Response of peach bark tissues to inoculation with epiphytic fungi alone and in combination with *Leucostoma cincta*

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Wounded peach bark was inoculated with the fungal epiphytes *Alternaria alternata* (Fr.:Fr.) Keissl., *Trichoderma harzianum* Rifai, and *Epicoccum nigrum* Link alone and in combination with the peach canker pathogen *Leucostoma cincta* (Fr.:Fr.) Höhn. Bark tissues were sampled over a time series for light and fluorescence microscopic examination to determine the influence of inoculations on the ontogeny of tissues associated with wound healing, specifically the extent of formation of the polysaccharide-impregnated zone, the ligno-suberized boundary layer, and the necrophylactic (wound) periderm. None of the inoculations with epiphytic fungi were associated with as quick a formation of wound-related tissues as observed in the noninoculated control wounds. Inoculation of wounds with *T. harzianum* resulted in delayed formation, but not prevention, of tissues critical to normal wound healing. Combined inoculations of *L. cincta* and epiphytes resulting in wound healing similar to that of inoculations with the epiphyte alone were observed consistently. Inoculation of wounds with *E. nigrum* alone, or in combination with *L. cincta*, did not cause any deleterious effects on the wound responses examined in this study.

Key words: *Alternaria*, biological control, *Epicoccum*, *Trichoderma*, wounding.

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Les auteurs ont inoculé l'écorce blessé de pêchers avec les champignons épiphytes *Alternaria alternata* (Fr.:Fr.) Keissl., *Trichoderma harzianum* Rifai et *Epicoccum nigrum* Link seuls et en combinaison avec le champignon pathogène responsable du chancre du pêcher, le *Leucostoma cincta* (Fr.:Fr.) Höhn. Les tissus de l'écorce ont été échantillonnés selon une chronoséquence et éxaminés en microscopie en fluorescence afin de déterminer l'influence des inoculations sur l'ontogénèse des tissus associés à la cicatrisation des blessures, surtout l'importance de la formation de la région imprégnée de polysaccharides, la zone frontière lignification-subérisation et le périderme nécrophyllactique (traumatique). Aucun des champignons n'est associé avec la formation plus rapide de tissus cicatriciels comparativement à ce qu'on peut observer dans les blessures témoins. L'inoculation des blessures avec le *T. harzianum* retarde la formation, sans la prévenir, des tissus critiques pour une cicatrisation normale. Partout, l'inoculation concommittante du *L. cincta* et des épiphytes a conduit à des cicatrisations semblables à celles observées avec les épiphytes seules. L'inoculation des blessures avec le *E. nigrum*, ou en combinaison avec le *L. cincta*, n'a entrainé aucun effet négatif sur les réactions aux blessures examinées dans cette étude.

Mots clés : *Alternaria*, lutte biologique, *Epicoccum*, *Trichoderma*, blessure.

[Traduit par la rédaction]

Introduction

Leucostoma persoonii Höhn. (anamorph = *Leucocytospora leucostoma* (Sacc.) Höhn.) and *Leucostoma cincta* (Fr.:Fr.) Höhn. (anamorph = *Leucocytospora cincta* (Sacc.) Höhn.) are the causal agents of perennial canker and twig dieback in peach, *Prunus persica* (L.) Batsch. These fungal pathogens initiate disease in bark wounded by pruning, leaf abscission, winter injury, and insect boring (20). The disease appears as perennial cankers on trunks, scaffold limbs, and branches, and causes crop losses through reduction in bearing surface and premature tree death. All commercial peach cultivars are susceptible to these pathogens (2, 7, 14). Chemical controls are limited to a few protectant fungicides, none of which have been found to be very effective (10, 11). Resistance to infection, invasion, and colonization is associated with rate of formation of new tissues associated with defense and repair in the bark (2, 4, 5, 7). The formation of tissue zones, predominant in either polysaccharides (gum and callose) or lignin and suberin, has been demonstrated in peach bark either wounded or wounded and inoculated with *L. cincta* or *L. persoonii* (2, 4). Rate of formation of new tissue layers in mature bark and leaf scars also is influenced strongly by temperature (3, 8).

