Detection of Cytospora Species in Twig Elements of Peach and its Relation to the Incidence of Perennial Canker

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ABSTRACT

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Nontreated and captafol- or benomyl-treated twig elements (buds, leaf scars, and internodes) of field-grown Redhaven peach trees were monitored at 2- to 3-wk intervals to detect increases in the occurrence of *Cytospora* spp. during the infection period from November to April. There was no significant effect of fungicides on the number of isolations of *Cytospora* spp. from twig elements until late winter. The infection rate (IR) was reduced more by captafol than by benomyl. Applications of captafol during leaf fall or during late winter 1976-1977 significantly reduced canker incidence in the spring of 1977. Benomyl applied at time of leaf fall or late-winter 1976-1977 was effective in reducing canker incidence in the spring of 1977. Benomyl applied at leaf fall in 1975, however, was not effective in reducing the disease in the spring of 1976. Simple correlation coefficients (r) were higher between leaf-scar isolations and canker incidence than between bud or internode isolations and canker incidence at two dates in 1977 (bud break and 10 days after bud break). The contribution of the leaf-scar element to the incremental proportions of variance (ΔR^2) values for canker incidence always was higher than that of other twig elements at bud break and 10 days after bud break. Leaf-scar isolations had higher ΔR^2 values in canker incidence at bud break than 10 days after bud break. Bud isolations produced higher ΔR^2 values in canker incidence 10 days after bud break than at bud break.

Additional key words: *Cytospora cincta*, *C. leucostoma*, multiple regression methods, *Valsa* spp.

Perennial canker occurs throughout the Midwest wherever peach trees [*Prunus persica* (L.) Batsch] are grown. In Illinois, this disease is often the limiting factor in production. The disease is caused by two species of fungi, *Leucostoma cincta* (Pers. ex Fr.) Höhn [=*Valsa cincta* (Pers. ex Fr.)] and *L. persoonii* (Nits.) Höhn [=*Valsa leucostoma* (Pers. ex Fr.) Fr.]. The conidial stages [*Cytospora cincta* (Pers.) Fr. and *C. leucostoma* (Pers.) Fr., respectively] are commonly encountered in the field. The disease often is destructive in young orchards where it causes the premature death of trees. Older infected trees gradually lose productivity and longevity is decreased.

Girdling and dieback of 1-yr-old twigs in the spring is an important phase of perennial canker (4, 6, 8). Most twig cankers are nodal and arise from leaf scars (6, 8) or dead buds (2, 4, 5, 6) and infections occur between leaf fall in October and bud break in March.

Little is known about the effects of fungicides on the activity of *Cytospora* sp. during the infection period from November to April. The purposes of this study were: (i) to monitor changes in the occurrence of *Cytospora* spp. on buds, leaf scars, and internodes over time and as affected by fungicide treatment; and (ii) to discover the relationship between the occurrence of *Cytospora* spp. and perennial canker on 1-yr-old peach twigs.

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MATERIALS AND METHODS

Experiments were conducted from 1975-1977 in an orchard of the Plant Pathology Research Center, University of Illinois, Urbana. A block of Redhaven peach trees planted in 1961 and heavily infected with perennial canker was used in these studies. Normal orchard management was maintained throughout the experiments.

Effect of fungicide application during leaf fall on reduction of canker incidence.—Peach trees were sprayed to run-off with either captafol (Difolatan 4F, 33% flowable, Chevron Chemical Co., San Francisco, CA 94104 or benomyl (Benlate 50% WP, E. I. du Pont de Nemours and Co., Wilmington, DE 19898 using a hand gun operated at 14 Kg/cm^2. Each tree received approximately 14 liters of spray, equivalent to 5,110 liter/ha applied on a commercial basis with an airblast sprayer. Sprays were applied on 14 October 1975 and 20 October 1976 at time of 50% leaf fall. Nonsprayed trees served as controls. Fungicide treatments were arranged in a split-plot design with three replications in the 1975-1976 assay period and five replications in the 1976-1977 assay period. In both years, treatments were assigned at

random to the whole unit in a randomized complete-block design; sampling dates were assigned as subunits within each whole unit.

