Cold Predisposition of Dormant Peach Twigs to Nodal Cankers Caused by Leucostoma spp.

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ABSTRACT

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Flower bud mortality in selected peach cultivars was correlated with a sequence of low temperatures from −17 to −25 C when dormant twigs were collected in midwinter and so exposed. In repeated trials, samples frozen at various low temperatures contracted a significantly higher number of nodal cankers compared with nonfrozen field samples when artificially inoculated with the canker fungi, *Leucostoma cincta* and *L. persoonii*. Among field samples of three peach cultivars collected in late winter, those showing higher incidence of flower bud mortality also showed increased susceptibility to artificially induced nodal cankers. Water leachates of frozen flower buds greatly influenced conidial germination of *L. cincta* and germ tube growth of both *L. cincta* and *L. persoonii*. These results lend support to the view that winter-killed flower buds predispose peach twigs to nodal cankers, which may develop into perennial cankers.

Additional key words: Leucocytospora cincta (=*Cytospora cincta*), *Leucocytospora leucostoma* (=*Cytospora leucostoma*), perennial peach canker.

Perennial canker caused by *Leucostoma cincta* (Fr.) Hohn. (Imperfect state, *Leucocytospora cincta* [Sacc.] Hohn. [=*Cytospora cincta* Sacc.]) and *L. persoonii* (Nits.) Hohn. (Imperfect state, *Leucocytospora leucostoma* [Pers.] Hohn. [=*Cytospora leucostoma* Sacc.]) has been an important disease of peach (*Prunus persica* [L.] Batsch) in Ontario and elsewhere in the northern regions of its culture (1,4,8,9,13,18,20,21). Trunks and scaffold branches often are girdled by extensive cankers and the trees so affected are doomed to a short orchard life. Cankers are first apparent in the spring at nodes, fruit pedicels, and oriental fruit moth (*Grapholitha molesta* [Busck]) injury sites at twig terminals.

Leaf scars (14,19,22) or winter-killed flower buds (1,17) or both have been implicated in nodal canker infection. Many large perennial cankers have their origin in such nodal cankers and then spread to successive subtending branches.

Winter injury to peach flower buds, twigs, and bark is a recurring problem on the northern fringe of peach culture. Several reports have associated low temperature injury with either the infection of stone fruit trees by perennial canker (2,3,5,22) or the aggravation of existing cankers (5,9). Similarly, freezing stress was reported to predispose *Euonymous alatus* stems to girdling stem canker by *Nectria cinnabarina,* a nonaggressive facultative parasite (15). Frost injury was shown to predispose pear blossoms to blossom blight by ***Pseudomonas syringae*** **van Hall (10). In peach canker,** injured flower buds have been suspected to be among the first infection sites, but their close proximity to leaf scar makes it difficult to distinguish the two potential sites. This study was designed to show whether cold injury to dormant flower buds predisposed peach twigs to nodal canker caused by *Leucostoma* spp.

MATERIALS AND METHODS

Twig sampling and freezing.—Peach cultivars regarded as examples of cold hardy ('Bailey'), medium hardy ('Redhaven'), and tender ('Loring' and 'Redglobe') reactions were used for the purposes of these experiments. Terminal twigs of mature peach trees, about 45 cm long, were pruned on various dates in February and March and were stored at 3 C overnight. Natural flower bud mortality was assessed on five to ten twig samples for each cultivar by dissecting the buds and counting the percentage of those browned. The other twigs were placed in wooden boxes lined with polystyrene foam and transferred to a freezing chamber programmed to cool at the rate of 5 C/hr. Each box was supplied with a thermocouple inserted into a twig and then connected to a Thermo Electric multipoint potentiometer (Model FMWST6C). In the 23 February experiment, 'Bailey,' 'Loring,' and 'Redhaven' peach cultivars were sampled; a temperature interval of 2 C over the entire range of −17 to −25 C was used. Individual boxes containing bundles of twigs of different cultivars were removed at each test temperature and the material was thawed at 3 C overnight in polyethylene bags before assessing flower bud mortality.

Inoculation.—Samples of five to ten twigs, trimmed to about 30 cm, were inoculated by immersing for 10 min in a water suspension of conidia in 30 × 3.8-cm tubes, being careful to avoid immersion of the cut ends. The inoculum contained about 3.0×10^6 conidia/ml (10 Klett units

against a No. 42 blue filter in a Klett-Summerson colorimeter) of *L. cincta* or *L. persoonii* which had been either produced on Leonian's malt extract agar (LMA) (7) in 1-liter flasks for 4–5 months, or obtained from pycnidia on cankered twigs in the orchard. The twigs were then withdrawn, drained, enclosed in polyethylene bags, and incubated at 20 C for 3–4 weeks. Cankers at nodes with zonations characteristic of Leucostoma lesions were counted. They were later confirmed by isolation from some of the cankers using LMA plates. The canker data were analyzed by analysis of variance (16).

