefgh	6095 cdefg
IR 323-2	.105 abcdef	215.4 efgh	5926 defg
IR 586-3	.121 ab	211.1 abcdef	6315 abcd
IR 587-1	.086 cdefg	214.4 defgh	5958 defg

[v]Mean of 5 single-tree replications.

[w]Slope of linear regression of Gompertz-transformed percent infection data.

[x]Estimated from the linear regression of Gompertz-transformed percent infection data.

[y]Area = $\Sigma((R_i + R_{i+1})/2)(t_{i+1} - t_i)$, where t_i = day of the year at evaluation i and R_i = percent of leaves infected at evaluation i (i = 1-9).

[z]Mean separation by Duncan's multiple range test ($p = 0.05$).

TABLE 2.--Resistance of *Prunus* species to infection by *Coccomyces hiemalis*.

Species	Cultivars and selections evaluated[v] (no.)	Infection rate[w]	Date of 50% infection (day of the year)[x]	Infection severity (area under inf curve)[y]
P. avium	9	.078	212.6	6202
P. cerasus	6	.103	211.6	6260
P. gondouinii	6	.091	212.7	6144
P. fruticosa	4	.105	213.5	6073
Significance of planned non-orthogonal comparisons				
P. cerasus versus *P. avium*		**[z]	n.s.	n.s.
P. cerasus versus *P. gondouinii*		*	n.s.	n.s.
P. cerasus versus *P. fruticosa*		n.s.	n.s.	*
P. gondouinii versus *P. avium*		*	n.s.	n.s.
P. fruticosa versus *P. avium*		**	n.s.	n.s.

[v]Five single-tree replications per cultivar.

[w]Slope of linear regression of Gompertz-transformed percent infection data.

[x]Estimated from linear regression of Gompertz-transformed percent infection data.

[y]Area under infection curve = $\Sigma((R_i + R_{i+1})/2)(t_{i+1} + t_i)$, where t_i = day of the year at evaluation i and R_i = percent of leaves infected at evaluation i (i = 1-9).

[z]n.s., *, ** = not significant, significant at $p = 0.05$, and significant at $p = 0.01$, respectively, for single degree of freedom comparison from analysis of variance.

TABLE 3.--Resistance of cherry cultivars and selections to defoliation by Coccomyces hiemalis.[v]

Cultivar	Defoliation rate[w]	Date of 50% defoliation (day of the year)[x]	Defoliation severity (area under def curve)[y]
Prunus avium			
Black Tartarian	.064 abcdefg[z]	234.2 abcd	4045 cdef
Emporer Francis	.052 efgh	246.5 defg	2828 h
Governor Wood	.057 defgh	236.7 bcde	3724 def
Hedelfingen	.038 ghi	246.8 defg	2874 gh
Lambert	.088 ab	238.9 cdefg	3471 fg
Napoleon	.064 abcdefg	234.4 abcd	3755 def
Schmidt	.034 hi	247.4 efg	2803 h
Windsor	.055 defgh	246.6 defg	2879 gh
Yellow Glass	.049 fghi	249.3 fg	2716 h
P. cerasus			
Early Richmond	.064 abcdefg	228.0 abc	4551 abc
English Morello	.077 abcde	227.4 abc	4644 abc
Meteor	.060 cdefgh	228.7 abc	4377 abcd
Montmorency	.090 a	224.5 ab	4936 ab
North Star	.044 fghi	237.5 cdef	3736 def
SHT-2	.055 defgh	235.2 abcde	3623 ef
P. gondouinii			
Brassington Duke	.063 abcdefg	237.9 cdefg	3686 ef
Kansas Sweet	.067 abcdef	238.0 cdefg	3542 f
Krassa Severa	.085 abc	222.8 a	4994 a
May Duke	.065 abcdefg	230.1 abc	4378 abcd
SHT-3	.062 bcdefg	231.3 abc	4219 cde
Wczesna Z Prin	.082 abcd	227.5 abc	4594 abc
P. fruticosa			
Dwarf Rich	.058 cdefgh	240.0 cdefg	3555 f
IR 323-2	.066 abcdef	229.4 abc	4342 bcd
IR 586-3	.062 bcdefg	234.5 abcd	3996 cdef
IR 587-1	.025 i	250.3 g	2934 gh

[v]Mean of 5 single-tree replications.

[w]Slope of linear regression of Gompertz-transformed percent defoliation data.

[x]Estimated from linear regression of Gompertz-transformed percent defoliation data.

[y]Area under defoliation curve = $\Sigma((D_i + D_{i+1})/2)(t_{i+1} - t_i)$, where t_i = day of year at evaluation i and D_i = percent of leaves defoliated at evaluation i (i - 1-9).

[z]Mean separation by Duncan's multiple range test ($p = 0.05$).

TABLE 4.--Resistance of *Prunus* species to defoliation by *Coccomyces hiemalis*.

Species	Cultivars and selections evaluated[v] (no.)	Defoliation rate[w]	Date of 50% defoliation (day of the year)[x]	Defoliation severity (area under def curve)[y]
P. avium	9	.056	242.3	3233
P. cerasus	6	.065	230.2	4311
P. condouinii	6	.071	231.3	4235
P. fruticosa	4	.052	238.6	3707
Significance of planned non-orthogonal comparisons				
P. serasus vs *P. avium*		*[z]	**	**
P. cerasus vs *P. gondouinii*		n.s.	n.s.	n.s.
P. cerasus vs *P. fruticosa*		*	**	**
P. gondouinii vs *P. avium*		**	**	**
P. fruticosa vs *P. avium*		n.s.	n.s.	**

[v]Mean of five single-tree replications per cultivar.

[w]Slope of linear regression of Gompertz-transformed percent defoliation data.

[x]Estimated from linear regression of Gompertz-transformed percent defoliation data.

[y]Area under percent defoliation curve = $\Sigma((D_i + D_{i+1})/2)(t_{i+1} + t_i)$, where t_i = day of the year at evaluation i, and D_i = percent defoliation at evaluation i (i = 1-9).

[z]n.s., *, ** = not significant, significant at $p = 0.05$, and significant at $p = 0.01$, respectively, for single degree of freedom comparison from analysis of variance.

Higher rates of infection were not always associated with earlier dates of 50% infection. For example, 'Yellow Glass' had a slightly higher infection rate than 'Schmidt', but the estimated date of 50% infection was more than 7 days earlier for 'Schmidt' than for 'Yellow Glass.' Similarly, the infection rate for 'North Star' was considerably higher than P. cerasus 'Montmorency', but the estimated date of 50% infection was more than 6 days earlier for 'Montmorency' than for 'North Star.'

The average infection rate for P. avium cultivars was significantly lower than that of the other three species (Table 2). However, date of 50% infection and infection severity did not differ significantly between P. avium and the other species. These results reflect the higher average percent infection in P. avium cultivars early in the season, compared with a lower percent infection for P. avium later in the season (Figure 1).

Rates of defoliation differed more than 3-fold among these cultivars, from a low of 0.025 for P. fruticosa 'IR 587-1' to 0.090 for 'Montmorency' (Table 3). Dates of 50% defoliation range from earlier than day 223 for the duke cherry 'Krassa Severa' to later than day 250 for 'IR 587-1'. Defoliation severity (area under defoliation curve) was highest for 'Krassa Severa' (4994) and lowest for 'Yellow Glass' (2716). In general, cultivars with low defoliation severity values had lower defoliation rates and later dates of 50% defoliation. Simple correlations calculated on a cultivar mean basis between defoliation severity and either defoliation rate ($r = 0.743$) or date of of 50% defoliation ($r = -0.986$) were highly significant ($p < 0.01$).

However, P. avium 'Lambert' had a relatively high defoliation rate (0.088) but a moderately low defoliation severity value (3471). The defoliation curve for this cultivar was characterized by a delay in the onset of defoliation, followed by a relatively rapid increase in defoliation later in the season.

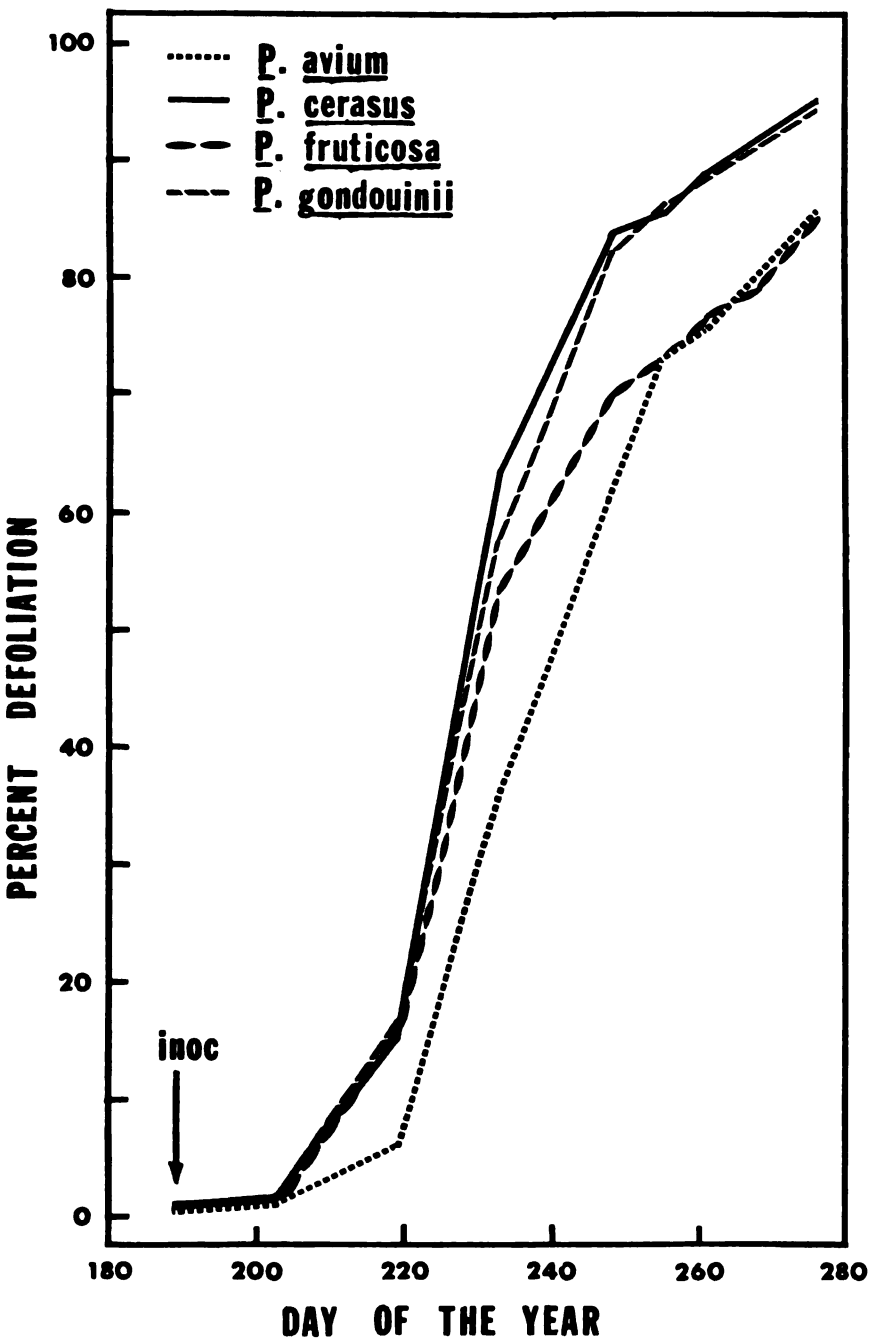
Parameters that estimated resistance to infection were poorly correlated with defoliation severity. Only the date of 50% infection was significantly correlated with defoliation severity ($r = -0.426$, $p = 0.05$). In addition, some cultivars estimated to be least resistant to infection were among the most resistant to defoliation, and vice-versa (Tables 1 and 2). For example, 'Schmidt' had the highest infection severity of all 25 cultivars, and one of the lowest defoliation severity values.

Differences between species in resistance to defoliation were greater than differences in resistance to infection (compare Figures 1 and 2). P. avium was significantly more resistant than either P. cerasus or P. gondouinii in all three defoliation parameters, and more resistant than P. fruticosa in terms of defoliation severity (Table 4). P. cerasus was significantly less resistant than P. fruticosa in all three parameters.

Discussion

The results of this study provide valuable information on selection of parents for breeding cherries with increased resistance to C. hiemalis. How resistant cultivars can be developed depends not

Figure 2. Mean percent defoliation curves for cultivars of four cherry species inoculated with Coccomyces hiemalis on day 189 (inoc). Curves are the mean of 9, 6, 6, and 4 cultivars for Prunus avium, P. cerasus, P. gondouinii and P. fruticosa, respectively.


P. avium
P. cerasus
P. fruticosa
P. gondouinii
PERCENT DEFOLIATION
100
80
60
40
20
0
inoc
180
200
220
240
260
280
DAY OF THE YEAR

only upon identification of a source of resistance, but also on the number and nature of genes controlling resistance. Preliminary studies indicate that cultivars of sweet, sour and duke cherry differ in breeding value for resistance to C. hiemalis (8,17).

Evaluation of resistance to C. hiemalis should accurately assess resistance to defoliation. Severe defoliation by C. hiemalis reduces the yield, growth and hardiness of cherry trees up to two seasons following defoliation (7,9). Reduction in growth can, in turn, reduce the development of fruiting spurs important to future productivity (12). Of the three defoliation parameters evaluated in this study, defoliation severity probably best measures the impact of defoliation on the tree. Defoliation severity, calculated as the area under the defoliation curve, assesses not only the extent of defoliation, but also when during the season that defoliation occurs. Defoliation early in the season would be more detrimental to the tree than defoliation later, even if the total severity of defoliation was the same. Unfortunately, accurate determination of defoliation severity requires that several evaluations be made for each tree throughout the season. Measurements of defoliation severity, date of 50% defoliation, or defoliation rate probably are not practical for use in a breeding program except for advanced selection evaluation in replicated trials. Evaluation of components of resistance in young seedlings under controlled conditions has been proposed as an alternate method of resistance screening (16), but initial results indicate that heritabilities of individual components are too low to

apply this method to initial single-plant selection (17). Initial screening of individual seedlings can probably be done most efficiently by a single evaluation per season in the field when the mean level of defoliation appears to be 50%, which should allow for greatest dispersion of the genotypes around the mean. Establishment of a uniform point source of inoculum in each tree should provide sufficient disease pressure in most seasons.

Although no cultivar in this study was completely resistant, genetic variation in the level of resistance to C. hiemalis was present within all four cherry species. In addition, the differences between cultivars in this study probably underestimate the magnitude of differences between the same cultivars in orchard plantings. Conidia are likely to splash from heavily infected trees to adjacent trees during wind-driven rain in this closely spaced planting (5). This type of plot-to-plot interference can diminish true differences in resistance between cultivars (20).

The need for increased resistance to C. hiemalis is especially critical in sour cherry. The sour cherry industry in Michigan and the rest of the United States is based almost entirely on the cultivar 'Montmorency' and strains derived from it (1). 'Montmorency' was very susceptible to defoliation in this study (Table 3). The sour cherry cultivar 'North Star', in contrast, showed much greater resistance to defoliation. In addition, initial studies on the inheritance of resistance in sour cherry indicate that 'North Star' is a good parent for obtaining progeny with resistance to C. hiemalis (17). The introduction of commercially acceptable cultivars with a

level of resistance comparable to 'North Star' should reduce the number of fungicide sprays needed each season to control leaf spot. Currently, about six fungicide applications per season are recommended to Michigan sour cherry growers to control this disease (10).

An alternate approach to increasing resistance in sour cherry is interspecific hybridization with the more resistant sweet cherries. There is indication, however, that this approach may be complicated by non-additive gene action. The mean resistance to defoliation of the duke cherries in this study was very similar to that of the sour cherries (Table 4). If these duke cherry cultivars are representative of hybrids between sweet and sour cherry, then the genes for resistance in sweet cherry may be recessive to genes in sour cherry. This hypothesis should be tested by creating hybrids between sweet and sour cherries. Levels of resistance in hybrid progenies should be compared to within-species progenies as well as clonal parent material.