Forty-four each of buds, leaf scars, or internodes (twig elements) from each replication were excised with a scapel from 1-yr-old twigs at 2- to 3-wk intervals from 10 November 1975 to 2 April 1976 and from 11 November 1976 to 31 March 1977. The twig elements were bioassayed for the presence of *Cytospora* spp. by surface disinfesting the components in a 1% sodium hypochlorite solution for 5 min, then in 70% ethanol for 2 min, and finally rinsing them in sterile distilled water. Then they were plated on Difco potato-dextrose agar (PDA) and incubated at room temperature (24 C). The fungi growing from the twig elements were recorded separately after 2 wk.

Canker incidence.—One-yr-old nonbranched twigs were examined for *Cytospora* spp. lesions during floral tube abscission (14 May 1976 and 6 May 1977). One-hundred twigs (20 twigs per replicate) were evaluated in 1977 whereas 70 twigs (14 per replicate) were evaluated in 1976. Isolations from infected tissues were made on PDA. Disease incidence was expressed as the percentage of twigs with Cytospora infection.

Effect of fungicide applications in late winter on reduction of canker incidence.—Thirty-nine days prior to bud break (10 February 1977) and 18 days prior to any detectable increase in the number of isolations of *Cytospora* spp. in the 1976-1977 period, trees were sprayed with one of the following: captafol at 0.20 kg/100 liter or benomyl at 0.03 kg/100 liter. Ten days after bud break (31 March) twig elements were bioassayed as previously described for differences in the occurrence of *Cytospora* spp. as affected by late-winter fungicide applications. Canker incidence data was taken at floral tube abscission (6 May).

Determination of Cytospora species distribution; test for randomness.—Twenty-two samples each of buds, leaf scars, and internodes from each of nine trees were bioassayed to determine if the presence of *Cytospora* spp. in a given element would increase the probability that other Cytospora infections would occur in nearby elements. Nine quadrats containing one peach tree each were sampled. Data for four dates (28 February, 9, 21, and 31 March 1977) were subjected to a Chi-square (χ^2) test to determine goodness of fit to a Poisson distribution (9).

To determine whether the numbers of isolations from buds, leaf scars, and internodes were correlated with each other and with canker incidence, simple correlation coefficients (r) were calculated for two different time periods: bud break (21 March 1977) and 10 days after bud break.

Relationship between isolations of Cytospora species from various twig elements and the incidence of perennial canker.—Isolations were made from the twig elements, buds, leaf scars, and internodes and regression analysis methods were used to determine the relationship between relative number of isolates from the twig elements and the incidence of perennial canker.

Nontransformed (arithmetic) and transformed (semi-logarithmic, and log-log) isolation data from buds, leaf scars, and internodes were entered into a multiple-regression computer program with canker incidence as a dependent variable. Numbers of isolations from each twig element were independent variables: X_1 = leaf scar isolations, X_2 = bud isolations, and X_3 = internode isolations. The multiple regression method was used to derive coefficients of determination (R^2) for the predictor variables X_1, X_2, and X_3. The resulting composite was defined by the multiple regression equation:

$$\hat{Y} = a + b_1X_1 + b_2X_2 + b_3X_3$$

The proportions of variance for canker incidence "accounted for" by each twig element were measured by R^2. The term "accounted for" is used to indicate a statistical relationship, not a cause-effect relationship. The effect of a predictor variable was defined as the amount by which the squared multiple correlation (R^2) decreased if the variable was removed from the regression equation.

Rates of increase (IR) in the number of isolations of *Cytospora spp.* were calculated using linear regression methods. Semilog-transformed [$\log_e 1/(1-x)$] data for isolations for 11 November 1976 to 17 February 1977 (99 days) and from 17 February to 31 March 1977 (44 days) were entered into a linear regression model and slopes (b = IR) were calculated for each treatment. Infection rate (IR) was not calculated for internodes because the number of isolations was small during these periods.

TABLE 1. Mean number[a] of *Cytospora* spp. isolations from buds, leaf scars, and internodes of 1-yr-old peach (*Prunus persica* 'Redhaven') on potato-dextrose agar at various dates following applications of benomyl or captafol at 50% leaf fall

	1975					1976					
	Nov		Dec			Jan	Feb	Mar		Apr	
Treatment[b]	10	20	3	17	31	13	12	4[c]	19	2	Total
Nonsprayed	0.3	0.7	0.3	0.3	0.3	0.7	0	9.7	4.3	4.7	21.3
Benomyl	1	0	0.3	0	2	0.7	0	5.3	3	4.3	16.7
Captafol	0.3	0.7	0.3	0.3	0.3	0	0	0.3	0	1.3	3.7
FLSD[d] = 0.93											

[a]Based on 132 isolations (44 each of buds, leaf scars, and internodes) from each of three replications/treatment.
[b]Benomyl = 0.03 kg/100 liter; captafol = 0.20 kg/100 liter.
[c]Time of bud break.
[d]Fisher's least significant difference ($P = 0.05$) for comparison between any two means.