TABLE 1. Flower bud mortality at sequence of low temperatures in three peach cultivars and their correlation coefficients

Temperature (C)	% Flower bud mortality (mean ± SE)[a]		
	Bailey	Redhaven	Loring
Field sample[b]	3.0 ± 2.1	16.5 ± 3.5	36.5 ± 7.9
−17	39.6 ± 5.3	59.3 ± 6.4	49.5 ± 7.8
−19	49.0 ± 2.7	66.2 ± 5.0	56.7 ± 4.5
−21	49.6 ± 3.4	92.5 ± 4.3	89.2 ± 3.7
−23	85.0 ± 6.8	94.9 ± 1.6	86.4 ± 2.7
−25	93.5 ± 3.0	95.8 ± 2.0	98.9 ± 1.1
r[c]	0.908*	0.964**	0.815*

[a]Each value is mean of ten-twig sample and its standard error.
[b]Twigs sampled on 23 February 1973.
[c]Significance of r: $P = 0.05$ (single asterisk) and $P = 0.01$ (double asterisk).

Water leachates of flower buds.—Water leachates of live and frozen flower buds for conidial germination studies were prepared. Dormant twigs of 'Bailey' or 'Redhaven' cultivars were pruned in January, frozen at −25 C for 3 hr, and thawed at 3 C for 48 hr. A portion of the frozen sample was examined to ensure nearly 100% flower bud mortality. Twenty frozen or live buds were shaken in 10 ml of sterile distilled water for 3 hr, and the leachate subsequently was sterilized by passing through a Millipore membrane filter (pore size, 0.45 μm).

Conidial germination.—Three drops of a conidial suspension in sterile distilled water with a reading of 75 Klett units (against a No. 42 blue filter) was added to 2 ml of leachate. Generally, the suspension had a conidial concentration of about 3.0×10^6 ml as determined by a hemacytometer. Drops of such a preparation in live or frozen bud leachate were placed on the surface of 2% Difco Purified Agar or LMA. The plates were incubated at 20 C (*L. cincta*) or 25 C (*L. persoonii*), optimum temperatures for the respective fungi, for 24 to 96 hr. Percent germination was recorded at 36 and 48 hr by counting germination among 100 conidia in each of four replicates per treatment. Germ tubes of 100 germinated conidia per treatment were measured at 24-, 36-, and 48-hr intervals.

TABLE 2. Nodal cankers in three peach cultivars resulting from inoculating 1-yr-old dormant twigs exposed to low temperatures with laboratory and field inoculum of *Leucostoma cincta* and *L. persoonii*

Cultivar[a]	Temp. (C)	No. of cankers/5 twigs					Variance ratio[d]
		Leucostoma		Field	Not	Total	Field sample vs
		cincta[b]	*persoonii*[b]	inoculum[c]	inoculated		−17 C to −25 C
Bailey	FS[e]	1	4	0	1	6	
	−17	3	11	16	4	34	
	−19	6	15	20	2	43	F=20.94**[f]
	−21	6	12	16	2	36	
	−23	7	22	20	3	52	
	−25	12	17	26	4	59	
Redhaven	FS	3	0	3	0	6	
	−17	6	8	8	0	22	
	−19	9	20	14	0	43	
	−21	10	17	19	0	46	F=13.75**
	−23	8	10	12	0	30	
	−25	9	7	15	3	34	
Loring	FS	6	5	7	0	18	
	−17	5	11	10	12	38	
	−19	7	18	14	8	47	F= 9.13**
	−21	9	13	22	16	60	
	−23	11	16	21	6	54	
	−25	8	14	30	3	55	

[a]Twigs sampled on 23 February 1973.
[b]Culture-grown conidial inoculum.
[c]From pycnidia on cankered peach trees.
[d]Analysis of variance after partitioning treatments sum of squares (Snedecor and Cochran, 1967).
[e]FS = field sample.
[f]Double asterisks (**) indicate statistical significance, $P = 0.01$.

RESULTS

Peach twigs sampled during middle and late winter showed cultivar differences in natural flower bud mortality similar to known differences in cold hardiness (Layne, *personal communication*). Such differences were also evident when they were exposed to a sequence of low temperatures. When peach twigs were sampled on 23 February 1973 and exposed to a temperature range of −17 to −25 C at 2 C intervals, flower bud mortality was considerably lower in 'Bailey' than in 'Redhaven' or 'Loring' at comparable temperatures up to −21 C (Table 1). The data in Table 1 also show significant increases in flower bud mortality in all three peach cultivars, with decreasing temperatures in the freezing range.