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CHAPTER II

COMPONENTS OF PARTIAL RESISTANCE TO *COCCOMYCES HIEMALIS* IN *PRUNUS* SPECIES

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COMPONENTS OF PARTIAL RESISTANCE TO *COCCOMYCES HIEMALIS* IN *PRUNUS* SPECIES

Abstract

Cultivars of *Prunus avium*, *P. cerasus* and *P. gondouinii* differing in levels of partial resistance to *Coccomyces hiemalis* were evaluated for components of resistance in the greenhouse in two separate experiments. In the first experiment, components were evaluated over four leaf ages (6.5, 10.5, 14.5 and 18.5 days old) in six *P. cerasus* cultivars and two *P. gondouinii* cultivars. Numbers of lesions per cm^2 of inoculated leaf (infection frequency) at 6 (but not 16) days after inoculation and proportion of lesions present at 6 days differed among cultivars. Lesion areas and spores per lesion, both measured 20 days after inoculation, differed over leaf ages and among cultivars. Spores per cm^2 of inoculated leaf, the combined effect of infection frequency and spores per lesion, differed nearly 5-fold among cultivars. Cultivar x leaf age interactions were present for lesion area, spores per lesion and spores per cm^2 of inoculated leaf area. Measurement of spores per lesion

were predicted from measurements of lesion areas, proportion of lesions present and leaf age (R^2 = 0.68). In the second experiment, components of resistance were evaluated over three leaf ages (7.5, 19.5, and 31.5 days old) in ten P. avium cultivars, five P. cerasus cultivars and five P. gondouinii cultivars. Infection efficiency (ratio of lesions produced to inoculum applied), days to 50% of lesions present, rate of lesion appearance, lesion area, spores per lesion at 9, 18 and 36 days after inoculation, and reproductive efficiency (ratio of spores produced to inoculum applied) differed among cultivars and over leaf ages. Cultivar by leaf age interactions were present for infection efficiency, days to 50% of lesions present, spores per lesion at all three dates, and reproductive efficiency. Much of the interaction for each component was attributed to differences between the three species. Measurements of lesion area, lesions per leaf, days to 50% of lesions present and leaf age predicted spores per lesion at 9 days (R^2 = 0.33), 18 days (R^2 = 0.70) and 36 days (R^2 = 0.86) after inoculation. Field resistance to defoliation in these cultivars was highly correlated with the components days to 50% of lesions present (r = -0.70), rate of lesion appearance (r = 0.79), lesion area (r = 0.81), spores per lesion (r = 0.78) and reproductive efficiency (r = 0.82).

Introduction

Cherry leaf spot, caused by Coccomyces hiemalis Higg., is a widespread fungal disease of cultivated cherries. Resistance to C. hiemalis has been reported in cultivars of sweet cherry (Prunus avium L.), sour cherry (P. cerasus L.) and duke cherry (P. gondouinii Rehd.) (1,2,7,12,14,16). P. avium cultivars are generally more resistant to defoliation by C. hiemalis than either P. gondouinii or P. cerasus cultivars (12). Most cultivars in these studies were susceptible to infection by C. hiemalis, but differed quantitatively in percent of leaves infected and/or defoliated. This type of resistance has been termed partial resistance (10).

Factors that directly or indirectly measure the ability of the pathogen to reproduce on the host are called components of resistance, and are often strongly correlated with differences in partial resistance in the field (9). The purpose of this study was to identify components of resistance in cultivars of P. avium, P. cerasus and P. gondouinii. In addition, the relationship of these components to previously reported disease severity of the same cultivars (12) was examined.

Materials and Methods

Plant material. Cultivars and selections of P. avium, P. cerasus and P. gondouinii were chip budded onto seedling rootstocks of P. mahaleb L. Budwood of all cultivars was obtained as previously described (12).

Trees for each experiment were grown in 3.7-L containers in a greenhouse at 15-30 C. Trees for experiment one were grown in a mixture of soil and perlite (2:1, v/v), and fertilized biweekly with a 5.3 g/L solution of 20% N-20% P_2O_3-20% K_2O fertilizer (Robert B. Peters Co., Inc., Allentown, PA 18104). Trees for experiment two were grown in a mixture of sand, peat moss, and perlite (1:1:1, v/v) and fertilized once with 5.25 g of 19% N-6% P_2O_5-12% K_2O controlled release fertilizer (Osmocote, Sierra Chemical Co., Milpitas, CA 95035) per container plus 0.75 g of sustained released micronutrient mixture (Esmigran, Mallinckrodt Inc., St. Louis, MO 63147) per container.

All trees were grown as a single vegetative shoot by cutting back to a single vegetative bud and removing lateral shoots at weekly intervals. Because there is a relationship of leaf age to infection frequency by C. hiemalis in P. cerasus (5), the age of each leaf was calculated from the date of unfolding, i.e., when the laminar blades were separated by an angle greater than 90°. All leaves unfolding within a 4-day period were assigned to the same age group. The age of a leaf was the average number of days from unfolding to inoculation. Thus, each tree had a range of leaf ages present at the time of inoculation.

Inoculum. A single-conidium isolate of C. hiemalis (isolate B) from naturally infected P. cerasus 'Montmorency' leaves in a commercial orchard near Decatur, Michigan, was used in both experiments. The isolate was maintained by periodically inoculating young leaves of 'Montmorency' trees grown in the greenhouse, or by freezing

leaves bearing sporulating lesions at -20 C for a maximum of 6 months. Inoculum used for each experiment was washed from 2- to 3-week old lesions on leaves that had not been frozen.

Experiment 1. Components of resistance to C. hiemalis were determined for six cultivars of P. cerasus and two of P. gondouinii in a split-plot design. Cultivars were the whole units, blocked by time of inoculation into three single-tree replications, and leaf ages were subunits. A single leaf in each of four age groups, 6.5, 10.5, 14.5, and 18.5 days, was inoculated on each tree. The area of each leaf was measured with an area meter (Model LI-3000, Lambda Instrument Corp., Lincoln, NE 68504) on the day of inoculation.

Leaves were inoculated with a conidial suspension of C. hiemalis in distilled-deionized water. The suspension was adjusted to 10^5 conidia per ml with a hemacytometer. The conidial suspension was sprayed uniformly onto the undersurface of each leaf with an atomizer (The DeVilbis Co., Somerset, PA 15501) operated at 0.7 bar. Within 5 min after inoculation, trees were placed in a mist chamber at 19 to 21 C for 48 hr. Trees were then incubated in a cheesecloth tent on a greenhouse bench covered with 6 cm of sand. The cheesecloth and sand were wetted daily to maintain a high relative humidity around the plants. The mean temperature during incubation was 23 C (range 17-30 C).

Lesions per leaf were counted at 6 and 16 days after inoculation. Infection frequency was expressed as the number of lesions per cm^2 of leaf area at inoculation. The proportion of lesions on day 6

was the number of lesions on day 6 divided by the number of lesions on day 16. The leaves were removed 20 days after inoculation and frozen at -20 C for further analysis.

Sizes of lesions were measured at 20 days with a binocular dissecting microscope fitted with a calibrated ocular micrometer. Length by width measurements were made on five randomly chosen lesions per leaf, and the lesion area was calculated as a rectangle, square or right triangle.

Spore production was measured at 20 days by washing conidia from each leaf into 40 ml water. Six samples from each spore wash were counted with a hemacytometer and spore production was expressed as spores per lesion basis and as spores per cm^2 of leaf area at inoculation.

Log_{10} transformations were made on lesion areas, spores per lesion and spores per cm^2 data prior to analysis of variance to eliminate proportionality between means and their standard deviations (8). The relationship of spores per lesion to other variables was examined by stepwise multiple regression analysis (3).

Experiment 2. Components of resistance to C. hiemalis were determined for ten cultivars of P. avium, five of P. cerasus and five of P. gondouinii in a split-plot design. Cultivars were the whole units, blocked by time of inoculation with C. hiemalis into four single tree replicates, and three leaf age groups, 7.5, 19.5 and 31.5 days, were subunits.

A single leaf in each age group on each tree was inoculated with a Schein quantitative inoculator (11) modified by addition of an electronic timer to operate the solenoid valve. Two circular areas 2.1 cm^2 were inoculated on each leaf. A DeVilbiss atomizer containing a 10^5 conidia/ml suspension of C. hiemalis was positioned 43 cm from the leaf undersurface and operated for 1 sec at 1.4 bar (1.4 atm). The number of conidia deposited on each leaf was estimated by periodically inoculating sections (1.5 by 1.5 cm) of membrane filters (0.45 μ pore size) placed on 2% water agar. Four sections were inoculated per replication and incubated in glass petri dishes in the mist chamber containing the inoculated trees for 48 hr. Germinating and total numbers of spores were counted in five random light microscope fields at 200x (0.88 mm diameter) for each section. Average number of conidia deposited on filter sections varied from 9600 to 11300 for the four replications. Germination of conidia on filter sections exceeded 90% for all replications.

Immediately after inoculation the trees were placed into a mist chamber for 48 hr at 18 to 21 C. Trees were then incubated as described for experiment 1 at 17 C (range 7-24 C).

Lesions per leaf were counted 3 days after inoculation and at 1 to 3 day intervals for 20 days. Total lesions per leaf were determined 36 days after inoculation. Infection efficiency was calculated as the number of lesions per leaf divided by the number of spores applied to each leaf (11). Rate and time of lesion appearance were estimated from lesion count data by an asymptotic curve, $Y = 1\text{-}e^{-(bX + a)}$, using the equivalent form, $\ln(\frac{1}{1-Y}) = bX + a$, to

fit a linear regression line where X = number of days after inoculation, Y = lesions present at day X as a proportion of the total, b = slope of the regression line, and a = intercept of the regression line. Estimates of days to 50% of lesions present and rate of lesion appearance (slope of the regression line) were made for each leaf and subjected to analysis of variance, using $\log_e$ transformed values for date of 50% lesions.

Lesion areas were determined 36 days after inoculation as described for experiment 1, and the data were $\log_{10}$ transformed prior to analysis of variance.

Spore production was measured at 9, 18 and 36 days after inoculation. At 9 and 18 days, conidia were washed from each inoculated leaf into 9 ml distilled deionized water with a DeVilbiss atomizer operated at 0.7 bar. One ml of 2% (w/v) formaldehyde plus 1% (v/v) polyoxyethylene sorbitan monolaurate (Tween 20, Sigma Chem. Co., St. Louis, MO 63178) in distilled deionized water was added to each spore suspension to preserve the spores and minimize adherence of spores to glass surfaces. At 36 days, conidia were removed by immersing the inoculated area of each leaf into 5 ml of 2% (w/v) formaldehyde plus 0.1% (v/v) Tween 20 in distilled deionized water. Samples were stored at 2 C until counted.

Numbers of conidia in each sample were estimated by measuring absorbance of the spore suspensions at 700 nm with a dual beam spectrophotometer (model DB-G, Beckman Instruments, Inc., Fullerton, CA 92634). A standard curve relating absorbance to hemacytometer

counts was developed for each set of samples measured on a given day. Hemacytometer counts were estimated from absorbance by a power curve, $Y = bA^m$, using the equivalent form, $\log_{10} Y = \log_{10} b + m (\log_{10} A)$, to fit a linear regression line where A = absorbance at 700nm and Y = hemacytometer count, b = intercept and m = slope of linear regression line.

Spore production was expressed as spores per lesion and as reproductive efficiency (defined as the number of spores produced divided by the number of spores applied as inoculum). Both measurements were $\log_{10}$ transformed prior to analysis of variance. In addition, the relationship of spores per lesion to other variables was examined by stepwise multiple regression analysis.

Cultivar and cultivar x leaf age interactions in experiment 2 were partitioned into planned orthogonal comparisons (8) of P. avium cultivars versus the combination of P. cerasus and P. gondouinii cultivars and P. cerasus cultivars versus P. gondouinii cultivars. The basis for these comparisons was the reported higher level of resistance among P. avium cultivars relative to P. cerasus and P. gondouinii cultivars (12).

Correlations between these components of resistance and evaluations of field resistance were determined for 19 of the 20 cultivars in experiment 2 (field resistance evaluations were not available for P. avium 'Angela'). Simple correlations were made between each component of resistance and both the infection severity and the defoliation severity reported for these cultivars (12).

Results

Experiment 1. Infection frequency at 6 (but not 16) days after inoculation, size of lesions, numbers of spores per lesion and per cm^2 of inoculated leaf differed significantly among the eight cultivars (Table 1). The proportion of lesions present 6 days after inoculation was significantly lower for P. cerasus 'SHT-2' than for the other P. cerasus cultivars.

Size of lesions showed a highly significant quadratic trend with increasing leaf age. Lesions were largest in 14.5-day-old leaves; however, a significant cultivar x leaf age interaction indicated that not all cultivars showed the same trend.

Numbers of spores per lesion decreased linearly with increasing leaf age; however, cultivar x leaf age interactions were present. Much of this interaction was due to the significantly lower number of spores per lesion in the 6.5-day-old leaves of North Star (2.63×10^4) and Kansas Sweet (2.40×10^4) compared to 10.5-day-old leaves (3.39×10^4 and 3.20×10^4 for North Star and Kansas Sweet, respectively). In contrast, numbers of spores were highest for lesions in 6.5-day-old leaves of all other cultivars (average of 6.74×10^4), and lower in 10.5-day-old leaves (5.20×10^4).

Numbers of spores per cm^2 of inoculated leaf varied 5-fold from 4.79×10^4 for Kansas Sweet to 2.57×10^5 for Meteor (Table 1). Cultivar x leaf age interactions were also present, mainly due to the low number of spores per lesion in 6.5-day-old leaves of North Star and Kansas Sweet. Number of spores per cm^2 decreased linearly with increasing leaf age in inoculated leaves of all other cultivars.

TABLE 1.--Components of resistance to *Coccomyces hiemalis* in cultivars of *Prunus cerasus* and *P. gondouinii*

Cultivar[u]	Infection frequency[v]		Proportion of lesions[w] (%)	Lesion area (mm^2)	Sporulation	
	6 days (lesions per cm^2)	16 days (lesions per cm^2)			Spores per lesion (log)	Spores per cm^2 (log)
P. cerasus						
Early Richmond	1.86 bc[y]	2.58	77.4 a	0.62 b	4.77 ab	5.08 abc
English Morello	3.76 a	5.44	67.3 a	0.64 b	4.68 bc	5.37 ab
Meteor	1.76 bc	2.45	70.3 a	1.49 a	5.05 a	5.41 a
Montmorency	3.77 a	5.00	80.7 a	0.70 b	4.77 ab	5.37 ab
North Star	3.36 ab	5.29	62.4 a	0.33 cd	4.44 bc	5.07 abc
SHT-2	2.44 abc	5.37	34.5 b	0.30 cd	4.38 c	4.95 bc
P. gondouinii						
Kansas Sweet	1.15 c	1.96	56.3 ab	0.28 d	4.47 bc	4.68 c
SHT-3	1.32 c	2.25	54.6 ab	0.46 bc	4.55 bc	4.76 c
Significance of F-test from analysis of variance						
Cultivars	**	n.s.	*	**	**	**
Leaf age	n.s.	n.s.	n.s.	*	**	n.s.
Cult x lf age	n.s.	n.s.	n.s.	*	**	**

[u]Mean of three single-tree replications, averaged over four leaf ages.

[v]Lesions per cm^2 leaf area at inoculation, measured at 6 and 16 days after inoculation.

[w]Ratio of 6-day infection frequency to 16-day infection frequency x 100.

[x]Spores per cm^2 leaf area at inoculation.

[y]Mean separation within columns by Duncan's multiple range test, $p = 0.05$.

[z]n.s., *, ** = not significant, significant at $p = 0.05$, and significant at $p = 0.01$, respectively.

Sporulation was related to the proportion of lesions present 6 days after inoculation and size of lesions. Simple correlations of log spores per lesion with log lesion area ($r = 0.76$) and proportion of lesions present ($r = 0.55$) were highly significant. A multiple regression equation including lesion area, proportion of lesions present 6 days after inoculation, and leaf age accounted for 67.9% of the total variation in spores per lesion.

Experiment 2. Highly significant differences existed among the cultivars for each component of resistance (Tables 2 and 3). Leaf age effects were also significant or highly significant for each component. Cultivar x leaf age interactions were detected for time of 50% lesion appearance, numbers of spores per lesion on each date of sampling, infection efficiency, and reproductive efficiency.

The average infection efficiency for the 20 cultivars was 0.91% with a range from 0.48% (P. gondouinii 'Brassington Duke') to 1.51% (P. avium 'Governor Wood'). Planned comparisons between species averaged over all leaf ages did not differ significantly. However, a significant portion of the cultivar x leaf age interaction could be attributed to comparisons between species (Figure 1). P. avium cultivars showed a generally linear trend of decreasing infection efficiency with increasing leaf age, while the trends with P. gondouinii and P. cerasus cultivars were strongly nonlinear. Infection efficiencies were highest in 19.5-day-old leaves of P. cerasus cultivars, but were slightly higher in 7.5- than in 19.5-day-old leaves of

TABLE 2.--Infection efficiency and lesion development in cultivars of *Prunus* species inoculated with *Coccomyces hiemalis*.

