Induction of Cankers on Pear Tree Branches by *Neofabraea alba* and *N. perennans*, and Fungicide Effects on Conidial Production on Cankers

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ABSTRACT

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Cankers formed on pear branches after inoculations with mycelia of *Neofabraea alba* or *N. perennans*, causal agents of bull's eye rot of pear fruit. The highest proportions of successful infections followed inoculations made in fall and winter. Cankers induced by *N. perennans* were larger than those induced by *N. alba*. Small, superficial cankers were obtained after inoculations with conidia of *N. perennans* on wounded branches of pear trees. Sporulation of both pathogens on mycelial-induced cankers occurred throughout the year, with the largest amount of conidia produced at the end of summer and during fall. Sporulation on cankers induced by *N. perennans* spanned at least 2 years. Copper sulfate reduced sporulation on cankers induced by *N. alba*, while copper sulfate, trifloxystrobin, and ziram applied to sporulating cankers reduced germination of conidia of *N. perennans*.

Additional keyword: epidemiology

Bull's eye rot is an important postharvest disease affecting both apple and pear fruit. However, while bull's eye rot pathogens cause canker diseases on apple trees, the association of bull's eye rot pathogens with canker diseases of pear trees has not been well defined.

In apple, *Neofabraea malicorticis* (Jacks.) Nannf. causes anthracnose canker following direct penetration of sound wood (7,10,20). Cankers develop during the fall and to a lesser extent in winter. In spring, they resume development, and by summer cankers stop growing and the surrounding bark cracks, delimiting the canker. *N. perennans* (Kienholz) Dugan, R.G. Roberts & G.G. Grove causes perennial canker on apple trees (15). The pathogen requires wounds to penetrate branch tissue and creates a canker that enlarges from year to year as the result of new infections occurring at the margin of the old canker (5,13,20). Pruning wounds are the main points of infection (15,31). *N. alba* (Guthrie) colonizes healthy woody tissues poorly when inoculated on apple branches

and requires wounds to infect (2,3,11). It has been found on dead bark, causing anthracnose of leaves and twigs, on dead pruning snags and fruiting spurs (28), and grows saprophytically on leaves (27). This species caused a serious outbreak of coin canker of ash trees in Michigan (23) and was recently identified as causing bull's eye rot of apple and pear fruit in the Pacific Northwest (14). Cankers induced by these pathogens or their saprophytic growth serve as the source of inoculum for apple fruit infections known as bull's eye rot or lenticel rot (2,11,15,26). Conidia are splash dispersed to fruit that are susceptible to infection through most of the growing season. Apple and pear fruit can become infected as early as 1 month after bloom, and susceptibility increases as the growing season progresses (1,12,25).

Previous reports briefly list *Pyrus* as a host for anthracnose or perennial canker diseases (30,31), or as susceptible to canker development following artificial inoculation (18,20,22), but details regarding pear tree susceptibility and natural disease development are lacking. The objectives of this study were to determine the susceptibility of pear trees to canker formation induced by *N. perennans* and *N. alba* and to assess conidial production on artificially induced cankers. In addition, the effects of selected fungicides on canker sporulation and conidial germination were evaluated.

MATERIALS AND METHODS

All trees used for inoculations were located in the orchard of the Southern Ore-

gon Research and Extension Center, near Medford. Bosc pear trees and Granny Smith apple trees were planted in 1994, and d'Anjou pear trees were planted in 1968. The d'Anjou trees were irrigated with over-tree sprinklers, while others were irrigated with under-tree sprinklers.

Origin of isolates. *N. perennans* MA-0001 was isolated from an Asian pear in Medford, OR, and *N. perennans* HR-238 was isolated from a d'Anjou pear in Hood River, OR. *N. alba* MB-0128 and MB-0140 were isolated from Bosc pears in Medford. All isolates were grown on acidified potato dextrose agar (aPDA) at room temperature.

