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Title: Studies on the Etiology and Epidemiology of Bull's Eye Rot of Pears.

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The objectives of this study were: (1) to determine the etiology of bull's eye rot on pears grown in Oregon and Washington; (2) to determine periods of greatest susceptibility of pear wood to canker formation by the fungal pathogens *Neofabraea alba* and *N. perennans*; (3) to monitor conidial production in cankers; (4) to determine the timing of fruit infection; and (5) to determine the effect of environmental factors, cultural practices and chemical treatments on the development of bull's eye rot of pears. *N. alba, N. perennans* and *N.* sp. *nova* were identified in isolates obtained from bull's eye rot on pear fruit, using species-specific primers in a PCR reaction. *N. alba* was also found to be associated with naturally occurring small cankers and pruning stubs on pear trees.

Pear trees were inoculated at monthly intervals with mycelia of *N. alba* and *N. perennans* to determine susceptibility to canker formation. Susceptibility was

highest during autumn and winter months, with larger cankers bearing conspicuous acervuli produced after inoculations from October to February. Small cankers resulted from conidial inoculations with *N. perennans* on superficially wounded pear branches. Cankers induced after mycelial inoculations sporulated throughout the year with highest amounts of conidia produced from September to December. Pear fruit became naturally infected throughout the growing season, with increasing infection levels close to harvest. Contradictory effects of temperature on bull's eye rot development by *N. perennans* were observed between 2001 and 2002, where the highest levels of disease were found at 10°C and at 30°C, respectively. Wetness duration did not affect bull's eye rot development, while the concentration of conidia correlated positively with disease development. Over-tree irrigation and late harvest resulted in higher disease levels than under-tree irrigation and early to mid season harvest. The fungicides trifloxystrobin and ziram protected inoculated fruit for about one month, while copper sulfate reduced the sporulation rate of cankers induced by *N. alba*. Thiabendazole applied as a postharvest dip reduced bull's eye rot on inoculated pears.

Studies on the Etiology and Epidemiology of Bull's Eye Rot of Pears

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Jose Luis Henriquez, Author

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DEDICATION

This thesis is dedicated to my wife Lorena and my children, Felipe and Morin, for all their unconditional support and patience during these years. They have been my partners in this big adventure and they have been the inspiration needed to complete the journey.

STUDIES ON THE ETIOLOGY AND EPIDEMIOLOGY OF BULL'S EYE ROT OF PEARS

1. INTRODUCTION

Bull's eye rot of apples and pears is an important postharvest disease in the Pacific Northwest (PNW) and is found in most of the fruit-growing areas of British Columbia, Oregon and Washington. The disease is also important in Europe; in Germany bull's eye rot is considered the main postharvest disease of apples (Kennel, cited by Leibinger *et al.*, 1997). The disease is considered of minor importance in the eastern United States (Spotts, 1990). There is extensive literature since 1900 on this disease on apples but there are few reports of the disease on pears, especially regarding epidemiology and etiology. Most of the actual knowledge of the disease on pears has been extrapolated from research conducted on apples.

The disease is also known in Europe as bitter rot (Corke, 1956; Sharples, 1959; Tan and Burchill, 1972; Wilkinson, 1954), lenticel rot (Wilkinson, 1954) and *Gloeosporium* rot (Snowdon, 1990), and as ripe spot in Australia and New Zealand (Brook, cited by Snowdon, 1990).

Several fungi incite bull's eye rot in pome fruit. The main pathogens associated with the disease in the PNW are believed to be *Neofabraea (Pezicula) malicorticis* (Jacks.) Nannf., *N. (P.) perennans* (Kienholz), and *N. alba* (Guthrie) which was recently identified by Gariepy (2002) from isolates of bull's eye rot obtained in the present work and loaned for the development of a molecular

diagnostic protocol. The two former pathogens are normally found producing cankers on limbs of apple trees. *N. alba* is also associated with the disease in the East coast of North America (Lockhart and Ross, 1961; de Jong et al., 2001) as well as in Europe (Edney, 1956). An undescribed species has been recently reported (de Jong et al., 2001) from two isolates, one causing bull's eye rot of apples and the second causing limb cankers on apple trees.

1.1 Neofabraea canker diseases of apple trees

N. malicorticis causes anthracnose canker of apple which is also known as northwestern anthracnose or black spot canker. This disease was first reported in 1900 in the PNW and has been reported in California, Idaho, Illinois, Maine, Massachusetts, Michigan, Nebraska (Grove, 1990), Pennsylvania and West Virginia (McColloch and Watson, 1966) in the United States, and in Denmark, Great Britain, Holland and New Zealand (Grove, 1990), Poland (Borecki et al., 1970) and Spain (Palazon et al., 1984). The anthracnose cankers start developing in the fall, appearing as small circular spots, reddish brown to black in color when forming on smooth bark. Beneath these spots a water-soaked appearance extends to the cambium. The activity of the cankers slows down or stops in winter but resumes in the spring, when cankers enlarge, becoming elliptical and somewhat depressed or sunken. In early summer cankers cease enlargement and the bark dries and cracks around the advancing edges. Conical pustules develop in the affected area and by mid-summer they open to expose the acervuli of the fungus. By the following fall, a callus ridge borders the cankered area. In the second year

the bark usually cracks away from the edges and falls apart, leaving a scar (Heald, 1920). Old cankers are usually characterized by a fiddle-string appearance, which results from the failure of the pathogen to attack the bast fibers (Kienholz, 1939). The cankers grow for a single season but sporulation may persist for several years (Dugan *et al.*, 1993; Grove, 1990). The apple anthracnose fungus infects during fall and winter, penetrating the bark directly. It is a virulent pathogen which does not usually enters through wounds (Zeller and Childs, 1925). Sporulation occurs during cool and moist conditions, conidia are exuded in a gelatinous matrix and are rain-splash dispersed (Grove, 1990). Borecki et al. (1970) reported *N. malicorticis* causing apple fruit rot in Poland but rarely causing cankers on apple trees.

N. perennans is the causal agent of perennial canker, also known as false anthracnose or target canker. It was first reported in the PNW in 1925 (Zeller and Childs, 1925). Apparently, the disease was present several years before but was considered to be a phase of the anthracnose pathogen (Mc Larty, 1933). It is found in British Columbia, California, Idaho, Montana, England and continental Europe (Grove, 1990). *N perennans* has also been reported inciting bull's eye rot in eastern North America (Mc Colloch and Watson, 1966) and in Europe (Wilkinson, 1945). The perennial canker pathogen penetrates the wood through wounds and creates a canker that enlarges from year to year. This led to the name of the disease, despite the fact that the canker is not technically perennial; this condition arises as the result of new infections that take place at the margins of the old canker (Childs, 1929; Kienholz, 1939). Infection of uninjured woody tissues has been reported in

Great Britain, where the pathogen was considered weakly parasitic and readily affected by weather conditions (Corke, 1956). Spore release is greatest in late fall and winter, corresponding to the time when host susceptibility is highest (Grove et al., 1992). Pruning wounds are the main points of infection, and the fungus can penetrate current-year wood as well as older wood in trunks and scaffold branches. Frost cracks, woolly aphid injuries, broken twigs and spurs, and picker's scars and injuries during harvest are other points of infection reported for the perennial canker fungus. Cankers develop on previous year's wood up to branches of 12 to 15 cm in diameter and they are rarely found below the main crotches in the scaffold branches (Grove, 1990; Zeller and Childs, 1925). New cankers are elliptical, sunken, with a coloration that varies from orange to purple or brown. Later, the affected area is surrounded by a raised layer of callus tissue that isolates it from the healthy tissue (Grove, 1990). Cankers are frequently infested by the woolly apple aphid (*Eriosoma lanigerum* (Hausmann), which feed on the calluses, irritating the tissues and producing galls that are killed by freezing temperatures during the winter. Concurrently, winter precipitation keeps the dead tissues moist and promotes sporulation of the pathogen so that reinfections readily occur and the canker enlarges rapidly in the spring, acquiring the typical concentrical shape (Childs, 1929; Dugan *et al.*, 1993a; Gussow, 1930; McLarty, 1933). Black erumpent acervuli are evenly distributed over the surface of first year cankers and form on the most recently colonized tissues in older cankers (Grove, 1990).

Anthracnose canker in the PNW is more frequent in the moist fruit producing areas west of the Cascade Mountains, whereas perennial canker is

prevalent east of the mountains where dryer conditions prevail during the summer and winters are more severe (Grove, 1990; Kienholz, 1939). The geographic ranges of both diseases are reported to overlap in some areas, including the Hood River and White Salmon Valleys, but normally in the areas where one is abundant, the other is rare or absent (Kienholz, 1939). Both kinds of cankers serve as inoculum sources for the bull's eye rot of fruit, which is considered the most damaging aspect of these diseases (Grove, 1990).

Both pathogens are very conspicuous on apple trees by the cankers produced on twigs, main branches and tree trunks. Neither anthracnose nor perennial canker is common as tree cankers on pear trees, but production of spores of both fungi have been observed on superficial bark injuries, and fruit infections are commonly found in trees in which cankers are not observed (Kienholz, 1951). Pear trees are generally resistant to cankers, and the fungi seem to colonize only injured bark and dead outer bark (Fisher and Reeves, 1928; Spotts, 1990). Zeller and Childs (1925) reported that perennial cankers on Bartlett pear trees were limited to last year's wood, such as water sprouts or suckers from the base of the trunk. Similarly, Fisher (1925) reported that the apple cultivar Winesap was resistant to the canker, or only rarely infected, while the fruit was highly susceptible to the rot. Cankers on Bartlett trees and other pear varieties, presumably caused by *N. perennans,* were found in orchards in Spokane Washington, after an extremely cold winter (Fisher, 1925).

Neofabraea alba is considered a weak parasite (Bompeix, 1974) to purely saprophytic (Bompeix, 1988; Edney, 1956). It poorly colonized live woody tissues

when inoculated on apple-tree branches and required wounds to infect them (Bompeix and Bondoux, 1974). Low levels of infection and formation of small cankers that did not produce spores were reported by Corke (1956), who suggested that the frequent occurrence on fruit was due to the saprophytic growth of the fungus on the trees. Saprophytic growth of *N. alba* on apple leaves has also been observed. The fungus grew epiphytically on both leaf surfaces and colonized and sporulated only when growing on damaged and dead leaf tissues (Tan and Burchill, 1972). *Neofabraea alba* is found mainly on dead wood and rarely parasitizes healthy wood (Edney, 1983). It has been found on dead bark, causing anthracnose of leaves and twigs, and on dead pruning stubs and fruiting spurs (Verkley, 1999). Nevertheless, this pathogen has been recently reported as a serious disease of ash trees, causing coin canker in Michigan (Rossman et al., 2002).

N. alba is considered as an important pathogen of apples in Europe, particularly in Germany, Holland and Great Britain, but of minor importance in pears (Edney, 1983). It is one of the most important postharvest rots of apples in France, with variations according to season and localities (Bompeix and Cholodowski-Faivre, 2000). It is considered of lesser importance, compared to *N. perennans*, as a cause of apple rots in Britain (Corke, 1956). Montgomery (1958) reported that *N. alba* was responsible for 7 to 96 percent of the total rot caused by *Neofabraea* spp. *N. alba* was the principal cause of lenticelar rot in England during 1937-1939, with *N. perennans* being isolated only occasionally, but by the 1950's *N. perennans* was prevalent (Wilkinson, 1954), associated with an increase in

branch infections cause by the second pathogen. *N. alba* is seldom found rotting apple fruits in Poland (Borecki et al., 1970). It has been observed rotting apples but not pears in Spain (Palazon and Rodriguez, cited by Palazon et al., 1984), while the same authors reported *N. perennans* on pears but not on apples. There is a differential susceptibility of fruit and woody tissues to infection by *N. alba*, where high levels of fruit rot originate in orchards lacking conspicuous wood infection (Bompeix, 1974).

The putative new species of *Neofabraea* recently found in a phylogenetic study by de Jong et al. (2001), was identified from two isolates, one obtained from a canker on an apple tree in Nova Scotia, which was originally labeled as *N. perennans*, based on differences in morphology as compared to the prevalent *Neofabraea* canker in the region, assumed to be *N. malicorticis*. The second isolate, was obtained from a rotted apple in Portugal and was originally identified as *N. malicorticis*. It also was recently found in a molecular study in Australia from herbaria material of a branch canker that was originally identified as *N. malicorticis* (Dr. James Cunnington, personal communication[1]).

1.2 Bull's eye rot of pome fruits

The most common bull's eye rot on apples in the PNW is believed to be that caused by the perennial canker pathogen *N. perennans* (Chollet and Sprague, 1958), which is considered of lesser importance on pears (Edney, 1983). Conidia

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[1]Dr. James Cunnington, Institute for Horticultural Development. Vic. 3156. Australia.

of *N. perennans* and *N. malicorticis* are produced in cankers on apple trees for two or more years (Barss, 1925; Mc Larty, 1933) throughout the year peaking during fall and winter. They are exuded from acervuli in an opaque, gelatinous matrix and dispersed to the fruit by the impact of water drops (Fisher, 1925; Grove *et al.*, 1992). Rain splash is the main mechanism of dispersal (Grove, 1990, McLarty, 1933), but water splash from over-tree sprinkler irrigation has also been observed (Grove et al., 1992). Higher infection of fruits has been observed when the fruit is exposed to rains at harvest time (Fisher, 1925). Conidia of the perennial canker pathogen are not dispersed by the apple woolly aphid (Grove *et al.*, 1992). Long distance dispersal, resulting in a high percent of storage rot from infection coming from a heavily cankered neighboring orchard, has also been reported (Fisher, 1925).

Fruit is susceptible to infection throughout the growing season from petal fall to harvest, and its susceptibility increases as the growing season progresses (Edney, 1958; Spotts, 1985a). Bompeix (1978a), nevertheless, observed three different periods of susceptibility of apples to lenticelar rots caused by *Neofabraea alba* and *N. malicorticis*. In the first period, the "refractory phase", which occurs shortly after petal fall and proceeds during cellular multiplication, infections were impossible due to the inherent resistance of fruits given by organic acids and phenolics. Susceptibility of the fruits begins in the "intermediate" phase at the end of cellular multiplication and the beginning of cellular enlargement, increasing closer to harvest. Symptom development occurs during the phase of "sensibility". Edney (1956), also reported that resistance to infection is high in young fruit but

falls off as the fruit approaches maturity. Severe fruit infection is associated with rainy conditions during harvest (Childs, 1929; Spotts, 1990), and preharvest rain reduces the resistance of fruit to infections by *N. alba* (Edney et al., 1977). Fruit maturity results in a reduction of the mechanical resistance of the fruits, an increase in pH, which activates fungal enzymes and a reduction of phenolics and other compounds (Bompeix, 1978a). In years with higher levels of preharvest rainfall, fruit infections by *N. perennans* are higher following severe winter damage to the trees (Kienholz, 1951).

The fruit is attacked mainly through the lenticels, but cracks in the skin are also susceptible to attack (Edney, 1956; Kienholz, 1951). Infection may also take place around the stem and calyx (Spotts, 1990). In an *in vitro* experiment, conidia of both pathogens adhered chiefly to lenticels and cracks in the cuticle (Dugan *et al.*, 1993a). Cultivars with open lenticels have been shown to be more susceptible to infections by *N. perennans and N. alba*, while those with closed lenticels were resistant (Bompeix, 1968; Edney et al., 1977). Bompeix (1968) observed a direct relationship of lenticel openings and rot development by *N. alba* in Golden Delicious apples. Less than 25% of the lenticels were open after 125 days of storage. A rapid increase in the percent of opened lenticels occurred thereafter, correlating with the development of fruit rotting which increases with prolonged storage. Wilkinson (1945) suggested that lenticels of Allington Pippin apples were damaged by non-parasitic agents and that fungal spores germinating in the lenticel craters rapidly penetrated through the weakened lenticelar tissues.

Infestation of fruits with bull's eye rot pathogens occur in the orchard and can take place very early in the season, symptoms of infection do not normally appear in the orchard but develop only after four or more months of storage (Bompeix, 1976, 1978a; Spotts, 1990). Occasionally, fruit rot symptoms on late apple varieties caused by *N. malicorticis* are observed while fruits are still on the tree at harvest time (Barss, 1925; Edney, 1956; Fischer, 1925; Wilkinson, 1954). Acervuli may develop on these lesions. The rate of rot development in storage might be related to time of infection; early infections have been shown to take longer to develop than later ones (Edney, 1958). Infections of *N. alba* normally appear later in storage than those of *N. malicorticis*. *N. alba* is more sensitive to phenolics in the fruit and will develop when phenolics decrease as fruit maturation advances (Bompeix, 1978a; Montgomery, 1958).

According to Bompeix (1978b), conidia of bull's eye pathogens can attach to the surface of the fruit and rest there until well after harvest. Conidia germinate after 2 to 3 months of cold storage. Under a constant high relative humidity, mycelia colonize the lenticel cavities and start slowly growing into the surrounding tissues. Symptoms then appear after several months as fruit senesces. *N. alba* also can be present at harvest as mycelium inside lenticelar cavities as well as spores on the surface of fruits (Bompeix et al., 2000). Edney (1958) inoculated apples with *N. perennans* in the orchard before harvest, and he observed germination of conidia and formation of appresoria that remained dormant until storage. He also observed germ tubes arising from appresoria attached to suberized cells which

could not be directly penetrated by the fungus; therefore penetration may take place at a site different than where the appresoria are formed.

Symptoms of *N. malicorticis* infections begin appearing on unripened fruit earlier than those of *N. alba*. The ripening process seems to activate the development of *N. alba* and speed up existing infections of *N. malicorticis* (Bompeix, 1978b; Montgomery, 1958).

Bull's eye lesions appear first as small, circular, light brown spots, which rapidly enlarge and finally may destroy the entire fruit. The spots are usually flat to slightly sunken, and most often brown with a clear or tan center. Acervuli develop on older lesions. They are wet and cream to white colored and often arranged in concentric rings. The rotted tissue is firm and does not readily separate from healthy tissue. Lesions of *N. malicorticis* tend to be more zonate than those of *N. alba* (Snowdon, 1990). *N. malicorticis* also has been reported as one of several fungi inciting moldy core of apples (Brien 1937, cited by Snowdon, 1990).

Bull's eye rot does not spread from fruit to fruit in storage. However, spread of conidia could happen with any processing which involves drenching or dipping the fruit in water (Bompeix, 1988).

1.3 The taxonomy of bull's eye rot pathogens

The taxonomy of bull's eye rot pathogens has been controversial. The perennial canker pathogen has been considered a variant of the organism causing anthracnose canker (Mc Larty, 1933). Bull's eye rot pathogens, belonging to the genus *Neofabraea* (*Pezicula*), are Ascomycetous fungi in the Series Discomycetes,

characterized by the production of apothecioid ascocarps, and grouped in the family Dermataceae of the Order Helotiales (Hawksworth et al., 1983).

Cordley (1900) was the first to identify the conidial state of the anthracnose pathogen as *Gloeosporium malicorticis*, in Oregon. Around the same time Peck described the anthracnose pathogen as *Macrophoma curvispora* Peck, although that name was never accepted, even having precedence to the one by Cordley (Kienholz, 1939; Verkley, 1999). Jackson (1913) discovered the teleomorph and created the genus *Neofabraea* to accommodate it as *N. malicorticis* Jacks. Zeller and Childs (1925) described the anamorph of the perennial canker pathogen as *Gloeosporium perennans*, but Wollenwebr in 1939 transferred it to *Cryptosporiopsis* as *C. perennans* (Verkley, 1999), an anamorphic genus typical of *Pezicula* teleomorphs. On 1932, Nannfeldt transferred the genus *Neofabraea* to *Pezicula*, to accomodate *Neofabraea corticola* (Edg.) Jorgensen, a European species considered typical of the genus *Pezicula* (Kienholz, 1939). He also transferred *G. malicorticis* to *Cryptosporiopsis malicorticis* (Cordley). In 1939 Kienholz discovered the teleomorph of the perennial canker pathogen and described it as *Neofabraea perennans*. Guthrie (1959) described the occurrence of the teleomorph of *Gleosporium album* Osterw. in England and named it *Pezicula alba*. The accepted name for the anamorph of *P. alba* is *Phlyctema vagabunda* Desm., which has more than 10 *Gloeosporium* synonyms. Dugan et al. (1993) transferred *N. perennans* to *Pezicula* for taxonomic consistency. The anthracnose and perennial canker organisms have long been considered different organisms only in North America but as the same species in Europe (Bompeix and

Cholodowski-Faivre, 2000; Edney, 1983; Guthrie, 1959; Snowdon, 1990). Sutton (1980) lists *G. perennans* and *C. perennans* as synonyms of *C. malicorticis*, but he recognized that the anamorphs were not typical of the genus and that the differences could be related with teleomorphic states belonging to the genus *Neofabraea* rather than *Pezicula*. Verkley (1999), in a world monograph of the genus *Pezicula*, matched morphological characters with molecular data (RFLPs of nuclear rDNA) and revalidated the genus *Neofabraea* including 4 species; *N. malicorticis, N. perennans, N. alba and N. krawtzewii*. Nevertheless, he was unable to differentiate specifically *N. perennans* from *N. malicorticis* and stated the need for a genetic characterization of the genus *Neofabraea*. Abeln et al. (2000), in a phylogenetic analysis of *Pezicula* by partial sequencing of ribosomal RNA genes, concluded that *Neofabraea* is a separate evolutionary lineage and should not be included among *Pezicula*. De Jong et al. (2001), in a phylogenetic study, showed the occurrence of four different species of *Neofabraea* causing apple tree cankers and bull's eye rot, including *N. malicorticis, N. perennans, N. alba* and a putative new species. These authors found small genetic differences between *N. perennans* and *N. malicorticis* but they were consistent and transcended geography. They considered their data as revealing true evolutionary separation and merited the recognition of these taxa as two separate species.

The currently recognized species of the genus *Neofabraea* and their accepted anamorphic states, according to Verkley (1999), are listed in Table 1. *N. krawtzewii*, isolated from dead bark of *Populus* spp (Thompson, 1939), is the only species not associated with apple cankers or bull's eye rot of pome fruit.

Table 1. The genus *Neofabraea* according to Verkley (1999).

Teleomorph	Anamorph	Geographic distribution
N. alba	*Phlyctema vagabunda*	Eastern North America, Europe, New Zealand, Australia, Tasmania and South Africa
N. krawtzewii	*Cryptosporiopsis* sp	North America, Northern Europe, Siberia and Japan.
N. malicorticis	*C. curvispora*	North America, Europe and New Zealand.
N. perennans	*C. perennans*	North America, Europe.

1.4 Overwintering of bull's eye rot pathogens, host range and teleomorphic states.

N. malicorticis and *N. perennans* overwinter as mycelium and conidia in cankers and infected fruits left from the previous season (Grove, 1990; Grove et al., 1992). The mycelium of *N. alba* overwinters on pruning stubs, dead buds and mummified fruitlets, within the outer scales of flower buds, and on senescent leaves (Tan and Burchill, 1972). The saprophytic capacity of *N. alba* and *N. perennans* allows them to colonize fallen leaves that could act as another source of inoculum for the disease (Edney, 1956; Tan and Burchill, 1972).

The sexual stage of bull's eye pathogens is rarely found, and its role in the disease cycle is not clear (Grove, 1990). Conidia rather than ascospores have been found by spore trapping over several years (Bompeix, 1988). Widespread dispersal of the disease, possibly for several miles, may take place by the discharge of ascospores from apothecia developing on old pruning wood (Sharples, 1959; Tan and Burchill, 1972). Ascospores of the anthracnose canker develop on apothecia

the second year after infection. Apothecia are formed in the same cavity where the acervuli formed the preceding year. Ascospore release occurs after rain fall and ascospores are dispersed by wind. They are apparently capable of producing infections in the same way as conidia (Heald, 1920). Apothecia of *N. alba* have been found on dead pruning stubs on apple trees, often produced on the remains of acervuli. Conidia and ascospores were found in the same stroma (Guthrie, 1959). Apothecia also have been observed on dead apple leaves and dead bodies of red spider mites (Tan and Burchill, 1972).

Both canker-inducing pathogens are known to have similar host range which includes apple, pear, crab apple, quince, peach, serviceberry, apricot, cherry, flowering quince, hawthorn, and mountain ash (Grove, 1990). *N. alba* has been reported on *Malus* spp., *Pyrus*, *Olea europaea*, *Euonymus*, *Rubus*, *Sambucus*, *Aconitum*, *Erigeron* (Verkley, 1999) and *Fraxinus* spp. (Rossman et al., 2002).

1.5 Morphology of bull's eye rot pathogens

Morphological features among these pathogens are quite similar and the main structures used to separate them are microconidia, macroconidia and ascospores. Microconidia (5-8 (-13) x 1-1.5(-2) μm) are produced in culture only by *N. malicorticis* and *N. perennans*. They are cylindrical straight or slightly bent in the lower part, apex round, with a truncate base, hyaline and with granular contents.

Macroconidia of *N. malicorticis* measure 15-35 x 3-6 μm, they are aseptate, hyaline, sickle to U-shaped, ends somewhat pointed or rounded, with granular contents and containing a variable number of oil droplets at maturity.

They are produced on simple or branched conidiophores borne in closely packed subepidermal to erumpent acervuli that are circular or irregular and often merged, pulvinate and liberating creamy masses of conidia when moistened. Apothecia are erumpent through the upper dark tissue of the acervular stroma of the previous year, solitary or in clusters, sessile to short stalked, gray to flesh colored and measuring 0.5 to 1 mm in diameter. The receptacle is surrounded by older black stroma. Asci are cylindrical-clavate, with a truncate-rounded apex, inoperculate and short-pedicellated, measuring 75-150 x 10-20 μm, and containing 8 ascospores. Ascospores are unicellular, ellipsoidal, hyaline and coarsely granular, measuring 12.5-26 x 5-9 μm. They are straight or curved with ends rounded or somewhat pointed. They become 1-3(-5)-septate before germination (Verkley, 1999).

Macroconidia of *N. perennans* measure 12-25 x 3-6 μm, they are unicellular, becoming 1-2 septate at germination, straight or weakly curved, with pointed or rounded ends, hyaline, thin walled and with granular contents or oil droplets (Verkley, 1999). They are borne on simple or branched conidiophores produced in subepidermal to erumpent acervuli. Asci are clavate and inoperculate measuring 86-168 x 8-14 μm. Ascospores are unicellular, ellipsoidal, hyaline and coarsely granular or guttulate, measuring 13-22.5 X 4.5-8 μm (Grove, 1990). Apothecia are similar to those of *N. malicorticis* (Kienholz, 1939).

Macroconidia of *N. alba* are cylindrical to fusiform allantoid, weakly to strongly curved, ends rounded or somewhat pointed, 0-septate, measuring 14-30 x 2-4 um. Conidiophores are simple or branched, born on eustromatic conidiomata,

which can be acervular to pulvinate to almost pycnidial, first immersed but later breaking through the bark. Apothecia develop from conidial stromata, sessile and not sharply delimited from conidiogenous tissues, pale gray or buff with a disc measuring up to 1 mm. Asci are cylindrical-clavate measuring 125-150 x 13-24 um, eight-spored. Ascospores are elongated, ellipsoid to fusoid; straight to slightly curved with rounded to pointed ends, hyaline, 0-septate becoming 3-5(-6)-septate and measuring 20-30 x 7-10 um (Verkley, 1999).

Besides the morphological similarities between the canker pathogens, where the curved shape of macroconidia of *N. malicorticis* contrasts with the straight shape of those of *N. perennans*, identification of apple anthracnose and perennial canker can be supported by several characteristics (Table 2).

Table 2. Some differential characters between anthracnose canker (*Neofabraea malicorticis*) and perennial canker (*N. perennans*) of apple trees reported to help in identification (Kienholz, 1939).