Species	Cultivar[u]	Infection efficiency[v] (%)	50% of lesions[w] (days)	Rate of lesion appearance[x]	Lesion area (mm^2)
P. avium	Angela	0.67 de[y]	9.88 g	0.37 de	0.20 hijk
	Black Tartarian	0.89 cde	7.34 ef	0.43 de	0.18 ijk
	Emporer Francis	0.72 de	7.56 f	0.43 de	0.14 jk
	Governor Wood	1.51 a	7.56 f	0.47 de	0.27 hij
	Hedelfingen	0.71 de	5.93 cde	0.53 cd	0.26 hij
	Lambert	0.83 cde	6.67 def	0.51 cd	0.33 ghi
	Napoleon	1.10 abcd	7.16 ef	0.54 cd	0.31 ghi
	Schmidt	0.68 de	9.96 g	0.24 e	0.13 k
	Windsor	1.10 abcd	7.03 ef	0.35 de	0.18 ijk
	Yellow Glass	0.87 cde	6.46 def	0.49 de	0.28 ghi
P. cerasus	Early Richmond	0.92 bcde	4.32 a	0.88 a	1.81 ab
	English Morello	0.93 bcde	5.03 abc	0.83 a	1.01 bcd
	Meteor	1.40 ab	4.23 a	0.82 a	2.36 a
	Montmorency	0.95 bcde	4.63 ab	0.74 abc	1.32 abc
	North Star	0.88 cde	5.43 bcd	0.49 de	0.51 efg
P. gondouinii	Brassington Duke	0.48 e	6.59 def	0.57 bcd	0.68 def
	Kansas Sweet	0.77 cde	5.84 cde	0.57 bcd	0.78 cde
	Krassa Severa	1.24 abc	4.30 a	0.88 a	1.95 a
	May Duke	0.89 cde	6.76 def	0.42 de	0.39 fgh
	Wczesna Z Prin	0.69 de	4.77 ab	0.79 ab	0.90 cde

TABLE 2.--Continued

Species	Cultivar	Infection efficiency[v] (%)	50% of lesions[w] (days)	Rate of lesion appearance[x]	Lesion area (mm^2)
		Significance of F-test from analysis of variance			
Cultivars		**[z]	**	**	**
Leaf age		**	**	*	**
Cultivar x leaf age		**	*	n.s.	n.s.

[u]Mean of four single-tree replications averaged over three leaf ages.

[v]Number of lesions/number of inoculum x 100, 36 days after inoculation.

[w]Estimated from asymptotic regression of proportion of total lesions against days after inoculation.

[x]Slope of asymptotic regression.

[y]Mean separation within columns by Duncan's multiple range test, $p = 0.05$.

[z]n.s., *, ** = not significant, significant at $p = 0.05$, and significant at $p = 0.01$, respectively.

TABLE 3.--Spore production and reproductive efficiency in cultivars of Prunus species inoculated with Coccomyces hiemalis.

Species	Cultivar[v]	Spores per lesion (log)			Reproductive efficiency (log)[x]
		9 days[w]	18 days	36 days	
P. avium	Angela	2.05 de[y]	2.63 fg	3.14 ghi	0.74 hi
	Black Tartarian	2.27 bcd	2.62 fg	2.77 hi	0.55 ij
	Emporer Francis	2.52 abcd	2.88 ef	2.96 hi	0.57 ij
	Governor Wood	2.24 bcde	2.71 fg	2.87 hi	0.84 hi
	Hedelfingen	2.60 abcd	3.17 de	3.46 fg	0.83 hi
	Lambert	2.62 abcd	3.15 de	3.46 fg	1.15 gh
	Napoleon	2.43 abcd	3.24 cde	3.48 fg	1.18 gh
	Schmidt	1.57 ef	2.34 g	2.69 i	0.20 j
	Windsor	2.17 cde	2.58 fg	2.80 hi	0.74 hi
	Yellow Glass	2.22 bcde	2.87 ef	3.19 gh	0.88 hi
P. cerasus	Early Richmond	2.89 ab	3.88 a	4.99 a	2.79 ab
	English Morello	2.98 a	3.61 abc	4.52 bcd	2.27 cd
	Meteor	2.91 ab	3.67 ab	4.81 abc	2.90 a
	Montmorency	2.90 ab	3.86 a	4.86 ab	2.57 abc
	North Star	1.98 de	3.17 de	3.91 e	1.75 ef
P. gondouinii	Brassington Duke	2.17 cde	3.42 bcd	4.33 d	1.89 def
	Kansas Sweet	2.05 de	3.45 bcd	4.41 cd	2.16 cde
	Krassa Severa	3.08 a	3.81 ab	4.97 a	2.84 ab
	May Duke	1.25 f	2.76 f	3.69 ef	1.48 fg
	Wczesna Z Prin	2.80 abc	3.85 a	4.80 abc	2.41 bc

TABLE 3.--Continued.

Species	Cultivar	Spores per lesion (log)			Reproductive efficiency (log)[X]
		9 days[W]	18 days	36 days	
	Significance of F-test from analysis of variance				
Cultivars		**[Z]	**	**	**
Leaf age		**	**	**	**
Cultivar x leaf age		*	**	**	**

[V]Mean of four single-tree replications averaged over three leaf ages.

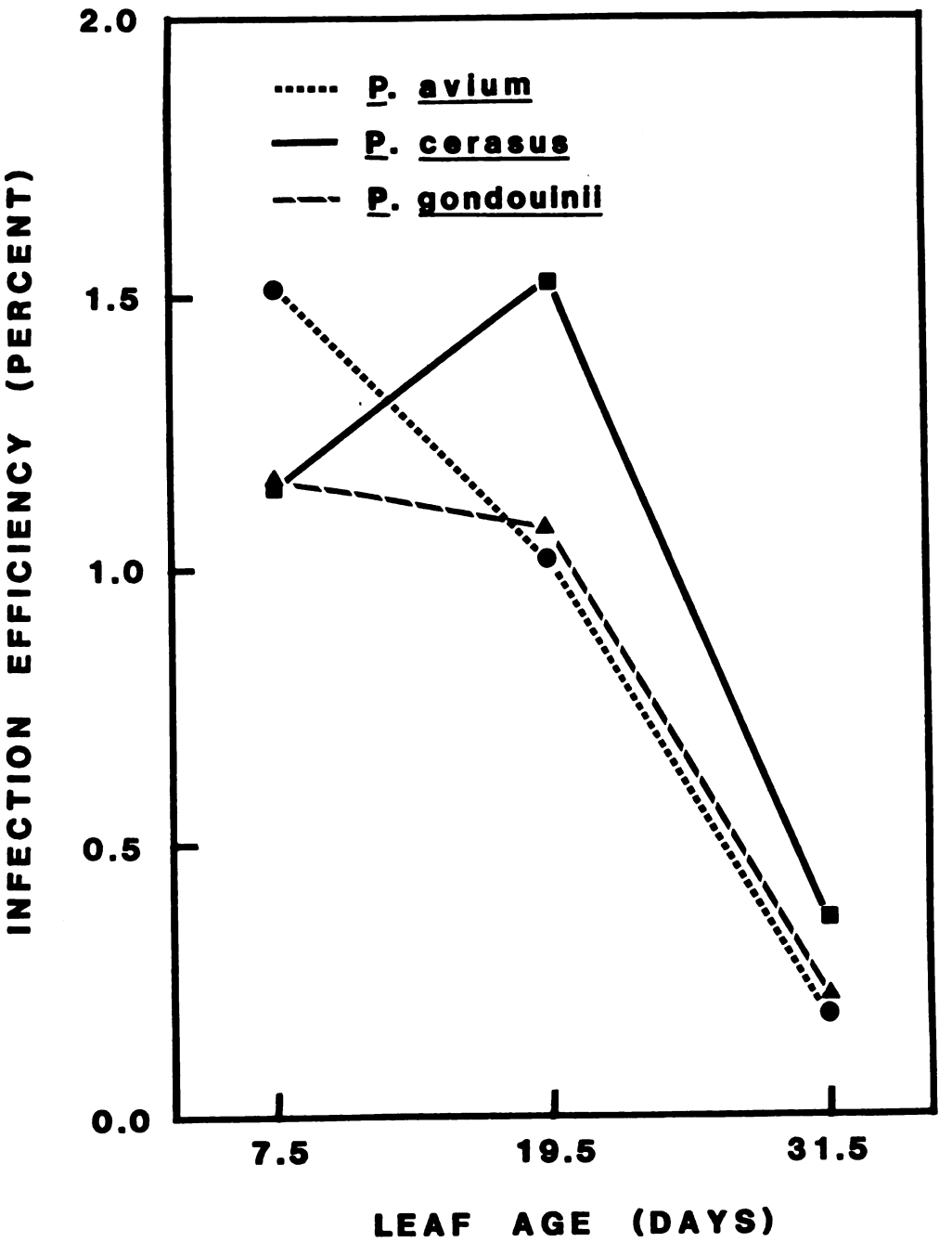
[W]Days after inoculation.

[X]Ratio of total spore production per leaf 36 days after inoculation to inoculum applied.

[Y]Mean separation within columns by Duncan's multiple range test, $p = 0.05$.

[Z]n.s., *, ** = not significant, significant at $p = 0.05$, and significant at $p = 0.01$, respectively.

Figure 1. Relationship of infection efficiency to leaf age in cultivars of three Prunus species inoculated with Coccomyces hiemalis.


2.0
1.5
1.0
0.5
0.0
P. avium
P. cerasus
P. gondouinii
INFECTION EFFICIENCY (PERCENT)
7.5
19.5
31.5
LEAF AGE (DAYS)

P. gondouinii cultivars. All cultivars showed a large reduction in infection efficiency in 31.5-day-old leaves.

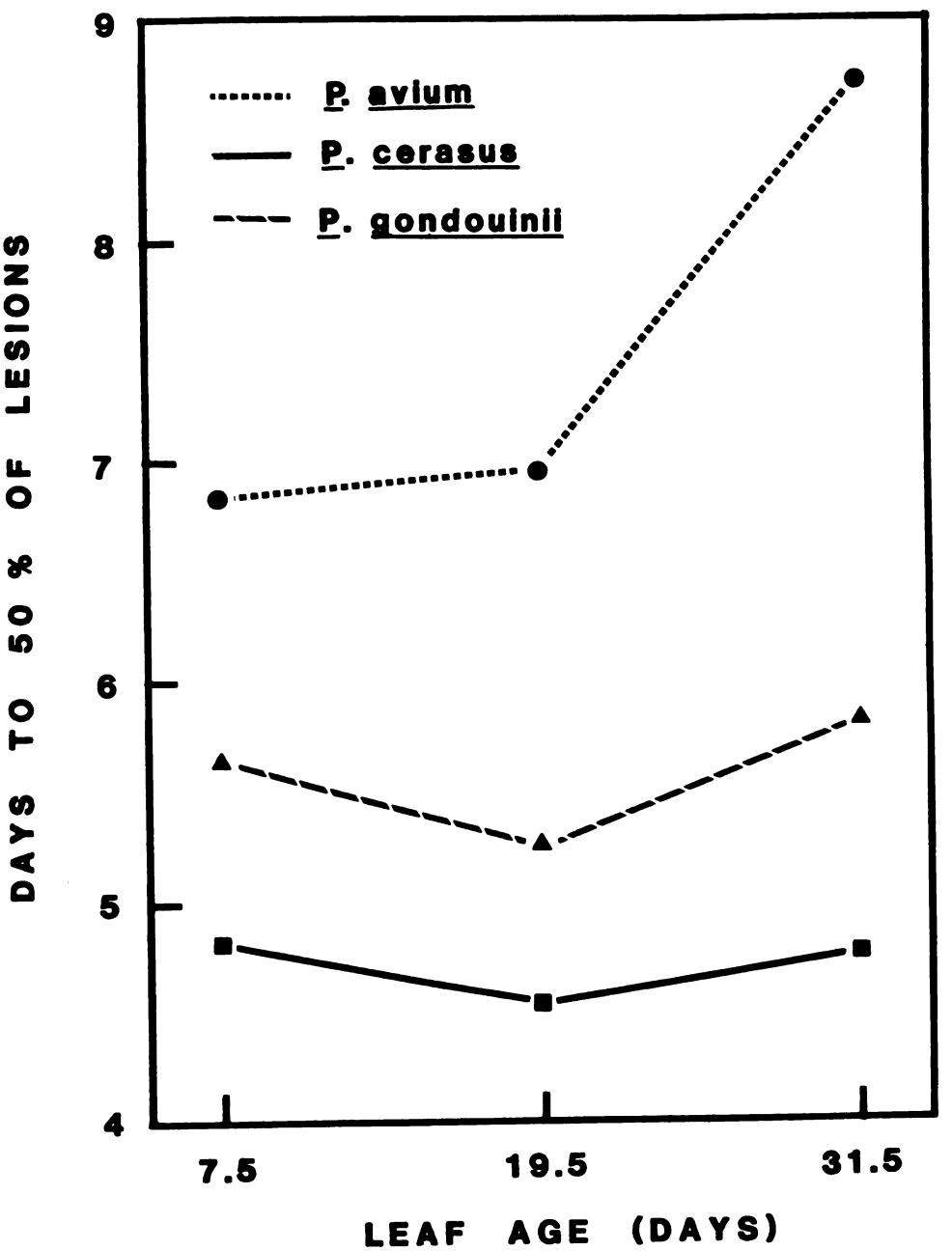
Differences among cultivars in number of days to 50% of lesions present were highly significant. Comparisons between species were significant at each leaf age, and were greatest in 3.15-day-old leaves (Figure 2). The cultivar x leaf age interaction was largely due to differences among species, rather than differences within species.

Rate of lesion appearance was significantly slower ($p = 0.01$) for P. avium cultivars (0.44) than for P. cerasus (0.75) and P. gondouinii (0.65) cultivars. Differences between P. cerasus and P. gondouinii cultivars were not significant. The rate of lesion appearance decreased linearly with increasing leaf age when averaged over all cultivars.

Average lesion areas 36 days after inoculation were significantly smaller ($p = 0.01$) for P. avium cultivars (0.22 mm^2) compared to P. cerasus (1.24 mm^2) and P. gondouinii (0.88 mm^2) cultivars. Differences between P. cerasus and P. gondouinii cultivars were also highly significant. Averaged over all cultivars, log lesion area increased linearly with increasing leaf age.

Large differences were observed among cultivars in spore production per lesion at all three dates of sampling (Table 3). The significantly lower ($p = 0.01$) spore production in lesions of P. avium cultivars compared to P. cerasus and P. gondouinii cultivars accounted for much of the difference among cultivars. In addition, spore

Figure 2. Relationship of time of lesion appearance to leaf age in cultivars of three Prunus species inoculated with Coccomyces hiemalis.


9
8
7
6
5
4
DAYS TO 50 % OF LESIONS
P. avium
P. cerasus
P. gondouinii
7.5
19.5
31.5
LEAF AGE (DAYS)

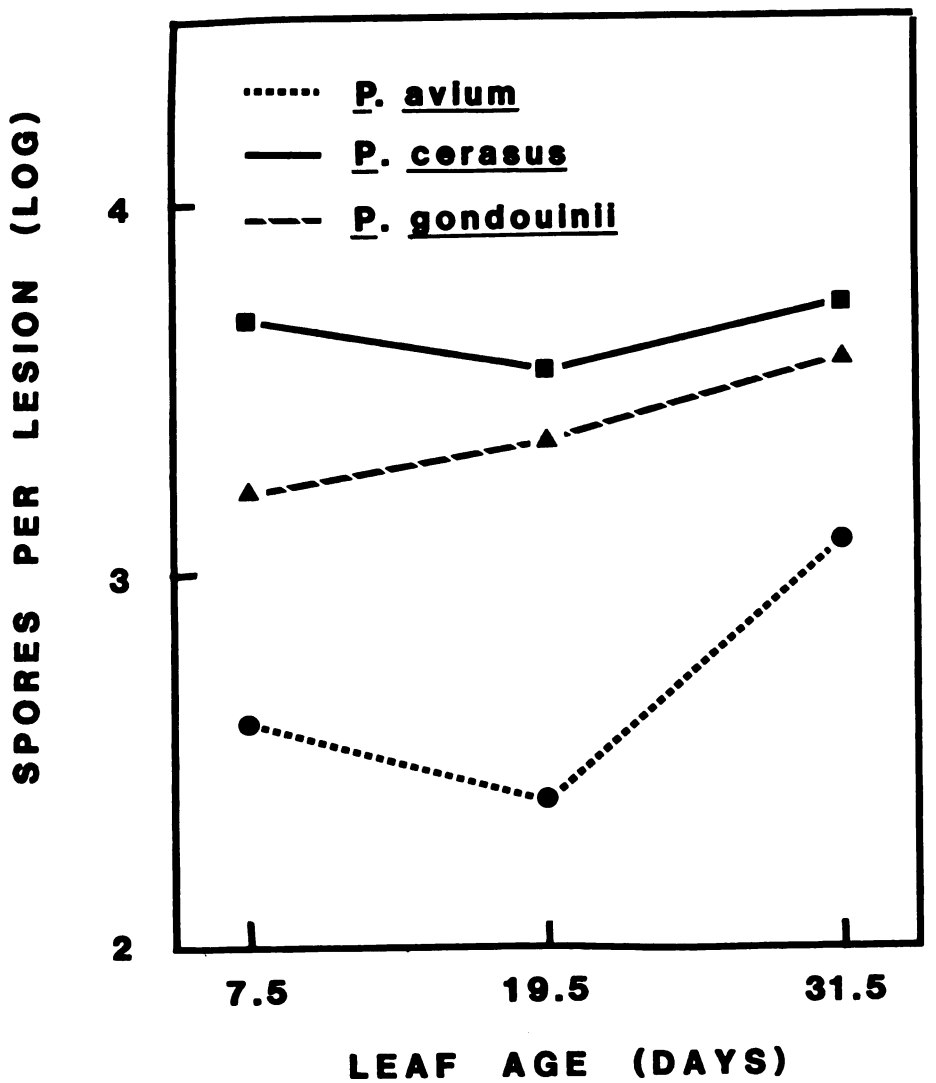
production in P. cerasus cultivars was significantly greater than that in P. gondouinii cultivars.