Mycelial inoculations. All isolates were inoculated on current season, 2-year-old, and three or more year-old branches on each of four Bosc pear trees, three d'Anjou pear trees, and three Granny Smith apple trees. The section of the branch to be inoculated was first surface-disinfested with 95% ethanol. A 3- to 4-mm² mycelial plug, from the margin of 30-day-old cultures, was placed under a V-shaped cut made into the wood to the depth of secondary xylem. Control inoculations were performed by the same method using a sterile plug of aPDA. After inoculation, wounds were wrapped with Parafilm (Pechiney Plastic Packaging, Menasha, WI), which was removed 1 month later. Canker progression was observed monthly.

Inoculations of Bosc and Granny Smith trees with *N. perennans* were performed at monthly intervals from September 2000 to March 2003. Inoculations with *N. alba* were performed from December 2001 to March 2003. Inoculations of d'Anjou trees with *N. perennans* and *N. alba* were conducted from January 2002 to March 2003.

Conidial inoculations. In order to determine if wounding was required for infection by *N. perennans*, Bosc pear trees were inoculated at monthly intervals from October 2001 to March 2002 with suspensions containing isolates MA-0001 and HR-238 at 5×10^4 conidia/ml. Conidial suspensions were prepared by washing cultures of the pathogens with sterile distilled water (SDW) and adjusting the concentrations using a hemacytometer. Three sets of two branches (one current season branch and one older branch) were superficially wounded with a 5-mm cork borer. The same number of branches was inoculated without wounding. The conidial sus-

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pensions were applied to the wound area with a hand sprayer, and control inoculations consisted of wounded branches sprayed with SDW. Three replicate trees were inoculated each time, and both isolates and control were inoculated on all trees.

Monitoring conidial production. Cankers induced by mycelial inoculations were used to monitor conidial production in the orchard. For each pathogen isolate, five cankers on Bosc trees and three cankers on Granny Smith and d'Anjou trees were washed every 2 weeks. A 130-mm-diameter plastic funnel was placed under each canker and secured to the branch with wires. Each canker was washed with 20 ml of distilled water dispensed with a hypodermic syringe. The cankers were initially soaked with about 5 ml for 30 s, then the rest of the water was applied. Wash water

was collected in a plastic tube placed at the end of the funnel and transported to the laboratory, where conidial concentrations were measured using a hemacytometer and compound microscope. Counts were recorded as the number of conidia per milliliter of wash water.

Canker washes were performed from July 2001 to March 2003 on cankers induced during 2000. A second set of cankers, resulting from inoculations performed during 2001 to 2002, was monitored beginning 14 February 2002, where cankers induced by *N. alba* were included. A fire blight outbreak in 2002 resulted in the reduction in the number of cankers monitored. Additional canker washes were performed on d'Anjou trees, where three cankers induced by each isolate of *N. perennans* and *N. alba* were washed from June 2002 to March 2003.

Effect of fungicide on sporulation on artificially induced cankers. Cankers artificially induced by *N. perennans* isolate MA-0001 and *N. alba* isolate MB-0128 on d'Anjou trees were used to test the effect of selected fungicides on sporulation and spore germination. Copper sulfate, trifloxystrobin, and ziram were applied with a hand sprayer at a rate of 3,000 mg, 47 mg, and 3,638 mg per liter, respectively. Copper sprays are sometimes applied to pear trees for fire blight or *Pseudomonas* blight control, trifloxystrobin for pear scab and powdery mildew control, and ziram for reduction of postharvest decay (24). Three cankers per isolate were sprayed with each fungicide to runoff on 18 October 2002. Two spore washes were conducted before fungicides were sprayed to identify pretreatment sporulation levels. Four washes were conducted after fungicides were applied. Cankers were washed as described above, and conidia were counted using a hemacytometer and compound microscope. Samples from each canker were also plated on water agar to observe the effect on germination. Plates were incubated at 4°C for 1 week, and germination was observed under the compound microscope. A conidium with a germ tube longer than twice its length was considered to be germinated. Germination data were arcsine transformed to stabilize variances and were analyzed with ANOVA. Means were separated with Tukey's honest significant difference.

