Optimum Sample Size for Detecting Virulence Differences in *Leucostoma* Isolates from Peach

G. C. ADAMS, Assistant Professor, and S. A. HAMMAR, Research Assistant, Department of Botany and Plant Pathology, and A. IEZZONI, Associate Professor, Department of Horticulture, Michigan State University, East Lansing 48824

ABSTRACT

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A wide range in virulence was found among 28 isolates of *Leucostoma persoonii* and *L. cincta* after inoculation of each isolate on one branch per tree in 10 peach trees. An optimum experimental design for detecting differences in virulence among isolates of *Leucostoma* spp. was developed on the basis of measurement of variance in susceptibility of inoculated trees and branches. Inoculating more than one branch per tree had a minimal effect on reducing experimental error, but increasing the number of trees inoculated to more than six significantly reduced the statistical variance. Inoculating six to nine trees, one branch per tree, gave high precision in the detection of differences in isolate virulence while utilizing few trees and reducing labor. The experimental design could detect a minimum difference of 4.45 cm ($P = 0.05$) in canker size (virulence) between two isolates with 90% assurance that the difference in virulence was genuine. Criteria for differentiating *L. cincta* and *L. persoonii* in culture were examined, and a system based on colony margin and color, size of pycnidia, and growth at 37 C was found to be suitable. Isolates of *L. cincta* and *L. persoonii* were comparable in virulence, and the geographic origin of isolates tested was not correlated with the level of virulence.

Cytospora canker of peach caused by *Leucostoma cincta* (Fr.) Höhn. (imperfect state, *Leucocytospora cincta* (Sacc.) Höhn.) and *Leucostoma persoonii* (Nits.) Höhn. (imperfect state, *Leucocytospora leucostoma* (Pers.) Höhn.) is a destructive disease of peach (*Prunus persica* (L.) Batsch) in Michigan and elsewhere (19). The disease is often the limiting factor in peach production as well as the major impetus behind replanting and establishing new orchards (23). Cytospora canker is also an important disease of prune, apricot, cherry, and plum (8,17). The disease is characterized by premature leaf senescence, twig and branch dieback, and extensive perennial cankers on the trunk, branches, and scaffold limbs (16,20,35). The relative virulence of isolates of *L. persoonii* and *L. cincta* has been reported to differ greatly. In one study (38), two of 10 isolates of *L. persoonii* on peach were avirulent. In another study (17), one of four isolates of *L. cincta* was avirulent. Two of 76 isolates (mostly *L. persoonii*) inoculated on French prune produced cankers greater than twice the size formed by 70% of the remaining isolates (1). *L. persoonii* has been reported to be of low virulence compared with *L. cincta* on peach (17,37). In other research, however, *L. persoonii* was more virulent in warm weather (mean temperature greater than 16 C) and *L. cincta* was more virulent in cool weather (2,19,36).

Cytospora canker is difficult to control. Once a tree is infected, fungicides are ineffective (18,28) and pruning out the infection is not always practical. All of the currently grown peach cultivars are susceptible to Cytospora canker (4,10). The most advantageous means of controlling Cytospora canker of peach would be the introduction of disease-resistant cultivars with acceptable horticultural characteristics. A breeding program for disease resistance in peach entails a long-term commitment that is costly in expense and labor. A test screening peach for resistance to *Leucostoma* spp. must permit separation of the genomic variation of the plant from the genomic variation of the pathogen as well as the environmental variation. This is important for calculating heritability of the resistance. But most important, the resistance must hold up to pathogen variability in the field. Understanding the relative virulence of different isolates of the pathogen identifies strains with high levels of virulence for use in screening tests. Categorizing isolates into virulence classes is also requisite to identifying race-specific resistance and to eliminating selection for such resistance from the breeding program.