Several reports have indicated that the peach canker pathogens may be sensitive to competition by other microorganisms (1, 12, 13, 18, 19). Antagonism of *Leucostoma* spp. by other bark-inhabiting fungi may be a useful biological method in the arsenal of integrated techniques now employed to manage this disease (6). The mechanisms by which fungi, such as *Alternaria* spp., *Trichoderma harzianum* Rifai, and *Epicoccum nigrum* Link, exert influence over the peach canker pathogens has not been defined adequately and may include hyperparasitism, antibiosis, and (or) competition for nutrients (18). No one has suggested the possibility that bark epiphytes may cause alterations in the infection court beyond changes in nutrient status. For example, colonization of wounds by epiphytes could cause a more rapid death of exposed tissues thus stimulating the production of new layers of defensive tissues, including the ligno-suberized boundary layer and necrophylactic periderm (2). Given the importance of wounds as infection courts for *Leucostoma* spp., the establishment of epiphytic microbes in the wound would seem to be of paramount importance if biological control is to be successful. The objective of the present study was to determine the influence of some bark fungal epiphytes, inoculated alone and in combination with *L. cincta*, on several histological parameters associated with defense and repair in wounded peach bark.

Materials and methods

Experiment 1

On 29 May 1984, six branches on each of two 'Earlired' peach trees were surface sterilized with 70% ethanol, wounded with a 4 mm diameter cork borer, and inoculated with mycelial plugs (2% malt extract agar) of 14-day-old epiphyte cultures of *Alternaria alternata* (Fr.:Fr.) Keissl. and *T. harzianum* alone and in combination with 7-day-old cultures of *L. cincta*, which were applied at the time of epiphyte inoculation. *Leucostoma cincta*, the pathogen isolated most commonly from leaf scars, was chosen for use in these tests because of previous experience with this pathogen in relation to biological control of leaf scar infections (1). Epiphytic fungi, all isolated from apparently healthy peach leaf scar tissue, were selected based on preliminary experiments designed to demonstrate antagonism in dual cultures with *L. cincta* (1). Six epiphyte plus pathogen permutations were tested, including the control, which consisted of noninfested malt extract agar. For epiphyte plus pathogen treatments, malt agar plugs of the epiphytic fungi were placed mycelium-side down in the cork-borer wounds and the mycelial plug of *L. cincta* was placed mycelium-side down on top of the epiphyte mycelial plug. For epiphyte or pathogen treatments alone, malt agar plugs of the fungi were placed mycelium-side down in the fresh wounds. The inoculated site was then wrapped in Parafilm. Treatments from each tree at each postwounding time were sampled for histological examination at 3, 6, 9, and 14 days after inoculation. Samples were fixed in formalin – acetic acid – alcohol and prepared for light and fluorescence microscopic examination as described previously (2). Data were recorded for nine histological features based on the deposition of reaction zones and new tissues in peach bark as described previously (2). The histological features measured were as follows: depth and width of the polysaccharide zone, the timing and tissue depth at which the ligno-suberized layer (LSL) occurred, the thickness and numbers of cells in this layer, the longitudinal length of the LSL tissue and whether or not the tissue was complete around the perimeter of the inoculation site, and the presence and number of phellem cells in the wound periderm and whether or not it was complete around the perimeter of the inoculation site. Where epiphyte or pathogen treatments resulted in significant delays in wound response relative to the control, some degree of fungal pathogenicity was presumed.