Character	*N. malicorticis*	*N. perennans*
Geographic distribution	West of Cascade Range	East of Cascade Range
Chemical sensitivity	Sensitive to copper fungicides	Not sensitive to copper fungicides
Infection	Sound wood Independent of wooly apple aphid and low temperature damage	Injured wood Dependent on wooly apple aphid and low temperature damage
Canker shape	Fiddle string appearance	Target shape

N. alba, having curved macroconidia, can be differentiated from the other *Neofabraea* species by the lack of microconidia. Germinating macroconidia of *N. alba* do not produce microconidia as those of *N. perennans* (Wilkinson, 1954).

1.6 Effect of storage conditions on the development of bull's eye rot.

The storage of fruit under modified and controlled atmospheres (CA), intended to extend the postharvest life of the fruit by reducing the respiration rate, has also been used directly or indirectly to reduce postharvest rots. On these storage systems the fruit is kept in atmospheres with low concentrations of oxygen and relatively high concentrations of carbon dioxide (Sommer, 1982). The growth of many organisms is retarded in CA storage, but the mixture of gases that provides the best results in extending the life of fruit in storage is not necessarily the best to reduce the development of storage rots. Some pathogens are not affected at all, and others are even favored by CA storage (Lockhart, 1969). A reduction of Gloeosporium rot on Cox's Orange Pippin on apples in CA storage has been observed, where differential results between samples from same lots were shown to be caused by differences in the composition of *Neofabraea* species. Rot development by *N. alba* was reduced in CA storage but the same treatment did not effect rot development by *N. perennans* (Montgomery, 1958). Reduction in the rotting of Cox's Orange Pippin apples by *Neofabraea* spp. under elevated concentrations of carbon dioxide and reduced concentrations of oxygen also was observed by Edney (1964) who noted a greater effect on *N. alba* than on *N perennans*. He also observed a reduction in production of pectolytic enzymes by

these pathogens, which was greater for *N. alba*. Bompeix (1978b) observed no effect of CA (5% CO_2 and 3% O_2) on the growth of mycelium of *N. alba* and *N. perennans in vitro* but observed a greater effect on the incidence of *N. alba* on inoculated apple fruits compared to *N. malicorticis*. Latency of infections was proved for *N. alba,* and it was concluded that such latency is a result of the physiological evolution of the fruit retarded by CA.

Both high concentrations of CO_2 (above 5%) and its complete absence greatly suppress fungal growth (Sommer, 1982). Growth of *N. alba in vitro* was reduced by decreasing concentrations of oxygen, but stimulated with the addition of CO_2. Mycelial growth of *N. alba in vitro* was greater in the presence of CO_2 than without this gas, and greater at 10% than at 5% CO_2. Nevertheless, the incidence of rots of McIntosh apples caused by *Neofabraea* sp. was reduced in the presence of 5% CO_2 compared to 0% CO_2 (Lockhart, 1967). *N. malicorticis* lesion development was prevented on apple fruits stored at 12.8% CO_2 (Sitton and Patterson, 1992), while lesion development was more rapid at 3.6-4.2% O_2 but significantly reduced at 0.5-0.7% O_2. No effect on the level of decay by *N. malicorticis* wounded inoculated on d'Anjou pears was obtained when the fruit was stored at 1% O_2. Nevertheless, fruit stems were greener and more resistant to invasion by the pathogen than those fruit stored in conventional storage (Chen et al., 1981).

1.7 Bull's eye rot control.

Elimination of cankers on apple trees has been long utilized to reduce bull's eye inoculum in the orchard (Childs, 1929; Fisher, 1925; Grove, 1990; Gussow, 1930; Kienholz, 1951). A summer spray of Bordeaux mixture, before fall rains, was effective in reducing both anthracnose canker and bull's eye incidence on apples (Barss, 1925). Fall application of copper fungicides was used to prevent attack of anthracnose canker, but these fungicides did not have effect on perennial canker (Childs, 1929; Kienholz, 1951). Some measures that promote the recovery of injured tissues, minimize infection courts for the perennial canker pathogen, or reduce the amount of inoculum, that have been recommended include: removal of old bark with water under high pressure or by hand scrapping and brushing out the cankers; controlling wooly apple aphids; delaying pruning until the risk of freezing is over; keeping trees vigorous, but avoiding over-succulence; and spraying copper fungicides to reduce reinfection of injured tissue (Kienholz, 1951).

Repacking of fruit in packinghouses may occur due to the development of bull's eye rot, and holding fruit for late shipments is always hazardous. Storing the fruit loose in cold storage and packing immediately before shipment has helped to reduce repacking costs and sort out rots (Kienhloz, 1956).

Tree cankers do not develop commonly on pear trees or some apple varieties such as Winesap, and since the pathogens colonize diverse plant materials and thrive as saprophytes, removal of cankers is of limited value. Orchard sprays are the main avenue to control bull's eye rot (Kienholz, 1951; Sharples and Somers, 1959). Early recommendations of orchard sprays to prevent infections by

bull's eye pathogens included an early spray of ziram applied together with the first cover spray for codling moth control to prevent early infections. A second spray of the same material was recommended to be applied before rainfall close to harvest; one or more sprays were advised if large amounts of rainfall occurred (Kienhloz, 1956). Ziram (first use commercially in 1952), captan, dichlone and several copper fungicides were the first materials tested to control bull's eye rot on apples and pears (Kienholz, 1956). A spray of maneb in late September was as good as ziram in reducing the amount of bull's eye rot in Winesap apples (Chollet and Sprague, 1958). A spray of phenylmercuric chloride in 2 percent linseed oil emulsion reduced sporulation from stem cankers of *N. perennans* by 75 percent during the period of fruit development (Sharples and Somers, 1959). The same mercuric fungicide was used in winter sprays to reduce the inoculum and bull's eye rot incidence in the subsequent season (Edney et al, 1961). Sprays of phenylmercuric chloride and dichlorophen reduced sporulation of *N. alba* on wood infections and resulted in less fruit rot development, although an increase in spore production was observed as the action of the fungicides declined. Further sprays were considered to reduce the overall level of infection in the orchard. (Burchill and Edney, 1963).

Variation in the effectiveness of fungicide sprays on the control of bull's eye rot is related to variations in rainfall before and after fungicides are sprayed. A reduction in the interval between sprays during wet growing seasons for more effective control has been suggested (Moore and Edney, 1959). In a fungicide trial aimed at reducing storage rot due to *N. alba* and storage scab caused by *Venturia*

inaequalis, delan gave the best and most consistent control of bull's eye rot, dodine did not consistently give good control, and varying results were obtained with folpet, which was better than captan (Ross, 1964).

Good control of cankers produced after inoculations with *N. alba, N. malicorticis* and *Pezicula corticola* was achieved with the fungicides benomyl and cercobin in a test on nursery apple tress in Poland, where captan, dodine and copper oxychloride were not effective (Borecki, 1970). Benomyl also reduced the production of spores from cankers of *N. perennans* as compared to untreated cankers (Corke and Sneh, 1979). Benzimidazole fungicides (benomyl and thiabendazole) have been reported to provide good control of bull's eye rot when sprayed in the orchard during early summer for control of apple scab (Burchill and Edney, 1972). Benzimidazole fungicides strongly reduced the incidence of bull's eye rot in Europe in the 1970's, but changes in the spray programs and resistance to benzimidazole fungicides in *N. alba* resulted in the reappearance of the problem (Bompeix and Cholodowski-Faivre, 1997).

Ziram or captan sprays at petal fall and before harvest and benomyl dip treatments after harvest have shown promise in controlling bull's eye rot (Grove, 1990). Control of bull's eye rot of pears in the PNW by orchard sprays of fungicides is still based on the use of ziram, which is normally applied 2 - 4 weeks before harvest (David Sugar, personal communication). Benzimidazole fungicides have been mostly eliminated from use in the orchard in the PNW to prevent pathogen resistance development, as thiabendazole is the main chemical tool for

postharvest treatments. Captan is not registered for orchard application in pear production

The triazole and imidazole fungicides are not considered effective against *N. alba*, while anilinopyrimidines and phenylpyrrol strobilurines are more effective (Bompeix et al., 2000).

Post-harvest treatments have the advantage of allowing better control of treatment conditions. Dipping or drenching fruits with thiabendazole or benomyl are the most common post-harvest treatment to reduce storage rots in pome fruits (Bompeix, 1988). Burchill and Edney (1972) reported that thiabendazole reduced the incidence of bull's eye rot when applied as a postharvest fruit dip of Cox's Orange Pippin apples. Postharvest fungicide application is effective for control but may not reach the fungus if it is established deep in lenticels as result of early-season infections (Grove, 1990).

New and alternative technologies to control the fruit rots in postharvest have been studied. Natural products like terpene compounds extracted from plants have been promising in the control of plant pathogens. Among these compounds, carvone, extracted from mint plants, gave promising results when used in combination with hot water at 45°C for 2 minutes in preventing bull's eye rot of Belchard apples caused by *N. alba* (Bompeix and Cholodowski-Faivre, 1997). Similar results were obtained combining eugenol (extracted from *Eugenia caryophyllata*) with hot water at 50°C for 3 minutes (Bompeix and Cholodowski-Faivre, 2000). Hot water treatments alone are not industrially feasible due to long

exposure times and differential susceptibility of different apple cultivars to the treatment (Bompeix et al., 2000).

Biological control of bull's eye rot also has been studied. Postharvest applications of the yeasts *Cryptococcus laurentii, C. infirmo-miniatus* and *Rhodotorula glutinis,* used alone or in combination with a low dose of the fungicide thiabendazole significantly reduced the incidence and severity of bull's eye rot on d'Anjou pears inoculated with *N. malicorticis* (Chand-Goyal and Spotts, 1996). *C. laurentii* plus *C. infirmo-miniatus* and *R. glutinis* plus half of the commercial dose of thiabendazole had the same level of control than the fungicide alone at normal rate and significantly reduced bull's eye rot incidence in a trial at commercial scale, where the treatments were sprayed to the fruit in the packing line. None of the treatments significantly reduced bull's eye rot of Golden Delicious apples, where disease incidence was very low (Chand-Goyal and Spotts, 1997). Leibinger et al. (1997) applied mixtures of antagonistic microorganisms in the orchard before harvest and observed a reduction of postharvest rots of apples and concluded that early applications should be useful in reducing diseases of apples in storage.

2. ETIOLOGY OF BULL'S EYE ROT OF PEARS

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2.1 Abstract

A collection of *Neofabraea* isolates from pears grown in Washington, Oregon, and California were screened with species-specific primers in a multiplex PCR reaction. *N. alba* was most frequently identified in samples from Oregon and California, while *N. perennans* was most frequently found in samples from Washington. *N.* sp. *nova* was identified in samples from Medford, Oregon, increasing the known geographic distribution of this undescribed species. *N. alba* was identified in small cankers and pruning stubs of pear trees in a PCR reaction. Bull's eye pathogens were isolated from 9 different European pear cultivars, Asian pear and quince. Overall, *N. alba* was the most prevalent species in 2001 while *N. perennans* was more prevalent in 2002.

2.2 Introduction

Bull's eye rot of apples and pears occurs in the Pacific Northwest (PNW) of the United States, Europe and other growing areas. Fruit can be infected in the orchard at any time during the growing season, and spores or appresoria remain dormant until several months of postharvest storage (Bompeix, 1978a, 1978b; Edney, 1958; Spotts, 1990). Bull's eye pathogens produce cankers of branches and twigs or develop saprophytically on dead wood, from which spores are produced and water splashed to the fruits (Barss, 1925; Mc Larty, 1933; Fisher, 1925; Grove, 1990; Grove et al., 1992).

Four different species of the genus *Neofabraea* are known to cause bull's eye rot of apples (De Jong et al., 2001). *N. alba* is the main pathogen causing

bull's eye rot in continental Europe, especially in Germany, Holland, Great Britain and France (Bompeix and Cholodowski, 2000; Edney, 1983), and has been recently isolated from pear fruits in the PNW during the present research (Gariepy, 2002). It is weakly parasitic to saprophytic on the wood of the host trees (Bompeix, 1974, 1988; Corke, 1956), it is also weakly parasitic on fruits and starts developing with the maturation of the fruit and develops lesions later than the other species in storage (Bompeix, 1978a; Montgomery, 1958). It has been previously reported as a minor disease in eastern North America causing bull's eye rot of apples (Spotts, 1990).

N. malicorticis causes anthracnose canker of apple trees and is found principally in the more humid areas of the PNW. It is a virulent pathogen able to infect sound wood directly (Kienholz, 1939). The rots caused by the anthracnose pathogen develop on immature fruits and can even be present at harvest time (Barss, 1925; Edney, 1956; Fisher, 1925; Wilkinson, 1954). *N. perennans* causes perennial canker of apple trees. It requires wounds to infect the wood and the development of new cankers initiated on wounds in the border of old cankers give it a characteristic target shape (Childs, 1929; Kienholz, 1939). Fruit rots caused by *N. perennans* also develop on immature fruit and symptoms normally appear before those of *N. alba* (Edney, 1956). *N. malicorticis* and *N. perennans* are considered a single species in Europe (Bompeix and Cholodowski-Faivre, 2000; Edney, 1983; Guthrie, 1959, Snowdon, 1990), whereas they are considered as two different species in North America based on the differences in macroconidia morphology, canker type, sensitivity to fungicides and geographical distribution

(Kienholz, 1939). A putative new species, yet to be described, has been recently reported in a phylogenetic study of *Neofabraea* species causing tree cankers and bull's eye rot of apples (De Jong, 2001). It was described from an isolate causing tree cankers in Nova Scotia and from another causing bull's eye rot of apples in Portugal. *N. sp. nova* also has been recently found in a molecular study in Australia where it was originally identified as *N. malicorticis* (Dr. James Cunnington, personal communication[1]). There is no canker disease described on pear trees due to the bull's eye pathogens, and cankers have been reported very infrequently (Childs, 1925). Pear trees are considered resistant to cankers, and the fungi seem to colonize injured or dead bark (Fisher and Reeves, 1928; Spotts, 1990). There are few reports of the disease on pears and most of what is known about it has been inferred from the knowledge on apples. *N. perennans* has been reported on pears in Spain (Palazon and Rodriguez cited by Palazon et al., 1984) and *N.malicorticis* reported on Bartlett pears in the United States (Sommer, 1982, cited by Spotts, 1990) and on *Pyrus* in British Columbia (De Jong et al., 2001). Morphological differences among bull's eye pathogens are subtle and usually overlap, making specific identification difficult and inaccurate. Several comparative studies on morphology have been performed, especially to differentiate *N. malicorticis* from *N. perennans* (Dugan et al., 1993a, 1993b; Kienholz, 1939). Verkley (1999) in a world monograph of the genus *Peziculà,* matched morphological characters with molecular data (RFLPs of nuclear rDNA)

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and revalidated the genus *Neofabraea*. He was unable to differentiate *N. malicorticis* from *N. perennans* and stated the need for a genetic characterization of the genus. Abeln et al. (2000), conducted a phylogenetic study of *Pezicula, Dermea*, and *Neofabraea* by partial sequencing of ribosomal RNA genes, and concluded that *Neofabraea* is a separate evolutionary lineage and should not be included among *Pezicula*. Later, De Jong et al. (2001) identified four species of *Neofabraea* associated with apple tree cankers and bull's eye rot, including *N. alba, N malicorticis, N. perennans* and the putative new species. They found some genetic differences among *N. malicorticis* and *N. perennans*, which merited the recognition of these taxa as distinct species. Furthermore, they developed species-specific primers from sequencing of the ß-tubulin gene for the five species of *Neofabraea* known, including *N. krawtzewii*, a pathogen of poplar trees. Gariepy (2002) set the protocol for a multiplex PCR reaction using multiple primers for the specific identification of bull's eye isolates from mycelia and *in planta*. She identified the presence of *N. alba* and *N. perennans* on samples of rotten pears collected during the present study.

The objective of the present study was to determine the species of *Neofabraea* causing bull's eye rot of pears grown in Oregon and Washington, using species-specific primers in a multiplex PCR reaction. The sources of inoculum on pear trees also were investigated in a PCR reaction, where samples of woody tissues were screened.

2.3 Materials and methods

2.3.1 Collection and storage of isolates. Pears showing symptoms of bull's eye rot were collected from packinghouses in Medford and Hood River (including the White Salmon area of Washington) in Oregon. Additional samples were taken from the orchards of the Southern Oregon Research and Extension Center (SOREC), several commercial orchards in Medford, and from the Mid-Columbia Agricultural Research and Extension center (MCAREC) in Hood River. Cultures of fungi isolated from pears with bull's eye rot from elsewhere in Washington were provided by Dr. Chang Lin Xiao of Washington State University, Wenatchee and Dr. Peter Sanderson of the Washington Tree Fruit Research Commission in Wenatchee. Samples of symptomatic Winter Nelis fruits from Philo, California also were provided by Rachel Elkins of the University of California, Davis. Most isolates were obtained during the 2001 season.

Fruit from different pear cultivars from the orchards of SOREC were stored on commercial cardboard pear boxes lined with perforated polyethylene bags at 0 +/- 1°C for several months, to allow bull's eye to develop in order to determine cultivar susceptibility.

Direct isolations were performed from fruit that were surface sterilized in 0.525 % sodium hypochlorite. A piece of infected tissue from the edge of the lesions was placed on acid Potato Dextrose Agar (aPDA), acidified with lactic acid (1.7 ml l^{-1}). Alternatively, monosporic cultures were obtained when sporulation was present. Monosporic cultures were made by immersing an individual fruiting body in 100 – 300 µl of sterile distilled water and subsequently spreading the

suspension on water agar in a Petri dish. The cultures were then incubated at approximately 4°C for one to two weeks; individual germinating conidia were transferred to a Petri dish containing aPDA. Morphology of the germinating conidia, and presence or absence of microconidia were recorded. Isolates were stored at 4°C until processing.

An additional screening of 205 pears with bull's eye rot obtained from four orchards in Medford and one orchard in Hood River was performed in 2002. The causal agents were identified as *N. alba* or *N. perennans* based on morphology of the macroconidia. This identification was conducted to compare with the results of the molecular identification of isolates from the season 2001 and to observe possible seasonal variability among the species of bull's eye rot fungi infecting pears.

2.3.2 Sampling of woody tissues. Samples of dead bark, pruning stubs, previous-season fruit spurs and small superficial cankers were taken from a block of Bosc pear trees with a history of bull's eye rot in an orchard of SOREC in Medford. These woody tissues were subjected to DNA extraction. DNA was then screened using PCR and *Neofabraea* species-specific primers in order to determine possible inoculum sources on pear trees. Three to five sub-samples were pooled in order to increase the number of samples tested. Cankered branches that resulted from artificial inoculations with *N. alba* and *N. perennans* were used as positive controls.

2.3.3 DNA extraction. Total genomic DNA was extracted using the Fast DNA Kit (Qbiogene, Carlsbad, California). Approximately 200 mg of mycelium from

cultures on aPDA were transferred to Fast-Prep 2 ml tubes containing 800 ul of CLS-VF buffer and 200 ul of PPS (protein precipitating solution) for DNA extraction from plant tissue to eliminate inhibitors that could be present in the culture media. Samples were processed in a Fast-Prep machine (FP 120) for 30 seconds at speed 4, incubated for 15 minutes at room temperature, and extracted following the protocol for CLS-VF buffer. DNA extraction from pear tree woody tissues was conducted as described above but at speed 5, incubated for 30 minutes at room temperature and extracted according to the protocol for CLS-VF buffer. Then, a second extraction, using CLS-Y buffer, for the extraction of fungal DNA, was performed.

2.3.4 DNA Amplification. Amplification of DNA was performed in a multiplex reaction, with the addition of four species-specific primers at the same time, according with the protocol developed by Gariepy (2002). The species-specific primer sets use the same forward (upper) primer in combination with the specific reverse (lower) primer (Table 3). PCR reactions were conducted in 0.2 ul tubes in a GeneAmp PCR system 2700 (Applied Biosystems, Foster City, California). A total of 25 ul of reaction contained 0.5 ul of 10 mM dNTPs; 0.4 ul of 5 uM of the universal fungal primers UN-UP18S-42 and UN-Lo28S-22 (Bakkeren et al., 2000) which amplify a portion of the ITS region and were used as positive control to demonstrate the presence of fungal DNA after the extraction; 5 ul of 20 uM of Neofab-up Tub-100; and 5 ul of 5 uM solution of each of four species specific primers; 0.5 ul of Taq titanium DNA polymerase and 2.5 ul of 10x titanium taq PCR buffer (Clontech Lab., Palo Alto California); and distilled water to the final

volume. The PCR reaction was performed using four different annealing temperatures to achieve higher specificity. It consisted of an initial denaturation step of 3 minutes at 95°C followed by 40 cycles of 45 seconds at 95°C, 45 seconds at 4 different annealing temperatures and 2 minutes at 72°C with a final extension of 10 minutes at 72°C. The annealing temperatures started at 72°C for 5 cycles and decreased to 70°C and 68°C for 5 cycles each and to 66°C for 25 cycles. PCR products were visualized by ultra-violet fluorescence at 302 nm following agarose gel electrophoresis and ethidium bromide staining.

2.3.5 Agarose gel electrophoresis and visualization of PCR products. All PCR products were run on 1.5% agarose gels and stained with ethidium bromide to visualize the PCR products by UV transillumination. Band sizes resulting from the PCR reaction were estimated based on a low DNA mass ladder (Gibco/Invitrogen. Carlsbad, CA).

Table 2.1. *Neofabraea* species-specific primers and the expected size of the PCR products.

Specie	Upper Primer	Lower Primer	Fragment Size (bp)
N. alba	Neofab-up-Tub-100	Neo_alba-loTub-439	359
N. malicorticis	Neofab-up-Tub-100	Neo_mal-loTub-262	182
N. perennans	Neofab-up-Tub-100	Neo_per-loTub-382	302
N. sp. *nova*	Neofab-up-Tub-100	Neo-spnov-loTub-319	239

2.4 Results

2.4.1 Molecular identification of bull's eye rot isolates from pear fruits. A total of 450 fungi were isolated from pears showing symptoms of bull's eye rot and were subjected to molecular screening. DNA fragment sizes obtained were consistent with those expected to be amplified for the *Neofabraea* species (Figure 2.1, Table 2.1). Three species of *Neofabraea* were found causing bull's eye rot of pears (Table 2.2, Appendix).

Neofabraea alba was the most common species isolated, with 282 isolates identified. It was obtained from pears sampled in Oregon (Hood River and Medford) and California. *N. perennans* was the second most common species, and was identified from 95 isolates originating in Wenatchee, Hood River and Medford. Eighteen isolates of *N.* sp. *nova* were found, 17 of them isolated from 9 different orchards widespread in the Medford area and one from an orchard located in Grants Pass, 19 miles north-east of Medford in the Rogue River Valley. Fourteen isolates were identified as *Cylindrocarpon magnusianum* Wollenweber (specific identification performed by Dr. Julia Kerrigan, Dept. of Botany and Plant Pathology, Oregon State University). The *Neofabraea* specific primers did not amplify any fragment of DNA from these isolates, but universal bands were obtained. Identification of *C. magnusianum* was performed based on morphological characters, all the cultures produced abundant conidia. Twenty-one isolates gave a mixed reaction where DNA bands specific for both *N. alba* and *N. perennans* were amplified. Twenty isolates were not identified, despite being screened twice. The primer sets failed to amplify DNA fragments from these

isolates. In addition, 8 isolates from apples of unknown cultivars were identified as *N. alba* while one was identified as *N.* sp. *nova*. All of the apples were grown in the orchard of SOREC in Medford.

Table 2.2. Species of bull's eye rot pathogens isolated from pears and identified by amplification of a portion of the β-tubulin gene in a multiplex PCR reaction.

	Wenatchee, WA	Hood River, OR[1]	Medford, OR	California
# samples	33	71	342	4
N. alba	0	42	236	4
N. perennans	18	28	49	0
N. sp. *nova*	0	0	18	0
C. magnusianum	5	1	8	0
Not determined	0	0	20	0
Mixed reaction[2]	10	0	11	0

[1] Including the White Salmon area of Washington.
[2] Isolates giving one band for *N. alba* and one band for *N. perennans*.

Bull's eye pathogens were identified in 9 different pear (*Pyrus communis*) cultivars (Bosc, Comice, d'Anjou, Packam's Triumph, Red d'Anjou, Red Comice, Rogue Red, Seckel and Winter Nelis), on quince (*Cydonia oblonga*) and Asian pear (*Pyrus pyrifolia*, unknown cultivar).

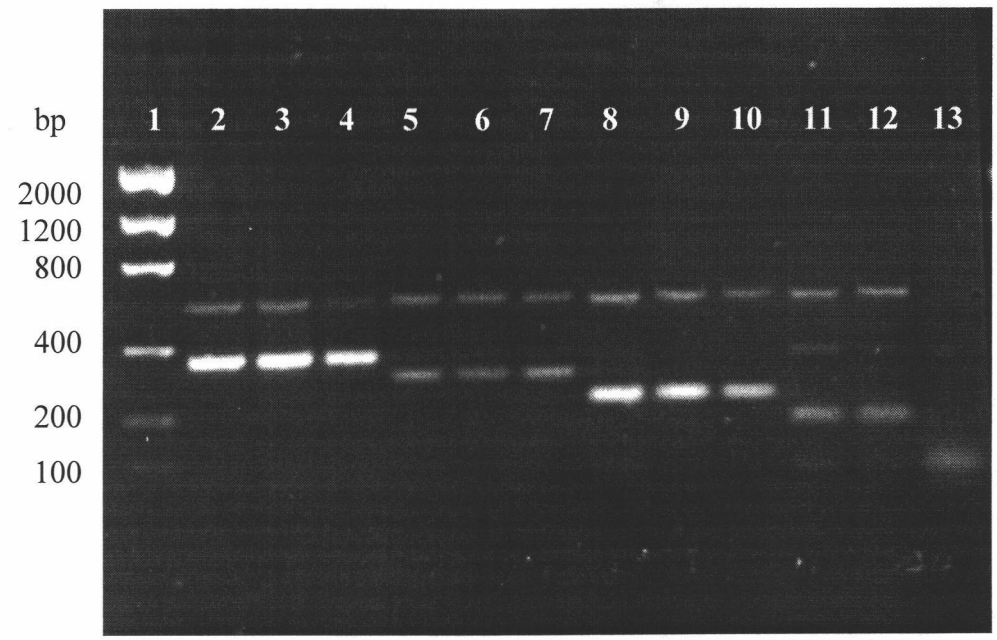

Figure 2.1. DNA fragments from PCR products of the *Neofabraea* species isolated from bull's eye rot lesions on pears. Lane 1 contains a low mass DNA ladder (Invitrogen, Inc. Rockville, Maryland); lanes 2, 3 and 4 *N. alba* (isolates MB-0202, MB-0203, MB-0204); lanes 5, 6 and 7 *N. perennans* (isolates MB-0274, MB-0278, MB-0279); lanes 8, 9 and 10 *N.* sp. *nova* (isolates MB-0222, MB-02109, MB-02127); lanes 11 and 12 *N. malicorticis* (isolates obtained from cankers of Newtown apple trees, Hood River, OR); lane 13 negative control. A ~600 bp band in lanes 2-12 corresponds to a fungal universal band used as control for positive DNA extraction.

Macroconidia of *N. perennans* produced microconidia abundantly when germinating in culture media. Germinating macroconidia of *N. alba* did not produce microconidia and their mycelia grew faster and branched more repeatedly than those of *N. perennans* (Figure 2.2). This criterion was used to make a preliminary identification of 194 isolates as *N. perennans* or *N. alba* when monocultures for molecular identification were performed. A 91.2% match was obtained between this morphological criteria and the molecular identification. However, 13 isolates identified preliminary as *N. perennans* were molecularly identified as *N.* sp. *nova*, lowering the matching level to 85.1%. Production of microconidia from germinating macroconidia of *N.* sp. *nova* was confirmed (Figure 2.2).