Others have reported differences in peach cultivar resistance to cankers and differences in isolate virulence (1,4,17, 36,38), but not variations caused by sampling errors (canker size on inoculated branches of a given tree) and replication errors (canker size on different inoculated trees of one genotype). Generally, four or fewer trees of one cultivar were inoculated at numerous loci or branches to determine cultivar or isolate responses (1,17,27,36,38). One concern in inoculating several loci on a few trees is that differences in host vigor (7) and in environmental stresses on hosts (1,5) have been shown to affect susceptibility of individual trees to Cytospora canker. For example, variations in the clay content of soils within one orchard can account for 88% of the variation in susceptibility to Cytospora canker among prune trees (2). Also, trees in an orchard are likely to be individually subjected to different severities of post-harvest moisture stresses, scale injury, and nematode infections that influence relative susceptibility to Cytospora canker (3,13,27). Thus, variation among trees of one genotype might mask differences between cultivars or fungal isolates if inadequate replications are tested. Another concern is that branches of different diameter or wood age might similarily increase variation and hinder accurate determination of cultivar response or isolate virulence. Thus, determining a reliable experimental design for testing differences in virulence among isolates of *Leucostoma* would influence the experimental designs for peach breeding programs.

The objective of this study was to determine the relative virulence of a number of isolates of *L. persoonii* and *L. cincta* on a uniform source of germ plasm. The contributions to variation in the experimental results are calculated for the error in sampling and the error in replications. From these data we developed recommendations for approximating the optimum experiment size and design for detecting a desired percentage difference in virulence among isolates of the pathogen.

MATERIALS AND METHODS

Twenty-eight isolates were obtained from Michigan, North Carolina, California, Pennsylvania, and West Virginia. Cultures were grown on Leonian's malt agar (LMA) (25) for 5 days at 25 C under cool-white fluorescent lamps before

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being used in inoculation experiments.

Designation of isolates as *L. cincta* or *L. persoonii* was based on comparing their cultural characteristics with descriptions by Hildebrand (19) and Willison (37). Cultures were grown for 30 days on LMA at 25 C under cool-white fluorescent lamps. Characteristics examined included 1) whether the colony margin was lobate with restricted growth or uniform with radial growth, 2) whether pycnidia were small (<1 mm) or large (1–3 mm), 3) the color of the colony, and 4) the ability to grow at 37 C. In addition, the cultural characteristics of the 28 isolates were compared (a year after the field experiments) to the cultural characteristics of single-ascospore isolates derived from perithecia of *L. persoonii* collected at the cv. Garnet Beauty site and *L. cincta* collected in Michigan on *Prunus* spp. by Proffer and Jones (29,34). The designations for the 28 isolates are based tentatively on the best current comparative cultural criteria available; further genetic and biochemical studies of the isolates are in progress (34).

The wound-freezing inoculation method of Scorza and Pusey (31) was used in field trials. The inoculation site was first cleaned with gauze soaked in 95% ETOH. The trees were wounded down to xylem depth with an empty hand-held stapling gun. The resulting 12 × 2 mm wound was frozen by being sprayed with 100% dichlorofluoromethane for 5 sec from a distance of approximately 15 cm. Inoculum consisted of a mycelial plug 5 mm in diameter. The inoculum was placed on the wounded bark and wrapped in Parafilm to prevent desiccation. For controls, branches on each tree were wounded as described except that a plug of LMA was substituted for the mycelial plug. One isolate (NC 9.2) was inoculated into seven trees on 10 separate branches per tree to test for within-tree variation (branch variance plots). Branches were measured with a caliper, and a portion of the branch with 2-yr-old wood and measuring 17 mm in diameter was inoculated.

Ten vigorous, randomly arranged 8-yr-old peach trees, cv. Garnet Beauty, were each inoculated with nine isolates of *L. cincta* and 15 isolates of *L. persoonii* in October 1985 (1985–1986 isolate virulence plot, trees 1–10). Twenty-four isolates and a control were placed on each tree, and there were 10 replications. In November 1987, 32 randomly arranged 6-yr-old peach trees, cv. Redhaven, were inoculated with nine *L. cincta* and 13 *L. persoonii* isolates (nine of those used in 1985 plus four others). These 32 trees were used in the 1987–1988 isolate virulence plots in three sets: 11 (1987–1988 trees 1–11) were inoculated with eight isolates, 11 (1987–1988 trees 12–22) were inoculated with eight different isolates, and 10 (1987–1988 trees 23–32) were inoculated with six different isolates. Both cultivars were on the rootstock Halford. Spacing was 4 m within and 8.5 m between rows for the Garnet Beauty trees and 1.5 m within and 4.5 m between rows for the Redhaven trees. Garnet Beauty trees had fewer natural cankers than Redhaven trees did, but all inoculated trees had a few cankers. Although only canker-free branches were inoculated, these were often attached to cankered scaffolds. Each inoculation was made on a separate 2-yr-old branch at a point on the branch 17 mm in diameter, one branch per isolate per tree. Branches were removed and canker length was measured (the length of necrotic area from the inoculation point to the farthest canker margin above the inoculation point) at the time of bud break and leaf elongation the following April. Statistical analysis of data included analysis of variance (ANOVA) of a randomized complete block design with trees as blocks (replicates). Isolates were ranked according to mean canker length using the least significant difference (LSD) at the $P = 0.05$ level.