Experiment 2

The first experiment was repeated on 12 June 1984, with modifications and additions as outlined below. Branches on nine 'Earlired' trees were surface sterilized, wounded, and inoculated with malt extract agar mycelial plugs of 5-day-old epiphyte cultures alone and in combination with 3-day-old cultures of *L. cincta*, which were applied 48 h later. The epiphyte *E. nigrum* was included as an additional treatment in this experiment. Trees were sampled for histological examination at 6, 9, and 14 days after inoculation by sampling three of the nine trees at each postwounding time. Samples were fixed in formalin – acetic acid – alcohol and prepared for microscopic examination. Data were recorded for the histological features described above.

Experiment 3

This experiment was initiated on 21 August 1984 and was conducted in a manner similar to the experiments described above, but with the modifications and additions outlined below. The experiment was conducted on 16 'Earlired' trees on one branch per tree and 12 inoculations per branch. All fungal cultures were 6 days old. The treatments were the same as described in experiment 2, with the modification of when the pathogen was applied in relation to the epiphyte. One set of combination treatments consisted of the epiphyte plus pathogen inoculated at the same time into fresh wounds, whereas a second set consisted of the epiphyte placed in the fresh wound followed by the pathogen 3 days later. Tissues were sampled at 6, 9, 14, and 21 days after wounding. The set of histological features examined was reduced to include those anatomical features thought to be most critical to the wound-healing and defense process in peach: the degree of development of the LSL and the degree of development of the wound periderm. These features were rated on a semiquantitative scale ranging from 0 to 3, where 0 represents tissue not formed, 1 represents tissue formed but not extensive, 2 represents tissue formed and extending externally toward the original periderm, and 3 represents tissue formed and in contact with the original periderm.

Experiment 4

This experiment was initiated on 9 October 1984 and was identical with experiment 3 except that histological features were assessed 21 and 28 days after wounding. Epiphyte cultures were 8 days old, and pathogen cultures were 3 days old.

Data analysis

Our original intention was to analyze the treatment effects with linear regression over time postwounding (7). However, tissue changes occurred either so rapidly (experiments 1 and 2) or so sporadically (experiments 3 and 4) that there were not enough degrees of freedom for regression analyses. Therefore, all data were analyzed with factorial analysis of variance based on a completely randomized design. Main effect means for the treatments were separated with Duncan's multiple range test (16). Where significant interactions of treatment with time postwounding were observed, or where only one time postwounding showed the tissue changes, separate analyses of treatment effects were conducted for each time postwounding. These additional analyses were necessary because of the dynamic nature of host tissue differentiation in response to wounding and inoculation and because of environmental influences.

Results

None of the epiphytic fungi, when placed in the potential infection court, stimulated the host to form defensive tissues more quickly than in noninoculated, control wounds. *Leucostoma cincta* and some of the epiphytes were associated with delaying, but not preventing, the formation of tissues critical to normal wound healing. The delay in formation of wound-related tissues was presumed to represent varying degrees of pathogenicity, the degree of which was measured indirectly in terms of the level of suppression of tissue formation. The suppressive effect of *L. cincta* on the formation of wound-related tissues was mitigated by some of the fungal epiphytes. Since none of the epiphytes stimulated the wound responses examined in this study, their mitigating effect was presumed to be a direct or indirect effect on the pathogenic activity of *L. cincta*. The apparent decreased pathogenicity of *L. cincta* when co-inoculated with epiphytes was observed frequently in these experiments. The results for the individual experiments are presented below.

Experiment 1

Inoculation of wounds with *L. cincta* alone caused infection as determined by copious production of gum, a typical feature of infection by *Leucostoma*. No other inoculations resulted in visible symptoms of infection. Wound responses occurred rapidly following the initial wounding, with complete LSL and wound periderm formation observed in the controls at 9 and 14 days postwounding, respectively. Neither epiphyte alone affected formation of the polysaccharide zone in a manner different from the control (Table 1). The depth and length of the zone were increased greatly in response to inoculation with *L. cincta* alone, and when *L. cincta* was inoculated with the