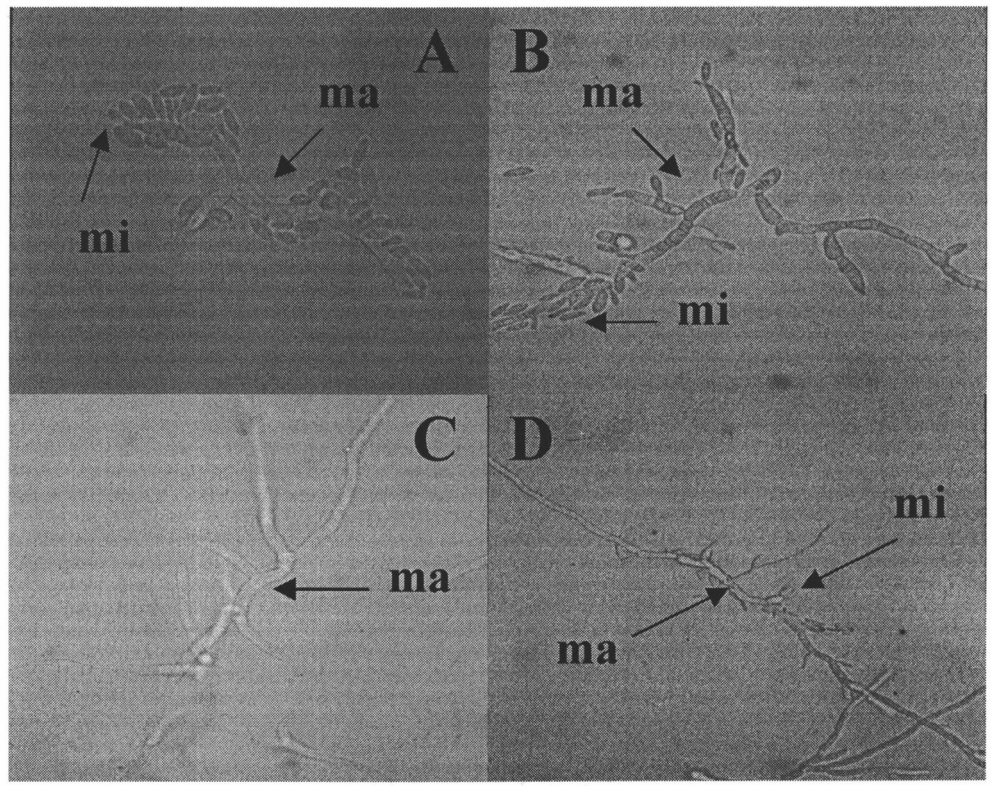

Figure 2.2. Germinating macroconidia and microconidia production of *Neofabraea* species causing bull's eye rot of apples and pears. A, *N. malicorticis*; B, *N. perennans*; C. *N. alba*; and D, *N.* sp. *nova*. Ma = macroconidia; mi = microconidia. (A, B, and C x 400; D x 250).

N. perennans was identified on 188 rotted fruits from the 2002 season while *N. alba* was found rotting only 17 fruits, based on identification performed using morphological characters. All orchards except one had a higher proportion of fruits infected by *N. perennans*. In contrast, the amount of fruit infected by *N. perennans* in 2001 was 19.2%, based on molecular identification (Table 1.3).

Table 2.3. Species of *Neofabraea* isolated from pear fruits with bull's eye rot in 2001 and 2002.

Orchard	*N. alba*	*N. perennans*
	2001 / 2002	2001 / 2002
Bybee (M)[2]	20 / 2	0 / 36
Fairlane (M)	17 / 4	0 / 5
Hanley (M)	85 / 4	14 / 39
Klamath (M)	13 / 1	0 / 15
MCAREC (HR)[3]	42 / 6	28 / 93
Total	177 /17	42 / 188

[1] Identification of isolates from season 2002 was based on the morphology of macroconidia and only distinguished between *N. alba* and *N. perennans*. Identification of isolates from season 2001 was done based on DNA differences.
[2] M = Medford; HR = Hood River
[3] Isolates from several orchards were screened in 2001 and only from one orchard in 2002.

2.4.2 Sources of inoculum on pear trees. A positive identification of *N. alba* in 5 pooled samples of small cankers and 4 pooled samples of pruning stubs was obtained (Figure 1.3). Each pooled sample consisted of 3 individual cankers and 5

pruning stubs. None of the pooled samples of dead bark, or previous year's fruiting spurs gave a positive reaction. Neither *N. malicorticis, N. perennans* nor *N.* sp. *nova* were found in any sample from pear trees.

2.5 Discussion

Neofabraea alba was the most common species isolated. This species has been listed previously in the PNW by Shaw (1972) by its anamorphic name (*Phlyctema vagabunda*) as found on *Malus silvestris* in Washington State. It was the prevalent species in the 2001 harvest season in Oregon and the only one present in samples from California, although few samples were obtained from the latter. It was not found in isolates from Wenatchee and appears to be more prevalent in the south range of the area of study.

It is likely *N. alba* has been present in the PNW for a long time. Fischer (1925) stated:

"Whether there may be another organism which produces a similar appearing rot is still a question, since some of the cultures isolated from these rots appear to be different, but inasmuch as they have failed to produce spores we cannot make definitive identification".

In fact, *N. alba* normally does not produce conidia directly on the mycelium and only produces conidia on conidiomata which were infrequently observed in a few isolates in old culture plates, stored for several months. The great majority of cultures of *N. alba* did not produce conidia. It is highly probable that this species has been confused with *N. malicorticis* since both have curved macroconidia. In fact, most of the references of bull's eye on apples and pears indicate *N.*

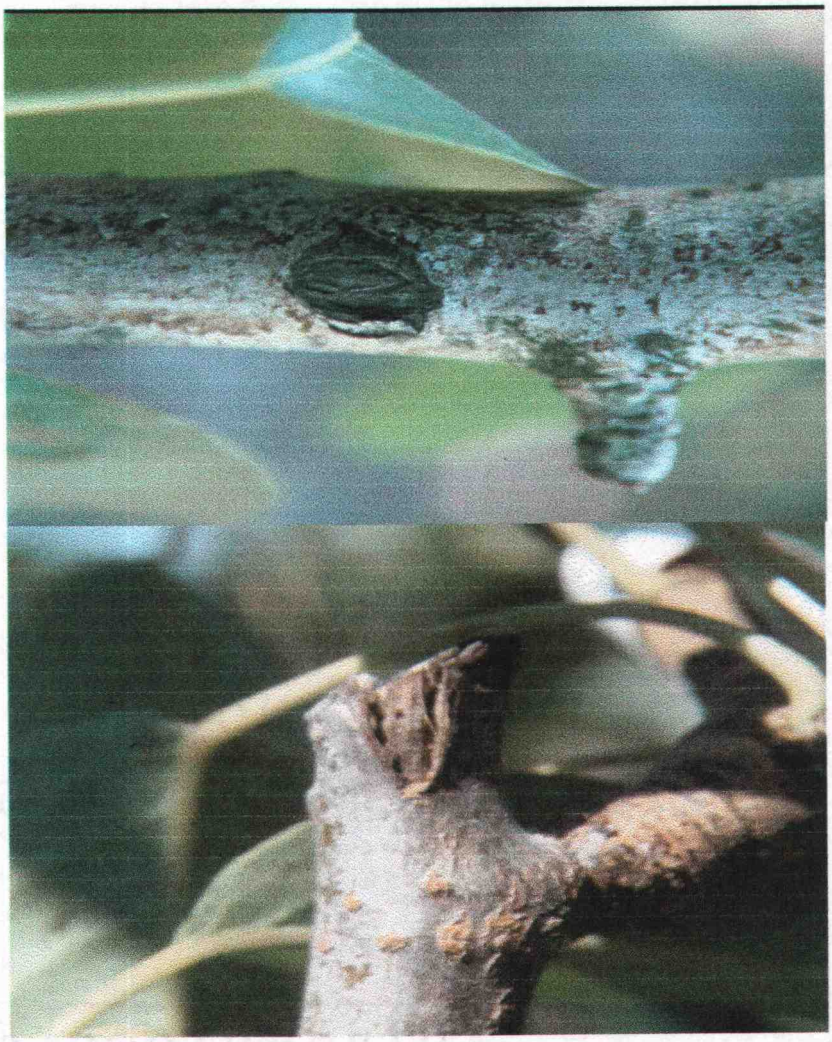

Figure 2.3. Woody tissues where *Neofabraea alba* was identified in a PCR reaction with species-specific primers. A; Small superficial canker on a branch of a pear tree cultivar Bosc. B; Pruning stub showing a small necrotic zone on a branch of a pear tree cultivar Bosc.

malicorticis as the causal agent, an identification probably based on the shape of the macroconidia (Salunkhe and Desai, 1986; Spotts, 1990; Wilson and Ogawa, 1979). The fact that *N. alba* was also identified causing bull's eye rot of apples grown in Medford, may indicate a greater role of this pathogen as a cause of bull's eye rot of apples in the PNW. This question could only be addressed by a thorough screening of *Neofabraea* isolates from apples.

N. perennans was the second most prevalent species associated with bull's eye rot of pears and its widespread distribution is consistent with what has been previously reported for apples (Kienholz, 1939).

The recently described *Neofabraea* species *nova* was found in 10 different orchards, in all of which at least one of the other species was present. In fact, in most of the orchards (six) all three species were found. This finding increases the known geographical distribution of this species, as it was previously known only from Nova Scotia, Canada, Portugal and Australia. *N.* sp. *nova* was in all cases the less frequent species found. *N.* sp. *nova* was not found in Washington or in Hood River, Oregon. This species could be more restricted in geographic distribution, but a larger number of samples from the areas were it was not found will be necessary in order to determine its presence or absence. Macroconidia of *N.* sp. *nova* were as straight as those of *N. perennans*. Microconidia were observed both in acervuli growing among macroconidia and produced by germinating macroconidia. Superficial mycelia grew over the bull's eye lesions caused by *N.* sp *nova*, as is observed for *N. perennans*.

Surprisingly, *N. malicorticis* was not found causing bull's eye rot of pears in this survey. Nevertheless, it was found in cankered branches of Newtown apples at MCAREC in Hood River, in a block contiguous to a block of pear trees, and in cankers on branches of Red Delicious apples from an orchard in Corvallis, Oregon. These samples of cankers, expected to be caused by *N. malicorticis* based on the morphology of the conidia present, were included as positive controls to check for the activity of the corresponding specific primer. *N. malicorticis* was also reported by De Jong et al. (2001) on *Pyrus* from British Columbia. *N. malicorticis* has been described as restricted to west of the Cascade Mountains (Kienholz, 1939). It is possible that this species is actually confined to the most humid areas of the PNW. A survey of a larger number of fruits from the Hood River-White Salmon and Willamette Valley, where *N. malicorticis* was found, would be needed to verify the presence or absence of *N. malicorticis* attacking pears.

Further studies, including gene sequencing and comparison with the sequences of the species already known will be necessary to determine the specific identity of those isolates that were not identified or gave double bands after PCR screening.

N. perennans, which is more aggressive than *N. alba*, was not found in any sample of woody tissues, specifically small cankers and pruning stubs from which it has been previously reported (Edney, 1956), besides it was identify on the positive controls utilized. *N. alba* was found both in small cankers and in pruning stubs (Figure 3) which fits the concept of a weakly pathogenic fungus colonizing

injured or dead tissue. *N. alba* also has an important saprophytic phase which would explain its higher prevalence on pears. *N. perennans* was less important than *N. alba* on apples in Great Britain in the 1930's but then become the most prevalent agent of bull's eye rot by the 1950's. Wilkinson (1954) speculated that this situation resulted from the progressive higher colonization of the orchards by a pathogen able to infect injured wood, especially pruning wounds, and then becoming more prevalent as a fruit rot. The fact that *N. perennans* does not induce cankers on pears trees under natural conditions could explain its relatively lower importance than *N. alba* in pear orchards. A climatic component could also play a role; *N. perennans* seems to have higher importance in the northern areas, declining to the south of the PNW. A similar situation is observed in Europe where *N. perennans* is more prevalent in Great Britain and Germany (Edney, 1956; Leibinger et al., 1997) while *N. alba* is more prevalent in Mediterranean Europe (Bompeix and Cholodowski-Faivre, 2000).

A climatic component may play an important role in the prevalence of a given species of *Neofabraea*. The morphological identification of bull's eye rots conducted in the 2002 harvest season, where the incidence of the disease was lower than during the preceding season, revealed a greater importance of *N. perennans* as the causal agent of bull's eye rot, causing more than 90% of the rots examined. Therefore, *N. alba* may be greatly affected by the seasonal climatic variations. A total of 57.15 mm of rain fell during the growing season 2001 (May 1 to September 30) in Medford, while 26.92 mm of rainfall was observed for the same period in the 2002 growing season (Figure 1.4). 87.1 mm of rainfall were

observed in Hood River during the 2001 growing season (May 1 to September 15) while 47.2 mm of rainfall were recorded for the same period in 2002 (Figure 1.5). Edney et al. (1977) reported that infection of apples by *N. alba* was influenced by the amount of water falling on the fruits before inoculations were performed, and infections by *N. perennans* were also enhanced. The combined effect of an increased susceptibility of fruits and greater inoculum dispersal may play an important role in the incidence of bull's eye rot. In the present study, rainfall was recorded close to harvest in September 2001, but not in 2002. Higher bull's eye rot incidence is associated with rainy conditions at harvest time (Fischer, 1925; Kienholz, 1956). However disease incidence cannot be due only to the effect of water falling over the fruit, since all of the orchards screened in Medford in 2002 had over-tree sprinkler irrigation, which would simulate rainfall. Therefore, some other factor or combination of factors must be affecting incidence of fruit infection.

Several isolates from bull's eye symptomatic pears were identified as *Cylindrocarpon magnusianum*. Isolates were obtained from Wenatchee, Washington, and Hood River and Medford, Oregon. All of these isolates were obtained by plating symptomatic tissues not bearing fructifications. This species has been previously reported as a miscellaneous fungus causing postharvest decay of apples (Rosenberger, 1990). The rot caused by *C. mali* (Allesch.) Wollenweber resembles bull's eye rot in the initial stages of development (Snowdon, 1990) and is one of several fungi causing lenticel rots of apples (Wilkinson, 1954).

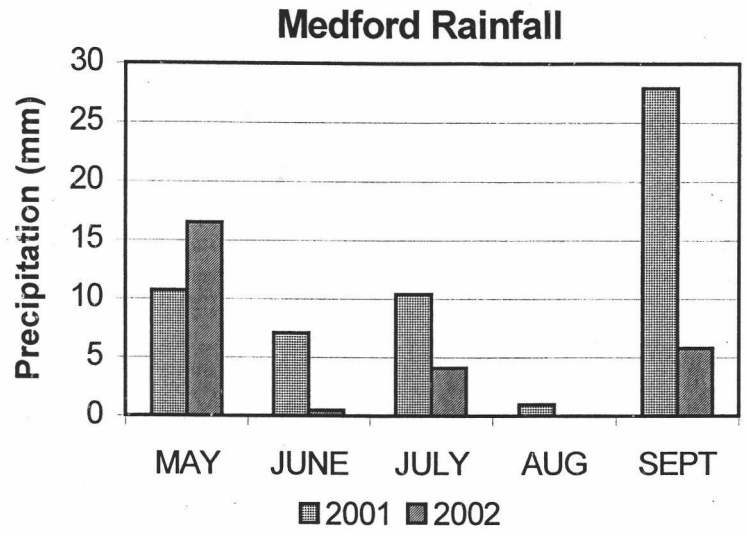

Figure 2.4. Precipitation recorded during the fruit growing seasons 2001 and 2002 in Medford, Oregon (Data obtained at the Agrimet Station located at SOREC, Medford. Bureau of Reclamation, Pacific Northwest Region).

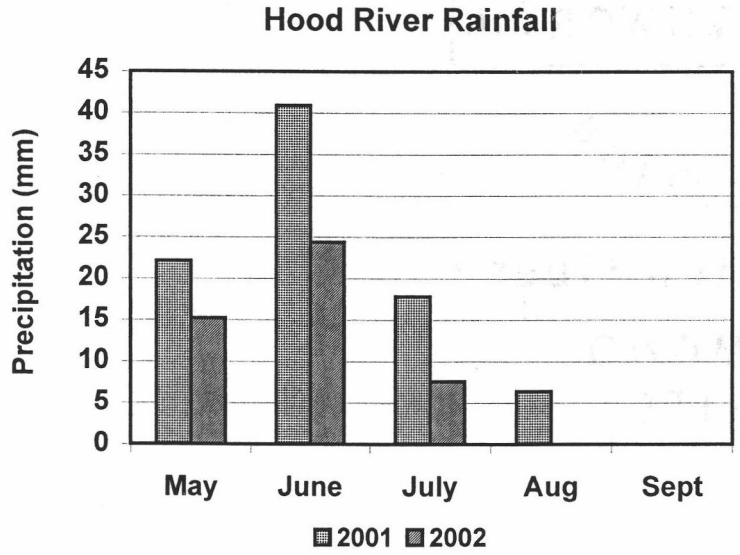

Figure 2.5. Precipitation recorded during the fruit growing seasons 2001 and 2002 in Hood River, Oregon (Data obtained at the Agrimet Station located at MCAREC, Hood River. Bureau of Reclamation, Pacific Northwest Region).

The production of microconidia by the germinating macroconidia of *N. perennans* but not by those of *N. alba* was consistent with a previous report (Wilkinson, 1954). Combined with the morphological differences between the macroconidia of these species, this criterion should be adequate to differentiate these two species. Nevertheless, not all lesions readily develop conidiomata and those of *N. alba* seem to take longer to develop. Furthermore, germinating macroconidia of *N.* sp. *nova* did produce microconidia, and their shape is similar to those of *N. perennans*, making the differentiation of these two species very challenging and probably restricted to the use of molecular techniques. Morphology of *N.* sp. *nova* still needs to be studied as well as epidemiology and symptomatology. Finally, germinating macroconidia of *N. malicorticis* produce microconidia as well, but it may be differentiated from *N. perennans* by the curvature of its macroconidia, geographical distribution, and host specificity.

Bull's eye pathogens were isolated from nine different cultivars of European pears. These results suggests that most pear cultivars are susceptible to infection by bull's eye pathogens, but due to the long time required for symptom development the rot is only commonly present in those cultivars stored for longer times like Bosc and d'Anjou. Bull's eye rot would not have enough time to develop in cultivars of short storage life such as Bartlett, where the fruit is marketed before symptoms could develop.

Presence of at least one pathogen was verified on woody tissues of pear trees. The saprophytic ability of *N. alba* may allow it to colonize diverse kinds of wood materials, making it more widespread in the trees and easier to detect in a

sampling as performed. A larger amount of samples would be necessary to detect the presence of the other two species. It was judged unnecessary in this study due to the known saprophytic ability of the pathogens that allows them to thrive diverse niches, and to the lack of clear and definitive symptoms to look for. Other protocols may be needed for the extraction of DNA of *Neofabraea* spp. from woody tissue that could allow to identify the species not recovered on this study. The positive identification of *N. alba* from woody tissues proved the validity of PCR for use on field samples to look for possible inoculum sources. The sources of inoculum identified are among those previously reported (Verkley, 1999).

The identification of three species causing bull's eye rot of pears brings new questions regarding their epidemiology and control. The different species coexist with each other and their relative importance from one season to another seems to be highly influenced by weather conditions. How cultural and chemical practices in the orchard affect fruit infections by each of the pathogens is important to know in order to achieve a satisfactory control of bull's eye rot of pears.

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3. ARTIFICIAL INDUCTION OF CANKERS OF *Neofabraea* spp. ON PEAR TREE BRANCHES, SEASONAL VARIATION OF CONIDIAL PRODUCTION AND TIMING OF FRUIT INFECTION IN THE ORCHARD

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3.1 Abstract

Cankers on pear branches resulted after monthly inoculations with mycelia of *Neofabraea alba* and *N. perennans*. Higher proportions of successful inoculations were obtained after fall and winter inoculations. Cankers induced by *N. perennans* were larger than those induced by *N. alba*. Small and superficial cankers were obtained after conidial inoculations of *N. perennans* on wounded branches of pear trees, resembling those naturally observed in the orchards. Sporulation of both pathogens on mycelial-induced cankers occurred throughout the year with the largest amount of conidia produced at the end of the summer and during the fall. Sporulation on cankers induced by *N. perennans* spanned two years. Copper sulfate reduced the sporulation rate of cankers induced by *N. alba*, while copper sulfate, trifloxystrobin and ziram affected the germination rate of conidia of *N. perennans*. Fruit infection onset was observed throughout the growing season with relative higher amounts close to harvest, coincidental with higher amounts of sporulation on artificially induced cankers.

3.2 Introduction

Cankers of different etiologies are produced on apple trees by bull's eye rot pathogens. *Neofabraea malicorticis* causes anthracnose canker following direct penetration of sound wood, resulting in cankers on twigs, branches, scaffold branches and trunks (Kienholz, 1939). The cankers develop during the fall and, to a lesser extent, winter. In spring, they resume development, becoming enlarged, elliptical and somewhat depressed. By summer, cankers stop growing and the

surrounding bark cracks, delimiting the canker. Old cankers have a "fiddle string" appearance (Kienholz, 1939). Cordley (1900) first described anthracnose canker in the Pacific Northwest (PNW) in 1900 and it is still an important disease of apple trees. It is also found in Europe (Grove, 1990; Borecki et al., 1970).

N. perennans causes perennial canker of apple trees, also known as target canker or false anthracnose (Grove, 1990). The pathogen requires wounds to penetrate the wood and creates a canker that can enlarge from year to year. Enlargement of cankers results from new infections occurring at the margin of the old canker (Childs, 1929; Kienholz, 1939). Galls produced in response to woolly apple aphid feeding on callus tissue around cankers are injured by winter frosts, opening avenues for new infections resulting in the perennation of the cankers (Childs, 1929; Dugan et al., 1993; Gussow, 1930; McLarty, 1933). Pruning wounds are the main points of infection, and the fungus can penetrate branches of different ages. Cankers are rarely found below the main crotches in the scaffold branches (Grove, 1990; Zeller and Childs, 1925). New cankers are elliptical, sunken, with an orange to purple coloration (Grove, 1990). Perennial canker was first reported in the PNW in 1925 (Zeller and Childs, 1925), and it also is found in Europe (Grove, 1990). Perennial canker is prevalent east of the Cascade Mountains where dry conditions prevail during the summer and winters are more severe (Grove, 1990; Kienholz, 1939).

N. alba has been described as weakly parasitic to purely saprophytic (Bompeix and Bondoux, 1974, 1988; Edney, 1956). It colonized healthy woody tissues poorly when inoculated on apple tree branches and required wounds to

infect them (Bompeix and Bondoux, 1974). It has been found on dead bark, causing anthracnose of leaves and twigs, and on dead pruning stubs and fruiting spurs (Verkley, 1999). This species was recently reported causing a serious outbreak of coin canker of ash trees in Michigan (Rossman et al., 2002). *N. alba* is also able to grow saprophytically on leaves (Tan and Burchill, 1972). *N. alba* was recently identified causing bull's eye rot of apple and pear fruit in the PNW from isolates collected during the present study (Gariepy, 2002).

An undescribed new species, *N.* sp. *nova*, was found in a phylogenetic study of the *Neofabraea* species associated with tree cankers and rot of apples (de Jong et al., 2001). It was also isolated from cankers on apple branches and from apple rots. No further characterization of this taxon is available.

In all cases, the canker phase of these pathogens or its saprophytic growth serves as the source of inoculum for fruit infections known as bull's eye rot or lenticel rot. The fruit rot is considered the most important phase of these diseases and causes economic losses in apples and pears in the PNW of North America and in Europe (Grove, 1990; Edney, 1956; Bompeix, 1988).

Pear trees are considered resistant to cankers and the fungi seem to colonize only injured bark and dead outer bark (Fisher and Reeves, 1928; Spotts, 1990). Spore production by *N. perennans* and *N. malicorticis* was observed in superficial bark injuries in which cankers were not observed (Kienholz, 1951). Nevertheless, pear fruit are susceptible to bull's eye rot and severe losses are observed in some years.

Several studies have been conducted using wood inoculations to determine the periods of susceptibility of apple trees to canker formation and to monitor spore production (Bompeix and Bondoux, 1974; Cooley and Miller, 1930; Corke, 1955, 1956; Grove et al., 1992; Wilkinson 1945). Inoculations of *N. perennans* on uninjured bark of apple trees did not produce cankers (Wilkinson, 1945). Wound inoculations with mycelia of *N. perennans* have been successful throughout the year, with cankers resulting more frequently when inoculations were performed during the fall and winter months (Cooley and Miller, 1930; Corke, 1956; Grove et al., 1992). Inoculations with spore suspensions resulted in less canker formation than those performed with mycelium (Corke, 1956). *N. malicorticis* colonized healthy tissue and quickly killed inoculated current season wood (Bompeix and Bondoux, 1974). In contrast, inoculations with *N. alba* infrequently resulted in canker formation. The pathogen mainly colonized injured tissues at the point of inoculation and development on healthy tissue was minimum (Bompeix and Bondoux, 1974). *N. alba* did not colonize inoculated current season wood. Development of the pathogen on wounded tissues was fastest from November to February (Bompeix and Bondoux, 1974). Successful inoculations with *N. alba*, leading to the formation of cankers, were obtained from July to September. Percentage of success was less than 20%. Inoculations with mycelium resulted in a greater infection rate than with spore suspensions; none of the cankers that formed produced conidia (Corke, 1956).

Corke (1956) reported that acervuli of *N. perennans* formed on cankers induced from inoculations performed during November through May. No

sporulation was detected on cankers induced during the summer months, and the highest sporulation was observed during September. Grove et al. (1992) reported that production of conidia of *N. perennans* in artificially induced cankers was highest during the fall and winter months and lower to null during the summer months. A similar pattern was observed on natural cankers. The latent period between inoculation and subsequent sporulation was of 5 to 6 months.

Fruits are susceptible to bull's eye rot throughout the growing season. They can become infected as early as one month after bloom but susceptibility increases as the growing season progresses (Bompeix, 1978; Edney, 1958; Spotts, 1985).

The objective of this study was to determine the time of year when trees are more susceptible to infection by *N. perennans* and *N. alba;* and when conidia are produced on artificially induced cankers. In addition, the effect of fungicides on sporulation on cankers and the timing of fruit infection in the orchard were assessed.

3.3 Materials and methods

3.3.1 Origin of isolates and trees utilized. Two isolates of *N. perennans* were used in this study. Isolate MA-0001 was isolated from an Asian pear of unknown cultivar grown in Medford. Isolate HR-238 was isolated from a d'Anjou pear in Hood River. Two isolates of *N. alba*, MB-0128 and MB-0140, were isolated from Bosc pears grown in the orchard of the Southern Oregon Research and Extension Center (SOREC) in Medford. Experiments with *N. alba* started in December 2001, after this species was positively identified as causing bull's eye rot of pears in

Medford and Hood River. All isolates were grown on acidified potato dextrose agar (aPDA) at room temperature.

All the trees used for inoculations were located in the orchard of SOREC. Bosc pear and Granny Smith apple trees were planted in 1994, and d'Anjou pear trees were planted in 1968. The block of d'Anjou trees was irrigated with over-tree sprinklers; the other blocks were irrigated with under-tree sprinklers.

3.3.2 Mycelial inoculations. Inoculations 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 apple trees were performed to compare with those conducted on pear trees and as an alternative source of conidia in the orchard in the event that conidia were not produced on pear trees. Inoculations of d'Anjou pear trees with *N. perennans and N. alba* were conducted from January 2002 to March 2003.

Each isolate was inoculated on four Bosc trees and on three d'Anjou and Granny Smith trees. Each isolate was inoculated in three locations on each tree: on current season, two-year-old and three or more-year-old branches. The section of the branch to be inoculated was first disinfected with a piece of cotton soaked in 95% ethanol. A 3 to 4 mm^2 mycelial plug was placed under a V-shape cut made into the wood to the depth of secondary xylem. Mycelia of each isolate were obtained from the margins of 30-day old cultures on aPDA. Control inoculations were performed with a sterile plug of aPDA. After inoculation, wounds were wrapped with Parafilm (Pechiney Plastic Packaging, Menasha, WI), which was removed one month later. Canker progression was observed monthly.