RESULTS

Isolations of *Cytospora* spp. from twig elements in 1975-1976 were higher in the spring during and after bud break for all treatments than from those made in the preceding fall and winter (Table 1). There were no significant differences ($P = 0.05$) between the number of isolations of *Cytospora* spp. from the twig elements from October 1975 through 12 February 1976. From March to 2 April, however, *Cytospora spp.* isolations from nontreated twig elements were consistently higher than were those from benomyl or captafol-treated twig elements (Table 1).

Results from the 1976-1977 bioassay period were similar to those obtained in 1975-1976. Significant increases in the occurrence of *Cytospora* spp. however, were detectable at least 3 wk prior to bud break (Table 2). Fewer isolations of *Cytospora* spp. were obtained from captafol- and benomyl-treated trees beginning in December than from nonsprayed trees, but the differences were not significant.

Canker incidence was higher in the 1977 season (36%) than in the 1976 season (15.7%). Captafol applied at time of 50% leaf fall or during late winter reduced canker incidence significantly over nonsprayed trees during the 1976-1977 period (Table 3). Benomyl applied at 50% leaf fall significantly ($P = 0.05$) reduced canker incidence in 1977, but not in 1976. Application of benomyl in late winter (1977) resulted in reduced canker incidence in the spring. There was no significant ($P=0.05$) difference in

TABLE 2. Mean number [a] of *Cytospora* spp. isolations from buds, leaf scars, and internodes of 1-yr-old peach (*Prunus persica* 'Redhaven') on potato-dextrose agar at various dates following applications of benomyl or captafol at 50% leaf fall

	1976			1977							
	Nov		Dec	Jan		Feb			Mar		
Treatment[b]	11	23	20	6	3	17	28	9	21[c]	31	Total
Nonsprayed	0.2	0.2	1.2	1.2	2.0	1.2	10.2	5.4	9.8	11	42.4
Benomyl	0.6	0.4	0.4	0.4	0.8	0.4	4	2.6	7.2	8.2	25.0
Captafol	0.4	0.4	0.4	0.4	0.2	0.2	1.8	1	2	1.8	8.6
FLSD[d] = 1.2											

[a]Based on 132 isolations (44 each of buds, leaf scars, and internodes) per each of five replications/treatment.
[b]Benomyl = 0.03 kg/100 liter; captafol = 0.20 kg/100 liter.
[c]Time of bud break.
[d]Fisher's least significant difference ($P = 0.05$) for comparison between any two means.

TABLE 3. Mean number of *Cytospora* spp. isolations from buds, leaf scars, and internodes at bud break and relative incidence of twig-canker infections by *Cytospora* spp. on 1-yr-old peach (*Prunus persica* 'Redhaven') twigs nontreated or treated with benomyl or captafol at 50% leaf fall or 39 days before bud break

Fungicide applications	Concentration in water (kg/100 liter)	Mean number isolations *Cytospora* spp.[a]		Relative incidence (%) of twig-canker infection (nonsprayed control = 100%)[b]	
		1976	1977	1976	1977
Captafol at 50% leaf fall	0.20	0.3	2.0	36	33
Benomyl at 50% leaf fall	0.03	5.3	7.2	91	44
Captafol 39 days before bud break	0.16	...	1.5	...	47
Benomyl 39 days before bud break	0.06	...	4.2	...	67
Nonsprayed Control	nontreated	9.7	11.0	100	100
FLSD[c]					
0.05		3.2	2.9	39	22
0.01		4.8	4.0	52	31

[a]Based on 132 isolations on potato-dextrose agar (44 each of buds, leaf scars, and internodes) from each of five replications per treatment; bud break occurred on 4 March and 21 March in 1976 and 1977, respectively; ... = data not available.
[b]Disease incidence is expressed as the percentage of twigs with cankers as indicated by positive *Cytospora* spp. isolations from lesions. Incidence of twig infection in nontreated check in 1976 and 1977 was 15.7% and 36%, respectively.
[c]FLSD= Fisher's least significant difference for comparison between any two means.

canker incidence when captafol was applied during late winter (39 days prior to bud break) or when it was applied at 50% leaf fall. The number of isolations from twig elements that received late-winter fungicide applications was less than from the nontreated twig components at bud break (Table 3).