Combined analysis of spores per lesion data over all three dates of sampling indicated that there were highly significant cultivar x leaf age and cultivar x date of sampling interactions. In addition, significant leaf age x date of sampling interactions were present.

A large portion of the cultivar x leaf age interaction could again be attributed to significant differences in the linear and non-linear trends of P. avium cultivars compared to P. cerasus and P. gondouinii cultivars (Figure 3). In addition, comparisons between P. cerasus and P. gondouinii indicate that P. gondouinii cultivars show a linear trend of increasing spores per lesion with increasing leaf age, whereas P. cerasus cultivars show little indication of a linear trend.

On the basis of the results of experiment 1, numbers of spores per lesion as a function of leaf age for P. cerasus 'North Star' were compared to the mean of the other P. cerasus cultivars. Numbers of spores in leaves of North Star were significantly less than those of the other cultivars at each leaf age. In North Star, numbers of spores per lesion increased logarithmically with increasing leaf age (4.57×10^2, 1.20×10^3, and 2.09×10^3 for 7.5-, 19.5- and 31.5-day-old leaves, respectively). However, numbers of spores were higher in 7.5-day-old leaves (8.91×10^3) than in 19.5-day-old (4.79×10^3) or 31.5-day-old leaves (7.08×10^3) of the other cultivars.

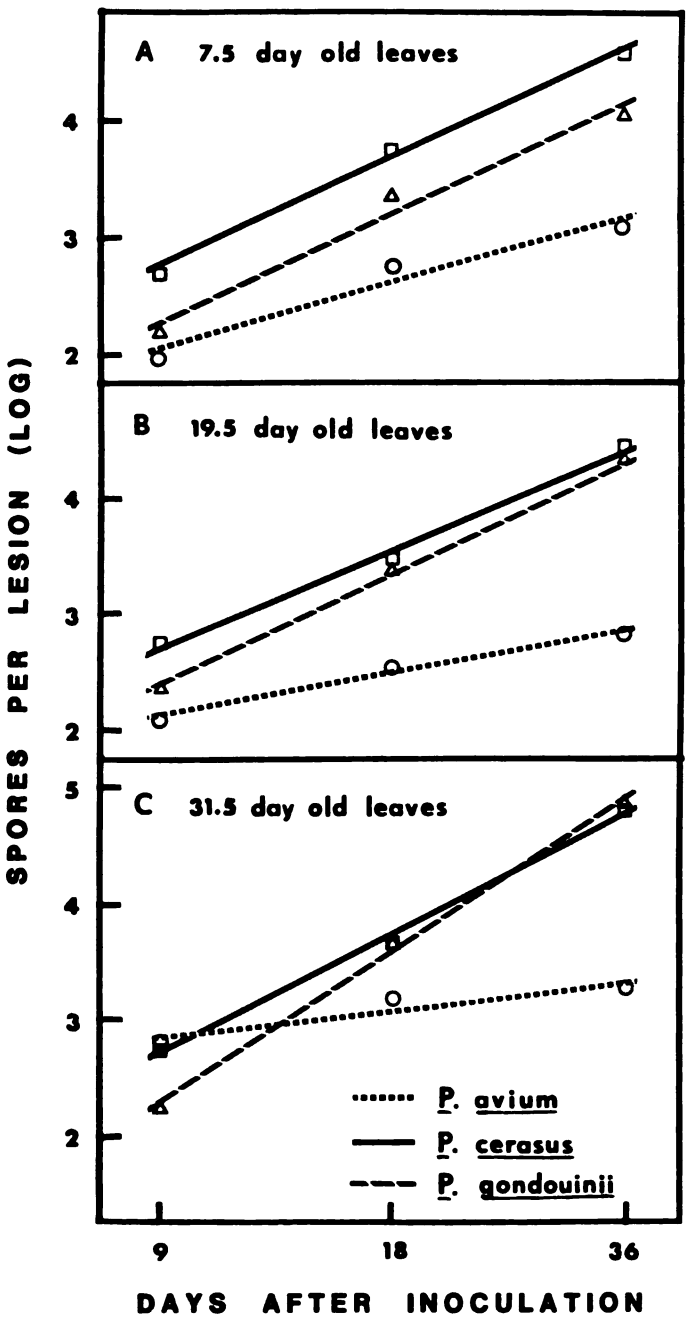
Figure 3. Relationship of spores per lesion averaged over three sampling times (9, 18 and 36 days after inoculation) to leaf age in cultivars of three Prunus species inoculated with Coccomyces hiemalis.


P. avium
P. cerasus
P. gondouinii
SPORES PER LESION (LOG)
4
3
2
7.5
19.5
31.5
LEAF AGE (DAYS)

The triple order interaction of cultivar x leaf age x date of sampling for spores per lesions was attributed in large part to between-species comparisons. The general trend was for sporulation to increase logarithmically between dates of sampling. However, numbers of spores per lesion were lowest for youngest age leaves (7.5-day-old) in all three species at 9 days after inoculation while they were lowest for intermediate age leaves (19.5-day-old) at 18 and 36 days after inoculation. Much of this interaction is explained by differences in the slopes of the linear regression of log spores per lesion against log days after inoculation for each species at each leaf age (Figure 4). Overall, the rate (slope of regression line) of increase in spores per lesion as a function of days after inoculation was significantly less ($p = 0.01$) for P. avium cultivars than for P. cerasus and P. gondouinii cultivars.

Reproductive efficiency (ratio of spores produced to inoculum applied) differed greatly among the three species at 36 days after inoculation. The average reproductive efficiency of P. avium cultivars (5.84) was significantly less ($p = 0.01$) than that of P. cerasus and P. gondouinii cultivars. The average reproductive efficiency of P. cerasus cultivars (285.23) was significantly greater ($p = 0.01$) than that of P. gondouinii cultivars (142.25). Log reproductive efficiency averaged over all cultivars decreased linearly ($p = 0.01$) with increasing leaf age; however, the slope of this linear trend differed among the three species (Figure 5). P. avium cultivars showed a much greater rate of decrease than P. cerasus and

Figure 4. Relationship of spores per lesion to days after inoculation for three leaf ages in cultivars of three Prunus species inoculated with Coccomyces hiemalis.


A 7.5 day old leaves
B 19.5 day old leaves
C 31.5 day old leaves
SPORES PER LESION (LOG)
DAYS AFTER INOCULATION
9
18
36
P. avium
P. cerasus
P. gondouinii

P. gondouinii cultivars. The trend with P. cerasus and P. gondouinii cultivars appeared to be nonlinear.

Numbers of spores per lesion were correlated with several other variables in experiment 2. Highly significant negative correlations were observed between either log time of 50% lesion appearance or log lesions per leaf, and log spores per lesion at 9, 18 and 36 days after inoculation. Highly significant positive correlations were observed between log lesion area, and log spores per lesion at each time of evaluation. Multiple regression equations that included the lesion area, lesions per leaf, time of 50% lesion appearance and leaf age as independent variables accounted for 33%, 70%, and 86% of the total variation in spores per lesion at 9, 18, and 36 days after inoculation, respectively.

Simple correlations with the reported defoliation severity of 19 of the 20 cultivars in experiment 2 (field resistance ratings not available for P. avium 'Angela') were highly significant for each component of resistance except infection efficiency (Table 4). Correlations with infection severity were not significant for all components of resistance.

Discussion

Diseases caused by pathogens such as C. hiemalis with more than one reproductive cycle per season are called "compound interest diseases" (15). The severity of a compound interest disease is the cumulative effect of environmental factors, the level of initial inoculum, genetic factors affecting reproduction of the pathogen

Figure 5. Relationship of reproductive efficiency (ratio of spore production to inoculum applied) to leaf age in cultivars of three Prunus species inoculated with Coccomyces hiemalis.

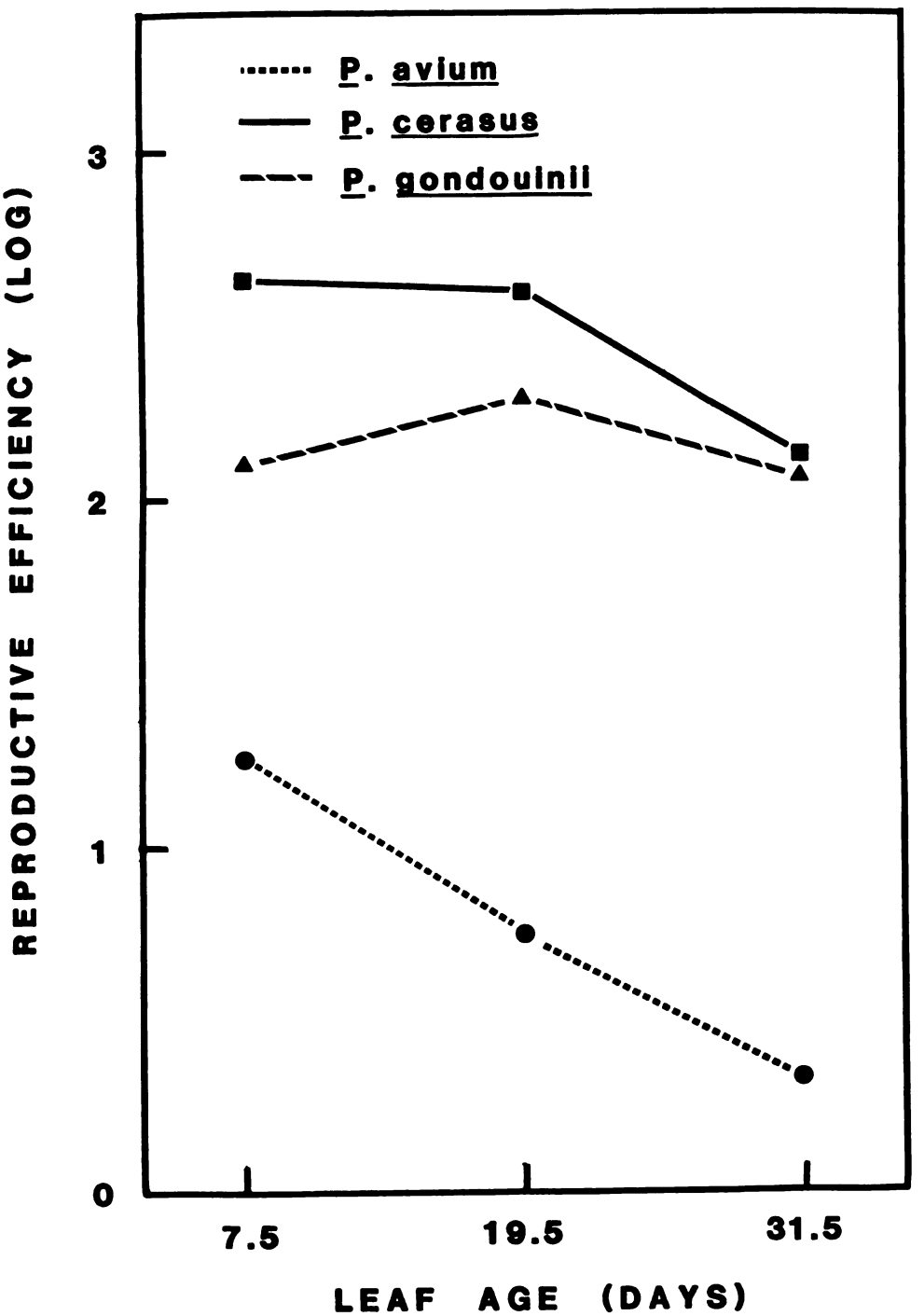

P. avium
P. cerasus
P. gondouinii
REPRODUCTIVE EFFICIENCY (LOG)
3
2
1
0
7.5
19.5
31.5
LEAF AGE (DAYS)

TABLE 4.--Correlations of components of resistance with the reported field resistance of 19 cherry cultivars.[u]

Component of resistance[v]	Field resistance parameter[w]	
	infection severity	defoliation severity
Infection efficiency	.099 n.s.[z]	.313 n.s.
Days to 50% of lesions	-.091 n.s.	-.704**
Rate of lesion appearance	.261 n.s.	.789**
Log (lesion area)	.359 n.s.	.808**
Log (spores per lesion)[x]	.231 n.s.	.782**
Log (reproductive efficiency)[y]	.191 n.s.	.815**

[u]Correlations included each cultivar in experiment 2, except P. avium 'Angela'.

[v]Mean of four replications over three leaf ages for each cultivar.

[w]Mean of five replications for each cultivar.

[x]Total spores per lesion 36 days after inoculation.

[y]36 days after inoculation.

[z]n.s., ** = correlation coefficient (r) not significant at $p = 0.05$ or significant at $p = 0.01$, respectively.

on its host (components of resistance and pathogenicity), and the ability of the host to endure the presence of the pathogen (tolerance). Prediction of disease severity for a compound interest disease requires an understanding of each of these factors and their interrelationships.

A model has been developed which predicts infection of Montmorency sour cherry from measurements of leaf wetness duration and air temperature (4). The components of resistance measured in this study should allow incorporation of a host resistance factor into the model. The model would then predict not only infection, but also the relative amount of inoculum present at the next infection period. The effect of changes in host resistance components on disease severity could be predicted in different simulated environments. These predictions could then be used to establish the level of resistance (or component of resistance) needed in a breeding program.

Prediction of leaf spot severity must also account for changes in resistance due to leaf age (6). The highly significant cultivar x leaf age interactions in the present study indicate that the effect of leaf age is not uniform over all genotypes. Most of this interaction could be attributed to differences between species (Figures 1 to 5), but some important differences within species remained. For example, sporulation in lesions on young leaves of North Star sour cherry was considerably lower than that in older leaves, but sporulation was generally highest in youngest leaves of other sour cherry cultivars. Thus, prediction of relative leaf spot

severity among cultivars or species requires that adjustment be made for changes in relative proportions of leaf age classes during the growing season (5). Also, the level of resistance of a cultivar under conditions of vigorous vegetative growth (where leaf emergence would cease later in the season) may be quite different from that under a less vigorous condition due to differences in the relative proportion of leaf age classes.

Identification of components of resistance contributing to observed differences in field resistance provides a systematic approach to breeding for partial resistance. The components can be selected in a breeding program following artificial inoculation under controlled conditions. However, associations between components must be considered. For example, a negative correlation was observed between spore production per lesion and lesion number per inoculated area in these studies. Selection based solely on fewer spores per lesion (or smaller lesion size) could lead to indirect selection for increased infection efficiency. A better selection criterion is reproductive efficiency, which is the combined effect of the components of infection efficiency and spores per lesion. Reduced reproductive efficiency could be due to reduced infection efficiency or reduced spore production or both. In addition, reproductive efficiency in these studies was measured more rapidly than any other component when a quantitative inoculator (11) was used and spore production was estimated by absorbance measurements of spore washes.

Improvement in the level of partial resistance in these cherry species should be possible by selection for components of

resistance. Genetic variation was large for each component in experiment 2, except infection efficiency. Reproductive efficiency differed 500-fold among the cultivars at 36 days after inoculation (Table 3). However, the rate of improvement in resistance will be affected by the number and nature of the genes controlling expression of the component selected. Initial studies of the inheritance of components of resistance in juvenile seedlings of P. cerasus and P. gondouinii indicate that heritabilities calculated on an individual plant basis are quite low (13), suggesting that the rate of improvement will be slow. This study should be considered only preliminary, though, because there were large differences from experiment to experiment in expression of resistance among progenies. More work is needed to determine the applicability of this approach to breeding leaf spot resistant cherries.

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CHAPTER III

INHERITANCE OF RESISTANCE TO *COCCOMYCES HIEMALIS* IN JUVENILE SEEDLINGS OF CHERRY

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Abstract

Components of resistance to *Coccomyces hiemalis* Higg. of infection efficiency, lesion area, spores per lesion and reproductive efficiency were studied in an incomplete diallel of four *Prunus cerasus* L. cultivars and one *P. gondouinii* Rehd. cultivar. A total of 342 progeny from 14 families plus clonal parent material were inoculated in a series of three experiments that differed in average age of plants. All components of resistance differed among both parents and families. Mean values for each component differed from experiment to experiment in both parents and progenies. Experiment by family interactions were present in all components except spores per lesion. No genetic variation between families was detected in the first experiment involving the youngest seedlings. General and specific combining ability effects were present among families in the second experiment. Only general combining ability effects were detected in the third experiment. *P. cerasus*

'North Star' had the highest breeding value for reducing spores per lesion and reproductive efficiency. Overall broad-sense heritabilities calculated on a single-plant basis were less than 0.5 for all components except lesion area in progeny.