TABLE 1. Effect of inoculations of peach bark with two epiphytic fungi alone and in combination with *Leucostoma cincta* on several histological parameters associated with defense and repair of injured tissues

	Polysaccharide zone*		Ligno-suberized layer†					Phellogen generation‡	
Treatment	Depth (μm)	Length (μm)	Depth (μm)	Length (μm)	Thickness (μm)	No. of cells	Progression	No. of cells	Progression
Control	15.3*c*	67.6*b*	47.0*b*	69.8*b*	6.0*a*	0.8*a*	1.4*ab*	2.0*a*	0.8*a*
L. cincta	109.2*a*	240.0*a*	95.0*a*	182.5*a*	0.0*b*	0.0*b*	0.8*c*	3.0*a*	2.5*a*
A. alternata	51.6*b*	96.0*b*	58.3*b*	102.3*b*	4.6*a*	0.6*a*	1.1*bc*	3.3*a*	1.3*a*
A. alternata + *L. cincta*	101.4*ab*	96.6*b*	67.3*ab*	132.3*ab*	7.3*a*	1.0*a*	1.0*a*	3.0*a*	1.7*a*
T. harzianum	22.8*c*	64.2*b*	73.3*ab*	79.3*b*	6.2*a*	0.8*a*	1.3*abc*	3.3*a*	1.3*a*
T. harzianum + *L. cincta*	83.6*ab*	81.4*b*	72.7*ab*	100.7*b*	5.7*a*	0.7*a*	1.6*ab*	2.0*a*	1.3*a*

NOTE: Values for polysaccharide zone measurements are from two replications at four postwounding times. Values for ligno-suberized layer and phellogen generation are from two replications at two postwounding times. Numbers of observations on the latter layers were limited by the presence or absence of the tissue and its rate of development. Means in the same column followed by different letters are significantly different according to Duncan's multiple range test ($P \leq 0.05$).

*Depth of the polysaccharide zone was measured in longitudinal sections from the outer edge of the zone nearest the wound surface to the inner edge of the zone nearest the cambium. Length was measured from the wound margin along the longitudinal axis.

†Depth of the ligno-suberized layer was measured in longitudinal sections from the wound surface to the outer edge of the tissue. Length was measured from the wound margin along the longitudinal axis. Mean number of cells was determined across the layer at a random location in the tissue. Thickness was measured across the layer at the same point where cells were counted. Progression was rated on a scale of 0 to 3: 0, no tissue present; 1, tissue present between wound surface and cambium only; 2, tissue present as in 1 and extending externally toward the original periderm; and 3, tissue present and in contact with the original periderm.

‡Mean number of cells was determined from a completely formed phellem layer. Progression was rated with the scale described in the previous footnote.

TABLE 2. Effect of inoculations of peach bark with three epiphytic fungi alone and in combination with *Leucostoma cincta* on several histological parameters associated with defense and repair of injured tissues

	Polysaccharide zone*		Ligno-suberized layer†					Phellogen generation‡	
Treatment	Depth (μm)	Length (μm)	Depth (μm)	Length (μm)	Thickness (μm)	No. of cells	Progression	No. of cells	Progression
Control	41.6*bcd*	84.4*bcd*	66.0*a*	109.0*a*	15.3*a*	2.0*a*	1.9*a*	2.2*a*	0.9*ab*
L. cincta	20.7*d*	62.8*d*	70.0*a*	107.5*a*	15.8*a*	1.7*a*	1.3*a*	2.0*a*	0.8*ab*
A. alternata	77.6*a*	139.6*a*	67.2*a*	92.0*a*	11.7*a*	1.4*a*	1.6*a*	2.2*a*	1.3*a*
A. alternata + *L. cincta*	38.3*bcd*	70.0*d*	66.8*a*	88.5*a*	16.5*a*	1.6*a*	1.4*a*	2.5*a*	0.7*b*
T. harzianum	68.8*ab*	127.2*abc*	76.4*ab*	134.2*a*	17.3*a*	1.7*a*	1.5*a*	1.6*a*	0.9*ab*
T. harzianum + *L. cincta*	63.8*abc*	133.6*ab*	74.8*ab*	151.0*a*	6.2*a*	1.1*a*	1.1*a*	1.6*a*	0.9*ab*
E. nigrum	29.9*cd*	78.8*cd*	70.0*a*	84.5*a*	10.3*a*	1.1*a*	1.8*a*	2.0*a*	1.0*ab*
E. nigrum + *L. cincta*	39.8*bcd*	94.1*abcd*	75.4*a*	139.6*a*	18.0*a*	1.6*a*	1.5*a*	1.8*a*	0.8*ab*