Isolate NC 9.2 contained double-stranded RNA (dsRNA) that formed a characteristic banding pattern in acrylamide gels after electrophoresis. NC 9.2 was included in the 1985 and 1987 trials as a marked strain to evaluate whether wounded and inoculated branches were successfully colonized by the applied inoculum rather than by wild strains. Cankered branches that had been inoculated the previous fall with isolate NC 9.2 were removed from the trees and brought into the laboratory. Pieces of tissue were removed along the side of the necrotic margin of the canker and soaked in a 0.525% hypochlorite solution for 3 min. The tissue was blotted dry with sterile paper towels and transferred to LMA. The resulting cultures were tested for the presence of dsRNA by extracting the dsRNA with a double cellulose column, electrophoresing the extract on a 5% polyacrylamide slab gel, and staining the gel with ethidium bromide (11,14).

The following methods were used to develop an experimental design providing the most efficient determination of the relative virulence of a pathogen. The experimental design requiring the least labor and providing the greatest reduction in sampling and experimental error was determined using a method from Sokal and Rohlf (32). The sampling error (*MSS*) and the mean square experimental error (*MSE*) from the ANOVA of data of the branch variance plots (Table 1A) were used to determine the minimal experiment size and optimum design for reducing component variance. This was achieved by calculating sampling variances expected for the experimental designs (Table 1B), using the following formulas (32,33): sampling error = *MSS*; replication error = (*MSE* − *MSS*)/number of branches inoculated per tree; variance of sampling mean = sampling error/(number of trees inoculated × number of branches inoculated per tree) + replication error/number of trees inoculated.

The experimental design is chosen from Table 1 with the number of trees and branches to be inoculated. When the size of the experiment is limited to a certain number of trees and branches, the ability of the experiment to detect differences in isolate virulence can be estimated.

Differences in isolate virulence (expressed as a difference in canker length in centimeters after inoculation with a fungal isolate) are detectable at a chosen magnitude with a chosen assurance that the detected differences are real. With the *MSE* from each set of trees in the 1985–1986 and 1987–1988 isolate virulence plots (Table 2) as estimates of variance, the desired assur-

Table 1. The optimum experimental design for determining the relative virulence of a pathogenic strain, using the least sampling variance per inoculated branches and trees

A. Analysis of variance table from branch variance plots

Source (one isolate)	df	MS	*F* significant at *P* less than:
Total	69		
Between trees (replications)	6	34.76 (MSE)	0.000
Within trees (branches)	63	1.94 (MSS)	

B. Sampling variances for different experimental designs

Number of branches inoculated per tree	Number of trees inoculated					
	1	3	6	9	12	15
1	5.22	1.74	1.05	0.58	0.43	0.35
2	4.10	1.41	0.89	0.47	0.35	0.28
3	3.93	1.31	0.84	0.43	0.32	0.26
4	3.77	1.25	0.81	0.41	0.31	0.25
5	3.67	1.22	0.79	0.40	0.30	0.24
10	3.47	1.15	0.76	0.36	0.29	0.23
20	3.37	1.12	0.75	0.36	0.28	0.22

ance level at 90% (0.90), and the level of significance at $P = 0.05$, the following formula and a table of t values are used to estimate detectable differences (33): centimeters of canker length (detectable difference in isolate virulence or cultivar resistance) = $[2(MSE)(t_0 + t_1)^2/\text{number of trees inoculated per isolate}]^{1/2}$, where t_0 is the t table (two-tailed) value for $P = 0.05$ using degrees of freedom associated with MSE, and t_1 is the t table (two-tailed) value for $P = 2(1 - 0.90) = 0.20$. The assurance level chosen is 0.90 and is used with the degrees of freedom associated with MSE.