3.3.3 Conidial inoculations. In order to determine if wounding was required for infection, Bosc trees were inoculated at monthly intervals from November 2001 to March 2002 with suspensions containing $5x10^4$ conidia/ml of *N. perennans* isolates MA-0001 or HR-238. Conidial suspensions were prepared by washing plates of 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 suspension was applied with a hand sprayer. Control inoculations consisted of wounded branches sprayed with SDW. Three trees were inoculated each time and both isolates were inoculated on all trees.

3.3.4 Monitoring conidial production. Well-developed cankers showing conspicuous acervuli were selected for monitoring spore production. Five cankers on Bosc trees and three cankers on Granny Smith, per each isolate, were washed every two weeks. A 130 mm diameter plastic funnel was placed under each canker and secured to the branches with wires. Each canker was washed with 20 ml of distilled water, measured and dispensed with a hypodermic syringe. The cankers were initially soaked with about 5 ml for 30 seconds and the rest of the water applied afterwards. Water from the washes was collected in a plastic tube placed at the end of the funnel. Samples were transported to the laboratory where the conidial concentration was measured with a hemacytometer. Two to three hundred microliters of each sample was plated on water agar (WA) and incubated at 4°C. Germinating conidia were transferred to aPDA and monosporic cultures compared

to those of the isolates inoculated. 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 using both isolates of *N. perennans*. A second set of cankers, resulting from inoculations performed during 2001-2002, was monitored beginning February 14 2002. These cankers were selected soon after inoculation and before evidence of acervuli was present, in order to determine the time at which sporulation on cankers began. Cankers induced by both *N. alba* and *N. perennans* were included. A serious fire blight outbreak in 2002 resulted in the reduction 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.

3.3.5 Effect of fungicides 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. Copper sulfate, trifloxystrobin, and ziram were applied with a hand sprayer at a rate of 3,000 mg l^{-1}; 47 mg l^{-1}; and 3,638 mg l^{-1}, respectively. Three cankers per isolate were sprayed with each fungicide to run off on October 18, 2002. Two spore washes were conducted before fungicides were sprayed to identify sporulation trends on each canker. Four washes were conducted after fungicides were applied. Cankers were washed as described before, and samples from each canker were plated on WA to observe the effect on germination. Plates were incubated at 4°C for one week and germination observed under the

compound microscope. Germ tubes longer than twice the length of conidia was the criterion used to determine germination. Germination data were arcsine transformed for stabilization of variances. All data were analyzed with ANOVA and means separated with Tukey's Honest Significant Difference.

3.3.6 Determination of the time of fruit infection in the orchard. In order to determine the time when fruit become infected with bull's eye pathogens, fruit were bagged shortly after fruit set creating a barrier to prevent fruit to become infested. The bags were removed for periods of one or two weeks, allowing exposure for infection, then the fruit were re-bagged until harvest. Individual fruits or clusters of fruits were covered with waxed paper bags that were closed and secured with wire twists. Bags broken due to the growth of the fruit or to external factors were replaced periodically. One study was conducted during the 2001 growing season in a block of Bosc trees planted from 1960 to 1964 with a history of bull's eye rot, located in the orchard of SOREC. Initial bagging of fruits was completed by May 16. Nine exposure periods were opened during the growing season and fruit were harvested on September 11. The experiment was repeated during 2002 using the same trees as before. Initial bagging was completed by June 26. Seven exposure periods were opened and the fruit were harvested on September 20. Harvested fruit were individually labeled with the tree of origin, grouped by exposure period, placed in cardboard boxes lined with perforated polyethylene bags and stored at ~ 0°C. Fruit were evaluated monthly for bull's eye incidence from December. The orchard was irrigated with over-tree sprinklers and the dates of each irrigation event were recorded.

Another study was conducted in a block of Bosc trees in the orchard of the MCAREC during the 2002 growing season. The orchard was irrigated with under-tree sprinklers. In this study whole branches on five trees were selected and the fruit bagged. Bags were removed and replaced creating sequential exposure periods for infestation of one or two weeks. Bagging of fruit began on July 22. The fruit was harvested on September 9 and stored at ~ 0°C.

Precipitation data for Medford were obtained from an AGRIMET weather station (Bureau of Reclamation, Pacific Northwest Region) located about 300 m from the orchard. Precipitation data for Hood River were obtained from an AGRIMET weather station located at MCAREC.

3.4 Results

3.4.1 Mycelial inoculations. Cankers resulted most commonly when inoculations were performed during the autumn and winter months. 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 to February. Branches of different ages were susceptible to cankering and the extent of the canker was determined by a combination of branch size and time of inoculation (data not shown). Current season branches inoculated with both *N. perennans* and *N. alba* were frequently girdled and necrosis distal to the canker was 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 at the canker margins. Branch growth delimited the canker that acquired a sunken shape and the margins cracked, separating from the healthy tissues. Small bumps appeared on the necrotic tissues that broke open exposing 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.

Bosc pear trees inoculated with *N. perennans* produced cankers on branches inoculated from September to April, except in 2002 when September inoculations did not result in cankers (Figure 3.1). Similarly, inoculations with isolate MA-0001 induced cankers from November 2001 to May 2002. Inoculations conducted in the fall and winter 2001-2002 resulted in a lower number of cankers than those performed during 2000-2001 and 2002-2003. Both isolates of *N. perennans* performed similarly. Inoculations of isolate HR-238 conducted in February 2002 failed to produce cankers.

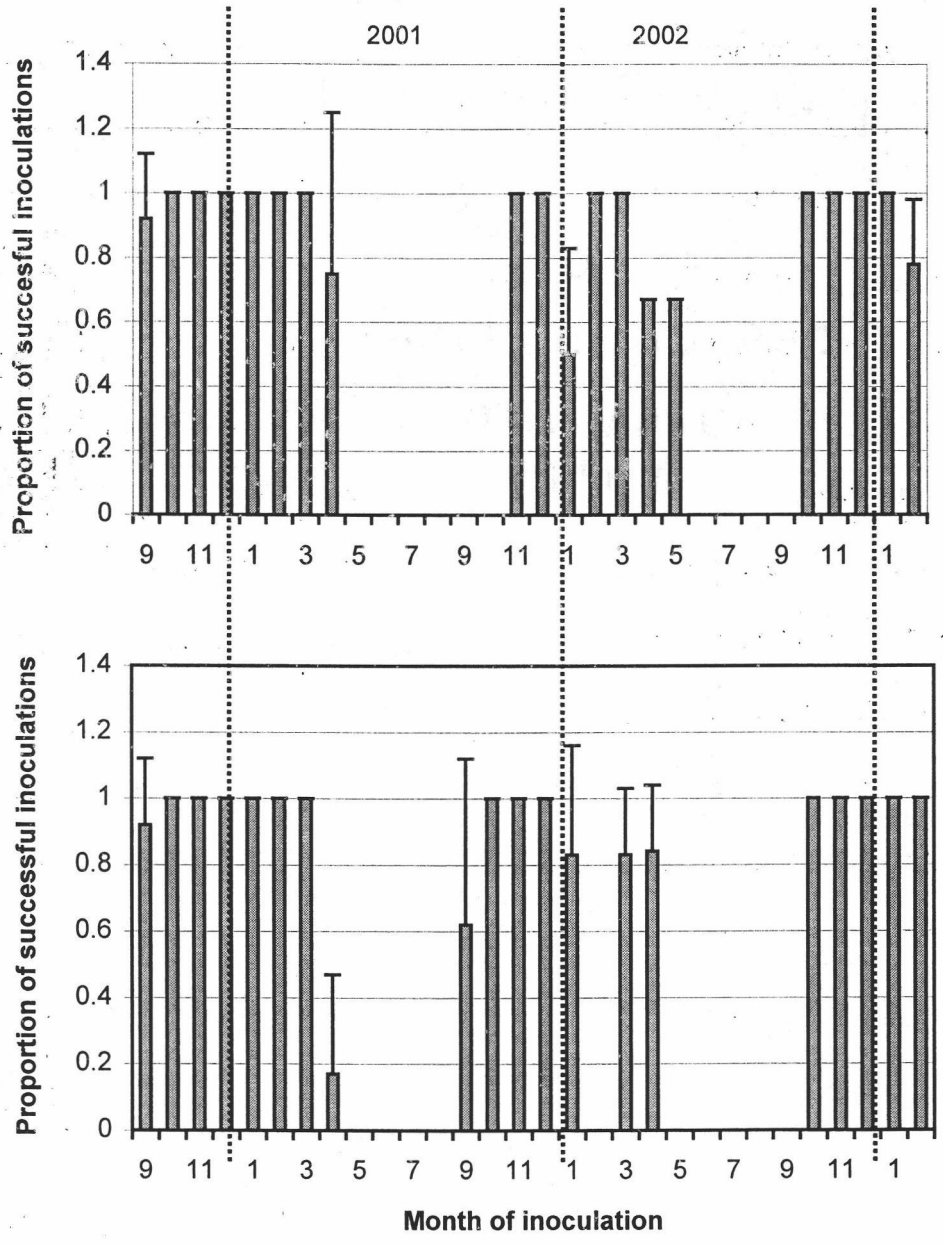

Figure 3.1. Proportion of wound inoculations of *Neofabraea perennans* isolates MA-0001 (top) and HR-238 (bottom) that resulted in canker formation in Bosc pear trees in Medford, Oregon. Values represent the average of four replicate trees with three inoculations each. Line bars denote standard deviations.

Bosc pear trees inoculated with *N. alba* produced cankers on branches inoculated from October to June (Figure 3.2). Isolates MB-0128 and MB-0140 performed similarly but isolate MB-0128 had a higher proportion of successful inoculations. Inoculations from December 2001 to June 2002 had a lower proportion of successful inoculations than those performed during October 2002 to February 2003. Cankers induced by *N. alba* were smaller than those induced by *N. perennans* and did not develop acervuli (Table 3.1).

Inoculations of *N. perennans* (Figure 3.3) and *N. alba* (Figure 3.4) 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*. The trees used in these inoculations were old trees with reduced vegetative growth in the lower branches. Girdling due to canker formation was frequently observed. Inoculations performed during September were lost due to fire blight.

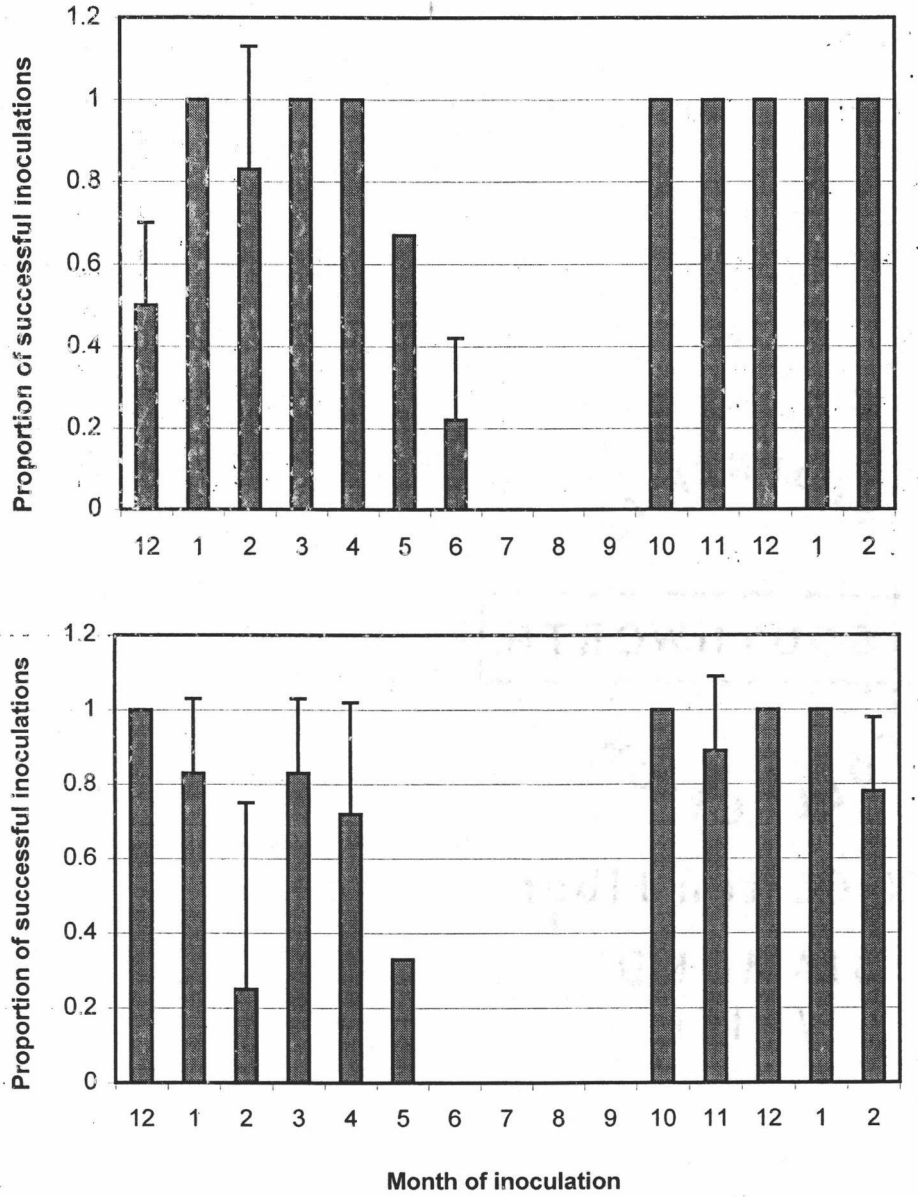

Figure 3.2. Proportion of wound inoculations of *Neofabraea alba* isolates MB-0128 (top) and MB-0140 (bottom) that resulted in canker formation in Bosc pear trees in Medford, Oregon. Values represent the average of five replicate trees with three inoculations each. Line bars denote standard deviations.

Table 3.1. Average length (mm) of cankers induced by *Neofabraea* spp. inoculated on branches of Bosc pear trees.[1]

Inoculation month	*N. perennans* isolate			*N. alba* isolate		
	MA-0001	HR-238	Average	MB-0128	MB-0140	Average
Dec. 2001	64.7	-[2]	64.7	13.3	22.5	17.9
Jan. 2002	27.5	34.2	30.8	37.3	20.0	28.7
Feb. 2002	48.4	0	48.4	23.2	33.3	28.2
Mar. 2002	-	-	-	-	-	-
Apr. 2002	23.5	22.4	23.0	15.3	13.4	14.4
May 2002	12.0	0	6.0	5.0	27.5	16.3
June 2002	0	0	0	12.5	0	6.3
July 2002	0	0	0	0	0	0
Aug. 2002	0	0	0	0	0	0
Sep. 2002	0	0	0	0	0	0
Oct. 2002	41.2	39.0	40.1	18.3	16.9	17.6
Nov. 2002	51.1	54.7	52.9	15.9	20.8	18.3
Dec. 2002	49.4	62.4	55.9	42.2	35.9	39.1
Jan. 2003	35.8	37.8	36.8	30.3	29.9	30.1
Feb. 2003	17.3	42.0	29.7	16.6	14.6	15.6

[1] Values are the average of three inoculations performed in four trees. Cankers were measured in June 2002 and May 2003.
[2] Data missing due to fire blight.

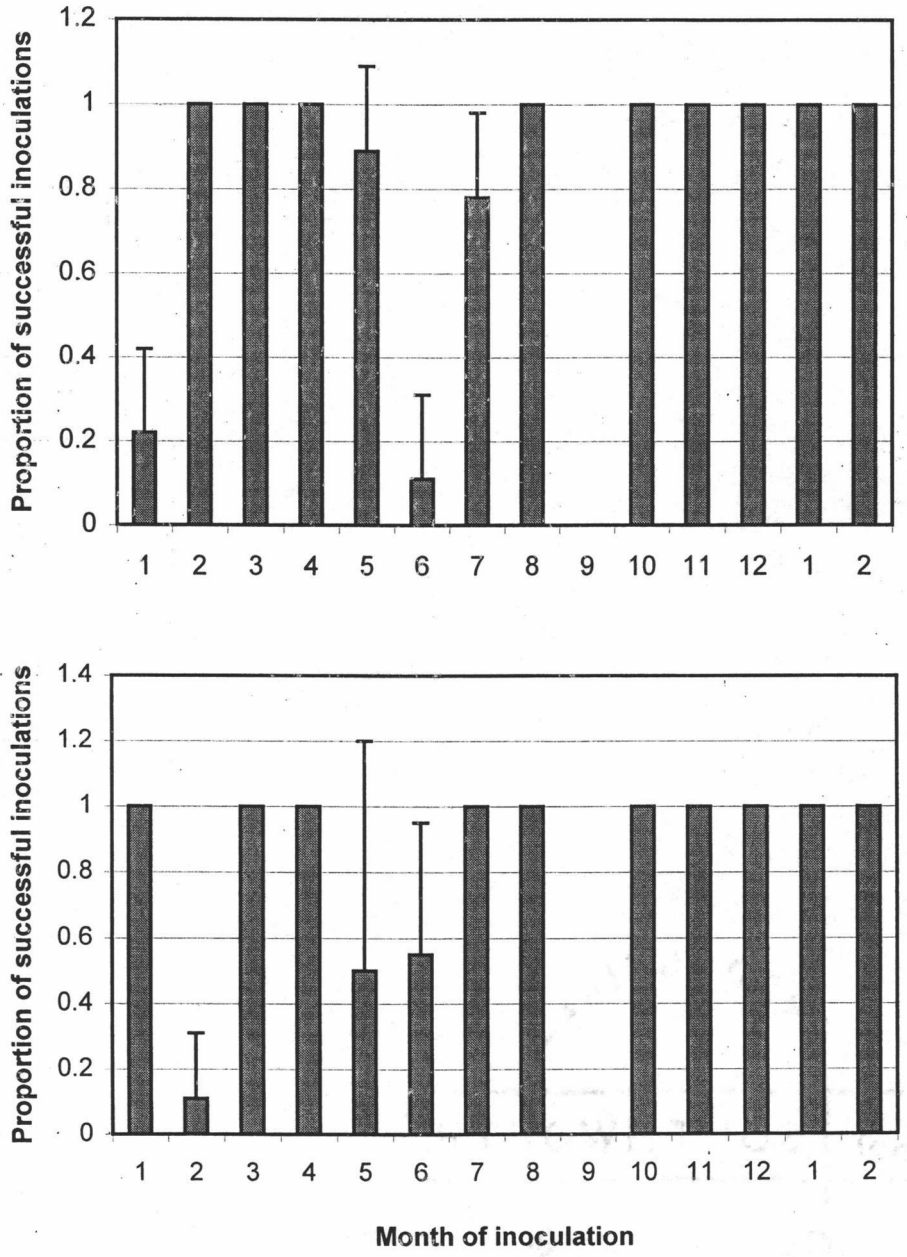

Figure 3.3. Proportion of wound inoculations of *Neofabraea perennans* isolates MA-0001 (top) and HR-238 (bottom) that resulted in canker formation in d'Anjou pear trees in Medford, Oregon. Values represent the average of five replicate trees with three inoculations each. Inoculations performed in September were lost due to fire blight infection. Line bars denote standard deviations.

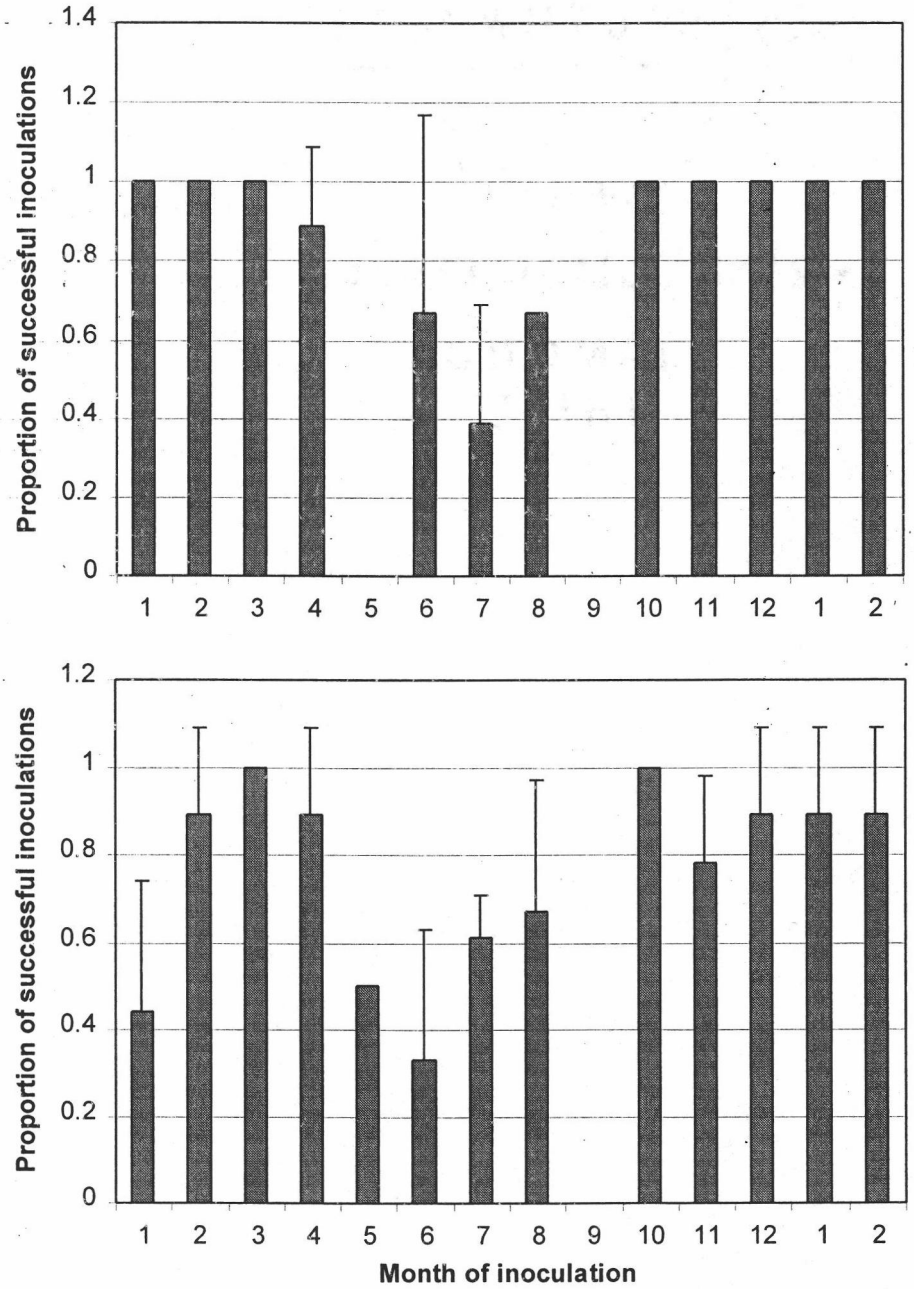

Figure 3.4. Proportion of wound inoculations of *Neofabraea alba* isolates MB-0128 (top) and MB-0140 (bottom) that resulted in canker formation in d'Anjou pear trees in Medford, Oregon. Values represent the average of five replicated trees with three inoculations each. Inoculations performed in September were lost due to fire blight infection. Line bars denote standard deviations.

Inoculations of Granny Smith trees with *N. perennans* induced cankers from August to May (Figure 3.5). Both isolates performed similarly, but HR-238 produced larger cankers and higher proportion of successful inoculations than MA-0001. Isolate HR-238 induced cankers after inoculations performed in June and July 2002, but these cankers were small and did not form acervuli.

Inoculations of Granny Smith trees with *N. alba* induced cankers from August to June (Figure 3.6). Both isolates performed similarly, but MB-0128 had a higher proportion of successful inoculations. Cankers were small and no acervuli were observed.

Of all the control inoculations made with sterile aPDA plugs only two resulted in cankers. The symptoms observed were different to those obtained by inoculation with *Neofabraea* spp. and were considered due to contamination with secondary agents

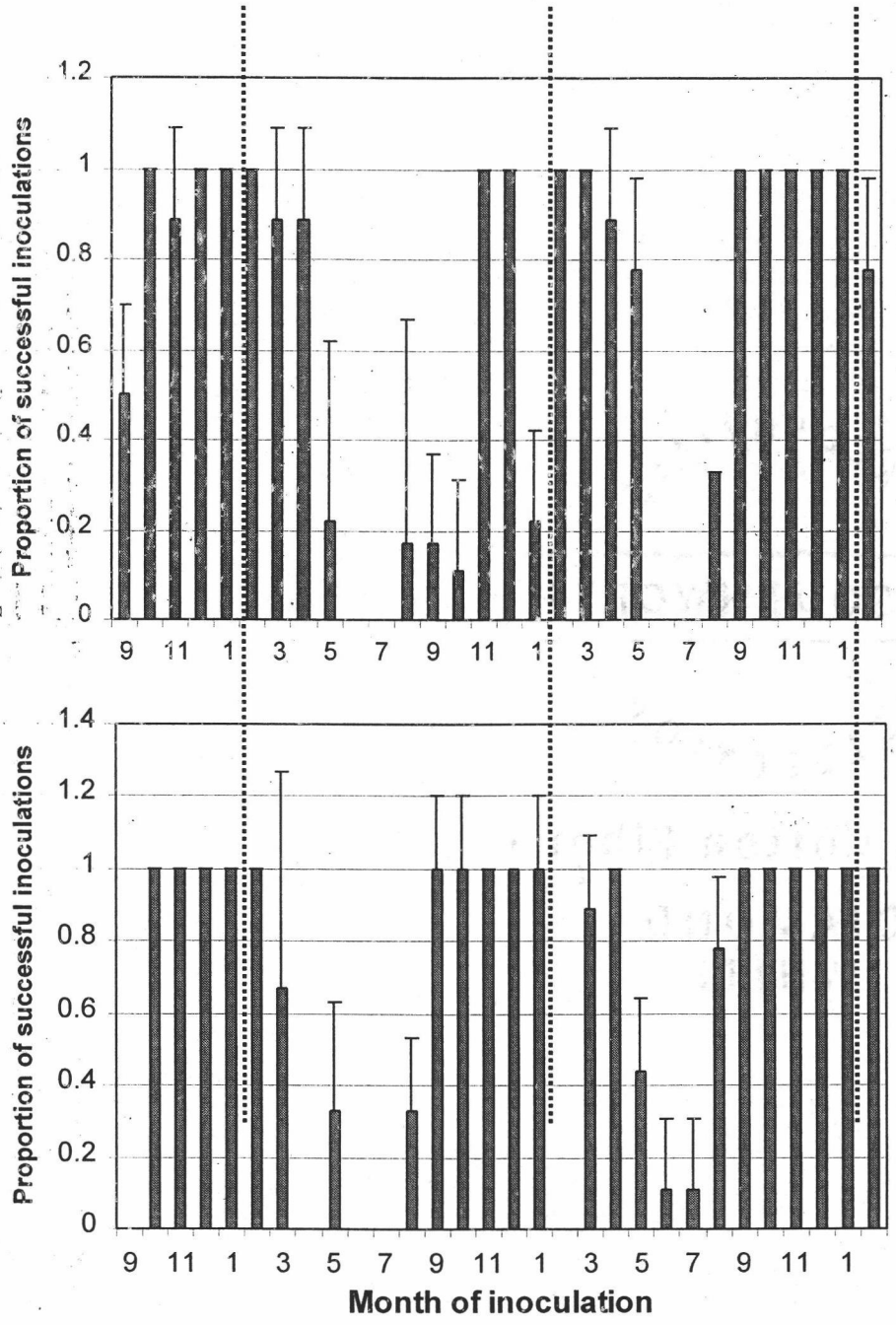

Figure 3.5. Proportion of wound inoculations of *Neofabraea perennans* isolates MA-0001 (top) and HR-238 (bottom) that resulted in canker formation in Granny Smith apple trees in Medford, Oregon. Values represent the average of three replicate trees with three inoculations each. Line bars denote standard deviations.