Nodal cankers resulting from culture-grown or field inoculum were significantly less ($P<0.01$) on nonfrozen twigs than on those frozen at temperatures of −17 to −25 C in each of 'Bailey,' 'Redhaven,' and 'Loring' (Table 2). Cankers in noninoculated samples reflect the level of field incidence already present at the time of sampling. Figure 1 which was drawn on the data from Tables 1 and 2, shows a significant regression of nodal cankers on percent flower bud mortality in Bailey ($P<0.01$) and 'Loring' ($P<0.05$). Values (r=0.784) for 'Redhaven' were close to significance ($P = 0.06$) with a different slope. Dormant twigs of 'Bailey,' 'Loring,' and 'Redglobe' (very cold tender) sampled on 10 March 1973 showed 1, 37, and 99% flower bud mortality, respectively. On inoculation, 'Bailey' had only one nodal canker, significantly ($P<0.01$) less than in others; 'Loring' had 35; and 'Redglobe' had 31, for ten-twig samples each (Table 3). Field observations in the spring showed incipient nodal cankers in close proximity to dead flower buds.

The association between freezing injury to flower buds and nodal cankers was investigated in terms of stimulation of conidial germination of the causal fungi, since conidia must germinate at, and the germ tubes must extend into, the infection courts before infection can take place. Conidial germination of *L. cincta* was significantly higher ($P< 0.05$) in water leachates of frozen buds than that of nonfrozen buds both at 36 and 48 hr (Table 4). On LMA, germination exceeded 90% in 36 hr, while in distilled water it was barely 4%, even in 48 hr.

Conidia were regarded as germinated when the germ tube exceeded half the diameter of the conidium. Evidence showed that higher germination took place in leachates of nonfrozen buds than in distilled water. For this reason, response of germ tube growth was examined at 24 and 36 hr. Germ tube growth of both *L. cincta* and *L. persoonii* in leachate from frozen buds of 'Bailey' significantly ($P < 0.001$) exceeded that in leachate from live buds in 24 hr (Table 5). Similarly, germ tube growth of *L. cincta* in leachate from frozen buds of 'Redhaven' significantly ($P < 0.001$) exceeded that in leachate from live buds, both at 24 and 36 hr.

DISCUSSION

The canker fungi *L. cincta* and *L. persoonii* are weakly parasitic wound pathogens. A number of investigators (1-3,5,9,22) have shown that winter injury is an important contributing factor in *Leucostoma* canker of peach and other stone fruits. It has been suggested that following winter injury, flower buds are invaded by canker fungi (1). Contrarily, unhealed leaf scars have also been implicated in serving as entry points for the canker fungi. Willison (22) stated that under weather conditions that limit periderm formation, the vessels of and the dead tissue below the leaf scar could serve as infection courts. Although he showed the relation between fall

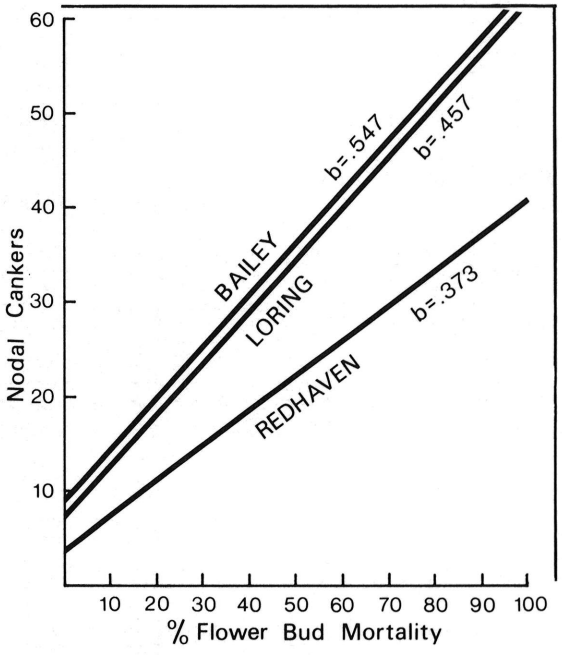

Fig. 1. Regression of number of nodal cankers (on 20 twigs) on percent flower bud mortality in three peach cultivars.