RESULTS

Among the 28 isolates tested, 19 were identified as *L. persoonii* and nine as *L. cincta*. Isolates of *L. persoonii* were characterized as having a lobate, restricted colony margin, an olivaceous or darker colony color after 30 days of growth on LMA, growth at 37 C or higher, and small (<1 mm) pycnidia (Fig. 1). Comparisons with cultures derived from single ascospores from stromatic perithecia identified as *L. persoonii* and *L. cincta* aided our identification of the species by cultural characteristics, although many isolates designated as *L. cincta* were not identical in cultural characteristics to ascospore-derived cultures. The nine *L. cincta* isolates were more variable (Table 3). Seven of the isolates had characteristic, uniformly radial colony margins; large pycnidia (1–3 mm), often having a cottony appearance; and an olivaceous to buff colony color (Fig. 1). The other two isolates had uniformly radial margins but lacked the characteristic pycnidia. Isolate MI F-4 (Table 3) had small (< 1 mm) pycnidia and isolate MI F-46 had no observable pycnidia after 30 days. These two isolates were identified as *L. cincta* on the basis of colony color, margin, and inability to grow at 37 C. Except for isolate MI G-3, isolates designated as *L. cincta* failed to grow at 37 C. Many of the *L. cincta* isolates grew at 32 C.

In the virulence screen, all inoculations except the controls resulted in canker formation. The isolates showed a wide range of virulence, which was based on mean canker length of nine to 11 replications (trees) (Table 4). The virulence of individual isolates differed during 1985–1986 on Garnet Beauty and 1987–1988 on Redhaven. For example, the cankers caused by MI 11.11 during 1987–1988 were more than twice as large as those caused by MI 11.11 during 1985–1986. Generally, the pathogens were more virulent during 1987–1988 on Redhaven than during 1985–1986 on Garnet Beauty, but peach were more severely damaged by cold injury at the Redhaven site. Two isolates of *L. cincta*—CA F-46 and NC 8.2—were highly virulent in both years of tests, with a mean canker length of 12.32 and 10.59 cm, respectively, while *L. cincta* isolates CA F-45 and NC 14.4A were low in virulence both years, at 6.05 and 4.73 cm, respectively. The geographic origin of an isolate was not a factor in its virulence. For instance, the four most virulent isolates as well as the four least virulent isolates were from Michigan, North Carolina, and California. No isolate was avirulent. The relative virulence of *L. cincta* did not differ appreciably from that of *L. persoonii*, and *L. cincta* isolates were dispersed evenly among the *L. persoonii* isolates in virulence ranking (Table 4). When the variation between trees was removed by ANOVA, the different *Leucostoma* isolates were revealed to have significant differences in virulence (Table 2).

There was considerably more variation in susceptibility to the pathogens among trees of a common cultivar (scion germ plasm) in an orchard than among branches within a tree (Table 1A). Branches within a single tree did not vary significantly in susceptibility (Table 1A).

Cultures reisolated from branches on 10 trees inoculated with strain NC 9.2 showed the typical dsRNA banding pattern of NC 9.2 in 18 of 20 instances. This indicated that some infections were colonized by wild isolates rather than by the applied inoculum; the amount of contamination appeared to be low, however. Wild isolates at these locations did not contain dsRNA, to our knowledge (15). Control inoculations became infected by wild isolates more often in 1987 than in 1986. Inoculations on young symptomless branches were contaminated by wild strains more frequently on trees with multiple perennial cankers than on trees with fewer cankers.

ANOVA of the data from the virulence plot and from the branch variance plot permit calculations revealing that increasing the number of branches inoculated per tree has an insignificant effect on reducing the sample variance (Table 1B). Increasing the number of trees inoculated greatly reduces the sample variance. Increasing the number of trees inoculated to more than nine, however, gives minor reductions in variance that do not warrant the