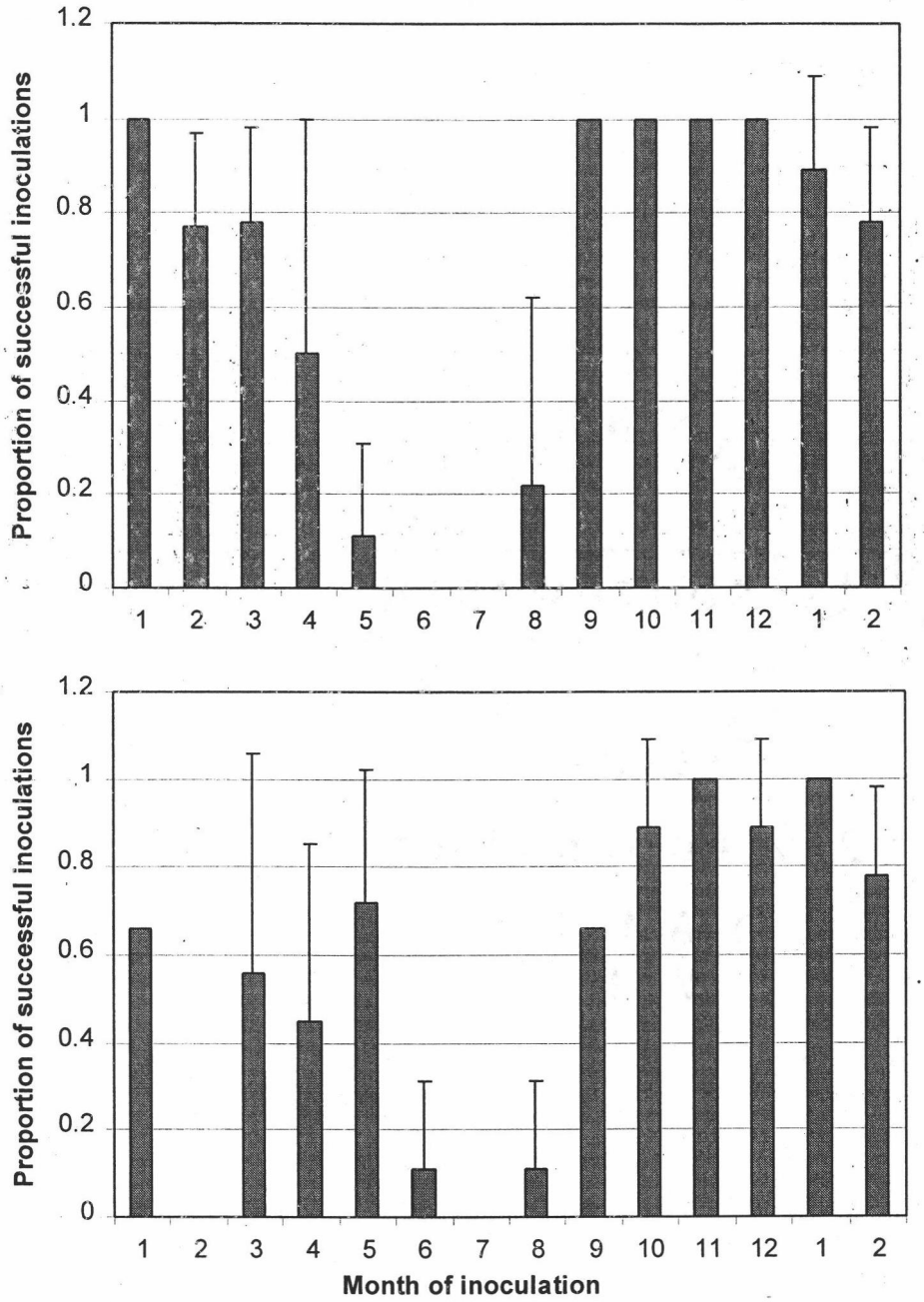

Figure 3.6. Proportion of wound inoculations of *Neofabraea alba* isolates MB-0128 (top) and MB-0140 (bottom) that resulted in canker formation in Granny Smith apple trees in Medford, Oregon. Values represent the average of five replicate trees with three inoculations each. Line bars denote standard deviations.

3.4.2 Conidial inoculations. Only conidial inoculations conducted on wounded branches resulted in canker formation (Table 3.2). Most of the cankers produced were small, superficial and without acervuli (Figure 3.7). Inoculations conducted in October, February and March induced the largest number of cankers. A small proportion of control inoculation resulted in very small cankers due most probably to natural infections.

Table 3.2. Proportion of inoculations with conidia of *Neofabraea perennans* isolates MA-0001 and HR-238 that resulted in cankers on branches of Bosc pear trees[1].

	MA – 0001		HR – 238		Control
Month	wounded	unwounded	wounded	unwounded	wounded
October	0.7[2]	0	0.4	0	0
November	0.	0	0.1	0	0
December	0	0	0.3	0	0.3
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

[1] Inoculations were performed from October 2001 to March 2002, with $5x10^4$ conidia/ml.
[2] Each value is the average of three replicate trees with three sets of two inoculations each.

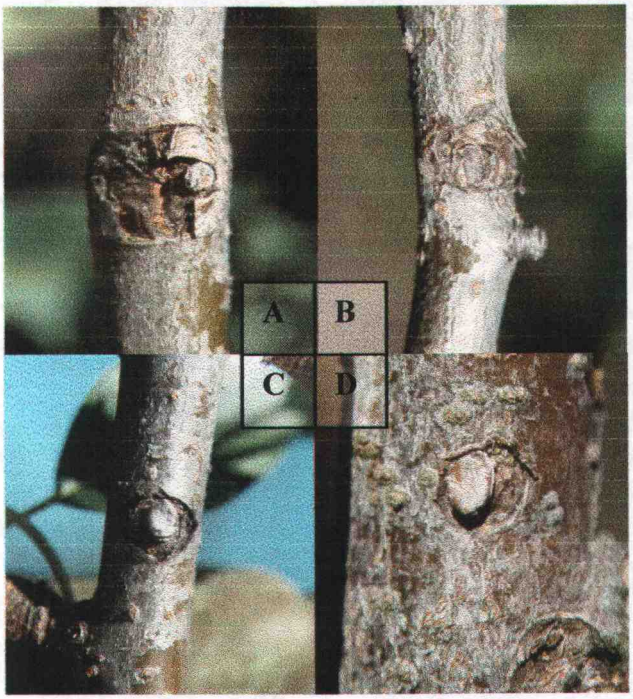

Figure 3.7. Cankers on branches of Bosc pear trees after conidial inoculation with *Neofabraea perennans*. A: isolate MA-0001; B: isolate HR-238; C and D: Control inoculations.

3.4.3 Monitoring conidial production. Production of conidia of *N. perennans* was observed almost year round with the highest amounts produced at the end of summer and during autumn. Production of conidia decreased thereafter reaching the lowest amounts in the summer and autumn of the second year and increasing again on winter. Both macro and microconidia were present.

In 2001, production of conidia of isolate MA-0001 on Bosc trees peaked during September and October (Figure 3.8). Production of conidia decreased to zero during July and resumed in August with a second peak on December, 2002. Production of conidia decreased to zero again by February 2003. Isolate HR-238 produced lower amounts of conidia but followed a similar pattern to isolate MA-

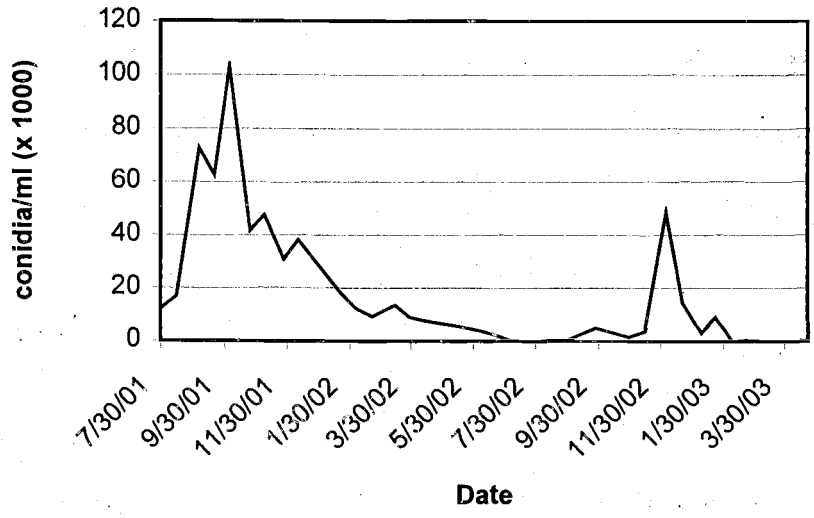

Figure 3.8. Sporulation of *Neofabraea perennans* isolate MA-0001 on artificially induced cankers on Bosc pear trees. Values represent the number of conidia per milliliter of wash water. Each value is the average of 5 cankers, except from June 20, 2002 where the values are the average of two cankers. The remainder of the cankers were lost due to fire blight.

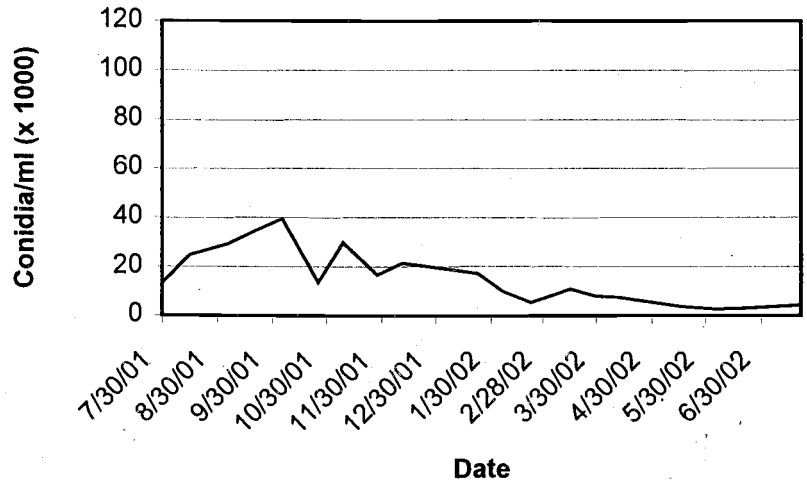

Figure 3.9. Sporulation of *Neofabraea perennans* isolate HR-238 on artificially induced cankers on Bosc pear trees. Values represent the number of conidia per milliliter of wash water. Each value is the average of 5 cankers.

0001 (Figure 3.9). Production of conidia had a maximum in October, 2001 (reaching about 40% of the amount produced by isolate MA-0001) and decreased steadily thereafter until reaching the lowest amount in June 20, 2002. No further monitoring of conidial production of isolate HR-238 was performed since all the trees were lost to fire blight.

In the second set of cankers washed, *N. perennans* isolates MA-0001 and HR-238 produced large initial amounts of conidia that decreased during the summer months and then increased in October, reaching maxima in December (Figure 3.10). Isolate MA-0001 produced more conidia than isolate HR-238.

Sporulation of *N. alba* isolates MB-0128 and MB-0140 on Bosc pear trees followed a similar pattern but the later isolate did not produce a large amount of conidia in the second peak (Figure 3.10). Production of conidia peaked in April (MB-0128) and March 2002 (MB-0140), decreasing in the summer months. Sporulation increased again during September and reached a maximum in December for both isolates.

On d'Anjou trees both isolates of *N. perennans* performed similarly (Figure 3.11). Conidial production reached a maximum in July, 2002, and then decreased progressively to almost zero by the end of the study in March. Meanwhile, both isolates of *N. alba* performed similarly with maximum sporulation in December 2002 (Figure 3.11).

On Granny Smith apple trees *N. perennans* produced both macro and microconidia throughout the period of study. Both isolates increase production of conidia in September 2001 and reached maxima in November 2001 (Figure 3.12).

Production of conidia decreased gradually thereafter and increased again by the end of August and early September 2002 and reached maximum in December.

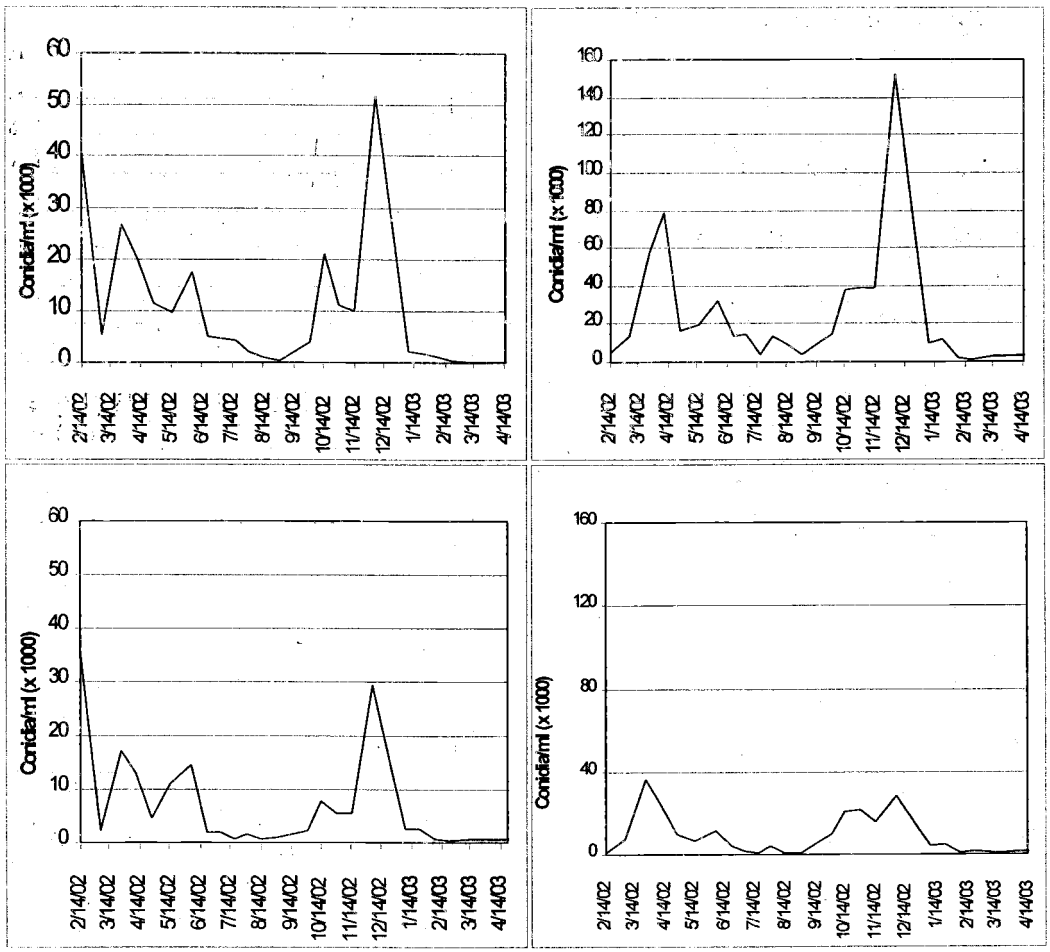

Figure 3.10. Sporulation of *Neofabraea perennans* isolates MA-0001 (top left) and HR-238 (bottom left) and *N. alba* isolates MB-0128 (top right) and MB-0140 (bottom right), on artificially induced cankers on Bosc pear trees. Values represent the number of conidia per milliliter of wash water. Each value is the average of 4 cankers.

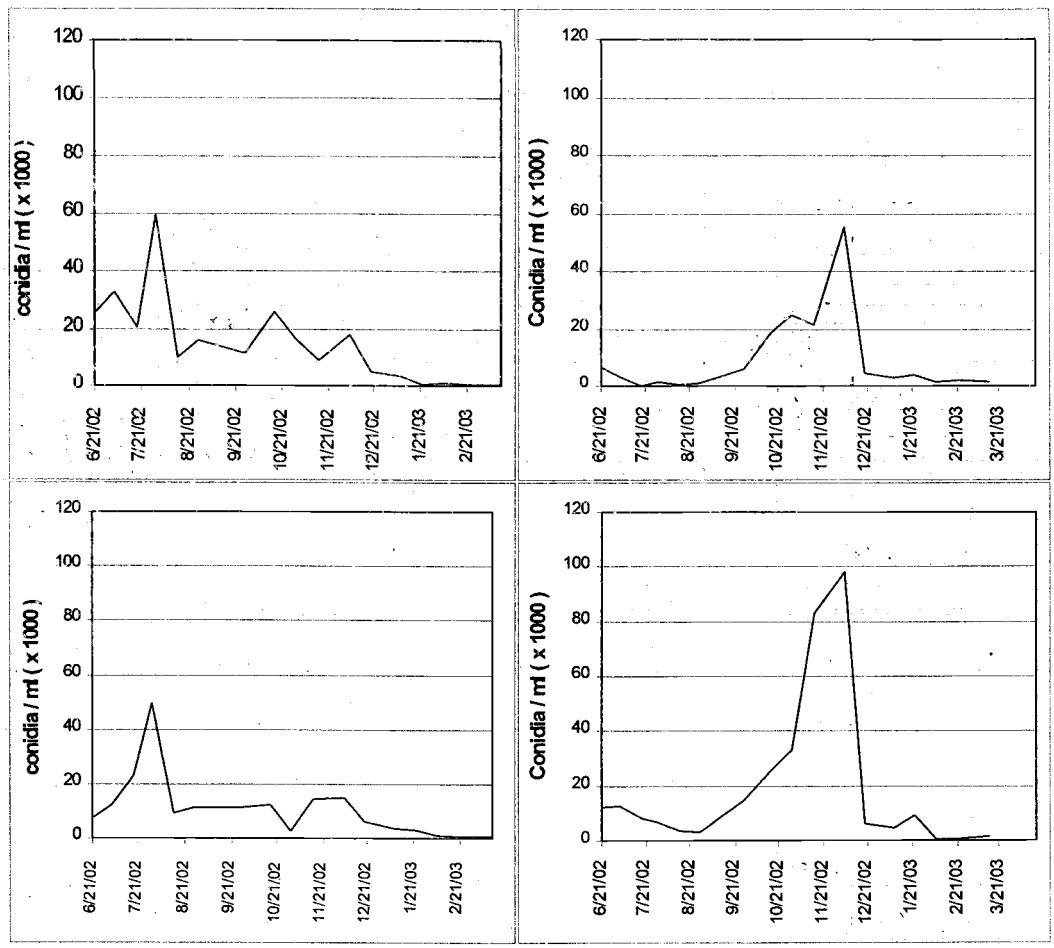

Figure 3.11. Sporulation of *Neofabraea perennans* isolates MA-0001 (top left) and HR-238 (bottom left) and *N. alba* isolates MB-0128 (top right) and MB-0140 (bottom right), on artificially induced cankers on d'Anjou pear trees. Values represent the number of conidia per milliliter of wash water. Each value is the average of 3 cankers.

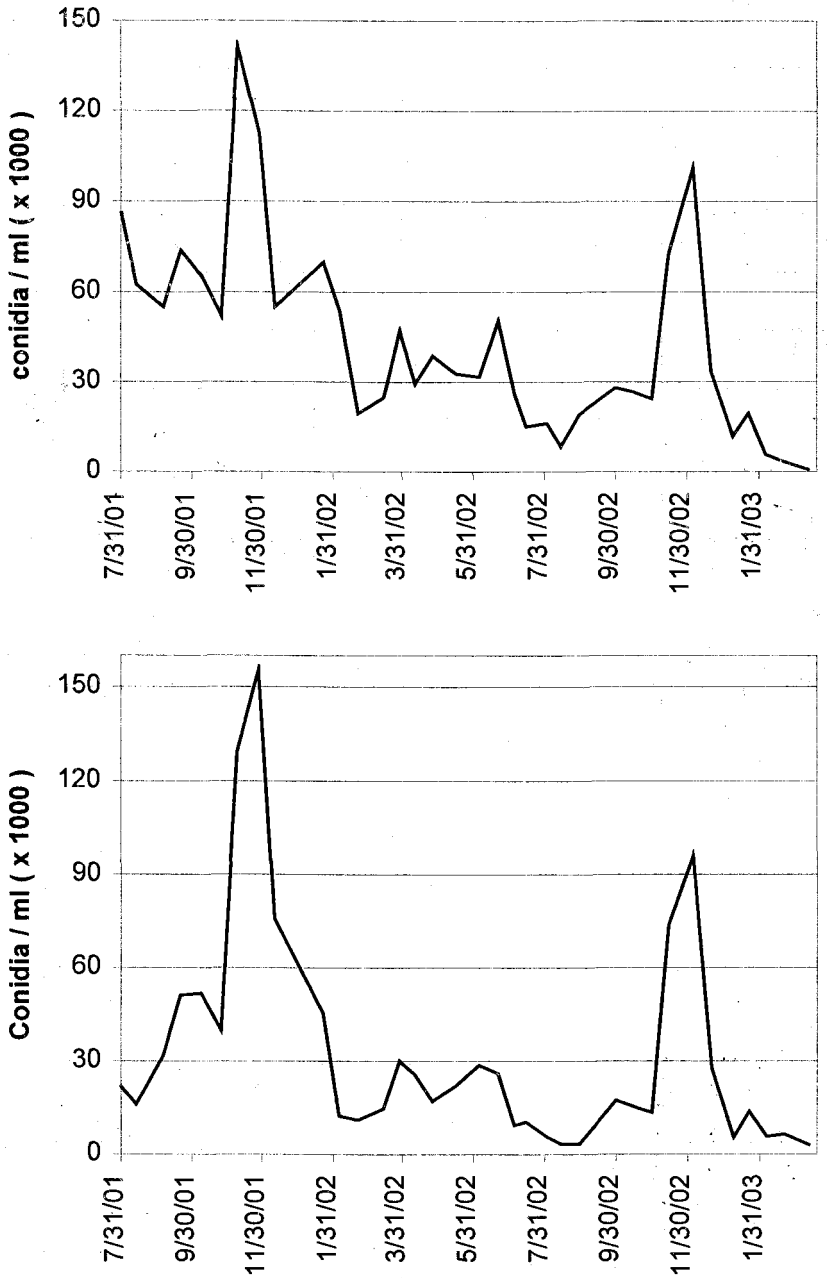

Figure 3.12. Sporulation of *Neofabraea perennans* isolates MA-0001 (top) and HR-238 (bottom) on artificially induced cankers on Granny Smith apple trees. Values represent the number of conidia per milliliter of wash water. Each value is the average of 3 cankers.

Production of conidia decreased thereafter until the end of the study, where the lowest amounts were recorded.

Both isolates of *N. alba*, MB-0128 and MB-0140 had a similar pattern of conidial production on Granny smith but the former isolate produced larger amounts (Figure 3.13). An initial increase in sporulation was observed from February to the beginning of June. Then, sporulation decreased during July and August and increased again from the end of September, reaching a maximum in October and November, for MB-0140 and MB-0128 respectively.

3.4.4 Effect of fungicides on sporulation on artificially induced cankers. Only copper sulfate significantly reduced conidial production of *N. alba* and none of the fungicides reduced sporulation of *N. perennans* (Table 3.3). 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 3.4). 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 was produced by the germinating macroconidia of *N. perennans*.

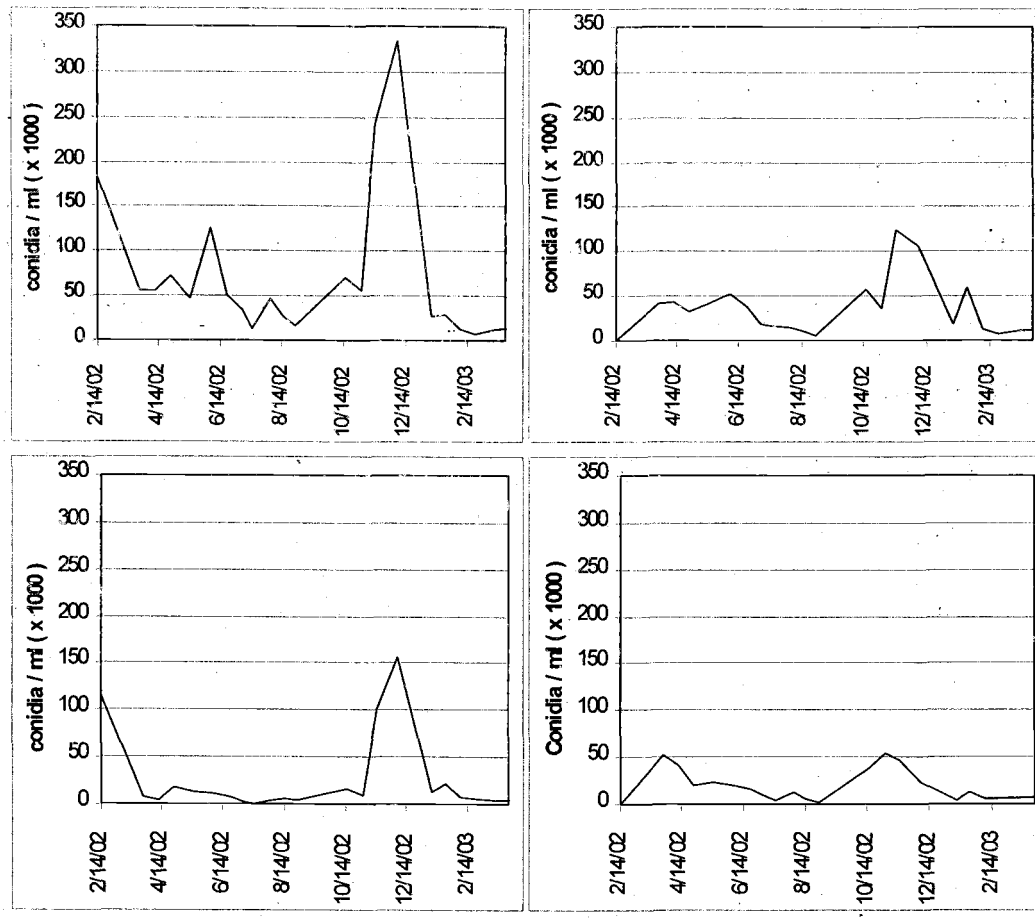

Figure 3.13. Sporulation of *Neofabraea perennans* isolates MA-0001 (top left) and HR-238 (bottom left) and *N. alba* isolates MB-0128 (top right) and MB-0140 (bottom right), on artificially induced cankers on Granny Smith apple trees. Values represent the number of conidia per milliliter of wash water. Each value is the average of 3 cankers.

Table 3.3. Effect of fungicides on conidial production of *Neofabraea alba* (MB-0128) and *N. perennans* (MA-0001) on artificially induced cankers on pear trees.[1]

	Canker washes					
N. perennans	9/27	10/17	10/30	11/14	12/5	12/19
check	11.3 a[2]	26.0 a	16.3 a	9.0 a	18.0 a	5.0 a
copper	14.3 a	12.6 a	4.0 a	4.7 a	16.0 a	8.0 a
trifloxystrobin	16.6 a	17.0 a	6.7 a	9.7 a	22.7 a	6.0 a
ziram	7.7 a	14.7 a	2.3 a	12.3 a	18.0 a	1.7 a
N. alba						
check	5.7 a	19.0 a	24.7 a	21.0 a	55.3 a	4.3 a
copper	3.7 a	12.0 a	3.0 b	18.7 a	61.3 a	8.3 a
trifloxystrobin	0.3 a	10.7 a	10.3 ab	13.7 a	21.3 a	1.7 a
ziram	2.3 a	12.7 a	7.0 ab	7.3 a	15.3 a	1.7 a

[1] Fungicides were sprayed on October 18. Values are the number of conidia/ml (x 1,000) of wash water.

[2] Values followed by the same letter in the column are not statistically different at a 5% confidence value according to Tukey's HSD.

Table 3.4. Percentage of germination of conidia of *Neofabraea perennans* isolate MA-0001 from canker washes after fungicide treatment.[1]

Treatment	Oct 30	Nov. 14	Dec. 5
Check	91.0 a[2]	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

[1] Fungicides were sprayed on October 18, 2002.
[2] Values followed by the same letter are not statistically different according to Tukey's HSD (critical value = 11.5).