Introduction

Cherry leaf spot, caused by Coccomyces hiemalis, is a serious fungal disease of sour cherry (Prunus cerasus) throughout the world (3). Yield, vegetative growth, and wood and bud hardiness of sour cherry are measurably reduced for up to two seasons following severe defoliation by C. hiemalis (5,8). 'Montmorency' sour cherry, the predominant cultivar in the Michigan sour cherry industry (2), is very susceptible to defoliation by C. hiemalis (14). Increased resistance to C. hiemalis is an important objective of the sour cherry breeding program at Michigan State University.

Previous work (4,9,13,14,17) has demonstrated that variation for resistance to C. hiemalis occurs within and among several species of cultivated cherries. Complete resistance was not observed in most of these studies. Instead, cultivars of sour cherry, sweet cherry (P. avium L.) and duke cherry (P. gondouinii) differed quantitatively in the levels of partial resistance to C. hiemalis. In addition, factors associated with disease development, called components of resistance, differed among cultivars of these species. Components of resistance that measured lesion development and sporulation in infected leaves were the best predictors of resistance in the field (15).

Little information exists on the inheritance of resistance to C. hiemalis in cultivated cherries. Enikeyev (6) reported that certain cultivars of sweet and sour cherry were better parents than others in breeding cherries resistant to C. hiemalis. Crosses involving cultivars of the European ground cherry (P. fruticosa Pall.) gave higher percentages of susceptible seedlings than crosses of P. cerasus or P. avium cultivars.

This research was undertaken to determine the inheritance of components of resistance to C. hiemalis in progenies of cultivars of P. cerasus and P. gondouinii.

Materials and Methods

Plant material. An incomplete diallel of unequal progeny numbers per cross was constructed in 1980 with 4 cultivars of P. cerasus ('English Morello', 'Meteor', 'North Star' and 'HTO 405') and 1 cultivar of P. gondouinii ('Kansas Sweet'). A total of 342 progeny from 14 crosses were used, with each parent represented in 4 to 6 crosses. Seeds from each cross were stratified at 1-4 C for 4-6 months until germination occurred. Germinated seeds were planted in peat pellets (Jiffy-7, Jiffy Products Ltd., Norway) covered with perlite in presterilized flats in a greenhouse at 18 ± 6 C. Seedlings were transplanted at the 1- to 3-leaf stage in January, 1981, into sand:peat:perlite (1:1:1, v/v) in 10-cm diameter clay pots, and fertilized with 5 g/L solution of 20% N-20% P_2O_5-20% K_2O fertilizer (Robert B. Peters, Co., Inc., Allentown, PA 18104) plus 1.3 g per pot of 19% N-6% P_2O_5-12% K_2O controlled release fertilizer (Osmocote,

Sierra Chemical Co., Milpitas, CA 95035). All seedlings were sprayed prior to emergence of the inoculated leaf with four weekly applications of 500 ppm gibberellic acid (Pro-Gibb, Abbott Laboratories, North Chicago, IL 60064).

Single-shoot trees of each parent cultivar except 'HTO 405' were grown as previously described (experiment 2 in (15)) in the same greenhouse as the seedlings. Parent performance of 'HTO 405', a bud mutation of P. cerasus 'Montmorency' from Hilltop Orchards and Nurseries, Inc., Hartford, MI 49057, was estimated with single-shoot trees of 'Montmorency'. Three trees of each cultivar were included as controls at each date of inoculation.

Inoculation. Seedlings were inoculated when 2- to 3-months old in three separate experiments. Progeny from each cross were divided into three approximately equal-number groups. Group one from each cross was inoculated on March 11, 1981; group two was inoculated on March 19, 1981; and group three was inoculated on March 27, 1981.

Previous studies have demonstrated that components of resistance to C. hiemalis in Prunus species are affected by leaf age (15). Therefore, a single leaf, 7- to 14-days old at the time of inoculation, was inoculated with a modified Schein quantitative inoculator as previously described (15). All plants were randomized prior to inoculation. An average of 6360, 2150 and 2870 conidia per leaf were applied in the March 11, March 19 and March 27 experiment, respectively. Plants were placed into a mist chamber at 18-21 C for 48 hr after inoculation, and then incubated in a cheesecloth tent in the greenhouse (15) at 20 ± 6 C.

Components of resistance. Infection efficiency, lesion area, spores per lesion and reproductive efficiency were determined 20 days after inoculation as previously described (15). Spore production was estimated from the absorbance at 700 nm of spore suspensions (10). A standard curve of hemacytometer counts of conidia to absorbance was established for each experiment. Examination of residual patterns and correlation coefficients indicated that spore counts in all three experiments were best estimated by the linear and cubic terms of absorbance measurements.

Data analysis. Analysis of variance for each component of resistance were performed on parent and progeny data from each experiment as well as the combined data for all experiments. Equal numbers of progeny per experiment was assumed for a given cross in the analysis of the combined progeny data (16, p. 477). Estimates of general combining ability (GCA) and specific combining ability (SCA) were made for each component of resistance in individual experiments by Gilbert's procedure (7) for incomplete diallels with unequal numbers of progeny per cross.

Broad-sense heritability estimates for each component of resistance were calculated on an individual-plant basis from the analysis of variance for parents and progenies in each experiment. Parent estimates were calculated as follows:

$$h^2_{BS} = \hat{\sigma}^2_{bc}/(\hat{\sigma}^2_{bc} + \hat{\sigma}^2_{wc})$$

where h^2_{BS} is the broad-sense heritability estimate, $\hat{\sigma}^2_{bc}$ is the between-clone component of variance and $\hat{\sigma}^2_{wc}$ is the within-clone component of variance.

Progeny broad-sense heritabilities were calculated using $\hat{\sigma}^2_{wc}$ as an estimate of environmental variance by the following equations:

$$h^2_{BS} = (\hat{\sigma}^2_{bp} + \hat{\sigma}^2_{wp})/(\hat{\sigma}^2_{bp} + \hat{\sigma}^2_{wp} + \hat{\sigma}^2_{wc})$$

where $\hat{\sigma}^2_{bp}$ is the between-progeny component of variance and $\hat{\sigma}^2_{wp}$ is the within-progeny component of variance. Coefficients of average progeny number in the expected mean squares were calculated assuming random effects for samples of unequal sizes (16, p. 289).

Broad-sense heritabilities of the combined data from all three experiments were corrected for clone by experiment and cross by experiment interactions. The component of variance due to the interaction of experiments with clones and experiments with crosses was included in the denominator of the equations for broad-sense heritabilities of parents and progenies, respectively.

Results

Cultivars differed significantly in all 4 components of resistance (Table 1). Cultivar by experiment interactions were not significant, but significant ($p = 0.01$) experiment to experiment variation was detected for each component. Although the relative ranking of cultivars differed from experiment to experiment, the components of infection efficiency, lesion area and reproductive efficiency were lowest overall in 'Kansas Sweet'. 'North Star' had

TABLE 1.--Components of resistance to Coccomyces hiemalis in parent cultivars of each experiment.

Experiment	Parent	Component of resistance[w]			
		infection efficiency[x] (%)	lesion area (mm^2)	spores per lesion (log)	reproductive efficiency[y] (log)
1	Montmorency	2.0 ab[z]	0.67 ab	4.86 ab	3.15 a
	North Star	2.4 ab	0.35 b	4.49 bc	2.85 a
	Meteor	2.4 ab	0.94 a	4.98 a	3.34 a
	English Morello	3.2 a	0.43 bc	4.57 bc	3.05 a
	Kansas Sweet	1.1 b	0.28 c	4.31 c	2.30 b
2	Montmorency	1.3 a	1.05 bc	5.14 ab	2.95 b
	North Star	2.7 a	0.94 c	4.84 c	3.23 ab
	Meteor	1.9 a	1.74 a	5.22 a	3.49 a
	English Morello	1.0 a	1.60 ab	5.00 abc	2.98 ab
	Kansas Sweet	1.2 a	1.07 bc	4.91 bc	2.81 b
3	Montmorency	3.4 ab	1.03 a	4.96 a	3.49 a
	North Star	4.0 ab	0.63 bc	4.61 a	3.21 ab
	Meteor	4.0 ab	0.92 ab	4.90 a	3.48 a
	English Morello	4.2 a	0.89 ab	4.83 a	3.43 ab
	Kansas Sweet	2.0 b	0.42 c	4.77 a	3.03 b
Mean	Montmorency	2.2 ab	0.92 b	4.98 a	3.19 ab
	North Star	3.0 a	0.64 c	4.65 b	3.09 b
	Meteor	2.8 a	1.20 a	5.03 a	3.44 a
	English Morello	2.8 a	0.97 ab	4.80 b	3.15 b
	Kansas Sweet	1.4 b	0.59 c	4.66 b	2.71 c

[w]Mean of three single-tree replications measured 20 days after inoculation.

[x]Number of lesions/number of inoculum x 100.

[y]Number of spores/number of inoculum.

[z]Mean separation within columns within experiments by Duncan's multiple range test.

the highest average infection efficiency but the lowest value for spores per lesion. The components of lesion area, spores per lesion and reproductive efficiency were highest overall in 'Meteor'.

Families also differed significantly ($p = 0.01$) for each component of resistance. Highly significant family by experiment interactions were present in each component of resistance except spores per lesion. Variation from experiment to experiment averaged over all families was highly significant for each component.

Analysis of progeny data from each experiment indicated that the amount of genetic variation between families differed from experiment to experiment. In experiment 1, mean values for each component of resistance differed little among families (Table 2), and estimates of total between-family variation, GCA and SCA were not significant (Table 5). Differences among families were much greater in experiments 2 and 3 for all components (Tables 3 & 4). Estimates of total between-family variation, GCA and SCA were significant for each component in experiment 2 (Table 6). Total between-family variation, GCA but not SCA, were significant in experiment 3 (Table 7).

Parent GCA estimates for each component showed little consistency from experiment to experiment (Table 8), but some trends were apparent. 'North Star' progenies had fewer spores per lesion in all experiments and lower reproductive efficiencies in experiments 2 and 3. Lesion areas and spores per lesion were highest in 'Meteor' progenies in experiments 2 and 3, while infection efficiencies were lowest for 'Meteor' in these same experiments.

TABLE 2.--Components of resistance to Coccomyces hiemalis in progeny of experiment 1.

Family	Number of progeny	Component of resistance (mean ± standard deviation)			
		Infection efficiency[x] (%)	Lesion area (mm^2)	Spores per lesion (log)	Reproductive efficiency[y] (log)
HTO 405 x HTO 405	10	2.53 ± 0.87	0.88 ± 0.39	4.65 ± 0.19	3.02 ± 0.13
HTO 405 x North Star	20	2.90 ± 0.76	0.70 ± 0.21	4.56 ± 0.23	3.01 ± 0.22
HTO 405 x Meteor	8	2.30 ± 0.83	0.97 ± 0.27	4.64 ± 0.11	2.97 ± 0.16
HTO 405 x Kansas Sweet	15	2.45 ± 1.03	0.89 ± 0.32	4.58 ± 0.22	2.93 ± 0.12
North Star x Meteor	7	2.01 ± 0.54	1.15 ± 0.37	4.62 ± 0.30	2.91 ± 0.25
North Star x Kansas Sweet	4	2.08 ± 1.15	1.11 ± 0.43	4.62 ± 0.27	2.86 ± 0.21
Meteor x HTO 405	7	2.14 ± 0.50	0.94 ± 0.25	4.63 ± 0.12	2.94 ± 0.16
Meteor x Meteor	10	2.78 ± 0.78	0.87 ± 0.27	4.64 ± 0.20	3.06 ± 0.20
Meteor x English Morello	6	2.83 ± 1.29	1.10 ± 0.78	4.51 ± 0.22	2.91 ± 0.10
Meteor x Kansas Sweet	2	2.47 ± 0.64	1.10 ± 0.69	4.64 ± 0.10	3.02 ± 0.02
English Morello x HTO 405	11	2.10 ± 0.89	1.04 ± 0.40	4.69 ± 0.17	2.97 ± 0.10
English Morello x North Star	21	2.38 ± 0.91	0.90 ± 0.32	4.61 ± 0.23	2.96 ± 0.21
English Morello x English Morello	10	2.66 ± 0.79	0.84 ± 0.38	4.59 ± 0.16	2.99 ± 0.16
English Morello x Kansas Sweet	3	1.93 ± 1.06	0.99 ± 0.47	4.73 ± 0.15	2.97 ± 0.08
All progeny	134	2.47 ± 0.88	0.91 ± 0.96	4.61 ± 0.20	2.97 ± 0.17
Mid-parent value[z]		2.32	0.57	4.70	3.03

[x]Number of lesions/number of inoculum x 100.

[y]Number of spores/number of inoculum.

[z]Mid-parent value based on clonal performance weighted by number of progeny per cross.

TABLE 3.--Components of resistance to Coccomyces hiemalis in progeny of experiment 2.

Family	Number of progeny	Component of resistance (mean ± standard deviation)			
		Infection efficiency[x] (%)	Lesion area (mm^2)	Spores per lesion (log)	Reproductive efficiency[y] (log)
HTO 405 x HTO 405	12	3.50 ± 1.42	1.32 ± 0.86	4.91 ± 0.18	3.43 ± 0.23
HTO 405 x North Star	18	3.31 ± 1.77	0.91 ± 0.43	4.67 ± 0.20	3.11 ± 0.35
HTO 405 x Meteor	8	3.01 ± 0.75	1.36 ± 0.50	4.71 ± 0.21	3.18 ± 0.22
HTO 405 x Kansas Sweet	11	2.42 ± 0.52	1.02 ± 0.48	4.81 1 0.06	3.18 ± 0.10
North Star x Meteor	4	1.83 ± 0.41	2.16 ± 0.54	4.77 ± 0.19	3.02 ± 0.10
North Star x Kansas Sweet	4	0.98 ± 0.41	2.64 ± 0.93	4.92 ± 0.05	2.88 ± 0.19
Meteor x HTO 405	6	2.38 ± 0.56	1.62 ± 0.68	4.80 ± 0.21	3.17 ± 0.23
Meteor x Meteor	11	1.06 ± 0.30	2.10 ± 1.20	5.04 ± 0.18	3.05 ± 0.19
Meteor x English Morello	4	2.00 ± 0.53	1.23 ± 0.61	4.77 ± 0.20	3.06 ± 0.14
Meteor x Kansas Sweet	2	4.65 ± 3.49	1.26 ± 0.14	4.94 ± 0.09	3.53 ± 0.45
English Morello x HTO 405	8	2.17 ± 1.35	1.23 ± 0.58	4.97 ± 0.09	3.24 ± 0.31
English Morello x North Star	7	2.74 ± 1.01	1.67 ± 0.61	4.95 ± 0.20	3.36 ± 0.23
English Morello x English Morello	12	4.24 ± 1.59	1.50 ± 0.83	4.74 ± 0.17	3.34 ± 0.16
English Morello x Kansas Sweet	1	1.58	0.31	4.48	2.68
All progeny	108	2.74 ± 1.52	1.42 ± 0.81	4.83 ± 0.20	3.20 ± 0.27
Mid-parent value[z]		1.57	1.29	5.06	3.14

[x]Number of lesions/number of inoculum x 100.

[y]Number of spores/number of inoculum.

[z]Mid-parent value based on clonal performance weighted by number of progeny per cross.

TABLE 4.--Components of resistance to Coccomyces hiemalis in progeny of experiment 3.