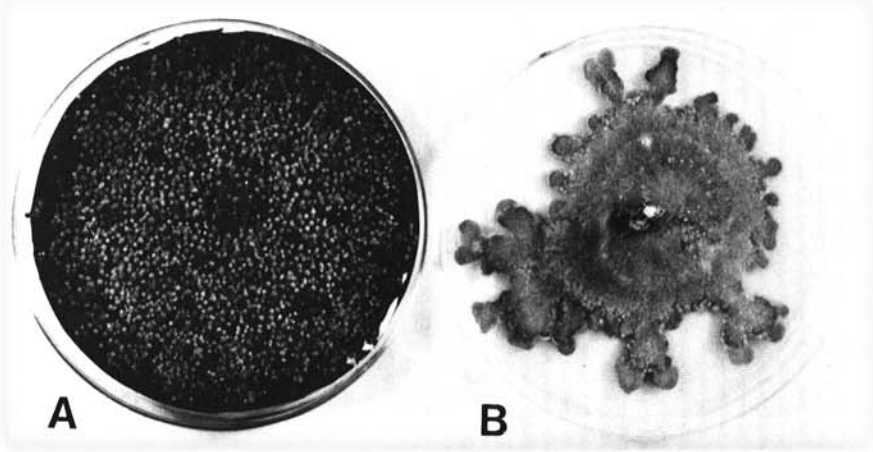

Fig. 1. Colony characteristics after 30 days of growth on Leonian's malt agar at 25 C under cool-white fluorescent lamps: **(A)** *Leucostoma cincta* with a uniformly radial colony margin and large (1–3) pycnidia and **(B)** *L. persoonii* with a lobate colony margin and small (<1 mm) pycnidia.

Table 2. Analysis of variance table for randomized complete block design of 1985 and 1987 isolate virulence plots

	1985–1986 peach cv. Garnet Beauty			1987–1988 peach cv. Redhaven								
	Trees 1–10			Trees 1–11			Trees 12–22			Trees 23–32		
Source of variation	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Peach tree	9	53.015	0.000	10	7.838	0.093	10	23.118	0.151	9	18.780	0.030
Isolates	23	36.033	0.000	8	52.089	0.000	8	208.536	0.000	6	91.229	0.000
Error	196	10.116		80	4.596		76	15.279		54	8.269	
Detectable difference ($P = 0.05$)[a] in centimeters	4.60			3.20			5.70			4.30		

[a] Sensitivity in detecting a difference in canker length with a 90% assurance that the difference is real.

increased labor or plant material. From our data, the experimental design of choice for the most efficient determination of the relative virulence of an isolate of the pathogen would be to inoculate one branch per tree and use between six and nine trees to screen a fungal isolate.

Inoculations on the four groups of trees (1985–1986 trees 1–10 and 1987–1988 trees 1–11, 12–22, and 23–32) showed differences among isolates of 3.2. 4.3, 4.6, and 5.2 cm, respectively, in virulence (canker length) at $P = 0.05$, with an assurance of 90% that the detectable difference was genuine (Table 2). This can be interpreted as the sensitivity to detect a minimum difference in virulence (canker length) among two isolates of 4.45 cm.

DISCUSSION

We interpret the analysis of the plot data to reveal the relative importance of sampling error and replication error. Calculations based on the data were used to estimate the optimum experimental size and design for screening isolates of *Leucostoma* spp. to determine their relative virulence. This experimental design should minimize the sources of experimental variance that would interfere with the precision of screening peach germ plasm for resistance to *Leucostoma* spp. Variance due to inoculation of different branches in a tree is of little consequence. Thus, inoculating more than one branch per tree gives little increased precision in differentiating isolate virulence or cultivar resistance. Variance due to inoculation of several trees (one branch per tree) of the same germ plasm is the major source of experimental error. Increasing the number of plants inoculated greatly increased the precision of the screening tests. The inoculation of more than six trees should be effective in detecting a true response of one peach genotype to the virulence of a tested isolate of the pathogen. The sources of experimental error under these conditions should be similar in other locales. In the plots utilized in our studies, there were no recognizable environmental factors that might have accounted for the variance seen among individual trees of the same germ plasm, except perhaps the background level of disease on individual trees. The Redhaven trees were more susceptible to the pathogens, however, and this difference might be caused by the more severe cold injury that occurs at the Clarksville orchard site. The 1985–1986 tests showed greater variability among trees than among isolates of the pathogen, whereas the three 1987–1988 tests showed greater pathogen variability.