RESULTS

Mycelial inoculations. Cankers on Bosc trees resulted most commonly when inoculations with *N. perennans* were performed during the autumn and winter months (Fig. 1). Inoculations early in the fall and in late winter and early spring induced smaller cankers that normally did not produce acervuli. However, larger cankers and abundant acervuli resulted from inoculations performed from October through February. A similar pattern of canker induction was observed after inoculations with *N. alba* (Fig. 1). Cankers induced by *N. alba* were generally smaller than those induced by *N. perennans* (Table 1) and did not developed conspicuous acervuli.

Branches of all ages inoculated with both pathogens were susceptible to canker induction, and the extent of the canker was determined by a combination of branch size and time of inoculation (data not shown), where the largest cankers resulted from autumn and winter inoculations on large (older) branches. Current season branches inoculated with both *N. perennans* and *N. alba* were frequently girdled, and necrosis distal to the canker was observed. Similar results were obtained in both seasons of study.

Both isolates of each pathogen performed similarly in each cultivar and spe-

Fig. 1. Proportion of wound inoculations with *Neofabraea perennans* isolate MA-0001 (top) and *N. alba* isolate MB-0128 (bottom) that resulted in canker formation in Bosc pear trees. Values represent the average of four replicate trees with three inoculations each.

cies of tree inoculated. Inoculations on Granny Smith apple trees resulted in a similar pattern of canker development as in Bosc pear trees, but apple inoculations successfully induced cankers in summer months (data not shown). Inoculations on d'Anjou pear trees were successful all year long with a lower proportion of cankers induced during the summer months, especially with *N. alba* (Fig. 2). Girdling due to canker formation was frequently observed.

Canker development began as a blackening of the tissues under the inoculation point. Necrosis subsequently extended farther in length than in width. Peeling of the bark occurred at the margin of the necrotic area. Branch growth delimited the canker, which acquired a sunken shape with cracked margins, separating from the healthy tissues. In the case of *N. perennans*, small bumps appeared on the necrotic tissues that opened to expose the acervuli. Most necrotic tissues separated completely from the healthy tissues by the second year and fell off the branch leaving a clean cavity. Nevertheless, sporulation continued on those lesions although acervuli were not apparent.

Two of the control inoculations made with sterile aPDA plugs resulted in cankers. However, the symptoms observed were clearly different than those obtained by inoculation with *Neofabraea* spp. and were considered to be caused by contamination with secondary agents.

Conidial inoculations. Only conidial inoculations conducted on wounded branches resulted in canker formation (Table 2). Most of the cankers produced were small, superficial, and without acervuli. Inoculations conducted in October, February, and March induced the largest number of cankers.

Conidial production. Cankers induced by *N. perennans* produced conidia through most of the year, with the highest amounts

Fig. 2. Proportion of wound inoculations of *Neofabraea perennans* isolate MA-0001 (top) and *N. alba* isolate MB-0128 (bottom) that resulted in canker formation in d'Anjou pear trees. Values represent the average of five replicate trees with three inoculations each. Inoculations performed in September were lost due to fire blight infection.

Table 1. Average length (mm) of cankers induced by *Neofabraea* spp. inoculated on branches of Bosc pear trees

Inoculation month	<i>N. perennans</i>			<i>N. alba</i>		
	MA-0001	HR-238	Average	MB-0128	MB-0140	Average
2001						
December	64.7 ^y	...	64.7	13.3	22.5	17.9
2002						
January	27.5	34.2	30.8	37.3	20.0	28.7
February	48.4	0	48.4	23.2	33.3	28.2
March	...	...	...	...	...	...
April	23.5	22.4	23.0	15.3	13.4	14.4
May	12.0	0	6.0	5.0	27.5	16.3
June	0	0	0	12.5	0	6.3
July	0	0	0	0	0	0
August	0	0	0	0	0	0
September	0	0	0	0	0	0
October	41.2	39.0	40.1	18.3	16.9	17.6
November	51.1	54.7	52.9	15.9	20.8	18.3
December	49.4	62.4	55.9	42.2	35.9	39.1
2003						
January	35.8	37.8	36.8	30.3	29.9	30.1
February	17.3	42.0	29.7	16.6	14.6	15.6

^y Values are the average of three inoculations performed in each of four replicate trees. Cankers were measured in June 2002 (*N. perennans*) and May 2003 (*N. alba*).