NOTE: Values for polysaccharide zone measurements are from three replications at three postwounding times. Values for ligno-suberized layer and phellogen generation are from three replications at two postwounding times. Numbers of observations on the latter layers were limited by the presence or absence of the tissue and its rate of development. Means in the same column followed by different letters are significantly different according to Duncan's multiple range test ($P \leq 0.05$).

*Depth of the polysaccharide zone was measured in longitudinal sections from the outer edge of the zone nearest the wound surface to the inner edge of the zone nearest the cambium. Length was measured from the wound margin along the longitudinal axis.

†Depth of the ligno-suberized layer was measured in longitudinal sections from the wound surface to the outer edge of the tissue. Length was measured from the wound margin along the longitudinal axis. Mean number of cells was determined across the layer at a random location in the tissue. Thickness was measured across the layer at the same point where cells were counted. Progression was rated on a scale of 0 to 3: 0, no tissue present; 1, tissue present between wound surface and cambium only; 2, tissue present as in 1 and extending externally toward the original periderm; and 3, tissue present and in contact with the original periderm.

‡Mean number of cells was determined from a completely formed phellem layer. Progression was rated with the scale described in the previous footnote.

epiphytes, the depth (but not the length) of the zone was increased relative to the control.

Neither epiphyte alone affected formation of the LSL (Table 1). *Leucostoma cincta* affected the formation of this layer by increasing its depth and length, decreasing its thickness and number of cells, and delaying its ontogeny. By adding epiphytes with *L. cincta*, the effect of *L. cincta* on the formation of this layer was mitigated. With *A. alternata* plus *L. cincta*, the thickness, number of cells, and ontogeny of the layer were significantly different from those obtained from *L. cincta* alone and not significantly different from those of the controls. With *T. harzianum*, the length, thickness, number of cells, and ontogeny were significantly different from those of inoculations with *L. cincta* alone and not significantly different from those of the control. None of the inoculations ultimately affected the ability of the plant to form wound periderm. Mean temperature during the postwounding period was 19.6°C.

Experiment 2

Formation of wound-related tissues occurred more quickly than in experiment 1, with the LSL and wound periderm exhibiting complete formation in the control wounds at 6 and 9 days postwounding, respectively. Position of the polysaccharide zone was affected by inoculations with *A. alternata* and *T. harzianum*, with the zone occurring either deeper, for *A. alternata*, or at a greater length, for both fungi, relative to the control (Table 2). There were no effects of the treatments on differentiation of the LSL. The progression of periderm differentiation was slowed in wounds co-inoculated with *A. alternata* plus *L. cincta* relative to inoculations with *A. alter-*

TABLE 3. Effect of inoculations of peach bark with three epiphytic fungi alone and in combination with *Leucostoma cincta* (applied at the same time as the epiphyte (0) or 3 days later (3)) on formation of the ligno-suberized layer and new periderm