Assuming that the identified sources of experimental error in our tests are similar to those of past tests, we find it difficult to interpret the results of experiments where numerous branches or loci were inoculated on four or fewer trees in screening tests. It is noteworthy that two studies evaluating resistance to *Leucostoma* spp. in peach trees (26,31) utilizing numerous replications found only very low or insignificant levels of disease resistance. These conclusions and similar conclusions from more extensive studies (6) give clear evidence that the existing level of resistance to *Leucostoma* spp. is quite low in North American germ plasm with acceptable horticultural characteristics.

Cytospora canker is caused by two species that are considered to be distinct in the northeast and north central states (22), although Lukezic et al (27) have questioned the validity of separating the two species. The important characteristics for identification are those of the sexual fruiting bodies (teleomorphs), which do not form under our laboratory conditions (24). Because of the relative scarcity of the teleomorph of the two species in nature, *L. cincta* and *L. persoonii* are commonly identified by cultural characteristics (19,21,36). Researchers generally have utilized the cultural characteristics described by Willison (37) as criteria for differentiating *L. cincta* from *L. persoonii*. In our study, we found the culture photographs (19,21) and optimum growth temperature studies (19) to be useful also for distinguishing between the two species. No characteristic was singly reliable in distinguishing cultures of the two species. The multiple characteristics used in this study, however, are suggested as usable until isozyme patterns (34), restriction fragment length polymorphisms, or more precise criteria become available.

Our data showed significant variation in virulence among isolates of *L. cincta* and *L. persoonii*. We have not found the significant numbers of avirulent isolates reported in other studies (17,38); only

Table 3. Origin, author-designated species, colony characteristics, and source of or reference for isolates of *Leucostoma* spp.

Origin[a]	Isolate[b]	Species designation	Colony color[c]	Colony margin[d]	Pycnidium size[e]	Growth at 37 C	Source or reference
NC	NC 8.2	*cincta*	Olivaceous buff	F	Large	−	Endert-Kirkpatrick (12)
NC	NC 9.2	*cincta*	Olivaceous	F	Large	−	Endert-Kirkpatrick (12)
NC	NC 14.2	*persoonii*	Oivaceous	L	Small	+	Endert-Kirkpatrick (12)
NC	NC 14.1	*cincta*	Greenish olivaceous	F	Large	−	Endert-Kirkpatrick (12)
WV	C-j-1	*persoonii*	Greenish olivaceous	L	Small	+	ATCC 58386
WV	C-MI-5	*persoonii*	Olivaceous	L	Small	+	C. L. Wilson
WV	C-jm-18	*persoonii*	Olivaceous	L	Small	+	C. L. Wilson
WV	C-S-20	*persoonii*	Isabelline	L	Small	+	C. L. Wilson
MI	H 10.9	*persoonii*	Greenish olivaceous	L	Small	+	S. A. Hammar
MI	H 9.11	*persoonii*	Iron gray	L	Small	+	S. A. Hammar
MI	H 9.5	*persoonii*	Greenish olivaceous	L	Small	+	S. A. Hammar
MI	H 6.15	*persoonii*	Olivaceous	L	Small	+	S. A. Hammar
MI	H 7.13	*persoonii*	Fuscous black	L	Small	+	S. A. Hammar
MI	G-3	*cincta*	Dark mouse gray	F	Large	+	S. A. Hammar
MI	R-1	*persoonii*	Olivaceous	L	Small	+	S. A. Hammar
MI	F-4	*cincta*	Buff	F	Small	−	S. A. Hammar
PA	P-1	*persoonii*	Gray olivaceous	F	Large	+	B. A. Snyder
MI	11.11	*persoonii*	Greenish olivaceous	L	Small	+	S. A. Hammar
MI	10.8	*persoonii*	Olivaceous	L	Small	+	S. A. Hammar
CA	CL-5	*cincta*	Greenish olivaceous	F	Large	−	J. M. Ogawa
CA	Ma-4	*persoonii*	Olivaceous	L	Small	+	J. M. Ogawa
CA	I-80	*cincta*	Olivaceous	F	Large	−	J. M. Ogawa
CA	F-45	*cincta*	Olivaceous	F	Large	−	H. English, ocl (1)
MI	F-46	*cincta*	Honey	F	Large	−	H. English, F-40 (1)

[a] NC = North Carolina, WV = West Virginia, MI = Michigan, PA = Pennsylvania, CA = California.
[b] All except F-45 and F-46 were from peach.
[c] See color chart in Rayner (30).
[d] F = full, uniformly radial; L = lobate.
[e] Small = <1 mm in diameter, large = 1–3 mm in diameter.