Infection rate (IR) for both buds and leaf scars was much higher in early spring than after leaf fall (Fig. 1 and 2). Incidence of infection was reduced by benomyl and captafol for both time periods. Captafol applied at 50% leaf fall resulted in the greatest reduction in IR from 17 February to 31 March 1977 for both buds and leaf scars.

Distribution of Cytospora spp.—Calculations of χ^2 for determination of goodness of fit to a Poisson distribution of *Cytospora* spp. in buds and leaf scars were 7.37 for three degrees of freedom and 9.97 for five degrees of freedom, respectively. The probabilities of these values lie between $P = 0.10$ and 0.05, which are not statistically significant. Therefore, the data conform to the Poisson distribution.

Relationship between isolations of Cytospora from various twig elements and the incidence of perennial canker.—Incremental proportions of variance (ΔR^2) for canker incidence "accounted for" by each twig element, over and above the contributions of the other two elements at two different assay dates are given (Table 4). The ΔR^2 values attributable to isolations from leaf scars always were greater than those isolations from buds or internodes at both assay dates. The sums of the individual ΔR^2 values "accounted for" by each twig element were much less than overall $\bar{R}^2$ values at both assay dates. Values of $\bar{R}^2$ were highest when semi-logarithmic transformation of the data [$\log_e 1/(1-x)$] was used (7).

Simple correlation coefficients (r) among twig elements and canker incidence showed higher values between leaf scars and incidence than between buds or internodes and canker incidence. The correlation coefficients (r) between leaf scar infection and canker incidence were 0.89 and 0.81 ($P = 0.01$) at bud break and 10 days after bud break, respectively. Correlation coefficients between canker incidence and the number of isolations from buds at bud break and 10 days post-bud break, respectively, were 0.30 (not significant) and 0.05 (significant, $P = 0.01$). There were no significant correlations among infections of buds, leaf scars, and internodes at either date.

DISCUSSION

The methods employed in this study revealed information on the correlation between the occurrence of *Cytospora* spp. in twig elements and canker incidence. Fungicide treatments were helpful in evaluation of the time of infection of *Cytospora* spp. at different times of the year. Applications of captafol and benomyl in late winter 1977 gave significant reduction in Cytopsora infection on twigs. In contrast, however, Northover (4) reported that with Loring peach trees captafol and benomyl applied in early spring did not reduce twig infection. This probably was due to the too-late application of fungicides after the period in which natural infection occurred in that season. Tekauz (5) found that combined autumn-spring sprays of Bordeaux mixture significantly reduced the number of natural infections on young twigs. He suggested that failure of the "spring only" spray program to reduce infection significantly

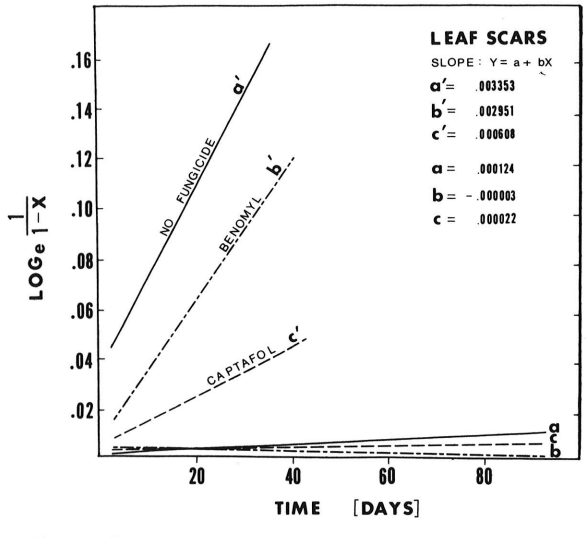

Fig. 1. Infection rate (IR) of *Cytospora* spp. in peach leaf scars nontreated or treated with benomyl or captafol at time of 50% leaf fall. Letters a, b, and c indicate the 99-day period from 11 November 1976 to 17 February 1977. Letters a', b', and c' indicate the 44-day period from 17 February 1977 to 31 March 1977 [X = infection incidence (J.E. van der Plank. 1963. Plant diseases: epidemics and control. Academic Press, New York and London. 349 p.)].