Treatment	Progression of ligno-suberized layer		Progression of new periderm	
	Experiment 3	Experiment 4	Experiment 3	Experiment 4
Control	3.0*a*	3.0*a*	2.0*a*	2.0*a*
L. cincta (0)	1.5*cd*	1.5*bc*	0.0*d*	0.5*bc*
L. cincta (3)	2.5*ab*	1.0*c*	1.0*cd*	0.0*c*
A. alternata	2.8*ab*	2.8*a*	1.2*bc*	1.8*a*
A. alternata + *L. cincta* (0)	1.0*d*	2.2*ab*	0.0*d*	1.2*ab*
A. alternata + *L. cincta* (3)	3.0*a*	2.2*ab*	2.2*ab*	1.2*ab*
T. harzianum	1.5*cd*	1.0*c*	0.0*d*	0.0*c*
T. harzianum + *L. cincta* (0)	2.0*bc*	1.0*c*	1.0*cd*	0.0*c*
T. harzianum + *L. cincta* (3)	2.2*abc*	1.2*c*	1.2*bc*	0.2*c*
E. nigrum	3.0*a*	2.5*a*	2.5*a*	1.5*a*
E. nigrum + *L. cincta* (0)	2.5*ab*	3.0*a*	1.8*abc*	2.0*a*
E. nigrum + *L. cincta* (3)	2.5*ab*	2.8*a*	1.8*abc*	1.8*a*

NOTE: Values for ligno-suberized layer and phellogen generation are from four replications at one postwounding time (9 and 21 days in experiments 3 and 4, respectively). Progression of the ligno-suberized layer and new periderm was rated on a scale of 0 to 3: 0, no tissue present; 1, tissue present between wound surface and cambium only; 2, tissue present as in 1 and extending externally toward the original periderm; and 3, tissue present and in contact with the origina periderm. Means in the same column followed by different letters are significantly different according to Duncan's multiple range test ($P \leq 0.05$).

nata alone. Mean temperature during the postwounding period was 17.9°C.

Experiment 3

Formation of wound-related tissues occurred at a rate similar to that in experiment 1, with the LSL and wound periderm exhibiting complete formation in the control at 9 and 14 days postwounding, respectively. Formation of LSL in fresh wounds inoculated with either the pathogen or the epiphytes alone was significantly different among treatments (Table 3). For example, treatments with *A. alternata* or *E. nigrum* were not significantly different from the control, whereas wounds inoculated with *L. cincta* or *T. harzianum* showed delayed formation of LSL. For treatments where epiphytes were inoculated with the pathogen into fresh wounds, formation of LSL was delayed relative to the control for *A. alternata* plus *L. cincta* and *T. harzianum* plus *L. cincta*, but not for *E. nigrum* plus *L. cincta*. In comparing inoculations of fresh wounds with the epiphyte plus pathogen combination to the epiphyte alone, the only epiphyte plus pathogen combination to exhibit delayed LSL formation relative to the epiphyte alone was *A. alternata* plus *L. cincta*. In comparing inoculations of fresh wounds with the epiphyte plus pathogen combination to the pathogen alone, only *E. nigrum* was associated with a significantly greater level of wound response, thus demonstrating its mitigation of pathogenicity of *L. cincta*. Where inoculations were made with the pathogen alone after 3 days, or where the pathogen was added to the epiphyte inoculum after 3 days, formation of LSL was similar to that of the control. Formation of LSL was greater in the 3-day samples relative to the corresponding fresh samples for wounds inoculated with the pathogen alone or with the combination *A. alternata* plus *L. cincta* (Table 3).