^z Data missing due to fire blight.

at the end of summer and during autumn. Sporulation decreased thereafter, reaching the lowest amounts during summer and autumn of the second year and increasing again in winter (Fig. 3). Inoculation with both isolates of *N. perennans* resulted in similar patterns of sporulation, and both macro- and microconidia were present. Sporulation of *N. alba* followed a similar pattern to that of *N. perennans* (Fig. 3).

An initial high amount of conidia of both pathogens was observed in the second set of cankers washed, which was conducted soon after wood inoculation aimed to detect the beginning of sporulation. Conidia were present even in the first wash conducted.

A different sporulation pattern was observed for *N. perennans* on cankers induced on d'Anjou trees. Conidial production reached a maximum in July 2002, and then decreased progressively to almost zero by the end of the study in March 2003 (Fig. 4). However, this situation was not observed in *N. alba*, which had the same sporulation pattern as in Bosc pear trees (Fig. 4).

Effect of fungicide on sporulation on artificially induced cankers. Only copper sulfate significantly reduced conidial production by *N. alba*, and none of the fungicides reduced sporulation by *N. perennans* (data not shown). Reduced sporulation was

observed only in the first wash after copper sulfate was sprayed. All three fungicides reduced germination of conidia of *N. perennans* from canker washes, with ziram causing the greatest reduction (Table 3). Germination of conidia of *N. alba* was not affected by fungicide treatment. Conidia of both pathogens from cankers sprayed with ziram produced short germ tubes with few ramifications, and no microconidia were produced by germinating macroconidia of *N. perennans*.

DISCUSSION

Susceptibility of pear trees to canker formation following inoculation with mycelium of *N. perennans* was highest during autumn and winter months as previously reported for apples (6,9,16). Cankers on pear trees were similar to those on apple trees, and no differences in canker formation were observed among pear tree cultivars. Nevertheless, cankers incited by *N. perennans* have not been reported as naturally occurring on pear trees. The severity of cankers, based on size and acervulus formation, generally decreased in inoculations conducted from February throughout spring. A greater susceptibility of the wood of d'Anjou trees to cankering was observed, where cankers developed even following summer inoculations. This ap-

Table 2. Proportion of inoculations with conidia of *Neofabraea perennans* isolates MA-0001 and HR-238 that resulted in cankers on Bosc pear trees

Month	MA-0001 ^y		HR-238		Control
	Wounded	Unwounded	Wounded	Unwounded	Wounded
2001					
October	0.7 ^z	0	0.4	0	0
November	0	0	0.1	0	0
December	0	0	0.3	0	0.3
2002					
January	0.4	0	0.4	0	0.2
February	0.6	0	0.5	0	0.1
March	0.8	0	0.9	0	0.1

^y Inoculations were performed using 5×10^4 conidia per milliliter.

^z Each value is the average of four replicated trees, each with three inoculations of each type.

Fig. 3. Sporulation of *Neofabraea perennans* isolates MA-0001 (top left) and HR-238 (bottom left), and *N. alba* isolates MB-0128 (top right) and MB-0140 (bottom right), on artificially induced cankers on pear trees cultivar Bosc. Values represent number of conidia per milliliter of wash water; each value is the average of five cankers.

parent higher susceptibility may have resulted from less vigorous branches being inoculated as compared with those of Bosc trees. Cankering of apple trees induced by *N. perennans* followed similar patterns to Bosc trees. Isolate HR-238 was somewhat more aggressive than MA-0001, inducing some cankering even in the summer of 2002.