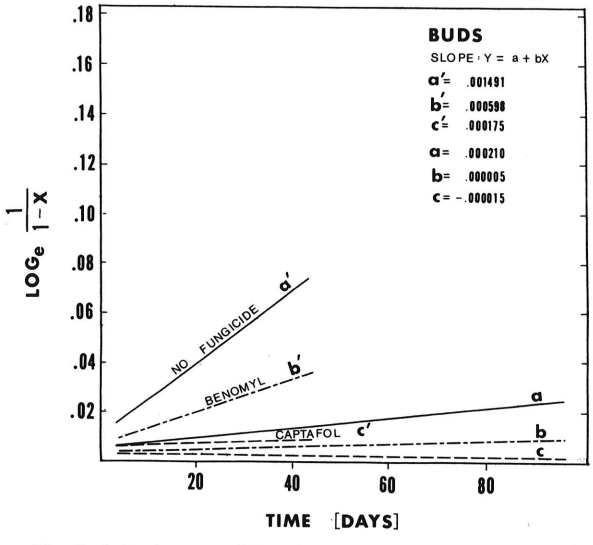

Fig. 2. Infection rate (IR) of *Cytospora* spp. in peach buds nontreated or treated with benomyl or captafol at time of 50% leaf fall. Letters a, b, and c indicate the 99-day period from 11 November 1976 to 17 February 1977. Letters a', b', and c' indicate the 44-day period from 17 February 1977 to 31 March 1977 [X = infection incidence (J.E. Vander Plank. 1963. Plant diseases: epidemics and control. Academic Press, New York and London. 349 p.)".

TABLE 4. Incremental proportions of variance (ΔR^2)[a] contributed by isolations at two different dates of *Cytospora* spp. from each twig element (leaf scars, buds, internodes) of peach (*Prunus persica* 'Redhaven') on twig canker incidence based on combined data from a 2-yr study

	Arithmetic		Semi-log transformation		Log-log transformation		
Twig element	Bud break	10 days post-bud break	Bud break	10 days post-bud break	Bud break	10 days post-bud break	N[b]
Leaf scars	0.2831	0.2401	0.2895	0.2480	0.1960	0.1989	24
Buds	0.0235	0.1079	0.0155	0.1045	0.1258	0.1369	24
Internodes	0.0004	0.0182	0.0037	0.0171	0.0008	0.0064	24
$\bar{R}$	0.797**[c]	0.849**	0.802**	0.850**	0.694**	0.749**	
$\bar{R}^2$	0.635	0.720	0.644	0.722	0.481	0.558	

[a]Amount by which the squared multiple correlation ($\bar{R}^2$) decreased if the variable was removed from the regression equation: $\hat{Y} = a + b_1X_1 + b_2X_2 + b_3X_3$.

[b]Number of observations in analysis.

[c]Asterisks (**) indicate statistical significance $P<0.01$.

implies that most infections occur in the autumn. This conclusion was based on the assumption that the spring sprays were applied prior to a period when natural infection occurred in that season. It appears, therefore, that fungicides for reduction of canker incidence, must be applied in late autumn or in winter before any increases in the occurrence of *Cytospora* spp. in twig elements are detectable. A sharp rise in the IR of leaf scars and buds that began in late February supports the contention that most infection takes place in late winter; i.e., well before bud-break. This conclusion agrees in part with the findings of Tekauz (5) that infection can take place through leaf scars at times other than at leaf fall. The low levels of infection prior to late winter probably reflect the number of buds and leaf scars that become infected in the autumn during and after leaf fall. The slightly better control from fall applications compared with late-winter applications supports this contention. Infection in the leaf scars and buds, however, does not necessarily result in the production of a lesion on the twig (5).

Cytospora spp. seem to be randomly distributed in buds, leaf scars, and internodes. Since there were no correlations between *Cytospora* spp. infection in any of the three twig elements, the presence of *Cytospora* spp. in an element on a twig apparently does not increase the probability that other nearby elements also will be infected.

The usefulness of the multiple regression method of analysis to measure the importance of the independent contribution of each predictor variable has been discussed (1, 3). In this study, the contribution of each type of infected twig element on the incidence of twig canker was measured by ranking the ΔR^2 values. The leaf-scar element had the highest ΔR^2 values for both bud break and 10 days after bud break. The results indicate that isolations from leaf scars is the most useful variable and buds the next most useful variable among twig elements (leaf scars, buds, and internodes) for predicting Cytospora canker incidence in Redhaven peach.

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