Periderm formation also was influenced by epiphyte and pathogen inoculations in this experiment (Table 3). Inoculations of fresh wounds with *A. alternata*, *L. cincta*, and *T. harzianum*, but not *E. nigrum*, exhibited delayed periderm formation relative to the control. *Alternaria alternata* or *T. harzianum* combined with *L. cincta* in fresh wounds also were associated with delayed periderm formation relative to the control, as was *T. harzianum* plus *L. cincta* and *L. cincta* alone when the pathogen was added to 3-day-old wounds. No periderm was observed in 9-day-old samples of fresh wounds inoculated with *A. alternata* plus *L. cincta*, *L. cincta* alone, and *T. harzianum* alone, relative to other treatments where periderm ranged from just initiated (for example, *T. harzianum* plus L. cincta) to almost completed (for example, *E. nigrum* alone). The effect of waiting 3 days to inoculate had no effect on periderm formation relative to the corresponding fresh wounds, except for the combination of *A. alternata* plus *L. cincta*, where the older wounds showed an increased level of periderm formation. In general, the best treatment for not interfering significantly with LSL and periderm formation, and for extenuating the effects of inoculation with *L. cincta*, was inoculation with *E. nigrum*. Mean temperature during the postwounding period was 20.3°C.

Experiment 4

Formation of the wound-related tissues occurred less rapidly in this experiment, with the LSL and wound periderm exhibiting complete formation in the control at 21 and 28 days postwounding, respectively. Very similar results to those described for experiment 3 were obtained in experiment 4, with one significant exception. The inhibition of LSL and periderm formation by the combination inoculation of *A. alternata* plus *L. cincta* in fresh wounds was less dramatic in experiment 4 than in the previous experiment (Table 3). As seen also in the previous experiment, *A. alternata* and *E. nigrum* alone did not interfere with LSL formation relative to the control, although LSL formation was slowed significantly by inoculation with both *T. harzianum* and *L. cincta* alone. Wounds inoculated with combinations of *T. harzianum* plus *L. cincta* in fresh or 3-day-old wounds also exhibited delayed LSL formation relative to the control and all the other epiphyte alone or combination inoculations. The effect of inoculating fresh versus 3-day-old wounds was not significant in this experiment, probably because of the lower ambient temperatures in October relative to those in August, when experiment 3 was conducted.

Periderm development was delayed or inhibited when wounds were inoculated with *L. cincta, T. harzianum*, or combinations of the latter fungus with *L. cincta* (Table 3). No other treatments adversely affected periderm development nor were any treatments associated with enhanced periderm development relative to the control. Mean temperature during the postwounding period was 11.8°C.

Discussion

None of the experiments performed in this study provided data to suggest that bark epiphytes can hasten the rate of formation of wound-related tissues. To the contrary, both *T. harzianum* and *A. alternata* acted to delay, but not prevent, formation of LSL and wound periderm in some experiments. In previous studies of wound response in peach bark, deposition of a polysaccharide substance was detected with periodic acid – Schiff's reagent in the walls of cells located in a zone about 300 μm from the wound surface (2). Polysaccharide deposition, which also included callose, occurred prior to the formation of a visible lignified zone. The first signs of lignification detectable with histochemical reagents (phloroglucinol–HCl) were apparent within 72 h and occurred internal to the area of polysaccharide deposition. The first lignified cells were detected in areas of the wound in closest proximity to the vascular cambium. Although most cell types exhibited lignification in response to wounding, the parenchymatous cells in phloem ray tissue often were the first to stain visibly with phloroglucinol–HCl.

In the later stages of wound response, a LSL formed, followed by phellogen generation and production of suberized phellem. Impermeability of the LSL is related temporally to the formation of intracellular suberin linings in cells present at the time of wounding, and which became lignified, and then suberized, following wounding (2). Suberin deposition in lignified cells occurred within 24 to 48 h after visible lignification. The LSL most often was located approximately 0.8 to 1.0 mm internal to the wound surface. Initial cells of this tissue can be detected within 4 to 7 days in wounds on actively growing trees in midsummer (3) and usually occur in an area of the wound with closest proximity to the vascular cambium. A meristematic layer formed immediately internal to and abutting the LSL and can usually be detected 24 to 48 h after the formation of the latter tissue. Wound (necrophylactic) periderm may be well formed by 10 days postwounding. Complete formation of the boundary zone and new periderm around the entire wound may take 14 to 21 days under ideal conditions; however, as boundary tissues continue to form in an outward direction, new phellogen cells form immediately internal to the established boundary tissue. As phellem is produced in an outward direction, the ligno-suberized boundary is crushed and diminishes in thickness.