Cankers resulted following inoculations with *N. alba* in all the experiments conducted. *N. alba* was able to colonize healthy tissues and grew beyond the inoculation point, in contrast with what has been reported previously for apple (3). It also colonized 1-year-old branches and girdled and killed some branches of d'Anjou trees. Successful inoculations with *N. alba* were those conducted in autumn and winter, except on d'Anjou trees, where cankers were also induced during summer. *N. alba*, however, induced smaller cankers than did *N. perennans*, and only inoculations from October to February resulted in conspicuous cankers. D'Anjou trees appeared to be more susceptible to *N. alba* than did Bosc. *N. alba* was previously identified in naturally occurring small cankers and pruning stubs on pear trees (19).

The necessity for wounds for the induction of cankers on pear trees by *N. perennans* was confirmed as previously reported

for apple trees (5,19,21,29). Sporulation from cankers induced by *N. perennans* was observed for a period of 20 months. Grove et al. (16) reported sporulation spanning almost 3 years on cankers on apple trees inoculated with *N. perennans*. The largest amount of conidia was produced from September to December, coincident with the highest susceptibility of wood to cankering. Both macro- and microconidia were present during the whole period of study, but macroconidia were more abundant during the peaks of sporulation (data not shown).

Sporulation of *N. perennans* on d'Anjou trees followed a different pattern than on Bosc or Granny Smith trees, with a peak in July that decreased thereafter without a significant increase during autumn. This pattern could be the result of a different

environment, where over-tree irrigation provided wetness during spring and summer that may have stimulated earlier sporulation compared with the other cultivars, where irrigation was delivered by under-tree sprinklers. Harley and Reeves (17) reported earlier production of conidia of perennial cankers in apple with over-tree irrigation.

Contrary to the reports by Corke (8,9), *N. alba* sporulated abundantly on artificially induced cankers. Sporulation of *N. alba* followed the same patterns as *N. perennans*, with low amounts observed during summer, increasing through the end of summer and reaching maxima during autumn.

Sporulation on cankers induced by *N. alba* was reduced only by copper sulfate, and the effect was sustained for approxi-

Table 3. Percent germination of conidia of *Neofabraea perennans* isolate MA-0001 from canker washes after fungicide treatment^y

Treatment	30 October	14 November	5 December
Check	91.0 a ^z	95.3 a	76.7 a
Copper	65.3 b	71.2 a	72.0 a
Trifloxystrobin	76.0 b	93.4 a	80.0 a
Ziram	16.0 c	86.7 a	77.3 a

^y Fungicides were applied 12 days before the first canker wash.

^z Values followed by the same letter are not different according to Tukey's honest significant difference.

Fig. 4. Sporulation of *Neofabraea perennans* isolates MA-0001 (top left) and HR-238 (bottom left), and *N. alba* isolates MB-0128 (top right) and MB-0140 (bottom right), on artificially induced cankers on pear trees cultivar d'Anjou. Values represent number of conidia per milliliter of wash water; each value is the average of three cankers.

mately 1 month. Copper fungicides have been reported to control apple anthracnose (*N. malicorticis*), but not perennial canker (*N. perennans*) (20). In the present study, the fungicides tested did not affect sporulation of *N. perennans*. An increase in the rate of sporulation of *N. alba* after the fungicide effect declined, reported by Burchill and Edney (4), was not observed in this study. Application of fungicides reduced the germination of conidia of *N. perennans*. Reduced sporulation and reduced viability of conidia should impact the incidence of bull's eye rot. Ziram had the greatest effect on conidial germination and induced abnormal growth of the germ tubes.

This study demonstrated that pear wood is susceptible to infection by *N. perennans* and *N. alba*, even though cankers caused by *N. perennans* may not occur naturally. Conidia of *N. alba* and *N. perennans* were produced on artificially induced cankers throughout the year, increasing at the end of the growing season and during harvest time, when fruit susceptibility is highest. Fungicides commonly applied in pear orchards had little effect on sporulation of the pathogens, but can affect conidial germination, which should in turn influence disease incidence.

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