3.4.5 Determination of the time of fruit infection in the orchard. Bull's eye rot occurred in Bosc pears from all periods exposed in the orchard of SOREC during 2001 (Figure 3.14). Rot incidence ranged from 5.2% to 12.5%. The highest incidence was observed in the latest two exposure periods and the control fruit. Every exposure period had one or two irrigation events of 12 hr each, while the control (exposure period 0) had 13 events. Rainfall was recorded during exposure periods 3, 4, 5, 8 and 9 (Figure 3.14). The highest amount of rainfall registered during the study occurred during exposure period 9, which had the highest incidence value. Sporulation of *N. perennans* increased from August reaching amounts of about 50,000 conidia/ml in canker washes during the last two exposure periods and during harvest time (Figure 3.14).

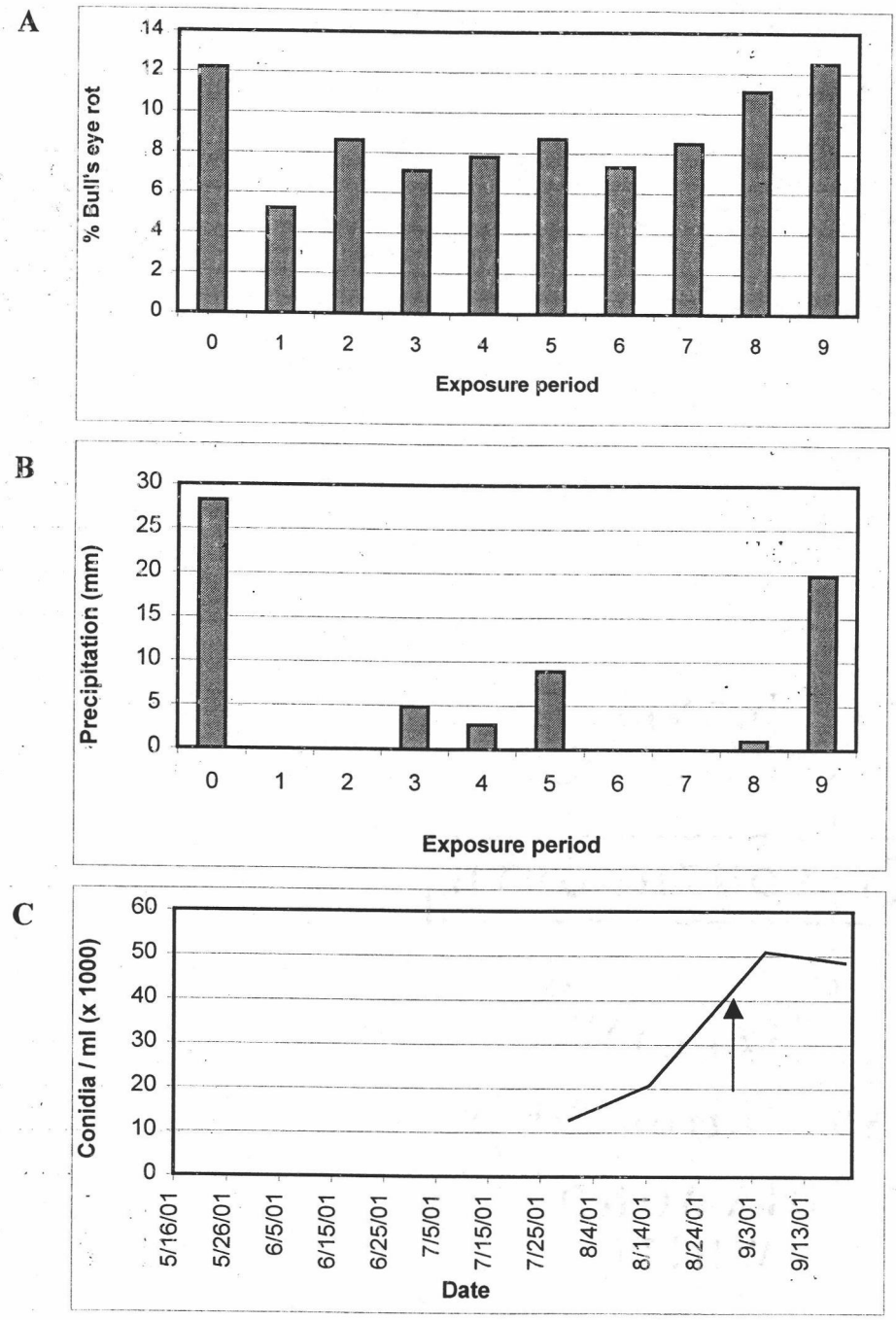

Figure 3.14. Timing of fruit infection in the orchard, 2001. **A**: Incidence of bull's eye rot on Bosc pear fruit corresponding to each of 9 exposure periods in the orchard of SOREC in Medford. Each period corresponded to a two-week exposure to infestation. Exposure period 0 corresponded to the non-bagged control fruit. Fruit was initially bagged on May 16 and harvested in September 11. **B**: Rainfall registered at the AGRIMET-station in Medford during the study period. **C**: Average number of conidia produced by two isolates of *N*eofabraea *perennans* on artificially induced cankers during the period of the study. Monitoring of conidia started in July 30, 2001. Arrow indicates fruit harvest.

Bull's eye rot was also found in Bosc pears from all the exposure periods opened during 2002 (Figure 3.15). Incidence varied from 0.3% in Exposure period 6 to 4.9% in the control; the highest incidence among exposure periods was in exposure period 7. Exposure periods 3 and 6 had one irrigation event; exposure periods 1, 2, 4, 5, and 7 had two irrigation events, while the control had a total of 14 events. Rainfall was registered during exposure periods 1, 3, and 7 with a total of 23.4 mm registered for the control fruit during the experiment. Sporulation of *N. perennans* and *N. alba* was relatively low on artificially induced cankers of Bosc trees, which increased after the fruit was harvested (Figure 3.16).

In Hood River, incidence of bull's eye rot on Bosc pears ranged from a 5.8% to 34.9% (Figure 3.17). Rot was observed in all exposure periods and had the highest incidence in exposure period 5. No rainfall was recorded during the experiment and 47 mm of rainfall were recorded from blooming to the beginning of the experiment.

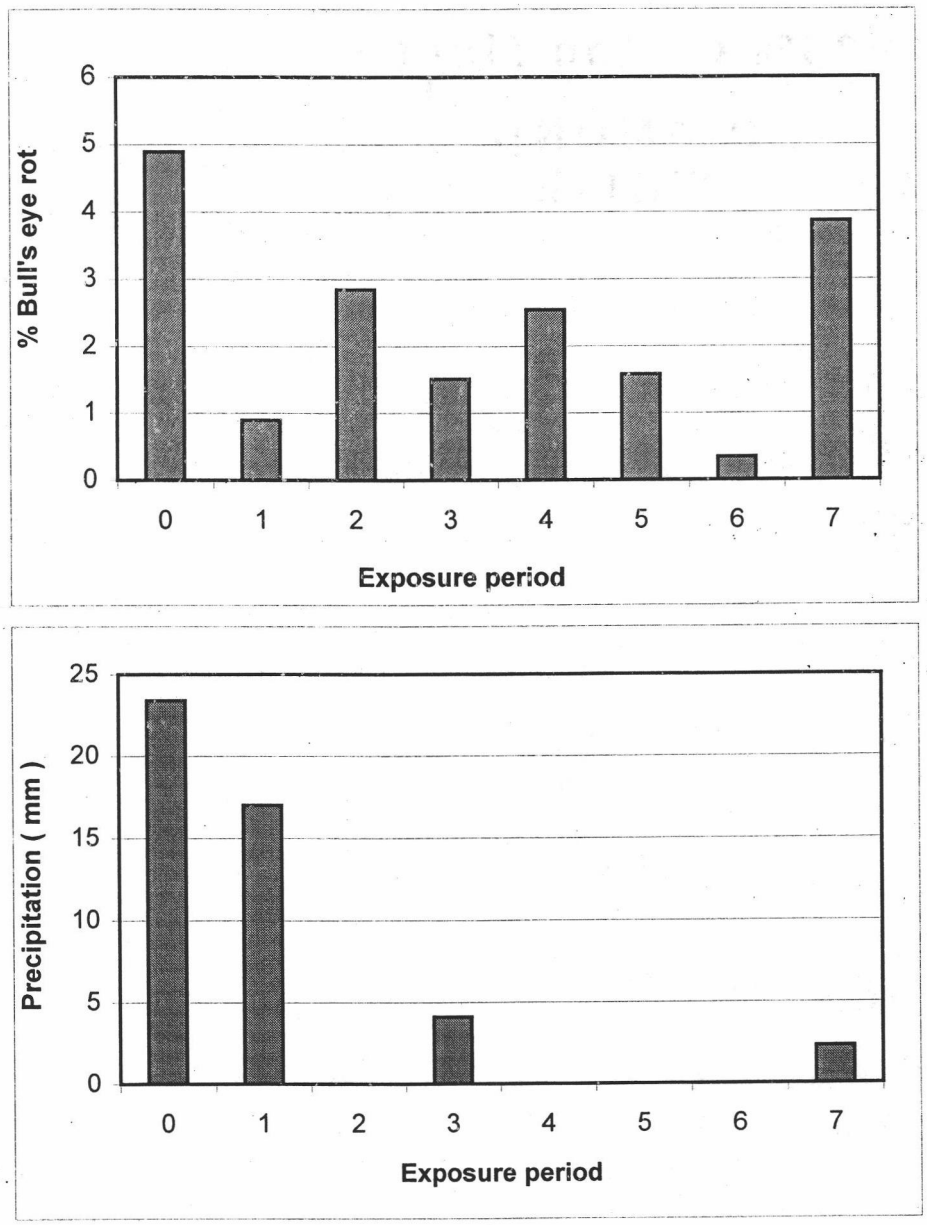

Figure 3.15. Timing of fruit infection in the orchard, 2002. Top: Incidence of bull's eye rot on Bosc pear fruit corresponding to each of 7 exposure periods in the orchard of SOREC in Medford. Each period corresponded to a two-week exposure to infestation. Exposure period 0 corresponded to the non-bagged control fruit. Fruit was initially bagged on June 26 and harvested in September 20. Bottom: Rainfall registered at the AGRIMET-station in Medford during the study period, amount on exposure period 0 is the accumulated since May 1 until harvest.

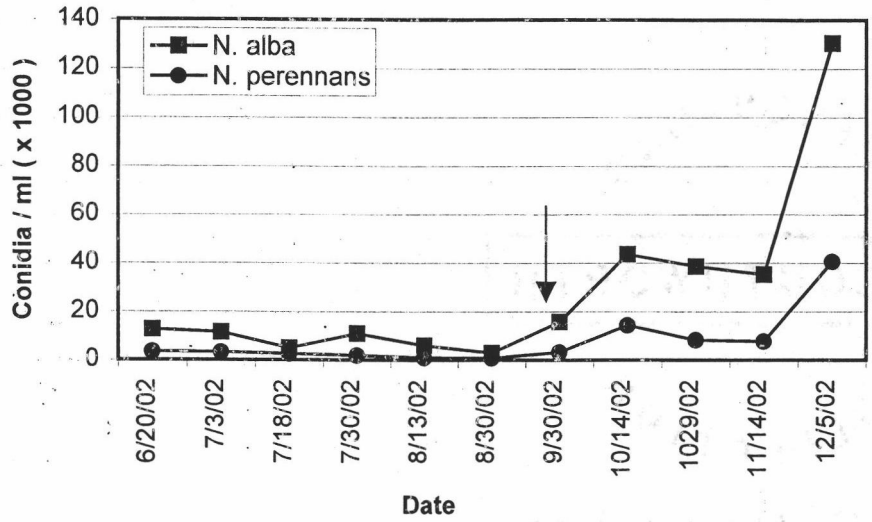

Figure 3.16. Sporulation of *Neofabraea alba* and *N. perennans*. Values represent the average of numbers of conidia of two isolates per each pathogen monitored on artificially induced cankers on Bosc pear trees. Arrow indicates fruit harvest.

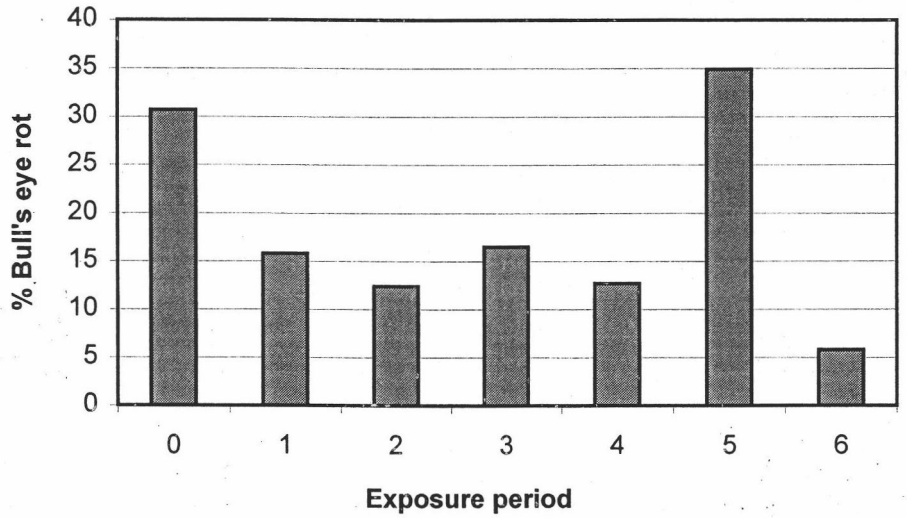

Figure 3.17. Timing of fruit infection in the orchard 2002. Incidence of bull's eye rot on Bosc pear fruit corresponding to each of 6 exposure periods in the orchard of MCAREC in Hood River. Each exposure period corresponded to a one-week period of fruit exposure to infection, except for a two-week period on exposure period 1. Exposure period 0 corresponded to the non-bagged control fruit. Fruit was initially bagged on July 22 and harvested in September 9.

3.5 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 (Cooley and Miller, 1930; Corke, 1956; Grove et al., 1992). Large cankers bearing abundant acervuli developed after inoculations performed from October to February. Cankers on pear trees were similar to those on apple trees and no differences were observed among pear tree cultivars. Nevertheless, the cankers obtained are not naturally found on pear trees; they were facilitated by deep inoculation of the fungus into the wood, with possible additional food sources provided by the aPDA plug. 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 higher susceptibility may have resulted from the less vigorous branches inoculated as compared with those of Bosc trees. Cankering of apple trees induced by *N. perennans* followed similar patterns as on Bosc trees. Isolate HR-238 was somewhat more aggressive than MA-0001, inducing some cankering even in the summer of 2002.

Cankers were obtained after 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 (Bompeix and Bondoux, 1974). It also colonized one-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 months, except on d'Anjou trees where cankers were induced during summer months also. This pathogen, however, induced smaller cankers than *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.

The requirement of wounds for the induction of cankers on pear trees by *N. perennans* was confirmed as previously reported on apple trees (Childs, 1929; Kienholz, 1939; Wilkinson, 1945). Cankers induced were small and superficial resembling those observed on pear trees.

Sporulation from cankers induced by *N. perennans* was observed for a period of 20 months. Grove et al. (1992) reported sporulation spanning almost three years on cankers on apples trees inoculated with *N perennans*. The largest amount of conidia was produced from September to December, coincidental with a high susceptibility of wood to cankering. Both macro and microconidia were present during the entire period of study, but macroconidia were more abundant during the peaks of sporulation.

Sporulation of *N. perennans* on d'Anjou trees followed a different pattern than on Bosc or Granny Smith trees, with a peak in July and decreasing thereafter without reaching a second increase by the autumn months. This pattern could be the result of a different environment where over-tree irrigation provided humidity during spring and summer that could stimulate earlier sporulation as compared with the other cultivars where irrigation was performed with under-tree sprinklers.

Harley and Reeves (1930) also reported earlier production of conidia of perennial cankers under over-tree irrigation.

Contrary to the report by Corke (1956), *Neofabraea alba* sporulated abundantly on artificially induced cankers. Sporulation of *N. alba* followed the same patterns of *N. perennans* with low amounts observed during the summer months, increasing through the end of summer and reaching maximums during the autumn months.

Sporulation on cankers induced by *N. alba* was reduced only by copper sulfate, but the effect was lost after approximately one month. Copper fungicides have been reported to control apple anthracnose (*N. malicorticis*), but not perennial canker (*N. perennans*) (Kienholz, 1939). In the present study the fungicides tested did not affect sporulation of *N. perennans*. Contrary to the report of Burchill and Edney (1963), increasing rates of sporulation of *N. alba* once the fungicide effect declined, was not observed in this study. Application of fungicides reduced the germination of conidia of *N. perennans*. Reduced viability of conidia should impact the incidence of bull's eye rot as well, and if this effect is complemented with a reduction in sporulation, better control of the disease could be achieved. Ziram had the greatest effect on conidial germination and induced abnormal growth of the germ tubes.

Bosc pears became infected throughout the growing seasons in the experimental orchard in Medford. Disease incidence observed in exposure period 1 indicates early fruit infestation and disease observed in subsequent exposure periods indicates new infestations that occurred as the season progressed toward

harvest. Increasing levels of disease were observed in the final exposure periods, which could be related to a higher susceptibility of the fruit when approaching maturity (Edney, 1958; Spotts, 1985), and to an increase in sporulation of the pathogen as observed on the artificially induced cankers of *N. perennans* on Bosc trees. In addition, rainfall was recorded during the final two exposure periods and may have increased conidial dispersal. Nevertheless, each exposure period received at least one irrigation, which should account for the majority of the infections and could have masked the effect of rainfall on bull's eye rot incidence.

Bull's eye rot was found in all the exposure periods opened during 2002 in Medford. In contrast to 2001, where the average incidence was 9.7 %, only 2.2 % was observed in 2002. Rainfall for the fruit-growing season was a 60% of that of 2001 and sporulation of both *N. perennans* and *N. alba* was reduced during the experiment and started to increase only after harvest. Accordingly, the lower disease incidence could have resulted from a lower amount of inoculum present and climatic conditions less favorable for infection. Even though over tree irrigation should replace the role of rain in conidial dispersal, other conditions present during rainfall could affect incidence of bull's eye rot. Incidence of bull's eye rot in all the exposure periods opened in Hood River should have resulted from rainfall occurring before the beginning of the experiment. The orchard was irrigated with under-tree sprinklers and the variation among exposure periods may be due to the experimental design where whole branches on individual trees were bagged at each exposure period, limiting the randomization within tree.

This study showed that pear wood is susceptible to infection by *Neofabraea* spp. Conidia of *N. alba* and *N. perennans* are produced on the trees almost constantly, increasing at the end of the growing season and during harvest time, where fruit susceptibility is highest. Fungicides commonly applied on pear orchards had little effect on sporulation of the pathogens, but can affect conidial germination that should in turn influence disease incidence. Pear fruit became infected throughout the growing season with relatively higher incidence close to harvest.

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4. EFFECT OF ENVIRONMENTAL FACTORS, CULTURAL PRACTICES, AND CHEMICAL TREATMENTS, ON THE DEVELOPMENT OF BULL'S EYE ROT OF PEARS

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4.1 Abstract

Bull's eye rot of pome fruits caused by *Neofabraea* spp. is characterized by infestations occurring in the orchard throughout the growing season while the rot develops later in postharvest. Spores are produced on the wood of trees and splashed to fruit. Preharvest fruit inoculations with *N. perennans* were performed to determine the effect of environmental, cultural and chemical factors on the development of bull's eye rot. Temperature had a contradictory effect; disease was greatest at 10°C in 2001 but greatest at 30°C in 2002. Wetness duration did not affect bull's eye rot development while the concentration of conidia correlated positively with bull's eye rot development. Disease incidence values of about 90% were obtained with concentration of conidia ranging from $25x10^3$ to $2x10^5$. Over-tree irrigation and late harvest resulted in higher bulls eye rot incidence than under-tree irrigation and early or mid season harvest. The fungicides trifloxystrobin and ziram protected inoculated fruit for about a month in 2001. Thiabendazole applied as a postharvest dip reduced bull's eye rot on inoculated pears.

4.2 Introduction

Bull's eye rot of apples and pears caused by *Neofabraea* spp. is a common disease in the Pacific Northwest, reaching important levels in some seasons. The pathogens cause cankers of branches or develop saprophytically on trees (Bompeix, 1988; Edney, 1956, 1983; Grove, 1990; Verkley, 1999). Sporulation occurs on the wood and the conidia are splash-dispersed to fruit. Fruit can become

infected from petal-fall to harvest but susceptibility is greatest close to harvest (Spotts, 1985a).

Research on bull's eye pathogens has focused on the canker phase and on chemical control of both the canker and the fruit phases. Furthermore, most research has been conducted on apples. The effects of environmental, cultural and chemical factors on bull's eye rot development have not been widely addressed.

Survival of conidia of *N. malicorticis* superficially inoculated on d'Anjou pears was greater at 10 and 20 °C than at 30 °C (Spotts, 1985b). Germination of conidia of *N. malicorticis* was greater at –1.1 °C than at 10 or 20 °C (Spotts and Peters, 1982). All detached pears inoculated with *N. malicorticis* developed bull's eye rot when incubated at –1.1 °C but none did when incubated at 20 °C (Spotts, 1985a). In contrast, *N. perennans* developed bull's eye rot on detached apple fruit incubated at 20 °C (Edney, 1956). Rot developed more slowly with decreasing temperatures and no rotting was observed at 25 °C. Bompeix (1974) observed that low temperatures induced sporulation of *N. alba*, with maximum sporulation at 10 – 12 °C. Light induced morphogenesis of pycnidia and acervuli and promoted mycelial growth of *Neofabraea* spp. (Bompeix, 1974).

Conidia of *N. malicorticis* germinated only at 100% relative humidity (RH) but not at 97 or 99 %, when placed on the surface of pears (Spotts and Peters, 1982). Nevertheless, 100% of wounded fruits were infected at 97, 99, and 100% RH.

Splashing rainfall is considered the main mechanism for spore dispersal of bull's eye rot pathogens. In addition, Grove et al. (1992) observed conidia being

splashed from over-tree irrigation. Harley and Reeves (1930) reported conidia produced earlier in the season on cankers under over-tree irrigation than under other irrigation systems.

The objectives of this study were to determine the effect of (1) temperature and wetness; and (2) harvest timing, irrigation method, and pre- and postharvest fungicides on the development of bull's eye rot of pears.

4.3 Materials and methods

4.3.1 Isolates utilized. Isolates MA-0001 and HR-238 of *N. perennans* and MB-0128 and MB-0140 of *N. alba* were utilized in this study. Isolate MA-0001 was isolated from an Asian pear in Medford, Oregon. Isolate HR-238 was isolated from a d'Anjou pear in Hood River, Oregon. Both isolates of *N. alba* were isolated from Bosc pears in Medford. Experiments with *N. alba* were conducted only in the second season of the study, after this species was identified as a causal agent of bull's eye rot of pears in Oregon (Chapter 2). All isolates were grown on acidified potato dextrose agar (aPDA) at room temperature. Conidial suspensions of *N. perennans* were prepared by washing 30-day-old cultures with sterile distilled water and concentrations were adjusted with a hemacytometer. Conidial suspensions of *N. alba* were prepared similarly using 30-day-old lesions on inoculated pears as a source of spores.

4.3.2 Inoculation method. Spore suspensions were applied with a hand sprayer to run off. Fruit were then covered with a polyethylene bag and wrapped with a sheet of aluminum foil, to secure the bag and avoid high temperature. The wrap and bag

were removed after the desired incubation time (wetness duration). At harvest, fruits were placed inside cardboard boxes lined with perforated polyethylene bags and stored at 0 +/- 1°C in air.

4.3.3 Effect of temperature and duration of wet period on bull's eye rot development.

Branches bearing numerous pears were enclosed inside limb cages that allowed for temperature control. Temperatures of 10, 20 and 30 °C and wet periods of 0.5, 1, 2.5, and 5 hr were studied. Fruit were inoculated with a conidial suspension containing $5x10^4$ conidia/ml of *N. perennans* isolate HR-238. Cages were closed after fruit inoculation and opened as needed to unwrap the various wetness treatments. One experiment was conducted on a block of d'Anjou pears on August 17-20, 2001. Each treatment combination was replicated three times with 4 to 7 fruit each. Fruit were harvested on September 4, 2001. Boxes were inspected periodically for bull's eye rot development. Fruit infected with gray mold and Mucor rot were removed. Disease incidence and severity (number of lesions per fruit) were determined.

The experiment was repeated on August 20 – 22, 2002, in a block of Bosc pear trees, and *N. alba* isolate MB-0128 was included. Each treatment combination was replicated three times with 5 to 10 fruits each. Fruit were harvested on September 9, 2002. Boxes were inspected periodically and bull's eye rot incidence and severity were determined.

4.3.4 Effect of conidial concentration on bull's eye rot development under field conditions.

Bosc pears were inoculated, as described above, with *N. perennans* isolate MA-0001 at conidial concentrations of $1.25x10^4$, $2.5x10^4$, $5x10^4$, $1x10^5$, and $2x10^5$ conidia /ml, with 4 hr of incubation, control fruit were sprayed with sterile distilled water. Inoculations were conducted in September 2001 and repeated in October 2002. *N. alba* isolate MB-0128 was included in 2002. The conidial concentration of $1.25x10^4$ conidia/ml was not included in the experiments of 2002. Five replicate trees were used and 10 fruits per treatment were inoculated on each tree. Fruit were harvested one week after inoculation. Disease incidence and severity were determined following 4-5 months storage at 0 +/- 1°C.

4.3.5 Effect of wetness duration on bull's eye rot development under field conditions.

Bosc pears were sprayed with *N. perennans* isolate MA-0001 at $5x10^4$ conidia /ml. Pears were then covered with plastic bags and aluminum foil as described above for 0, 0.5, 1, 2, 3, 4, and 5 hr in 2001 and 0, 2, 4, and 6 hr in 2002. Fruit in the 0 hr treatment were sprayed but not bagged. Disease incidence and severity were determined following 4-5 months storage at 0 +/- 1°C.

4.3.6 Effect of harvest time on the development of bull's eye rot of pears.

During February and March of 2002, Bosc pears from the 2001 harvest representing 24 different orchards were examined for incidence of bull's eye rot in a commercial packinghouse. Five boxes of approximately 100 pears each were randomly collected from the fruit of each orchard. The fruit had been harvested between 7 September and 10 October 2001, and stored at –1°C after packing. In a

complementary experiment during the 2002 harvest, five orchards were sampled either early (9-11 September), mid season (23-24 September) or late in the harvest period (8-9 October). One box containing 80-100 fruit was harvested from each of five trees in each orchard at each harvest timing. The fruit were then stored at 0°C and bull's eye rot incidence was evaluated after seven months.

4.3.7 Effect of sprinkler irrigation on the incidence of bull's eye rot of pears. A 0.35 ha block consisting of four rows of Bosc and four rows of Comice pear trees in the orchard of SOREC in Medford was utilized. The block was planted in 1984 and irrigated with over-tree sprinklers. Bull's eye rot was found in both pear cultivars during 2001. Over-tree sprinklers in the eastern half of the block were replaced with under-tree sprinklers while over-tree sprinklers were retained in the western half. One hundred fruit were harvested from each row of each irrigation treatment and cultivar in September 17, 2002. In addition, 18-20 Bosc pears were bagged in each tree before the first irrigation was performed. Individual fruit or clusters were enclosed in waxed paper bags and secured with wire twists to prevent bull's eye rot infestation. Three to four bags per tree were removed every two weeks, opening exposure periods for infestation and then fruit were rebagged. Six exposure periods were opened during the season. These fruit were also harvested on September 17, 2002. Evaluation was performed after seven months storage, when disease incidence was determined. Rainfall data were obtained from the Agrimet station (Bureau of Reclamation, Pacific Northwest Region) located at SOREC, Medford.