TABLE 3. Natural flower bud mortality and nodal cankers in three peach cultivars resulting from inoculating 1-yr-old dormant twigs with laboratory and field inoculum of *Leucostoma cincta* and *L. persoonii*

Cultivar[a]	% Flower bud mortality (mean ± SE)	No. of Cankers/10 twigs				
		Leucostoma		Field inoculum	Not inoculated	Total
		cincta	*persoonii*			
Bailey	1.0 ± 1.5	1	0	0	0	1
Loring	37.8 ± 9.1	10	9	10	6	35
Redglobe	99.4 ± 0.8	9	7	8	7	31

[a]Twigs sampled on 10 March 1973. Cankers on Bailey differ from those on Loring and Redglobe ($P < 0.001$).

temperatures, periderm formation in the leaf base, and the temporary presence of the inoculated fungus in the latter, he did not demonstrate actual lesion formation. He also stated that a combination of factors favoring an outbreak (of leaf scar infection) was of comparatively rare occurrence. Later, Weaver (19) showed that peach cultivars defoliating earlier in the fall had fewer cankers than those defoliating later. He did not show, however, that this relationship had anything to do with cankers at the leaf scar area. Furthermore, in carefully planned leaf scar inoculation experiments, Tekauz and Patrick (17) could not substantiate Weaver's conclusions that early defoliation greatly reduced canker infection. They secured a substantial reduction in twig cankers by removal of nodal elements (leaf scars and buds). Although they were able to get only 3% infection in bud inoculations, they still considered cold-injured buds as potential infection courts, because most of those they inoculated appeared to be healthy the following spring.

TABLE 4. Effect of water leachates of frozen and nonfrozen flower buds of peach cultivar 'Bailey' on germination of *Leucostoma cincta* conidia

Medium	% Germination of conidia[z]	
	36 hr	48 hr
Water leachate of frozen flower buds	75.75 b	80.25 a
Water leachate of nonfrozen flower buds	31.75 c	51.25 b
Leonian's malt-extract agar	92.75 a	
Distilled water	2.25 d	4.00 c

[z]Mean values in each column followed by same letter do not differ significantly ($P = 0.05$) by Duncan's multiple range test.

TABLE 5. Effect of water leachates of frozen and nonfrozen flower buds of peach cultivar 'Bailey' on conidial germ tube growth of *Leucostoma cincta* and *L. persoonii* in 24 hr

Leucostoma cincta[a]			*Leucostoma persoonii*[b]		
Germ tube length (μm)	% Frequency		Germ tube length (μm)	% Frequency	
	Frozen	Nonfrozen		Frozen	Nonfrozen
0.1–20	12	73	0.1–20	7	88
20.1–40	35	26	20.1–40	20	12
40.1–60	25	1	>40	73	0
>60	28	0			
X^2 for heterogeneity between frozen and nonfrozen bud leachates = 95.25***[c] for 3 df			X^2 for heterogeneity between frozen and nonfrozen bud leachates = 144.06***[c] for 2 df		

[a]Incubation at 20 C.
[b]Incubation at 25 C.
[c]Triple asterisks (***) indicate statistical significance, $P = 0.001$.

Strong evidence was obtained in the present report for predisposition of peach twigs to nodal cankers by *Leucostoma* spp., following cold injury. Although the sequential lower temperatures caused progressively increasing flower bud mortality, it did not result in a consistent increase in the number of nodal cankers. That *L. persoonii* was effective in producing nodal cankers was surprising, because *L. cincta* is most commonly encountered in isolations from such cankers in the spring in the Niagara Peninsula (17) and in southwestern Ontario (Dhanvantari, *unpublished data*).

Layne (6) has reported that cold injury to peach flower buds occurs most frequently between January and mid-March, and continues to a lesser degree until mid-May in southwestern Ontario. It was shown in this report that water leachates of freeze-injured flower buds stimulated conidial germ tube growth of both *L. persoonii* and *L. cincta*. Similar opportunities possibly exist for infection by both of the *Leucostoma* spp. Because cool spring temperatures favor relative growth (4) and pathogenicity (20) of *L. cincta* over those of *L. persoonii,* it may serve to explain why the former is encountered mostly in isolations made from nodal cankers in the spring.

Rohrbach and Luepschen (12) showed that a saturated atmosphere and a carbon source were necessary for germination of *L. persoonii* conidia, and further, that both germination and growth were optimal with mannitol and sucrose. Rohrbach (11) noticed the occurrence of these compounds in peach bark and increase in their amounts during winter. He suggested that these carbon compounds may enhance conidial germination in winter-injured tissue. Both *L. persoonii* and *L. cincta* required 100% relative humidity for substantial conidial germination and a carbon source as provided by dextrose or malt extract for germ tube growth (Dhanvantari, *unpublished data*).

The positive association between natural flower bud mortality and the number of nodal cankers that subsequently developed in this study (Table 3) may have important implications for the peach breeder inasmuch as it was also related to the relative cold hardiness of the cultivars tested. The experiments reported here, together with field observations and isolations, support the suggestion that nodal cankers are initiated by *Leucostoma* spp., in winter or early spring or both by conidia lodged in or near injured flower buds under conditions of moisture, temperature, and carbon nutrition critical for germination and nearly optimal for growth. *Leucostoma* pycnidia are usually abundant in older peach orchards, and conidia readily ooze out under wet conditions. Conceivably, these events take place and cankers develop at a time when the host is not in an actively growing condition to lay down a callus barrier.

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