Table 4. Virulence of isolates of *Leucostoma* spp. on 10 trees of peach cv. Garnet Beauty during 1985-1986 and 32 trees of peach cv. Redhaven during 1987-1988, ranked in order of decreasing canker length

Isolate	Origin	Species designation	Av. canker length (cm)[z]
Garnet Beauty			
R-1	Michigan	*persoonii*	11.00 a
8.2	North Carolina	*cincta*	9.98 ab
F-46	California	*cincta*	9.83 abc
CL-5	California	*cincta*	8.40 abcd
10.8	Michigan	*persoonii*	7.78 bcde
H 9.5	Michigan	*persoonii*	7.64 bcdef
C-j-1	West Virginia	*persoonii*	7.50 bcdefg
C-S-20	West Virginia	*persoonii*	7.24 bcdefg
G-3	Michigan	*cincta*	7.08 cdefg
F-4	Michigan	*cicnta*	6.95 defgh
Ma-4	California	*persoonii*	6.49 defgh
C-MI-5	West Virginia	*persoonii*	6.48 defgh
P-1	Pennsylvania	*persoonii*	6.06 defgh
C-jm-18	West Virginia	*persoonii*	5.94 defgh
H 10.9	Michigan	*persoonii*	5.46 efgh
9.2	North Carolina	*cincta*	5.34 efgh
14.1	North Carolina	*cincta*	5.11 efgh
F-45	California	*cincta*	4.92 fgh
14.4A	North Carolina	*persoonii*	4.81 gh
H 7.13	Michigan	*persoonii*	4.79 gh
H 6.15	Michigan	*persoonii*	4.75 gh
H 9.11	Michigan	*persoonii*	4.71 gh
11.11	Michigan	*persoonii*	4.18 h
I-80	California	*cincta*	4.02 h
Control (1-10)	...	1-10	0.50 i
Redhaven			
F-4	Michigan	*cincta*	16.55 a
F-46	California	*cincta*	14.82 a
P-1	Pennsylvania	*persoonii*	10.46 b
107	North Carolina	*persoonii*	9.11 b
R-1	Michigan	*persoonii*	9.10 b
G-3	Michigan	*cincta*	7.44 bc
Br107S	North Carolina	*persoonii*	7.44 bc
14.4A	North Carolina	*persoonii*	4.64 cd
Control (1-11)	...		3.08 d
8.2	North Carolina	*cincta*	11.19 a
C-j-1	West Virginia	*persoonii*	10.79 ab
KV-1	Michigan	*persoonii*	10.61 ab
11.11	Michigan	*persoonii*	10.43 ab
9.2	North Carolina	*cincta*	8.89 ab
C-S-20	West Virginia	*persoonii*	8.40 b
Control (12-22)	...		2.54 c
H 9.11	Michigan	*persoonii*	9.72 a
Ma-4	California	*persoonii*	8.78 ab
11.13	Michigan	*persoonii*	8.10 ab
I-80	California	*cincta*	8.03 ab
14.1	North Carolina	*cincta*	7.97 ab
CL-5	California	*cincta*	7.87 b
C-MI-5	West Virginia	*persoonii*	7.66 b
F-45	California	*cincta*	7.18 b
Control (23-32)	...		2.01 c

[z]Length of canker distal to inoculation point. Means followed by the same letters are not significantly different by the least significant difference test (LSD) ($P = 0.05$).

NC 14.4A was avirulent. However, the evident differences in virulence emphasize the importance of obtaining a measurement of the relative virulence of an isolate before using it to screen germ plasm. We did not observe the virulence differences between *L. cincta* and *L. persoonii* as reported in the literature (19,37). Hildebrand (19) noted that *L. cincta* always caused larger cankers than did *L. persoonii* when trees were inoculated during the cooler months of the year. Our tests showed an even distribution in virulence between the two species. The cooler temperatures of the Michigan winter and early spring did not favor the growth of *L. cincta* over *L. persoonii*. Similarly, Dhanvantari (9) found that in trees artificially inoculated in autumn, the two species of *Leucostoma* were equally virulent. The geographic origin of the isolates tested was not correlated with the level of virulence.

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