4.3.8 Effects of trifloxystrobin and ziram on the development of bull's eye rot of pears.

Bosc pears were inoculated with a suspension of $5x10^4$ conidia/ml of *N. perennans* isolate MA-0001, with 4 hr of incubation. Four to six fruits were inoculated, as described above, in five replicate trees per treatment in 2001. Inoculations were performed 7 and 1 day before fungicides were applied and 7, 14, 21, and 28 days after fungicide treatment. Ziram and trifloxystrobin were applied by hand sprayer at rates of 3,638 mg l^{-1} and 47 mg l^{-1}, respectively. Water was sprayed as the control. Fruit were harvested 2 weeks after the last inoculation. The experiment was performed differently in 2002. Fungicides and control water were sprayed 28, 21, 14, and 7 days before and 1 and 7 days after a single inoculation. *N. alba* isolate MB-0140 was also included. Ten to twelve fruit were inoculated in five replicates for each treatment. Replicates were randomly distributed in 36 trees. Fruit were harvested one week after the last inoculation, in 2001 and one and two weeks after the last spray for *N. alba* and *N. perennans*, respectively, in 2002. Disease incidence (I) and severity (S = number of lesions per fruit) were determined and a disease index was calculated according with the formula DI = (I x S)/100.

4.3.9 Effect of a postharvest dip of thiabendazole on the development of bull's eye rot of pears.

Bosc pears were inoculated in the orchard with *N. perennans* MA-0001 and *N. alba* MB-0140 with a conidial concentration of $5x10^4$ conidia/ml, with 4 hr incubation. Four weeks after inoculation the fruit were harvested and one-third of the fruit was immediately dipped in a solution of thiabendazole at a rate of 569 mg l^{-1} for 20 seconds. Fruit were then stored as described before. One month after

harvest another one-third of the fruit was dipped in the fungicide and the remainder was left untreated as the control. Each treatment had five replicates of 20 fruit. All fruit were stored for six months at 0°C, when bull's eye rot incidence and severity were determined.

4.3.10 Statistical analysis. Incidence data were arcsine transformed to stabilize variances. Square root transformation was used when incidence values were between 0 and 30 %. The data were analyzed through ANOVA and means separated with Fisher's Least Significant Difference (LSD).

4.4 Results

4.4.1 Effect of temperature and duration of wet period on bull's eye rot development.

Temperature had a significant effect on the development of bull's eye rot of pears caused by *N. perennans*. Bull's eye incidence and severity were greater at 10°C in 2001 (Table 4.1). However, the greatest incidence and severity was obtained at 30°C in 2002 (Table 4.2). Temperature also affected the development of bull's eye rot caused by *N. alba*, incidence was highest at both 10 and 30°C (Table 4.3). Temperature did not affect disease severity. Wetness duration and the interaction temperature-wetness did not have significant effects on the development of bull's eye rot.

Table 4.1. Effect of temperature and wetness on bull's eye rot incidence and severity caused by *Neofabraea perennans* inoculated on d'Anjou pears in 2001.

Disease Incidence[1]	Temperature (°C)			
Wetness (hr)	10	20	30	Wetness Main Effect
0.5	75.0	48.3	68.3	63.9 a[4]
1	87.5	31.7	24.4	47.9 a
2.5	80.0	26.7	33.3	46.7 a
5	78.6	55.6	32.1	55.4 a
Temperature Main Effect	80.3 b[3]	40.6 a	39.5 a	
Disease severity [2]	Temperature (°C)			
Wetness (hr)	10	20	30	Wetness Main Effect
0.5	5.2	1.2	0.9	2.4 a[4]
1	9.3	0.8	0.3	3.5 a
2.5	6.4	0.6	0.4	2.5 a
5	7.6	1.6	0.8	3.3 a
Temperature Main Effect	7.1 b[3]	1.1 a	0.6 a	

[1] Percent of fruit with bull's eye rot.
[2] Number of lesions per fruit.
[3] Values followed by the same letter in the row are not significantly different according to Fisher's Least Significant Difference.
[4] Values followed by the same letter in the column are not significantly different according to Fisher's Least Significant Difference.

Table 4.2. Effect of temperature and wetness on bull's eye rot incidence and severity caused by *Neofabraea perennans* inoculated on Bosc pears in 2002.

Disease Incidence[1]	Temperature (°C)			
Wetness (hr)	10	20	30	Wetness Main Effect
0.5	53.3	62.5	71.4	62.4 a[4]
1	83.3	71.4	100.0	84.9 a
2.5	71.3	78.0	100.0	83.1 a
5	74.6	59.7	95.8	76.7 a
Temperature Main Effect	70.6 a[3]	67.9 a	91.8 b	
Disease Severity[2]	Temperature (°C)			
Wetness (hr)	10	20	30	Wetness Main Effect
0.5	2.4	2.5	8.7	4.5 a[4]
1	2.6	2.6	7.9	4.4 a
2.5	2.8	3.5	11.1	5.8 a
5	2.2	2.6	13.1	6.0 a
Temperature Main Effect	2.5 a[3]	2.8 a	10.2 b	

[1] Percent of fruit with bull's eye rot.
[2] Number of lesions per fruit.
[3] Values followed by the same letter in the row are not significantly different according to Fisher's Least Significant Difference.
[4] Values followed by the same letter in the column are not significantly different according to Fisher's Least Significant Difference.

Table 4.3. Effect of temperature and wetness on bull's eye rot incidence and severity caused by *Neofabraea alba* inoculated on Bosc pears in 2002.

Disease Incidence[1]	Temperature (°C)			
Wetness (hr)	10	20	30	Wetness Main Effect
0.5	4.8	3.7	12.5	7.0 a[4]
1	6.7	0	4.8	3.8 a
2.5	38.4	0	16.4	18.3 a
5	7.0	7.4	4.8	6.4 a
Temperature Main Effect	14.2 b[3]	2.8 a	9.6 b	
Disease Severity[2]	Temperature (°C)			
Wetness (hr)	10	20	30	Wetness Main Effect
0.5	0.3	0.3	0.7	0.4 a[4]
1	0.7	0	0.3	0.3 a
2.5	1.5	0	1.0	0.8 a
5	0.7	0.3	0.3	0.4 a
Temperature Main Effect	0.8 a[3]	0.2 a	0.6 a	

[1] Percent of fruit with bull's eye rot.
[2] Number of lesions per fruit.
[3] Values followed by the same letter in the row are not significantly different according to Fisher's Least Significant Difference.
[4] Values followed by the same letter in the column are not significantly different according to Fisher's Least Significant Difference.

4.4.2 Effect of conidial concentration on bull's eye rot development under field conditions.

There were significant positive linear correlations between the concentration of conidia and the incidence and severity of bull's eye rot obtained after inoculations

with both pathogens (Table 4.4). Incidence levels of ~90% were obtained with conidial concentrations of $1x10^5$ and $2x10^5$ conidia/ml of *N. perennans* in 2001. Similar values were obtained with concentrations of $2.5 x10^4$, $5 x10^4$, $1.x10^5$, and $2x10^5$ conidia/ml in 2002. Severity always increased with increasing concentrations of conidia. A low number of fruits became infected with *N. alba* after conidial inoculations. The highest incidence (79%) was obtained with $2x10^5$ conidia/ml. Only 37.5% became infected with $1x10^5$ conidia/ml. Severity values with *N. alba* were low as well, reaching a maximum of 3.5 lesions/fruit at $2x10^5$ conidia/ml, compared with 11.8 lesions/fruit with *N. perennans* at the same concentration.

4.4.3 Effect of wetness duration on bull's eye rot development under field conditions.

Duration of wetness correlated positively with bull's eye severity caused by *N. perennans* in 2001 (Table 4.5). However, there were no differences in incidence or severity values among the different wetness durations tested. Wetness duration positively correlated with bull's eye incidence and severity of *N. perennans* in 2002, where all durations tested except 0 hr had similar incidence and severity values (Table 4.5). There were no correlations between wetness duration and incidence or severity caused by *N. alba*, were low levels of infection were observed. A 2.5% incidence on untreated fruit was observed in 2001 while bull's eye rot was not observed in 2002 (data not shown).

Table 4.4. Correlation coefficients (r) between conidial concentration and incidence and severity of bull's eye rot after fruit inoculations with *Neofabraea perennans* and *N. alba*.

Pathogen/season	r[1]	
	Incidence (%)	Severity (# lesions/fruit)
N. perennans 2001	0.861[1]	0.660
N. perennans 2002	0.777	0.771
N. alba 2002	0.824	0.609

[1] All coefficients are significant at P = 0.01.

Table 4.5. Correlation coefficients (r) between wetness duration and disease incidence and severity of bull's eye rot after field inoculations with *Neofabraea alba* and *N. perennans*.

	N. perennans 2001	
Wetness (hr)	Incidence[1]	Severity[2]
0	11.9 a	0.3 a
1	10.7 a	0.1 a
2	21.9 a	0.3 a
4	24.2 a	0.5 a
5	22.7 a	0.8 a
	r = 0.313	r = 0.398*[3]

	N. perennans 2002		*N. alba* 2002	
Wetness (hr)	Incidence	Severity	Incidence	Severity
0	2.5 a[4]	0.2 a	40.6 a	1.9 a
2	86.1 b	8.9 b	15.0 a	1.0 a
4	97.5 b	9.2 b	17.9 a	1.7 a
6	95.0 b	6.7 b	38.2 a	1.9 a
	r = 0.313*	r = 0.398*	r = 0.041	r =0.768

[1] Percent of fruit with bull's eye rot.
[2] Number of bull's eye lesions per fruit.
[3] Coefficients of correlation followed by an asterisk are significant at p = 0.05.
[4] Values followed by the same letter in the column are not significantly different according to Fisher's Least Significant Difference.

4.4.4 Effect of harvest timing on the development of bull's eye rot in pears.

Bull's eye rot was found in 18 of 24 orchards screened. Incidence values varied from 0.1 % in fruit harvested in September 7 to 6.4 % in fruit harvested on October 6. There was a trend for increasing bull's eye incidence during the second half of the harvest period (Figure 4.1). Higher bull's eye incidence on late harvested fruit was observed in two orchards in 2002 (Table 4.6). There were no differences between fruit harvested early or mid season in the orchards studied.

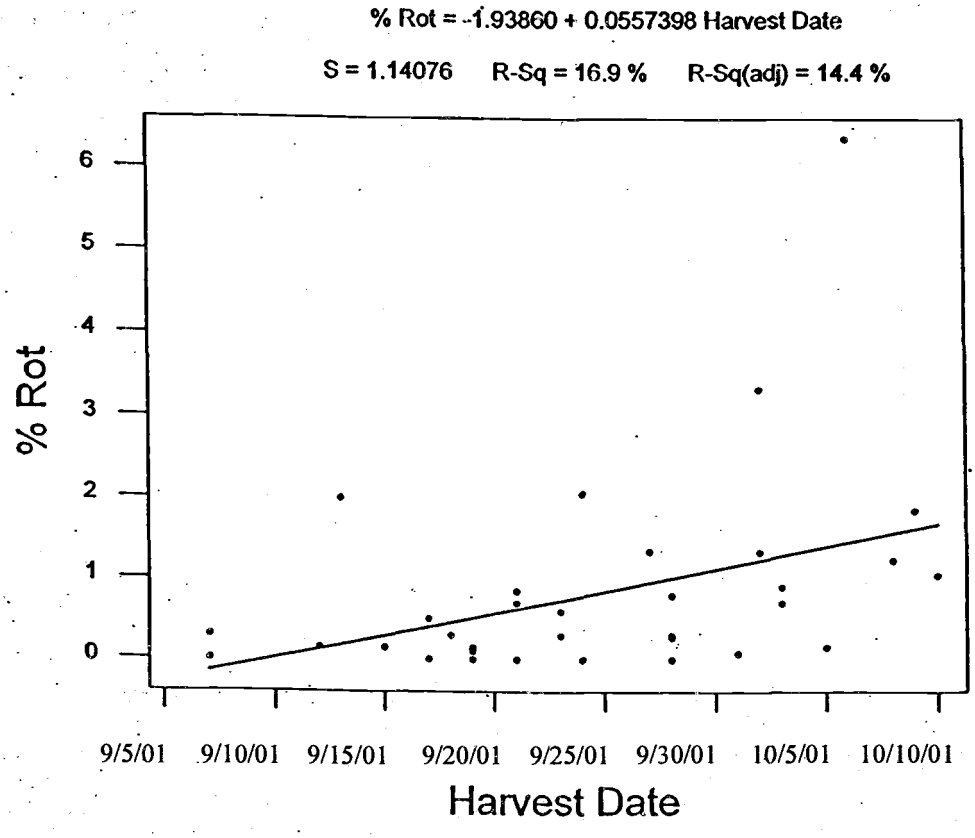

Figure 4.1. Relationship of harvest date to bull's eye rot incidence in fruit from 24 orchards in Medford, Oregon, following 5-6 months storage at –1°C.

Table 4.6. Bull's eye rot incidence (%) on Bosc pears on selected orchards harvested at three different times during the harvest season.

Orchard	Early (ff)[1]	Mid (ff)	Late (ff)	LSD
Bybee	3.3 a[2] (15.8)	2.8 a (13.7)	nd[3]	
Fairlane	3.3 a (15.7)	2.7 a (13.6)	2.2 a (13.0)	
Hanley	3.8 a (15.4)	2.0 a (13.2)	8.2 b (12.3)	0.62
Klamath	15.0 a (19.4)	6.8 a (16.6)	7.5 a (15.1)	
Medford Station	1.2 a (16.8)	1.4 a (15.0)	4.4 b (13.8)	0.85

[1] Fruit firmness (N).
[2] Values followed by the same letter in the row are not significantly different according to Fisher's Least Significant Difference (LSD).
[3] No data were obtained.

4.4.5 Effect of sprinkler irrigation on the incidence of bull's eye rot of pears.

3.7 % of the fruit developed bull's eye rot in Bosc pear trees that received over-tree irrigation, while bull's eye rot was not found in fruit from trees irrigated with under-tree sprinklers (Table 4.7). 1% bull's eye rot incidence was found in fruit from Comice trees with over-tree irrigation and 0.25% in fruit from trees with under-tree irrigation, but the difference was not significant ($p > 0.05$). Bull's eye rot was found in all the exposure periods opened in the over-tree irrigated block except for exposure period 2 (Figure 4.2). Only one fruit with bull's eye rot was found, in exposure period 4, in the block with under-tree sprinklers. Rainfall was recorded during exposure periods 1 and 4 (Figure 4.2).

Table 4.7. Effect of irrigation system on bull's eye rot incidence (%).

	Bosc	Comice
Over-tree	3.7 b[1]	1.0 a
Under-tree	0 a	0.25 a

[1] Values followed by the same letter in the column are not significantly different.

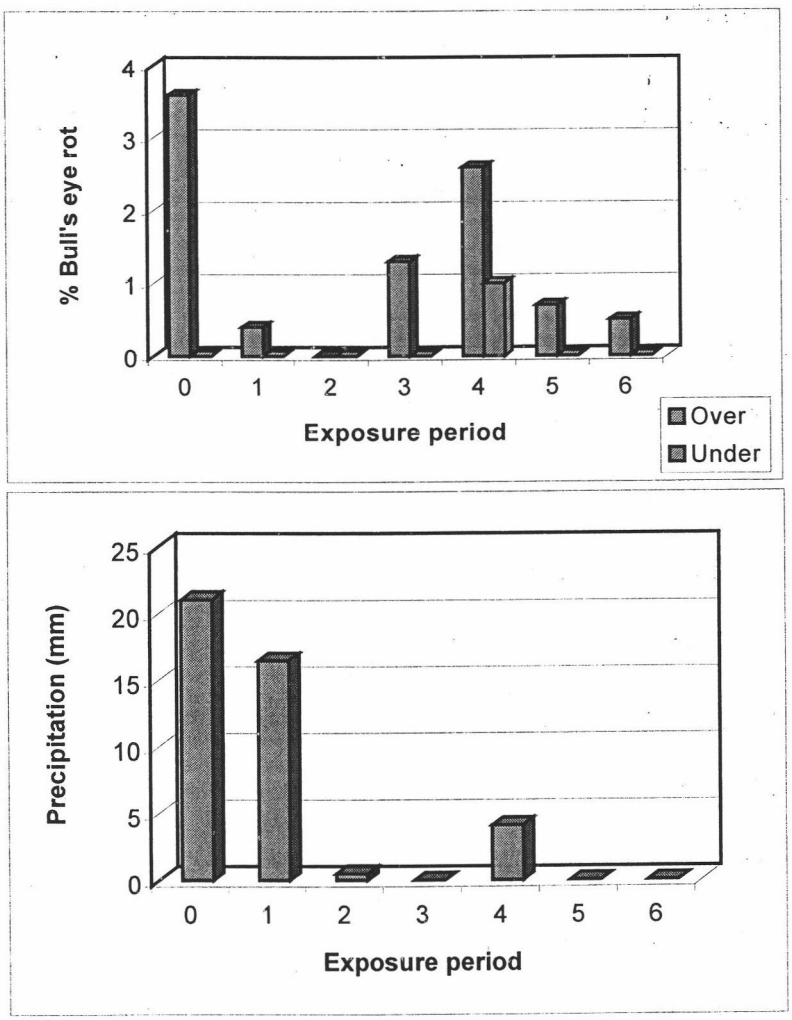

Figure 4.2. Top; Bull's eye rot incidence on six exposure periods opened during 2002 on Bosc trees irrigated with over-tree or under-tree sprinklers. Bottom; Rainfall recorded at the Agrimet station (Bureau of Reclamation, Pacific Northwest Region) in Medford during the 2002 growing season.

4.4.6 Effects of trifloxystrobin and ziram on the development of bull's eye rot of pears.

Both fungicides reduced the disease index of *N. perennans* in 2001 in all the inoculations performed except 7 days before fungicide treatments, where only trifloxystrobin reduced the disease index (Table 4.8). In 2002 only ziram applied 14 days before and 7 days after the inoculation reduced the disease index of *N. perennans*. Inoculations with *N. alba* resulted in very low disease, 18.2 % was the highest incidence observed in the water treatment. Therefore, significant differences among treatments were not obtained.

4.4.7 Effect of a postharvest dip of thiabendazole on the development of bull's eye rot of pears.

Thiabendazole treatments at harvest or 30 days after harvest reduced the incidence and severity of bull's eye rot of pears caused by *N. perennans* (Table 4.9). Fruit inoculated with *N. alba* resulted in very low levels of disease with an average incidence of 9% in the control. Neither disease incidence nor disease severity were affected by thiabendazole treatment (data not shown).

Table 4.8. Effect of selected fungicides in the disease index of bull's eye rot caused by *Neofabraea perennans* inoculated on Bosc pears.[1]

Season 2001	Treatments		
Inoculations[2]	Water	Trifloxystrobin	Ziram
- 7	4.3 a[3]	0.7 b	4.7 a
- 1	13.7 a	6.3 b	5.6 b
7	13.0 a	3.9 b	2.4 b
14	2.1 a	0.7 b	0.6 b
21	9.7 a	5.4 b	5.5 b
28	16.5 a	5.7 b	4.8 b
Season 2002			
Sprays[4]	Water	Trifloxystrobin	Ziram
28	5.4 a	6.4 a	2.4 a
21	9.0 a	7.2 a	3.7 a
14	11.5 a[3]	4.0 a	0.3 b
7	14.5 a	3.7 a	0.7 a
+1	7.5 a	2.7 a	1.2 a
+7	1.9 a	1.8 a	0.4 b

[1] Disease index was calculated by the formula DI = (Incidence x Severity)/100.
[2] Inoculations of *N. perennans* ($5x10^4$ conidia/ml) were conducted 1 and 7 days before and 7, 14, 21, and 28 days after fungicide spray.
[3] Values followed by the same letter in the row are not significantly different according to Fisher's Least Significant Difference (LSD).
[4] Fungicides were sprayed 28, 21, 14, 7 days before and 1, and 7 days after inoculation of *N. perennans* ($5x10^4$ conidia/ml).

Table 4.9. Effect of a postharvest thiabendazole (TBZ) dip on bull's eye rot incidence and severity caused by *Neofabraea perennans*.[1]

Treatment	Incidence (%)	Severity (# lesions/fruit)
Untreated control	96.0 a[2]	6.6 a
TBZ at harvest	43.5 b	0.6 b
TBZ 30 days after harvest	23.7 b	0.3 b

[1] Fruit was inoculated 4 weeks before harvest with conidial suspensions of $1x10^5$ conidia/ml and incubated for 4 hours.
[2] Values followed by the same letter in the column are not significantly different according to Fisher's Least Significant Difference (LSD).

4.5 Discussion

The effect of temperature on bull's eye rot development was not clear after two seasons of experiments. Higher incidence and severity values for infection by *N.* perennans found at 10°C in 2001 correspond to the findings of Spotts (1985b) where highest survival of conidia of *N. malicorticis* was observed at the lower temperatures. The results are congruent also with the concept of a pathogen whose main dispersal mechanism is by rain splashing, conditions where lower temperatures should prevail. Furthermore, lesions developed more frequently at lower temperatures on inoculated fruit. On the other hand, greater infection at higher temperatures, as observed in 2002, is congruent with a pathogen that usually infects fruit during summer and early fall, when higher temperatures usually prevail. Differences observed between the two seasons may be due to differential susceptibility of the cultivars used. Both cultivars are known to be

susceptible, but tests of comparative susceptibility have not been conducted. Nevertheless, high incidence of infection by *N. perennans* was observed at all temperatures tested.

Bull's eye rot developed independently of wetness duration, reaching at least 60% infection with 30 minutes of free water, under controlled or field conditions. Differences were observed only with *N. perennans* in 2002, where all wetness durations resulted in higher disease development than at 0 hours. Generally, bull's eye rot developed after conidia were sprayed on the fruit and allowed to dry immediately (0 hr). It took only 1-2 minutes for the fruit to dry. Four hours of wetness resulted in nearly 100 % bull's eye rot incidence in 2002, and was consider the best wetness duration to use in inoculation experiments. It is likely that water plays a bigger role on conidial dispersal of bull's eye pathogens than on infection itself.

Bull's eye rot development correlated positively with the concentration of conidia. Incidence of bull's eye rot caused by *N. perennans* of about 90% was observed with $10x10^4$ and $5x10^4$ conidia/ml or higher in 2001 and 2002, respectively. Higher concentrations of conidia of *N. alba* may be required to achieve incidence over 90%; only 79 % infection incidence was obtained with $2x10^5$ conidia/ml. However, the methodology for fruit inoculation utilized generally failed for *N. alba*. Edney et al. (1977) showed that rain increased the susceptibility of apples to infection by *N. alba*. Inoculations of previously washed fruit may help induce higher levels of disease and obtain more reliable infection by this pathogen.

The timing of fruit harvest may affect the incidence of bull's eye rot due to different factors. Pears are more susceptible to infection with greater maturity (Spotts, 1985a); the likelihood of rainfall increases at the end of the summer and beginning of autumn in many fruit growing areas; and sporulation of bull's eye pathogens increases at about harvest time (Grove et al., 1992). A higher incidence of bull's eye on fruit picked late in the season was found in two orchards in this study, as well as a trend among orchards for greater bull's eye rot incidence with later harvest.

This study conclusively showed the impact of the irrigation system on the development of bull's eye rot. Grove et al. (1992) observed dispersal of conidia of *N. perennans* by the impact of water droplets from over-tree irrigation. Even though disease incidence was low in 2002, Bosc pears had 3.7% incidence of infection with over-tree sprinklers, contrasting with no incidence with under-tree sprinklers. The disease levels were too low in Comice to show significant differences. Thirteen orchards with over-tree sprinkler irrigation had an average incidence of bull's eye rot of 1.1% compared to 0.3% in orchards with under-tree sprinklers. Over-tree sprinklers have been used for long time as a tool to prevent frost damage on the crop. Replacing the frost control system or replacing over-tree sprinklers with under-tree sprinklers once the frost risk is over for the season would help reduce losses due to bull's eye rot.

The fungicides tested reduced bull's eye rot, protecting inoculated fruits for almost one month in the 2001 season. Trifloxystrobin showed curative activity of 7 days in 2001 but it was only one day for ziram. In 2002 both fungicides showed

disease indices lower than those of the water control. Fungicides were tested under extremely favorable conditions for the pathogen, challenging their performance. Greater effects should be expected under natural inoculation conditions. Both fungicides are currently applied in pear orchards to reduce postharvest rots.

Benzimidazole fungicides have been satisfactorily used as orchard sprays in the control of bull's eye pathogens (Bompeix, 1988), but the development of resistance has reduced the use of these fungicides in the field. Thiabendazole, used as a fruit drench or dip, has been one of the main tools for the control of postharvest diseases of pome fruits. The present study showed the reduction of incidence and severity of bull's eye rot with postharvest TBZ treatment. Although bull's eye pathogens remain dormant in the fruit while still in the orchard, they continue developing and producing fruit infections some time after harvest and in cold storage (Bompeix, 1978). TBZ acts by inhibiting cell division and will affect only actively growing fungal cells. When the fungicide is applied immediately after harvest, most bull's eye propagules may still be dormant and relatively insensitive to the fungicide. When the fungicide is applied one month after harvest, it may be available at a higher concentration when the pathogen reassumes activity. The precise time at which the pathogens reassume activity is not known. Fungicide residues degrade at low rates under cold storage conditions. If they are applied only at harvest and the fruit stored for long time, a lower level of control may result due to loss of fungicide activity with time. Experiments comparing TBZ treatments at several times after harvest would help to understand when the

pathogens resume activity and when the treatment would be more effective in reducing rot development.

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5 CONCLUSIONS

Three species of *Neofabraea* were identified as causal agents of bull's eye rot of pears in the main growing areas of Oregon, and in samples from Washington and California. *N. alba* was one of the most important species, isolated from pears from Hood River and Medford in Oregon and from California. *N. alba* is considered the main pathogen causing lenticel rots of apples and pears in continental Europe. It was previously reported as a minor disease causing bull's eye rot of apples in eastern North America. However, it has never before been recognized as one of the pathogens causing bull's eye rot of pome fruit in the Pacific Northwest, even though it may have been present from a long time.

N. perennans also was a common species causing bull's eye rot of pears in Washington and Oregon. It was the only species found in samples from Wenatchee, Washington, where perennial canker has been prevalent and anthracnose canker almost absent (Frank Dugan, USDA-ARS, Pullman, WA. personal communication).

The undescribed *N. sp. nova* was also identified in samples from Medford. This taxon, recently reported in a phylogenetic study of the genus, has been obtained from diverse regions, originally from Portugal and Nova Scotia and more recently from Australia. This species resembles *N. perennans* in morphology and further characterization is needed to differentiate it and understand its importance in pome fruits.

N. malicorticis, considered the main organism responsible for bull's eye rot of apples and pears in the Pacific Northwest, was not identified in pear isolates during this study. However, it was identified in cankers on apple branches from Oregon. A more comprehensive screening would be needed to determine if it indeed causes bull's eye rot on pear fruits. In the previous literature, *N. alba* was probably confused for *N. malicorticis* due to the similar morphology of their macroconidia.

It is possible that some other taxa could be present since some *Neofabraea*-like isolates in this study were not identified with PCR screening or they reacted simultaneously to primers specific for two *Neofabraea* species.

It appears that both *N. alba* and *N. perennans* are widespread species in the drier growing regions of the Pacific Northwest, while *N. malicorticis* may be restricted to more humid areas. It is difficult to make conclusions regarding broader geographic distribution of these taxa due to taxonomic confusion in the literature. In Europe *N. perennans* and *N. malicorticis* are considered synonyms. De Jong et al. (2001) identified a European isolate as *N. perennans* which was originally labeled as *N. malicorticis*. Similar results were obtained by Cunnington (Institute for Horticultural Development, Victoria 3156, Australia. Personal communication) screening isolates from Australia. Furthermore, Sutton (1980) in his treatment of the Coelomycetes depicted straight macroconidia for *N. malicorticis*, resembling that of *N. perennans*. Further genetic analysis is needed to clarify this situation.

The prevalence of a given species in pear orchards may also be under the influence of climatic components. In this study, 2001 was conducive to high levels of bull's eye rot and *N. alba* was the predominant species found. The following season, where conditions were less favorable for bull's eye rot, *N. perennans* was the predominant species.