The course of events described above was observed within the normal time frame in all of the control wounds from the experiments described herein (3, 7). Inoculation of wounds with *E. nigrum* alone or in combination with *L. cincta* did not cause any deleterious effects on the wound responses examined in this study. Inoculation of wounds with *T. harzianum* resulted in delayed formation, but not prevention, of tissues critical to normal wound healing. Combined inoculations of *L. cincta* and epiphytes resulting in wound healing similar to that of inoculations with the epiphyte alone were observed consistently. It is possible that the epiphytes acted to stimulate host defensive responses that could not be detected with light or fluorescence microscopy, or which may not have been visible following alcohol fixation and dehydration. Alternatively, it is possible that some of the epiphytic fungi used in these experiments acted either directly or indirectly to antagonize *L. cincta*. For example, our results support those of Royse and Reis (12), who found that inoculations of *L. cincta*, singly or in various combinations with *A. alternata, E. nigrum*, and *Coniothyrium olivaceum* Bon., inhibited disease development on peach twigs. *Epicoccum nigrum* and *C. olivaceum* were the most effective antagonists for reducing pathogenesis by *L. cincta* in the field. In laboratory studies, *E. nigrum* caused the largest zone of inhibition and the greatest inhibition of radial growth of *L. cincta* in dual culture. *Epicoccum nigrum* also caused the greatest reduction in canker length, relative to the control, when co-inoculated with *L. cincta*. The mechanism of inhibition of *L. cincta* by *E. nigrum* may be related to the production of antifungal compounds by *E. nigrum*. *Epicoccum nigrum* has been shown to form coils of hyphae around the hyphae of *L. cincta*, perhaps indicating an inhibition of *L. cincta* by direct hyphal antagonism (12).

Schulz (13) also tested several microorganisms for their ability to inhibit development of peach cankers. Good to excellent control was obtained with several fungi, including *Trichoderma koningii* Oudem., *T. harzianum, E. nigrum, C. olivaceum*, and several others. In general, the protection provided by these organisms appeared to increase the longer they were present (up to 4 days) before inoculation with the pathogen. However, the proper controls for these experiments were not included. For this type of study, inoculation with *L. cincta* into wounds of 1, 2, 3, and 4 days of age, along with inoculations with each antagonist alone, are required. Our results with epiphytes alone and in combination with *L. cincta* at 0 and 3 days after wounding verify the need for a range of control treatments in these kinds of experiments.

In our experiments, *T. harzianum* was associated with delayed ontogeny of the ligno-suberized boundary zone and the wound periderm. In addition, this fungus was not a particularly effective antagonist. Smiley *et al.* (15) studied the effects of several *Trichoderma* spp., including *T. harzianum*, on peach canker caused by *L. persoonii*. They observed that *Trichoderma aureoviride* caused a reduction in pathogenesis in one of their tests. They concluded that the activity of several *Trichoderma* spp. on *L. persoonii* involved direct hyphal antagonism and the possible production of diffusible antibiotics. They did not observe direct penetration of hyphae. Elad *et al.* (9) have demonstrated that the biological activity of *T. harzianum* is associated with the production of chitinase and β-1,3-glucanase by the fungus.

In summary, our results have shown that three common fungal epiphytes on peach bark did not stimulate wound response when introduced into sites of mechanical injury. In some instances, epiphytes reduced the rate of formation of new tissue layers. The fungal epiphyte *E. nigrum* did not exhibit any deleterious effects on wound response and was the most effective antagonist for reducing the pathogenic activity of *L. cincta*. The effect of host response to wounding and the influence of rate of wound healing in relation to the susceptibility of the infection court to fungal pathogens should be considered in efforts to control wound pathogens with biological agents.

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