Family	Number of progeny	Component of resistance (mean ± standard deviation)			
		Infection efficiency[x] (%)	Lesion area (mm^2)	Spores per lesion (log)	Reproductive efficiency[y] (log)
HTO 405 x HTO 405	11	6.21 ± 2.00	0.71 ± 0.21	4.67 ± 0.13	3.44 ± 0.16
HTO 405 x North Star	25	5.10 ± 1.32	0.68 ± 0.24	4.65 ± 0.25	3.34 ± 0.26
HTO 405 x Meteor	7	4.41 ± 0.84	0.98 ± 0.45	4.78 ± 0.15	3.42 ± 0.13
HTO 405 x Kansas Sweet	11	5.29 ± 2.01	0.75 ± 0.25	4.72 ± 0.19	3.42 ± 0.13
North Star x Meteor	5	4.75 ± 0.48	0.66 ± 0.18	4.41 ± 0.24	3.08 ± 0.26
North Star x Kansas Sweet	4	4.65 ± 1.17	0.90 ± 0.45	4.46 ± 0.13	3.12 ± 0.08
Meteor x HTO 405	5	4.26 ± 0.85	1.11 ± 0.53	4.83 ± 0.11	3.46 ± 0.15
Meteor x Meteor	11	3.95 ± 1.02	1.07 ± 0.20	4.93 ± 0.18	3.51 ± 0.11
Meteor x English Morello	4	4.56 ± 1.22	0.91 ± 0.27	4.86 ± 0.36	3.51 ± 0.26
Meteor x Kansas Sweet	1	4.46	0.74	4.75	3.40
English Morello x HTO 405	10	4.91 ± 1.12	0.70 ± 0.21	4.74 ± 0.25	3.42 ± 0.24
English Morello x English Morello	6	4.50 ± 1.02	0.79 ± 0.28	4.73 ± 0.18	3.37 ± 0.21
All progeny	100	4.91 ± 1.43	0.80 ± 0.31	4.71 ± 0.23	3.39 ± 0.22
Mid-parent value[z]		3.64	0.85	4.85	3.40

[x]Number of lesions/number of inoculum x 100.

[y]Number of spores/number of inoculum.

[z]Mid-parent value based on clonal performance weighted by number of progeny per cross.

TABLE 5.--Mean squares for components of resistance to Coccomyces hiemalis in progeny of experiment 1.

Source of variation	d.f.	Component of resistance			
		infection efficiency (%)	lesion area (units)[y]	spores per lesion (log)	reproductive efficiency (log)
Additive effects (GCA)	4	.2073 n.s.[z]	103880 n.s.	.0067 n.s.	.0179 n.s.
Interactions (SCA)	9	1.1784 n.s.	142660 n.s.	.0262 n.s.	.0225 n.s.
Total (between cross)	13	.8796 n.s.	130728 n.s.	.0202 n.s.	.0211 n.s.
Error (within cross)	120	.7614	104435	.0427	.0311
Coefficient of variation (%)		35.0	39.2	4.5	6.0

[y] 900 units = 1.0 mm^2.

[z] n.s. = not significant at $p = 0.05$.

TABLE 6.--Mean squares for components of resistance to Coccomyces hiemalis in progeny of experiment 2.

Source of variation	d.f.	Component of resistance			
		infection efficiency (%)	lesion area (units)[y]	spores per lesion (log)	reproductive efficiency (log)
Additive effects (GCA)	4	12.38**[z]	1838174**	.0769*	.2925**
Interactions (SCA)	9	5.88**	1178382**	.1554**	.1519**
Total (between crosses)	13	7.88**	1381396**	.1312**	.1951**
Error (within crosses)	94	1.55	420433	.0294	.0579
Coefficient of variation (%)		45.3	50.3	3.5	7.5

[y] 900 units - 1 mm^2.

[z] *, ** = significant at $p = 0.05$ and $p = 0.01$, respectively.

TABLE 7.--Mean squares for components of resistance to Coccomyces hiemalis in progeny of experiment 3.

Source of variation	d.f.	Component of resistance			
		infection efficiency (%)	lesion area (units)[y]	spores per lesion (log)	reproductive efficiency (log)
Additive effects (GCA)	4	7.36**[z]	337573**	.2561**	.1841**
Interactions (SCA)	7	1.09 n.s.	73886 n.s.	.0761 n.s.	.0564 n.s.
Total (between crosses)	11	3.37 n.s.	169772*	.1416**	.1028**
Error (within crosses)	88	1.87	64329	.0436	.0400
Coefficient of variation (%)		28.0	35.1	4.4	5.9

[y]900 units = 1 mm^2.

[z]n.s., *, ** = not significant, significant at $p = 0.05$, and significant at $p = 0.01$, respectively.

TABLE 8.--General combining ability (GCA) estimates for components of resistance to *Coccomyces hiemalis* in each experiment.

Experiment	Parent	Component of resistance GCA estimate (± standard error)			
		Infection efficiency[y] (%)	Lesion area (mm^2)	Spores per lesion (log)	Reproductive efficiency[z] (log)
1	HTO-405	1.27 ± 0.10	0.412 ± 0.039	2.31 ± 0.02	1.50 ± 0.02
	North Star	1.28 ± 0.13	0.408 ± 0.054	2.28 ± 0.03	1.48 ± 0.03
	Meteor	1.25 ± 0.11	0.510 ± 0.044	2.31 ± 0.03	1.50 ± 0.02
	English Morello	1.20 ± 0.10	0.479 ± 0.042	2.31 ± 0.02	1.49 ± 0.02
	Kansas Sweet	1.06 ± 0.19	0.529 ± 0.077	2.30 ± 0.04	1.44 ± 0.04
2	HTO-405	1.73 ± 0.14	0.477 ± 0.079	2.41 ± 0.02	1.67 ± 0.03
	North Star	1.22 ± 0.23	0.810 ± 0.134	2.36 ± 0.03	1.49 ± 0.04
	Meteor	0.71 ± 0.15	1.007 ± 0.089	2.47 ± 0.02	1.53 ± 0.03
	English Morello	1.79 ± 0.15	0.711 ± 0.089	2.41 ± 0.02	1.66 ± 0.03
	Kansas Sweet	0.79 ± 0.31	0.746 ± 0.177	2.43 ± 0.04	1.51 ± 0.06
3	HTO-405	2.88 ± 0.16	0.372 ± 0.032	2.37 ± 0.02	1.74 ± 0.02
	North Star	2.31 ± 0.26	0.296 ± 0.020	2.21 ± 0.04	1.55 ± 0.04
	Meteor	1.94 ± 0.17	0.542 ± 0.035	2.44 ± 0.03	1.73 ± 0.02
	English Morello	2.23 ± 0.23	0.377 ± 0.047	2.37 ± 0.03	1.69 ± 0.03
	Kansas Sweet	2.40 ± 0.36	0.424 ± 0.073	2.32 ± 0.05	1.65 ± 0.05

[y]Number of lesions/number of inoculum x 100.

[z]Number of spores/number of inoculum.

Broad-sense heritability estimates for both parents and progenies varied considerably from experiment to experiment (Table 9). Overall estimates on a single-plant basis are less than 0.5 for all components except lesion areas in progenies (h^2_{bs} = 0.68). Most of the genetic variance for lesion areas in all three experiments was attributed to the within-family rather than between-family variance component.

Discussion

Selection for components of resistance has been suggested as a means of increasing the level of partial resistance in cultivated cherries to C. hiemalis (15). Reproductive efficiency, which is the combined effect of the components of infection efficiency and spore production, is highly correlated with measurements of field resistance to defoliation in cherry. Reproductive efficiency can be measured rapidly by inoculation of individual plants with a quantitative inoculator and measurement of spore production from absorbance of spore suspensions. It is estimated that the total time required to measure leaf age, inoculate, and measure spore production is less than ten minutes for each plant. Susceptible genotypes, if identified by such a procedure, could be eliminated early in the breeding cycle, thus increasing efficiency of land utilization in the breeding program.

The adoption of this procedure to screen for partial resistance to C. hiemalis in cherry depends not only on development of methods that rapidly estimate components of resistance that contribute

TABLE 9.--Parent and progeny broad-sense heritability estimates for components of resistance to *Coccomyces hiemalis*[y]

Component of resistance		Experiment			
		1	2	3	overall[z]
Infection efficiency	Parent	.35	.10	.25	.23
	Progeny	.15	.56	.40	.28
Lesion area	Parent	.64	.46	.57	.39
	Progeny	.74	.84	.61	.68
Spores per lesion	Parent	.59	.55	.10	.47
	Progeny	.01	.62	.35	.40
Reproductive efficiency	Parent	.65	.39	.32	.43
	Progeny	.00	.20	.14	.02

[y]All heritabilities calculated on a single-plant basis; within-clone variance was used as an estimate of environmental variance in both parents and progenies.

[z]Estimated from combined data from experiments 1 to 3, corrected for experiment by clone and experiment by cross interactions.

significantly to resistance in the field. The genetics of components of resistance and factors affecting expression of that resistance are also very important. The large experiment by family interactions and the low heritabilities observed in this study question the suitability of this approach to breeding cherries resistant to leaf spot. Further work is needed to explain the nature of this interaction.

The low heritability estimates for components in experiment 1 relative to experiments 2 and 3 (Table 9) appear to be due to a lack of expression of resistance in that experiment rather than increased error. Although numbers of conidia inoculated and incubation conditions varied from experiment to experiments, differences among clonal parent cultivars were detected for most components of resistance in each experiment (Table 1). Coefficients of variation within families were similar for all three experiments (Tables 5 to 7), but the progeny means for families in experiment 1 differed little for any component of resistance (Table 2). This lack of expression of resistance could be related to age of the seedlings, which were youngest, on average, in experiment 1. There may be a minimum plant age for expression of resistance.

The mid-parent value for infection efficiency in each experiment (based on clonal performance) was considerably less than the overall progeny mean (Tables 2 to 4). This deviation could not be consistently attributed to specific families, and may be an effect of juvenility on expression of resistance. Increased susceptibility of juvenile tissue to infection has been reported for other host-pathogen combinations (12).

Progeny means for all components of resistance appear to be continuous in nature. No evidence of discrete classes were observed in any family. This would suggest that the genetic control of each component is polygenic. The low heritabilities support this conclusion. It appears unlikely that an individual component of resistance gene can be rapidly fixed in the breeding population. However, the significant GCA effects observed in experiments 2 and 3 indicate that selection of parents will be effective in increasing the level of partial resistance.

Of the five cultivars evaluated in this study, 'North Star' appears to have the best breeding value for increasing partial resistance to C. hiemalis. GCA estimates for spores per lesion and reproductive efficiency were lowest for this cultivar in experiments 2 and 3. These components, in turn, are good indicators of field resistance to defoliation (15). Crosses should be made involving this cultivar to increase the level of resistance in the breeding population. The method of identification of resistant genotypes resulting from such crosses remains to be determined. Precise field evaluation of partial resistance can be time consuming (14). The low heritabilities observed in this study indicate that individual plant selection by component of resistance evaluation will be ineffective. Selection for traits with low heritabiities can be improved by progeny tests or clonal family evaluations (1). It may be possible to adapt these improved selection methods to greenhouse component of resistance evaluations without greatly increasing the length of the breeding cycle.

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CHAPTER IV

VARIABILITY OF *COCCOMYCES HIEMALIS* TO COMPONENTS OF RESISTANCE IN *PRUNUS* SPECIES

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Abstract

Components of resistance to six isolates of *Coccomyces hiemalis* were evaluated in eight cherry cultivars representing three *Prunus* species. Eight-fold differences in infection efficiency were observed among *C. hiemalis* isolates averaged over all cultivars. Isolates also differed in size of lesions produced, numbers of spores per lesion and reproductive efficiency (ratio of spores produced to inoculum applied) on hosts 20 days after inoculation. Numbers of spores per lesion, averaged over all isolates, varied 20-fold among the cultivars. Infection efficiency, time of lesion appearance, lesion size and reproductive efficiency also differed among cultivars. Lesions on *P. avium* cultivars were smaller with fewer spores per lesion than lesions on other cultivars. Cultivar x isolate interactions were not significant for all components of resistance. No evidence of specific interaction of host resistance genes with pathogen virulence genes was observed.

Introduction

Coccomyces hiemalis Higg. causes a serious leaf spot disease of sour cherry (Prunus cerasus L.) and related cherry species in Michigan and other cherry production regions in the world (1). Severe infection by C. hiemalis results in premature defoliation of trees. This defoliation can measurably reduce yield, vegetative growth and wood and bud hardiness of sour cherry (2,5).

Genetic differences exist among cultivars of sour cherry, sweet cherry (P. avium L.) and duke cherry (P. gondouinii Rehd.) in resistance to infection and defoliation by C. hiemalis (15). Greenhouse studies indicated that these differences were due in part to components of resistance that affected lesion development and sporulation in infected leaves (16).

Specialization by C. hiemalis is known to occur at the species level of the host (4,8). However, little information exists on the relative ability of C. hiemalis isolates to infect cultivated cherries. Magie (12) observed large differences in the average ability of an isolate to infect and sporulate on cherry hosts, but no consistent differences were found in relative virulence on a limited number of hosts. The purpose of this research was to examine the variability of C. hiemalis isolates to previously identified differences in components of resistance in cultivars of three Prunus species.

Materials and Methods

Plant material. Cultivars used in this study were selected to represent a range in previously identified components of resistance (16). Trees of four cultivars of P. cerasus (Montmorency, North Star, Meteor and English Morello), one cultivar of P. gondouinii (Kansas Sweet), and three cultivars of P. avium (Governor Wood, Napoleon and Yellow Glass) were grown on P. mahaleb L. rootstocks in the greenhouse at 19 ± 6 C as previously described (experiment 2 in (16)), except that each tree was trained to have six lateral shoots.

Isolates of Coccomyces hiemalis. Six monoconidial isolates collected from a range of cherry hosts and geographic areas were used in this study. Each isolate came from sporulating lesions on naturally infected hosts. Isolates were grown on an agar media containing (per liter): KH_2PO_4, 1.9 g; $MgSO_4 \cdot 7H_2O$, 1.0 g, $CaCl_2$, 0.1 g; K_2HPO_4, 0.1 g; $CuSO_4 \cdot 5\ H_2O$, 1.0 mg; $MnSO_4 \cdot H_2O$, 0.06 mg; $ZnCl_2$, 0.04 mg; $Na_2\ MoO_4 \cdot 2H_2O$, 0.05 mg; H_3BO_3, 0.06 mg; $FeSO_4 \cdot 7\ H_2O$, 1.0 mg; Na_2EDTA, 1.2 mg; Thiamine HCl, 0.5 mg; Difco yeast extract, 4.0 g; and sucrose, 20 g. Isolates were grown in culture until sporulation occurred, and then maintained by periodically inoculating young leaves of the same hosts from which they were isolated. Leaves with sporulating lesions were frozen at -20 C up to 6 months between inoculations.

Inoculation. The experiment was conducted in a split-plot design replicated five times with cultivars as the main plot blocked

by the time of inoculation. Isolates were the subplot with a single leaf on each lateral shoot inoculated with one isolate. Only 11- to 14-day-old leaves were inoculated.

Conidia of each isolate were washed from 2- to 3-week-old sporulating lesions on infected leaves into distilled deionized water. Concentration of each isolate was adjusted to approximately 10^5 conidia per ml by measuring absorbance of conidial suspensions at 700 nm (16). A single 2.1 cm^2 area on each leaf was inoculated using a modified Schein quantitative inoculator (14). Numbers of conidia deposited per leaf for each isolate were estimated by counting conidia deposited on a single membrane filter section (16) for each replication. Average number of conidia deposited on filter sections were 1400, 2500, 2000, 1800, 2300 and 1800 for isolates A, B, C. D, E and F, respectively. Germination of all isolates on filter sections exceeded 90%.

Trees were placed into a mist chamber immediately after inoculation for 48 hr at 15 to 18 C. Trees were then incubated in a cheesecloth tent on a greenhouse bench (16) at 18 C (range 13 to 23 C).

Components of resistance. Infection efficiency, lesion area, and reproductive efficiency were determined 20 days after inoculation as previously described (16). Spores per lesion were determined by hemacytometer counts of spores washed from leaves 20 days after inoculation into 5 ml of 2% (w/v) formaldehyde plus 0.1% (v/v) polyoxyethylene sorbitan monolaurate (Tween 20, Sigma Chemical Co., St. Louis, MO 63178) in distilled deionized water. Lesion areas, spores

per lesion and reproductive efficiency measurements were $\log_{10}$ transformed prior to analysis of variance to eliminate proportionality between the means and their standard deviations (11).

Previous work indicated that the proportion of visible lesions approaches the total number of lesions asymptotically with time after inoculation (9,16). However, fitting an asymptotic curve to this data was unsatisfactory due to the large number of leaves with 10 or fewer total lesions. Therefore, the estimated time to 50% of lesions present was calculated by an adaptation of the Spearman-Kärber method (3) for estimating LD_{50} for quantal response data:

$$LP_{50} = \sum_{i=1}^{n} (p_{i+1} - p_i)((t_{i+1} + t_i)/2)$$

where LP_{50} is the natural log of the estimated number of days to 50% of lesions present; t_i is the natural log of days after inoculation at evaluation i; p_i is the proportion of lesions present at evaluation i; and i is 1 to 10. Lesions were counted at 1 to 2-day intervals from 4 to 20 days after inoculation.

Data analysis. Data for each component of resistance were subjected to analysis of variance. Evidence for the presence or absence of specific interactions between isolates and hosts for each component of resistance was determined by the significance of the interaction term in the analysis of variance (17).

Results

Significant interactions between isolates and hosts were not detected in this experiment for any of the components of resistance (Table 1). However, cultivars differed significantly for each component of resistance. In addition, significant differences were observed among isolates for infection efficiency, size of lesions, numbers of spores per lesion and reproductive efficiency.

Isolates differed more than 8-fold in average infection efficiency (Table 2). This large difference in infection efficiency accounted for most of the difference in reproductive efficiency, as isolates with high infection efficiencies (isolates B, C and D) also had high reproductive efficiencies. However, no consistent relationship was observed between the average infection efficiency and the size of lesions or numbers of spores per lesion, or between size of lesions and numbers of spores per lesion (Table 2). For example, average lesion area was largest for isolate A and smallest for isolate F, but these isolates did not differ significantly in infection efficiency. Furthermore, average lesion area did not differ significantly between isolates D and E, but numbers of spores per lesions did differ between these isolates.

Average infection efficiency varied 4-fold among the eight cultivars, from 0.30% for *P. gondouinii* 'Kansas Sweet' to 1.14% for *P. avium* 'Governor Wood' (Table 3). Numbers of spores per lesion varied nearly 20-fold among these cultivars, from 5.37×10^3 for *P. avium* 'Yellow Glass' to 1.02×10^5 for *P. cerasus* 'Meteor'. In

TABLE 1.--Mean squares for components of resistance to six isolates of Coccomyces hiemalis in eight cherry cultivars.[w]

Source	Degrees of Freedom	Component of resistance				
		Infection efficiency (%)	Time to 50% lesions (days)	Lesion area (log)[x]	Spores per lesion (log)	Reproductive efficiency (log)
Replication	4	2.80**[y]	2.60 n.s.	0.175 n.s.	1.05 n.s.	2.19*
Cultivar	7	1.94*	8.60**	2.439**	5.44**	5.23**
Error (a)	28	0.62	2.23	0.126	0.48	0.61
Pathogen isolate	5	11.42**	1.24 n.s.	0.120**	0.72**	9.51**
Cultivar x isolate	35	0.42 n.s.	1.32 n.s.	0.027 n.s.	0.24 n.s.	0.40 n.s.
Error (b)	140[z]	0.38	1.31	0.018	0.20	0.30

[w]Cultivars = Prunus cerasus 'Montmorency', P. cerasus 'North Star', P. cerasus 'Meteor', P. cerasus 'English Morello', P. gondouinii 'Kansas Sweet', P. avium 'Governor Wood', P. avium 'Napoleon', and P. avium 'Yellow Glass'.

[x]Measured in units where 900 units = 1 square millimeter.

[y]n.s., *, ** = not significant, significant at $p = 0.05$, and significant at $p = 0.01$, respectively.

[z]Degrees of freedom for error (b) = 120 for latent period, lesion area and spores per lesion due to missing values.

TABLE 2.--Response of six *Coccomyces hiemalis* isolates to components of resistance in cultivars of *Prunus* species.[v]

Isolate	Host	Source	Component of resistance				
			Infection efficiency[w] (%)	50% of lesions[x] (days)	Lesion area (mm^2)	Spores per lesion (log)	Reproductive efficiency[y] (log)
A	*P. mahaleb* seedling	South Haven, MI.	0.3 b[z]	6.2 a	0.74 a	4.50 ab	1.54 b
B	*P. cerasus* Montmorency	Decatur, MI.	1.3 a	6.1 a	0.53 c	4.37 bc	2.41 a
C	*P. cerasus* Meteor	East Lansing, MI.	1.1 a	6.0 a	0.61 b	4.33 bc	2.30 a
D	*P. cerasus* English Morello	Coloma, MI.	1.2 a	6.3 a	0.65 ab	4.40 bc	2.32 a
E	*P. cerasus* Meteor	Hamburg, IA.	0.3 b	5.9 a	0.68 ab	4.64 a	1.64 b
F	*P. cerasus* Montmorency	East Lansing, MI.	0.2 b	6.4 a	0.50 c	4.21 c	1.27 c

[v]Cultivars = *P. cerasus* Montmorency, *P. cerasus* North Star, *P. cerasus* Meteor, *P. cerasus* English Morello, *P. gondouinii* Kansas Sweet, *P. avium* Governor Wood, *P. avium* Napoleon and *P. avium* Yellow Glass. Values are the mean of five replications per cultivar.

[w]Number of lesions/number of inoculum x 100.

[x]Back-transformed from $LP_{50} = \sum_{i=1}^{n} (p_{i+1} - p_i)((t_{i+1} + t_i)/2)$, where LP_{50} is the natural log of days to 50% of lesions present, t_i is the natural log of days after inoculation at evaluation i, p_i is the proportion of lesions present at evaluation i, and i is 1 to 10.

[y]Number of spores/number of inoculum.

[z]Mean separations within columns by Duncan's multiple range test, $p = 0.05$.

TABLE 3.--Components of resistance to *Coccomyces hiemalis* in cultivars of three *Prunus* species.[v]

Species	Cultivar	Component of resistance				
		Infection efficiency[w] (%)	50% of lesions[x] (days)	Lesion area (mm^2)	Spores per lesion (log)	Reproductive efficiency[y] (log)
P. avium	Governor Wood	1.1 a[z]	6.3 b	0.31 d	3.90 de	1.82 bc
	Napoleon	0.6 bc	5.9 ab	0.33 d	4.16 cd	1.73 bc
	Yellow Glass	0.5 bc	7.3 c	0.26 d	3.73 e	1.26 d
P. cerasus	English Morello	0.9 ab	6.1 ab	1.04 b	4.74 ab	2.42 a
	Meteor	0.8 ab	5.3 a	1.80 a	5.01 a	2.43 a
	Montmorency	0.7 abc	6.1 ab	0.96 bc	4.72 ab	2.15 ab
	North Star	0.6 bc	6.0 ab	0.69 bc	4.49 bc	1.95 bc
P. gondouinii	Kansas Sweet	0.3 bc	6.2 b	0.62 c	4.58 b	1.52 cd

[v]Values are the mean of six isolates with five replications per isolate.

[w]Number of lesions/number of inoculum x 100.

[x]Back-transformed from $LP_{50} = \sum_{i=1}^{n} (p_{i+1} - p_i)((t_{i+1} + t_i)/2)$, where LP_{50} is the natural log of days to 50% of lesions present, t_i is the natural log of days after inoculation at evaluation i, p_i is the proportion of lesions present at evaluation i, and i is 1 to 10.

[y]Number of spores/number of inoculum.

[z]Mean separations within columns by Duncan's multiple range test, $p = 0.05$.

general, large numbers of spores per lesion were associated with a shorter time to lesion appearance, larger lesion areas and higher reproductive efficiency (Table 3).

Discussion

The relative differences in components of resistance among these cultivars averaged over all six isolates are reasonably consistent with the differences previously reported using one of these isolates (isolate B) (16). For example, the small lesions with fewer spores per lesion found in leaves of P. avium cultivars are typical for this species. In addition, the reduced sporulation and reproductive efficiency in leaves of 'Kansas Sweet' and 'North Star' compared to 'Montmorency', 'Meteor' and 'English Morello' confirm earlier observations made in young leaves of these cultivars. However, differences between P. avium cultivars and the other cultivars in days to 50% lesion appearance (Table 3) are less than that previously reported. This discrepancy may be due to differences in the methods used to calculate time of lesion appearance, or it may reflect experiment to experiment variation. In general, though, components of resistance in these Prunus species were accurately estimated by individual isolates of C. hiemalis. Identification of resistant cultivars by components of resistance analysis (16) would be greatly simplified by the use of single isolates.

The absence of significant cultivar x isolate interactions, plus large differences among both cultivars and isolates in components of resistance, suggest that resistance in these cherry species is race-nonspecific in nature (17). However, major limitations to this

work must be resolved before it can be concluded that partial resistance in cherry is stable against all genotypes of C. hiemalis. First, this study tested only a small sample of C. hiemalis genotypes. A larger number of isolates from a wider range of hosts and geographic locations should be tested. Second, the statistical test for interactions used in this study is an average test (11). Biologically significant cultivar x isolate interactions may be masked by a greater number of insignificant interactions. Prior knowledge about these isolates could have led to logical single degree of freedom comparisons within the 35 degrees of freedom for interaction variance in this study. Planned comparisons of this type have demonstrated significant cultivar x isolate interactions when none were detected by an average test over all interactions (10).

A more serious objection to the interaction test was raised by Parlevliet and Zadoks (13). A model in which genes for partial resistance interacted in a gene-for-gene manner with genes for virulence in the pathogen indicated that only a small portion of non-environmental variance would be attributed to the interaction variance. Most of the variance was present as main effects. They concluded that the absence of significant interactions is more an indication of polygenic resistance than evidence of race-nonspecific resistance.

The best test of the stability of a resistance source is its widespread use over a long period of time (6). Detection of erosion in partial resistance can be enhanced by continual monitoring of the pathogen population with a sensitive method of components of resistance evaluation (7).

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POSSIBLE SOURCES OF COMPLETE RESISTANCE TO *COCCOMYCES HIEMALIS* IN *PRUNUS* SPECIES

Abstract

Seedlings and clones representing 15 *Prunus* species were inoculated with a strain of *Coccomyces hiemalis* Higg. isolated from *P. cerasus* L. Complete resistance to this isolate was found in the *Padus* subgenus, the *Pseudocerasus* and *Mahaleb* sections of the *Cerasus* subgenus, and interspecific hybrids between these sections and the *Eucerasus* section of *Cerasus*. Lesions were absent or nonsporulating on inoculated leaves of *P. maackii* Rupr., *P. serotina* Ehrh., *P. virginiana* L., *P. sargenti* Rehd., *P. serrula* Franch., *P. serrulata* Lindl., *P. subhirtella pendula* Tanaka, *P. yedoensis* Mats., *P. hillieri* (*P. japonica* Thunb. x *P. sargenti* Rehd.), *P. avium* L. x *P. pseudocerasus* Lindl. ('Colt'), *P. pennsylvanica* L., and *P. dropmoreana* (*P. cerasus* x *P. pennsylvanica*). Sporulating lesions were present on inoculated leaves of *P. cerasus* 'Montmorency', *P. avium* 'Angela', and two clones of *P. mahaleb* L. Spores per lesion were greatest on 'Montmorency', intermediate on 'Angela' and *P. mahaleb*

'IR 758-1', and lowest on P. mahaleb 'IR 759-2'. Infection efficiency on 'IR 758-1' was reduced relative to 'Montmorency', Angela' and 'IR 759-2'.

Introduction

The Michigan sour cherry (Prunus cerasus l) industry is based almost entirely on one cultivar, 'Montmorency', and strains derived from it (2). 'Montmorency' is very susceptible to infection and defoliation by the cherry leaf spot fungus, Coccomyces hiemalis (15). Severe defoliation of sour cherry by C. hiemalis reduces yield, vegetative growth, and wood and bud hardiness for up to two seasons (4,6). Currently, about five fungicide applications per season are recommended to Michigan sour cherry growers to control this disease (7). An alternative to chemical control is the development of cultivars with resistance to C. hiemalis.

Sources of partial resistance are present in sour cherry, sweet cherry (P. avium) and duke cherry (P. gondouinii) (15). Components of resistance that affect lesion development and sporulation in infected leaves were strongly correlated with the level of resistance to defoliation in the field (16). However, expression of components of resistance in families of juvenile seedlings from P. cerasus and P. gondouinii cultivars was variable, and heritabilities of the components were low (17).

An alternate approach to breeding leaf-spot-resistant sour cherries would be to identify sources of complete resistance in related cherry species, and introduce this resistance into cultivated

cherries by interspecific hybridization. Keitt (9) identified several cherry species that remained free of leaf spot following inoculation with strains of C. hiemalis isolated from P. cerasus or P. avium. The purpose of this research was to evaluate additional cherry species as possible sources of resistance to C. hiemalis.

Materials and Methods

Plant material. Clones and seedlings representing a total of 15 species and interspecific hybrids from the Cerasus and Padus subgenera of Prunus (11) were used. Trees of all species except P. pennsylvanica, P. serotina and P. virginiana were propagated by chip-budding onto P. avium or P. mahaleb rootstacks. Trees of P. pennsylvanica were transplanted from a single clump of native trees growing in Van Buren County, MI. P. serotina and P. virginiana were grown from seed obtained from native trees in Ingham County and Ogemaw County, MI, respectively. Budwood of P. dropmoreana, a selection from the F_2-generation of P. cerasus 'Kozlov' x P. pennsylvanica (8), was obtained from Interstate Nurseries, Inc., Hamburg, IA 51640. Budwood of P. cerasus 'Montmorency' clone 'IR 309-2', P. avium 'Angela', and P. mahaleb clones 'IR 758-1' and 'IR 759-2' were obtained from the U.S.D.A. Interregional Project No. 2 repository in Prosser, WA 99350. Budwood of P. mackii, P. sargenti, P. serrula, P. serrulata 'Kwanzan', P. subhirtella pendula, P. yedoensis, P. japonica x P. sargenti (P. hillieri 'Hillier Spire') and P. avium x P. pseudocerasus ('Colt') were obtained from trees on the campus of Michigan State University, East Lansing, MI.

All trees were grown in sand:peat:perlite (1:1:1, v/v) in 3.7-liter containers in the greenhouse at 13-30 C. Trees were fertilized at bud break with 5.25 g of 19% N-6% P_2O_5-12% K_2O controlled release fertilizer (Osmocote, Sierra Chemical Co., Milpitas, Ca 95035) plus 0.75 g of sustained-release trace elements (Esmigran, Mallinckrodt Inc., St. Louis, MO 63147) per container. A second application of Esmigran (0.75 g) was made 1 month later. Trees were trained to a single shoot by removing lateral shoots weekly.

Inoculation with C. hiemalis. Four trees of each species were inoculated with a strain of C. hiemalis (isolate "B") isolated from a naturally-infected P. cerasus 'Montmorency' tree. Leaves in three leaf age classes (5- to 8-day-old, 17- to 20-day-old and 29- to 32-day-old) were inoculated on each tree with a modified Schein quantitative inoculator (16). About 800 conidia were applied to a 2.1 cm^2 area on each leaf. Trees were placed in a mist chamber immediately after inoculation for 48 hr at 18-21 C. Trees were then incubated in a greenhouse in a cheesecloth tent (16) at 13-27 C. Eight days after inoculation, the inoculated leaves were removed from each tree and placed into plastic boxes lined with moist paper towels for an additional 8 days at 22-30 C. Leaves were then frozen at -20 C until analysis.

The experiment was repeated on the same trees using about 2900 conidia per leaf. Trees were held in the mist chamber 48 hr at 21-24 C and then incubated for 20 days in the cheesecloth tent in the

the greenhouse at 16-30 C. Leaves were then removed from each tree and frozen at -20 C until analysis.

Evaluation of resistance. Number of lesions per leaf were counted and divided by the total number of conidia inoculated to determine infection efficiency (12). Spores were washed from leaves with visible lesions into 5 ml water containing 2% (w/v) formaldehyde plus 0.1% (v/v) polyoxyethylene sorbitan monolaurate (Tween 20). Number of conidia in 8 samples from each leaf wash were counted with a hemacytometer. Lesions were classified as non-sporulating if spore-bearing acervuli were not observed under a dissecting microscope at 40x and if the number of conidia washed from each leaf was less than the number applied as inoculum. Percent infection efficiency values were square root-transformed and spores per lesion counts were log transformed prior to analysis of variance (10).

Results and Discussion

A number of the species were immune to the isolate of C. hiemalis used in this study (Table 1). All members of the Padus subgenus and most members of the Pseudocerasus section of the Cerasus subgenus showed no visible lesions on any leaves following inoculation in both studies. Lesions produced on P. yedoensis and on a P. avium x P. pseudocerasus interspecific hybrid ('Colt') were non-sporulating. P. pennsylvanica of the Mahaleb section of Cerasus subgenus was also immune, and lesions produced on the P. cerasus x P. pennsylvanica hybrid (P. dropmoreana) were non-sporulating.

TABLE 1.--Resistance of cherry species to *Coccomyces hiemalis*[s]

Species	Clone	Rootstock	Infection efficiency[t] (%)	Lesion type[u]	Spores per lesion (log)
Pseudocerasus section, *Cerasus* subgenus					
P. sargenti	unnamed	*P. avium*	0.00 b[v,z]	--	--
P. serrula	unnamed	*P. avium*	0.00 b	--	--
P. serrulata	'Kwanzan'	*P. avium*	0.00 b	--	--
P. yedoensis	unnamed	*P. avium*	0.22 b	NS	--
P. subhirtella pendula	unnamed	*P. avium*	0.00 b[v]	--	--
Eucerasus section, *Cerasus* subgenus					
P. avium	'Angela'	*P. avium*	1.19 a	SP	3.35 b
P. cerasus 'Montmorency'	IR 309-2	*P. avium*	0.99 a	SP	3.85 ab
P. cerasus 'Montmorency'	IR 309-2	*P. mahaleb*	1.61 a	SP	4.52 a
Mahaleb section, *Cerasus* subgenus					
P. mahaleb	IR 758-1	*P. mahaleb*	0.27 b	SP	3.17 bc
P. mahaleb	IR 759-2	*P. mahaleb*	1.01 a	SP	2.46 c
P. pennsylvanica	unnamed	self-rooted	0.00 b	--	--

TABLE 1.--Continued

Species	Clone	Rootstock	Infection efficiency[t] (%)	Lesion type[u]	Spores per lesion (%)
Padus subgenus					
P. maackii	unnamed	P. mahaleb	0.00 b	--	--
P. serotina	seedlings	self-rooted	0.00 b	--	--
P. virginiana	seedlings	self-rooted	0.00 b	--	--
Interspecific hybrids between sections					
P. dropmoreana[x]	unnamed	P. mahaleb	1.03 a	NS	--
P. avium x P. pseudocerasus	'Colt'	P. avium	0.01 b[v]	NS	--
P. avium x P. pseudocerasus	'Colt'	P. mahaleb	0.04 b[w]	NS	--
P. hillieri[y]	'Hillier spire'	P. avium	0.00 b[v]	--	--

[s]Mean of two experiments, four replications per experiment, three leaves per replication.

[t]Number of lesions/number of inoculum x 100.

[u]Sporulating (SP) or nonsporulating (NS) lesions.

[v]Mean of three replications per experiment.

[w]Mean of two replications per experiment.

[x]P. dropmoreana = P. cerasus 'Kozlov' x P. pennsylvanica

[y]P. hillieri = P. japonica x P. sargenti

[z]Mean separations within columns by Duncan's multiple range test, p = 0.05.

However, at least three of these species are not immune to all strains of C. hiemalis. Keitt (9) observed that P. serotina, P. virginiana and P. pennsylvanica were resistant to isolates of C. hiemalis from P. cerasus or P. avium, but susceptible to strains isolated from naturally-infected members of their own species. Thus, introduction of resistance to strains from P. cerasus by interspecific hybridization could simultaneously introduce susceptibility to other strains of C. hiemalis. Species and hybrids immune to the isolate in this study should be tested against a wide range of isolates, and planted in several locations where environmental conditions are favorable for natural infection by C. hiemalis.

The average infection efficiency and relative spore production of this C. hiemalis isolate on P. cerasus 'Montmorency' and P. avium 'Angela' are consistent with that previously reported for these cultivars (16). Spore production differed significantly ($p = 0.01$) from experiment to experiment in those species with sporulating lesions, but species by experiment interactions were not significant, indicating that the relative sporulation between clones was uniform from experiment to experiment. The two P. mahaleb clones, which were reported resistant to C. hiemalis (5), were not completely resistant. However, infection efficiency in one clone (IR 758-1) was significantly lower than P. avium 'Angela' and P. cerasus 'Montmorency', and sporulation was significantly lower in the other clone (IR 759-2) (Table 1). Interspecific hybrids between P. avium and P. mahaleb that were developed as cherry rootstocks (18) may be potential sources of increased partial resistance to C. hiemalis.

Scab-immune apple cultivars have been successfully developed by crossing completely resistant crab apple species (Malus floribunda) with susceptible apple cultivars (M. pumila), followed by backcrosses to susceptible cultivars. Resistance, controlled by a single dominant gene, was selected in segregating progenies after each backcross (14). Application of a similar approach to the development of leaf spot-resistant cherry cultivars depends not only on the ability to create fertile interspecific hybrids and subsequent backcross progenies, but also upon the number and nature of the genes controlling resistance. If several genes control complete resistance, or if resistance is recessive to susceptibility, intercrossing and selection after each backcross generation may be necessary (1). This intercrossing and selection step after each backcross would greatly lengthen the time required to develop leaf spot-resistant cultivars with desirable horticultural characteristics.

A substantial number of interspecific hybrids between cultivated cherries and potentially leaf spot-immune species have been reported (3,13). Infertility in the F_1 generation of at least one of these hybrids, P. dropmoreana, has been overcome by colchicine treatment of flowers (8). The clone used in this study from the resulting F_2 appears to be immune to C. hiemalis (Table 1), and is fertile (T. M. Sjulin, unpublished). The number or nature of genes controlling resistance in this clone, and the feasibility of backcrosses to cultivated cherry is not known. It is hoped that interspecific hybridization in cherries appears to be promising and should

be pursued for development of leaf spot-immune cultivars, and also for hybrids to test as potential cherry rootstocks (3).

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SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

An important objective of the sour cherry (Prunus cerasus L.) breeding program at Michigan State University is the development of cultivars with increased resistance to Coccomyces hiemalis Higg. The results of this research provide valuable information contributing to that objective. The purpose of this section is to summarize the significant points of the research, and to suggest further work indicated by the results.

Summary of Results

Genetic variation for resistance was clearly demonstrated within and among several species of cultivated cherry in field evaluations. Cultivars of sour cherry, sweet cherry (P. avium L.), duke cherry (P. gondouinii Rehd.) and ground cherry (P. fruticosa Pall.) differed quantitatively in levels of partial resistance to both infection and defoliation (10). Sweet cherries, as a species, were considerably more resistant to defoliation than either sour cherries or duke cherries. Ground cherries were intermediate in resistance.

Greenhouse evaluations of components of resistance confirmed the increased resistance of sweet cherry observed in the field. Although numbers of lesions were similar for all three species, lesions on sweet cherry appeared later, were smaller and produced fewer spores than those on sour cherry or duke cherry (11). Measurements of lesion

development or sporulation were strongly correlated with resistance to defoliation, while numbers of lesions were not. This implies that resistance is expressed after lesion establishment by restricting the growth and reproduction of the pathogen in the host tissue. Since leaf spot is a disease with multiple cycles of infection per season, a reduction in spore production after each cycle of infection would significantly reduce the secondary spread of the disease.

The relative resistance within and among sweet, sour and duke cherries was uniform over a range of C. hiemalis isolates (13). There was no indication that host or geographic source of an isolate was related to its relative ability to infect or reproduce on the cultivars tested. It appeared that evaluations of resistance based on a single isolate were representative of most, if not all, isolates attacking sour cherry. However, variation in the pathogen population should be continuously monitored in a breeding program to detect shifts in the population since increased use of resistant sorts for sour cherry may lead to selection within the pathogen population for strains that are able to overcome the resistance.

Inheritance studies indicated that genetic control of resistance in sour cherry and duke cherry is polygenic at the level of individual components (12). Heritabilities of all components of resistance were low (most less than 0.5 on an individual plant basis), and there was no indication of discrete classes of resistance in the progenies. Cultivars did differ in the average resistance of their progeny, indicating that resistance was at least partially controlled by additive gene action. However, selection for components of

resistance in juvenile seedlings did not appear promising. The low heritabilities indicate that progress would be slow if individual plants were selected, and significant genotype by experiment interactions were not completely resolved.

A number of cherry species and interspecific hybrids were completely resistant to an isolate of C. hiemalis attacking sour cherry (14). These species were outside of the Eucerasus section of Prunus that include the cultivated cherries. The resistant species were in the Pseudocerasus section of Prunus (Japanese flowering cherries), the Mahaleb section (P. pennsylvanica L.) and the Padus subgenus. In addition, complete resistance was present in interspecific hybrids between the Eucerasus section and both the Pseudocerasus and Mahaleb sections. Selections of P. mahaleb L. were not completely resistant, but showed higher levels of partial resistance than sweet cherry or sour cherry cultivars.

Suggestions for Future Research

There are several approaches to breeding for leaf spot resistance in cherry. Probably no one approach is the best, and the most effective approach may be a combination of two or more separate schemes.

One approach is to select for increased partial resistance within sour cherry. The cultivars 'North Star' and 'SHT-2' appear to be valuable sources of resistance within sour cherry. These two cultivars were more resistant to leaf spot than other cultivars in both field (10) and greenhouse (11) studies. Furthermore, 'North

Star' was the best parent for obtaining progeny with increased resistance (12). However, the resistance genes in 'SHT-2' could be identical to those in 'North Star' since the pedigree of 'SHT-2' is unknown (10). A wider range of sour cherry germplasm should be evaluated to identify additional sources of partial resistance.

Since inheritance of resistance in sour cherry appears to be polygenic with at least some additive gene action, a recurrent selection scheme should be effective in increasing the average level of resistance in the breeding population (2). Intermating of the best parents or their progenies after each generation of selection will increase the frequency of resistance alleles in the population. As allele frequency increases, the chance of obtaining resistant individuals also increases.

The simplest recurrent selection scheme is mass selection in which individual plants are the selection unit (2). The selected individuals are intermated to produce the next generation of progeny. A recurrent mass selection scheme could be readily adapted to component of resistance evaluations of juvenile seedlings. Individual seedlings would be screened at an early age, preferably before transplanting to the field. The most resistant seedlings representing a predetermined portion of the total population would be saved and the remaining individuals discarded. The resistant seedlings would be evaluated for other characteristics and the best individuals intermated. This method of independent culling is not as efficient as the selection index approach for improving more than one

character (3), but the savings in time and space by early culling probably outweigh the lower efficiency.

Unfortunately, the low individual plant heritabilites for components of resistance (12) indicate that progress will be slow in a recurrent mass selection scheme. Recurrent selection based on clonal family performance can be more effective than mass selection when heritabilities are low (2). However, use of n propagules per genotype in the field reduces to 1/n the number of genotypes evaluated per unit of land, and increases the time and work required to complete each generation. These disadvantages would nullify the advantages of clonal family selection.

If clonal families could be rapidly propagated and evaluated in the greenhouse, then most of the disadvantages of clonal family selection would be avoided. This may be feasible with sour cherries. Propagules, ideally rooted cuttings, could be taken from individuals in the top 5 to 10% of the population, based on field resistance evaluations and total score from evaluation of other characters. Propagules would be forced in the greenhouse and artificially inoculated for component of resistance evaluations in the winter. These resistance evaluations can then be included in a selection index of total horticultural value weighted for each character on the basis of its economic importance and heritability (3). The best individuals would then be intermated the following spring to produce the next generation. This method of clonal family evaluation would improve estimates of phenotypic variance without lengthening the generation time or reducing field space available for seedling evaluation.

Another scheme is recurrent selection based on full-sib, half-sib or self-family performance. Of these three, half-sib families can be most easily obtained in sour cherries. The evaluation scheme would be similar to clonal family evaluation except that seed collected from the most promising (e.g., the top 5 to 10%) individuals would be the evaluation unit, instead of clonal families. For this evaluation, performance of open-pollinated half-sib seedling families, grown and evaluated in the greenhouse during the winter, would be included in the selection index. A major disadvantage of selection based on half-sib family performance is that only 1/2 of the total additive genetic variance is utilized for selection (2). However, if the open-pollinated families are actually highly self-pollinated, then the evaluation scheme should compete favorably with evaluation based on clonal family performance, since most of the additive genetic variance would be utilized (2).

Another approach to breeding sour cherries resistant to leaf spot is interspecific hybridization with sweet cherries. Sweet cherries are a source of increased partial resistance to C. hiemalis (10). In addition, sweet cherries may be an important source of genetic diversity for other characters, since sour cherry is believed to be an allotetraploid of sweet cherry and ground cherry (8).

Interspecific hybrids are readily produced between sweet and sour cherry. The resulting hybrids are either highly sterile triploids or tetraploid with partial fertility. Fertility in the tetraploids (the duke cherries), which are thought to arise from the

union of an unreduced sweet cherry gamete with a reduced sour cherry gamete (4) is related to the level of quadrivalent formation at meiosis (9). The higher the percentage of quadrivalents, the lower the fertility, probably due to nondisjunction. Quadrivalent formation may be due to differentiation in sour cherry of the chromosomes originally derived from sweet cherry ancestors. This differentiation could reduce homology with chromosomes of contemporary sweet cherry cultivars, making possible competitive pairing of both sets in sour cherry with those of sweet cherry. How seriously quadrivalent formation will impede introduction of specific characters such as disease resistance from sweet cherry into sour cherry is not known.

The relative advantage of sweet cherry x sour cherry hybridization versus selection with sour cherry for resistance depends also on the number and nature of genes controlling resistance. If few loci with fairly large individual effects control the difference in spore production between these two species, interspecific hybridization followed by backcrosses to sour cherry could be used. If resistance is polygenic or recessive, then the backcross method will not be very effective (1).

Another difficulty with interspecific hybridization is the difference in ploidy level between these two species. Sweet cherry is diploid (2n = 16) while sour cherry is tetraploid (2n = 32). A large number of hybrids will be highly sterile triploids (4). Unless fertile tetraploids can be differentiated from triploids at an early age, much time and space will be inefficiently used. Gamete selection in sweet cherry pollen to increase the frequency of unreduced

gametes would increase the number of tetraploids in hybrid progenies. Production of tetraploid sweet cherry parents by colchicine treatment (8) or selection for haploid sour cherry parents would eliminate differences in ploidy between the two species.

Before greenhouse component of resistance evaluations can be applied to a recurrent selection scheme, the nature of genotype by time of inoculation interactions observed in juvenile seedlings should be explained. One hypothesis previously suggested is an effect of plant age on expression of resistance (12). This hypothesis could be tested by inoculating the same group of seedlings at intervals after germination. The seedlings should represent several families of crosses among parents differing in levels of resistance. Clonal parent material should also be included to estimate environmental error.

Breeding objectives in addition to leaf spot resistance should be considered in the selection of sweet cherry parents for interspecific hybridization. Resistance to X-disease, a serious mycoplasma-induced disease of cherry, has been reported for P. avium 'Angela' (16). This cultivar showed moderately high resistance to C. hiemalis in component of resistance evaluations (11), and thus would appear to be a good parent for interspecific hybridization. The self-fertile sweet cherries recently developed from mutation work (7) are other potential parents. Use of self-fertile sweet cherries would prevent introduction of P. avium self-incompatibility genes into P. cerasus germplasm. Self-incompatibility could later

impede intercrosses or self-fertilization, or reduce the usefulness of potential selections by requiring a pollinizing cultivar.

P. mahaleb clones are another source of partial resistance (14). Incorporation of resistance from this species into sour cherry may be even more difficult than using P. avium, due to the greater genetic distance between P. mahaleb and P. cerasus as well as the poor fruit quality of P. mahaleb. However, P. mahaleb represents a different source of resistance alleles which may be valuable if changes in the pathogen population through mutation or selection erode other sources of resistance.

Breeding for complete resistance is yet another approach to the development of leaf spot resistant cherries. An advantage of complete resistance is the ease by which resistance can be detected in segregating progenies. If natural levels of infection are not sufficient for accurate evaluation of complete resistance, trees can be artificially inoculated. Inoculation of a single leaf per tree should be sufficient to detect most susceptible progeny (10). Any tree showing secondary spread of infection beyond the inoculated leaf would be considered susceptible.

Complete resistance in other host-pathogen systems is often controlled by a single gene which can be introduced from a donor parent into improved material by backcross procedures (1). With heterozygous material of long generation times such as sour cherries, the recurrent parent often differs from generation to generation, resulting in a modified backcross approach. Intercrossing and

selection among recurrent parents can lead to fixation of resistance genes in the breeding population.

Sources of complete resistance are not available at present in cultivated cherries (P. avium, P. cerasus, P. fruticosa and P. gondouinii). Complete resistance in some of these species has been reported in field resistance evaluations by Eastern European workers (6,15), but retesting by artificial inoculation is needed to determine if these selections are completely resistant, partially resistant or escapes. Evaluation of a broader range of germplasm in each of these species might discover possible sources of complete resistance. Collections of open-pollinated progeny from centers of diversity of each of these species could be rapidly screened in the greenhouse or field by artificial inoculation.

Introduction of complete resistance into sour cherry from species in other sections of Prunus should be attempted. Success of these attempts depends first on development of fertile interspecific hybrids. The P. dropmoreana clone evaluated in this research, a fertile F_2 of P. cerasus x P. pennsylvanica with complete resistance to C. hiemalis (14), appears to be a valuable source of resistance. Backcrosses to sour cherry should be attempted with this clone. Another promising source of complete resistance is the P. avium x P. pseudocerasus rootstock cultivar 'Colt'. At present, it is not known if 'Colt' will produce fertile progeny.

A major concern in any disease resistance breeding program is the appearance of pathogen strains that can overcome resistance. The most obvious cause for concern in breeding leaf spot resistant

cherries is the reported variability by the pathogen at the species level of the host (6). Species with complete resistance to strains of C. hiemalis attacking cultivated cherries may be fully susceptible to other strains. This should not deter attempts to breed leaf spot resistant sour cherries, however. Instead, several sources of resistance should be exploited simultaneously to provide multiple options to the plant breeder. Immediate gains can be made by evaluation of partial resistance in advanced selections of sour cherry. In addition, crosses should be made to incorporate higher levels of partial resistance from sweet cherry and complete resistance from other cherry species. Selections should be evaluated in as many field locations as possible, preferably through interregional cooperative projects. Evidence of erosion in resistance due to the appearance of compatible pathogen strains can be tested by sensitive greenhouse pathogen variability evaluations (13).

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