Both *N. alba* and *N. perennans* induced cankers when inoculated on apple and pear trees. Cankers resulted after mycelial inoculations performed during autumn and winter months. Larger cankers producing abundant acervuli of *N. perennans* resulted from inoculations conducted from October to February. Cankers induced on pear trees were similar to those induced on apple trees, although they were not observed to occur naturally on pear trees. Cankers induced by *N. alba* were smaller than those induced by *N. perennans*.

The requirement of wounds for the induction of cankers on pear trees by *N. perennans* was verified in this study, as previously observed on apple trees. Conidial inoculations on superficially wounded pear branches resulted in small superficial cankers that resembled those naturally occurring on pear trees. A positive identification of *N. alba* on natural cankers was obtained with PCR, but none of the other species was identified from natural cankers.

Conidia of *N. alba* and *N. perennans* were produced throughout the year on cankers induced on pear trees. The highest amounts of conidia were produced from late summer through autumn. A different pattern of conidial production was observed on cankers induced by *N. perennans* on trees under over-tree irrigation.

Higher production of conidia took place during summer months, declining towards winter.

Infection of pears by bull's eye rot pathogens spanned the whole growing season with a higher incidence close to harvest. A higher incidence of bull's eye rot close to harvest can be explained by the combination of higher susceptibility of the fruit, rainfall occurring in late summer, and an increase in sporulation of the pathogens.

The effect of temperature on development of bull's eye rot was contradictory after two seasons of studies. Infection was highest at 10°C in 2001 but highest at 30°C in 2002. None of the temperatures studied inhibited bull's eye rot development. Wetness duration did not affect the development of bull's eye rot of pears. Disease was observed even after spraying the fruit with a conidial suspension and allowing it to dry within a few minutes. Increasing times of incubation from 0.5 to 6 hr did not affect development of bull's eye rot. The principal role of water, therefore, seems to be in spore dispersal. Nevertheless, Edney et al. (1977) reported that rainfall occurring before inoculations was important in reducing resistance of fruit to infection by *N. alba.*

Disease incidence and severity increased with increasing conidial concentrations of bull's eye pathogens inoculated on Bosc pears. Concentrations of 5-10 $x10^4$ conidia/ml gave incidence values over 90%, which were appropriate for inoculations of *N. perennans* in the orchard. However, the methodology utilized failed for *N. alba* and a different system needs to be developed in order to perform orchard inoculations with this pathogen.

Over-tree irrigation resulted in a higher incidence of bull's eye rot in Bosc pears than under-tree irrigation. Conidia of bull's eye pathogens are water splashed to fruits. Earlier conidial production on perennial cankers under over-tree irrigation as reported by Harley and Reeves (1930), and as observed on d'Anjou trees in this study, should result in a higher load of conidia on the fruit surface and a higher likelihood of infection. However, even in an orchard with over-tree irrigation, seasonal variation in bull's eye rot incidence was observed, with 9.9% incidence in 2001 and 2.2% in 2002. If over-tree irrigation were the main cause of infection, bull's eye rot levels should be similar from one season to another.

Higher susceptibility of fruits with increasing maturity, higher sporulation levels in the orchard, and late summer rains can result in higher levels of bull's eye rot if fruit is harvested late in the season. This situation was observed in this study in two out of five orchards in 2002.

Only copper reduced the sporulation rate of cankers induced by *N. alba*, but none of the fungicides tested reduced sporulation of cankers induced by *N. perennans*. However all the fungicides reduced germination of conidia of *N. perennans*. Both trifloxystrobin and ziram reduced bull's eye rot after fruit inoculations with *N. perennans* in the orchard. Thiabendazole applied as a postharvest dip reduced bull's eye rot incidence. The fungicides tested in the orchard are currently used in orchards to reduce postharvest rots. TBZ is applied to fruit in packinghouses.

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APPENDIX

Identification of *Neofabraea* isolates from bull's eye rot on pears and apples grown in Washington, Oregon and California, using PCR with *Neofabraea* species-specific primers.

ISOLATE	HOST[1]	ORIGIN	CULTURE NAME
MB-9901[2]	Bosc	Medford - Hanley	*N.alba*
MA-0001[2]	Asian pear	Medford	*N. perennans*
HR-238[2]	Pear	Hood River	*N. perennans*
MB-0102[2]	Bosc	Medford	*N.alba*
MB-0103[2]	Bosc	Medford	*N.alba*
MC-0106[2]	Comice	Medford	*N.alba*
CW-0101[2]	Anjou	Cachemire, WA	*N. perennans*
MB-0128	Bosc	Medford - Hanley	*N.alba*
MB-0132	Bosc	Medford - Hanley	*N.alba*
MB-0133	Bosc	Medford - Hanley	*N.alba*
MB-0134	Bosc	Medford - Hanley	*N.alba*
MB-0135	Bosc	Medford - Hanley	*N.alba*
MB-0136	Bosc	Medford - Hanley	*N.alba*
MB-0137	Bosc	Medford - Hanley	*N.alba*
MB-0138	Bosc	Medford - Hanley	*N.alba*
MB-0139	Bosc	Medford - Hanley	*N.alba*
MB-0140[2]	Bosc	Medford - Hanley	*N.alba*
MB-0141	Bosc	Medford - Hanley	*N.alba*
MB-0142	Bosc	Medford - Hanley	*N.alba*
MB-0143	Bosc	Medford - Hanley	*N.alba*
MB-0144[2]	Bosc	Medford - Hanley	*N.alba*
HR 236[2]	n/a	Hood River	*N.alba*
HR 240[2]	d'Anjou	Hood River	*N. perennans*
HR 241[2]	n/a	Hood River	*N.alba*
HR 242[2]	Bosc	Hood River	*N.alba*
HR 243[2]	Bosc	Hood River	*N. perennans*
HR 244[2]	Bosc	Hood River	*N.alba*
HR 245[2]	Bosc	Hood River	*N.alba*
HR 246[2]	Bosc	Hood River	*N. perennans*
HR 247[2]	Bosc	Bingen, WA (Hood River)	*N. perennans*
HR 248[2]	Bosc	Hood River	*N.alba*
HR 249[2]	Bosc	Hood River	*N.alba*
HR 250[2]	Bosc	Hood River	*N. perennans*
HR 251[2]	Bosc	Hood River	*N.alba*
HR 253[2]	Bosc	Hood River	*N. perennans*
HR 268	d'Anjou	Hood River	*N.alba*
HR 269	d'Anjou	Hood River	*N.alba*
HR 270	d'Anjou	Hood River	*N.alba*
HR 271	d'Anjou	Hood River	*N.alba*

HR 272	d'Anjou	Hood River	*N.alba*
HR 273	d'Anjou	Hood River	*N.alba*
HR 274	d'Anjou	Hood River	*N.alba*
HR 275	Bosc	Hood River	*N.alba*
HR 276	Bosc	Hood River	*N.alba*
HR 277	Bosc	Hood River	*N.alba*
HR 278	Bosc	Hood River	*N.alba*
HR 279	Bosc	Hood River	*N.alba*
HR 280	Bosc	Hood River	*N.alba*
HR 281	Bosc	Hood River	*N. perennans*
HR 282	Bosc	Hood River	*N. perennans*
HR 283	Bosc	Hood River	*N. perennans*
HR 284	Bosc	Hood River	*N.alba*
HR 285	Bosc	Hood River	*N. perennans*
HR 286	Bosc	Hood River	*N.alba*
HR 287	Bosc	Hood River	*N.alba*
HR 288	Bosc	Hood River	*N. perennans*
HR 289	Bosc	Hood River	*N. perennans*
HR 290	Bosc	Hood River	*N. perennans*
HR 291	Bosc	Hood River	*N.alba*
HR 292	Bosc	Hood River	*N.alba*
HR 293	Bosc	Hood River	*N.alba*
HR 294	Bosc	Hood River	*N. perennans*
HR 295	Bosc	Hood River	*N.alba*
HR 296	Bosc	Hood River	*N. perennans*
HR 297	Bosc	Hood River	*Cylindrocarpon*[3]
HR 298	Bosc	Hood River	*N.alba*
HR 299	d'Anjou	Hood River	*N. perennans*
HR 300	d'Anjou	Hood River	*N.alba*
HR 301	Bosc	Hood River	*N.alba*
HR 302	Bosc	Hood River	*N. perennans*
HR 303	Bosc	Hood River	*N. perennans*
HR 304	Bosc	Hood River	*N.alba*
HR 305	Bosc	Hood River	*N.alba*
HR 306	Bosc	Hood River	*N. perennans*
HR 307	Bosc	Hood River	*N.alba*
HR 308	Bosc	Hood River	*N.alba*
HR 309	Bosc	Hood River	*N.alba*
HR 310	Bosc	Hood River	*N.alba*
HR 311	Bosc	Hood River	*N.alba*
HR 312	Bosc	Hood River	*N. perennans*
HR 313	Bosc	Hood River	*N. perennans*
HR 314	d'Anjou	Hood River	*N. perennans*
HR 315	d'Anjou	Hood River	*N.alba*
HR 316	Bosc	Hood River	*N. perennans*

HR 317	d'Anjou	Hood River	*N.alba*
HR 318	d'Anjou	Hood River	*N.alba*
HR 319	d'Anjou	Hood River	*N. perennans*
HR 320	Bosc	Hood River	*N. perennans*
HR 321	Bosc	Hood River	*N. perennans*
HR 322	Bosc	Hood River	*N. perennans*
HR 323	Bosc	Hood River	*N.alba*
MRR 0201	Rogue Red	Medford-Hanley	*N. perennans*
MRR 0202	Rogue Red	Medford-Hanley	*N.alba*
MRR 0203	Rogue Red	Medford-Hanley	*N.alba*
MRR 0204	Rogue Red	Medford-Hanley	*N.alba*
MRR 0205	Rogue Red	Medford-Hanley	*N.alba*
MRR 0206	Rogue Red	Medford-Hanley	*N.alba*
MRR 0207	Rogue Red	Medford-Hanley	*N.alba*
MRR 0208	Rogue Red	Medford-Hanley	*N.alba*
MRR 0209	Rogue Red	Medford-Hanley	*N.alba*
MRR 0210	Rogue Red	Medford-Hanley	*N.alba*
MRR 0211	Rogue Red	Medford-Hanley	*N.alba*
MRR 0212	Rogue Red	Medford-Hanley	*N.alba*
MRR 0213	Rogue Red	Medford-Hanley	*N.alba*
MRR 0214	Rogue Red	Medford-Hanley	*N.alba*
MRC 0201	Red Comice	Medford-Hanley	*N.alba*
MRC 0202	Red Comice	Medford-Hanley	*N. perennans*
MRC 0203	Red Comice	Medford-Hanley	*N. perennans*
WN 0201	Winter Nellis	California Gowan	*N.alba*
WN 0202	Winter Nellis	California Gowan	*N.alba*
WN 0203	Winter Nellis	California Gowan	*N.alba*
WN 0204	Winter Nellis	California Gowan	*N.alba*
WW 1208	d'Anjou	WA, Wenatchee Orch.B	mix[4]
WW 1209	d'Anjou	WA, Wenatchee Orch.B	mix[4]
WW 1210	d'Anjou	WA, Wenatchee Orch.Z	*Cylindrocarpon*[3]
WW 1211	d'Anjou	WA, Wenatchee Orch.Z	mix
WW 1212	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1213	d'Anjou	WA, Wenatchee Orch.X	mix[4]
WW 1214	d'Anjou	WA, Wenatchee Orch.X	*N. perennans*
WW 1263	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1264	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1265	d'Anjou	WA, Wenatchee	*Cylindrocarpon*[3]
WW 1266	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1267	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1268	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1269	d'Anjou	WA, Wenatchee	mix[4]
WW 1270	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1271	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1272	d'Anjou	WA, Wenatchee	*N. perennans*

WW 1273	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1274	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1275	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1276	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1277	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1278	d'Anjou	WA, Wenatchee	*N. perennans*
WW 1279	d'Anjou	WA, Wenatchee	mix[4]
MD 0201	d'Anjou	Medford - Hanley	*N. perennans*
MD 0202	d'Anjou	Medford - Hanley	*N. perennans*
MD 0203	d'Anjou	Medford - Hanley	*N. perennans*
MQ 0201	Quince	Medford -Old Station	*N.alba*
MQ 0202	Quince	Medford -Old Station	*N.alba*
MQ 0203	Quince	Medford - Hanley	*N.alba*
MQ 0204	Quince	Medford - Hanley	*N.alba*
MS 0201	Seckel	Medford - Hanley	*Cylindrocarpon*[3]
MS 0202	Seckel	Medford - Hanley	*N.alba*
MS 0203	Seckel	Medford - Hanley	*Cylindrocarpon*[3]
MP 0201	Packam's T	Medford - Hanley	*N. perennans*
MP 0202	Packam's T	Medford - Hanley	*N.alba*
MRA 0201	Red d'Anjou	Medford - Hanley	*N.alba*
MRA 0202	Red d'Anjou	Medford - Hanley	*N.alba*
MRA 0203	Red d'Anjou	Medford - Hanley	*N.alba*
MRA 0204	Red d'Anjou	Medford - Hanley	*N.alba*
MC 0201	Comice	Medford - Hanley	*N.alba*
MC 0202	Comice	Medford - Hanley	*N. sp nova*
MC 0203	Comice	Medford - Hanley	*N.alba*
MC 0204	Comice	Medford - Hanley	*N.alba*
MC 0205	Comice	Medford - Hanley	*N.alba*
MC 0206	Comice	Medford - Hanley	*N.alba*
MC 0207	Comice	Medford - Hanley	*N.alba*
MC 0208	Comice	Medford - Hanley	*N.alba*
MC 0209	Comice	Medford - Hanley	*N.alba*
MC 0210	Comice	Medford - Hanley	*N.alba*
MC 0211	Comice	Medford - Hanley	*N. alba*
MC 0212	Comice	Medford - Hanley	*N. sp nova*
MC 0213	Comice	Medford - Hanley	*Cylindrocarpon*[3]
MC 0214	Comice	Medford - Hanley	*Cylindrocarpon*[3]
MC 0215	Comice	Medford - Hanley	*N. alba*
MC 0216	Comice	Medford - Hanley	*N. alba*
MC 0217	Comice	Medford - Hanley	nd[5]
MC 0218	Comice	Medford - Hanley	*N. alba*
MC 0219	Comice	Medford - Hanley	*N. alba*
MC 0220	Comice	Medford - Hanley	*N. alba*
MB 0201	Bosc	Medford - Meadow	*N. alba*
MB 0202	Bosc	Medford - Meadow	*N. alba*

MB 0203	Bosc	Medford - Meadow	*N. alba*
MB 0204	Bosc	Medford - Meadow	*N. alba*
MB 0205	Bosc	Medford - Meadow	*N. alba*
MB 0206	Bosc	Medford - Blackoak	nd[5]
MB 0207	Bosc	Medford - Blackoak	nd[5]
MB 0208	Bosc	Medford - Blackoak	*N. sp nova*
MB 0209	Bosc	Medford - Blackoak	nd[5]
MB 0210	Bosc	Medford - Blackoak	*N. sp nova*
MB 0211	Bosc	Medford - Blackoak	nd[5]
MB 0212	Bosc	Medford - Blackoak	*N. perennans*
MB 0213	Bosc	Medford - Blackoak	nd[5]
MB 0214	Bosc	Medford - Blackoak	nd[5]
MB 0215	Bosc	Medford - Blackoak	*N. alba*
MB 0216	Bosc	Medford - Blackoak	nd[5]
MB 0217	Bosc	Medford - Blackoak	*N. sp nova*
MB 0218	Bosc	Medford - Blackoak	*N. perennans*
MB 0219	Bosc	Medford - Blackoak	*N. alba*
MB 0220	Bosc	Medford - Blackoak	*N. alba*
MB 0221	Bosc	Medford - Blackoak	nd[5]
MB 0222	Bosc	Medford - Blackoak	*N. sp nova*
MB 0223	Bosc	Medford - Blackoak	nd[5]
MB 0224	Bosc	Medford - Blackoak	*N. alba*
MB 0225	Bosc	Medford - Blackoak	*N. alba*
MB 0226	Bosc	Medford - Blackoak	*N. alba*
MB 0227	Bosc	Medford - Blackoak	*N. alba*
MB 0228	Bosc	Medford - Blackoak	*N. alba*
MB 0229	Bosc	Medford - Blackoak	*N. alba*
MB 0230	Bosc	Medford - Blackoak	*N. alba*
MB 0231	Bosc	Medford - Blackoak	*N. alba*
MB 0232	Bosc	Medford - Bear Creek	*N. alba*
MB 0233	Bosc	Medford - Beeson	*Cylindrocarpon*[3]
MB 0234	Bosc	Medford - Allen	*N. alba*
MB 0235	Bosc	Medford - South Stage	*N. alba*
MB 0236	Bosc	Medford - Wightman	*Cylindrocarpon*[3]
MB 0237	Bosc	Medford – Wright/Smith	*Cylindrocarpon*[3]
MB 0238	Bosc	Medford - South Stage	*N. alba*
MB 0239	Bosc	Medford - South Stage	*N. alba*
MB 0240	Bosc	Medford - South Stage	*N. alba*
MB 0241	Bosc	Medford - Blackoak	*N. perennans*
MB 0242	Bosc	Medford - Meyer	*N. sp nova*
MB 0243	Bosc	Medford - Meyer	*N. alba*
MB 0244	Bosc	Medford - Meyer	mix[4]
MB 0245	Bosc	Medford - Meyer	mix[4]
MB 0246	Bosc	Medford - Meyer	*N. alba*
MB 0247	Bosc	Medford - Meyer	*N. alba*

MB 0248	Bosc	Medford - Meyer	*N. alba*
MB 0249	Bosc	Medford - Meyer	*N. alba*
MB 0250	Bosc	Medford - Meyer	*N. alba*
MB 0251	Bosc	Medford - Meyer	*N. alba*
MB 0252	Bosc	Medford - Meyer	*N. alba*
MB 0253	Bosc	Medford - Bybee	*N. alba*
MB 0254	Bosc	Medford - Bybee	*N. alba*
MB 0255	Bosc	Medford - Bybee	*N. alba*
MB 0256	Bosc	Medford - Bybee	*N. alba*
MB 0257	Bosc	Medford - Bybee	*N. alba*
MB 0258	Bosc	Medford - Bybee	*N. alba*
MB 0259	Bosc	Medford - Bybee	*N. alba*
MB 0260	Bosc	Medford - Bybee	*N. alba*
MB 0261	Bosc	Medford - Bybee	*N. alba*
MB 0262	Bosc	Medford - Bybee	*N. alba*
MB 0263	Bosc	Medford - Bybee	*N. alba*
MB 0264	Bosc	Medford - Bybee	*N. alba*
MB 0265	Bosc	Medford - Bybee	*N. alba*
MB 0266	Bosc	Medford - Bybee	*N. alba*
MB 0267	Bosc	Medford - Bybee	*N. alba*
MB 0268	Bosc	Medford - Bybee	nd[5]
MB 0269	Bosc	Medford - Bybee	*N. alba*
MB 0270	Bosc	Medford - Bybee	*N. alba*
MB 0271	Bosc	Medford - Bybee	*N. alba*
MB 0272	Bosc	Medford - Bybee	*N. alba*
MB 0273	Bosc	Medford - Bybee	*N. alba*
MB 0274	Bosc	Medford - Budge	*N. perennans*
MB 0275	Bosc	Medford - Budge	*N. alba*
MB 0276	Bosc	Medford - Budge	*N. sp nova*
MB 0277	Bosc	Medford - Budge	*N. alba*
MB 0278	Bosc	Medford - Budge	*N. perennans*
MB 0279	Bosc	Medford - Budge	*N. perennans*
MB 0280	Bosc	Medford - Budge	*N. sp nova*
MB 0281	Bosc	Medford - Budge	*N. alba*
MB 0282	Bosc	Medford - Budge	*N. alba*
MB 0283	Bosc	Medford - Budge	*N. alba*
MB 0284	Bosc	Medford - Holloway	*N. alba*
MB 0285	Bosc	Medford - Holloway	*N. alba*
MB 0286	Bosc	Medford - Holloway	*N. alba*
MB 0287	Bosc	Medford - Highland	*N. alba*
MB 0288	Bosc	Medford - Highland	*N. alba*
MB 0289	Bosc	Medford - Highland	*N. alba*
MB 0290	Bosc	Medford - Highland	*N. alba*
MB 0291	Bosc	Medford - Highland	*N. alba*
MB 0292	Bosc	Medford - Highland	*N. alba*

MB 0293	Bosc	Medford - Coker Butte	*N. alba*
MB 0295	Bosc	Medford - Coker Butte	*N. alba*
MB 0296	Bosc	Medford - Coker Butte	*N. alba*
MB 0297	Bosc	Medford - Coker Butte	*N. alba*
MB 0298	Bosc	Medford - Coker Butte	nd[5]
MB 0299	Bosc	Medford - Coker Butte	*N. alba*
MB 02100	Bosc	Medford - Coker Butte	*N. alba*
MB 02101	Bosc	Medford - Coker Butte	*N. alba*
MB 02102	Bosc	Medford - Coker Butte	*N. alba*
MB 02103	Bosc	Medford - Coker Butte	*N. alba*
MB 02104	Bosc	Medford - Coker Butte	*N. alba*
MB 02105	Bosc	Medford - Coker Butte	*N. alba*
MB 02106	Bosc	Medford - Coker Butte	*N. perennans*
MB 02107	Bosc	Medford - Coker Butte	*N. alba*
MB 02108	Bosc	Medford - Coker Butte	*N. alba*
MB 02109	Bosc	Medford - Coker Butte	*N. sp nova*
MB 02110	Bosc	Medford - Coker Butte	*N. alba*
MB 02111	Bosc	Medford - Coker Butte	*N. alba*
MB 02112	Bosc	Medford - Coker Butte	*N. alba*
MB 02113	Bosc	Grants Pass - Christie	*N. perennans*
MB 02114	Bosc	Grants Pass - Christie	*N. perennans*
MB 02115	Bosc	Grants Pass - Christie	*N. perennans*
MB 02116	Bosc	Grants Pass - Christie	*N. perennans*
MB 02117	Bosc	Grants Pass - Christie	*N. perennans*
MB 02118	Bosc	Grants Pass - Christie	*N. perennans*
MB 02119	Bosc	Grants Pass - Christie	*N. perennans*
MB 02120	Bosc	Grants Pass - Christie	*N. perennans*
MB 02121	Bosc	Grants Pass - Christie	*N. perennans*
MB 02122	Bosc	Grants Pass - Christie	*N. perennans*
MB 02123	Bosc	Grants Pass - Christie	*N. perennans*
MB 02124	Bosc	Grants Pass - Christie	*N. perennans*
MB 02125	Bosc	Grants Pass - Christie	*N. perennans*
MB 02126	Bosc	Grants Pass - Christie	*N. perennans*
MB 02127	Bosc	Grants Pass - Christie	*N. sp nova*
MB 02128	Bosc	Grants Pass - Christie	*N. perennans*
MB 02129	Bosc	Medford - Hull	*N. alba*
MB 02130	Bosc	Medford - Hull	*N. alba*
MB 02131	Bosc	Medford - Hull	*N. alba*
MB 02132	Bosc	Medford - Hull	*N. perennans*
MB 02133	Bosc	Medford - Hull	*N. alba*
MB 02134	Bosc	Medford - Hull	*N. alba*
MB 02135	Bosc	Medford - Hull	*N. alba*
MB 02136	Bosc	Medford - Hull	*Cylindrocarpon*[3]
MB 02137	Bosc	Medford - Hull	nd[5]
MB 02138	Bosc	Medford - Hull	*N. perennans*

MB 02139	Bosc	Medford - Hull	*N. alba*
MB 02140	Bosc	Medford - Hull	*N. perennans*
MB 02141	Bosc	Medford - Hull	*N. sp nova*
MB 02142	Bosc	Medford - Hull	*N. perennans*
MB 02143	Bosc	Medford - Hull	*N. perennans*
MB 02144	Bosc	Medford - Hull	*N. alba*
MB 02145	Bosc	Medford - Hull	*N. alba*
MB 02146	Bosc	Medford - Klamath	*N. alba*
MB 02147	Bosc	Medford - Klamath	nd[5]
MB 02148	Bosc	Medford - Klamath	*N. alba*
MB 02149	Bosc	Medford - Klamath	nd[5]
MB 02150	Bosc	Medford - Klamath	*N. alba*
MB 02151	Bosc	Medford - Klamath	*N. alba*
MB 02152	Bosc	Medford - Klamath	nd[5]
MB 02153	Bosc	Medford - Klamath	*N. alba*
MB 02154	Bosc	Medford - Klamath	*N. sp nova*
MB 02155	Bosc	Medford - Klamath	nd[5]
MB 02156	Bosc	Medford - Klamath	*N. alba*
MB 02157	Bosc	Medford - Klamath	*N. alba*
MB 02158	Bosc	Medford - Klamath	*N. alba*
MB 02159	Bosc	Medford - Newbry	*N. alba*
MB 02160	Bosc	Medford - Klamath	*N. sp nova*
MB 02161	Bosc	Medford - Klamath	*N. sp nova*
MB 02162	Bosc	Medford - Klamath	*N. alba*
MB 02163	Bosc	Medford - Klamath	*N. alba*
MB 02164	Bosc	Medford - Klamath	*N. alba*
MB 02165	Bosc	Medford - Klamath	*N. alba*
MB 02166	Bosc	Medford - Fairlane	*N. alba*
MB 02167	Bosc	Medford - Fairlane	*N. alba*
MB 02168	Bosc	Medford - Fairlane	*N. alba*
MB 02169	Bosc	Medford - Fairlane	*N. alba*
MB 02170	Bosc	Medford - Fairlane	*N. alba*
MB 02171	Bosc	Medford - Fairlane	*N. alba*
MB 02172	Bosc	Medford - Fairlane	*N. alba*
MB 02173	Bosc	Medford - Fairlane	*N. alba*
MB 02174	Bosc	Medford - Fairlane	*N. alba*
MB 02175	Bosc	Medford - Fairlane	*N. alba*
MB 02176	Bosc	Medford - Fairlane	*N. alba*
MB 02177	Bosc	Medford - Fairlane	*N. alba*
MB 02178	Bosc	Medford - Fairlane	*N. alba*
MB 02179	Bosc	Medford - Fairlane	*N. alba*
MB 02180	Bosc	Medford - Fairlane	*N. alba*
MB 02181	Bosc	Medford - Fairlane	*N. alba*
MB 02182	Bosc	Medford - Fairlane	*N. sp nova*
MB 02183	Bosc	Medford - Fairlane	*N. alba*

MB 02184	Bosc	Medford - Hanley NC-140	*N. alba*
MB 02185	Bosc	Medford - Hanley NC-140	*N. alba*
MB 02186	Bosc	Medford - Hanley NC-140	*N. alba*
MB 02187	Bosc	Medford - Hanley NC-140	*N. alba*
MB 02188	Bosc	Medford - Hanley StonyPit	mix[4]
MB 02189	Bosc	Medford - Old Station	*N. sp nova*
MB 02190	Bosc	Medford - Old Station	*N. perennans*
MB 02191	Bosc	Medford - Old Station	*N. perennans*
MB 02192	Bosc	Medford - Old Station	*N. perennans*
MB 02193	Bosc	Medford - Old Station	*N. perennans*
MB 02194	Bosc	Medford - Old Station	*N. perennans*
MB 02195	Bosc	Medford - Old Station	*N. perennans*
MB 02196	Bosc	Medford - Old Station	*N. perennans*
MB 02197	Bosc	Medford - RoxyAnn	*N. alba*
MB 02198	Bosc	Medford - RoxyAnn	*N. alba*
MB 02199	Bosc	Medford - RoxyAnn	*N. alba*
MB 02200	Bosc	Medford - RoxyAnn	*N. alba*
MB 02201	Bosc	Medford - RoxyAnn	*N. alba*
MB 02202	Bosc	Medford - RoxyAnn	*N. alba*
MB 02203	Bosc	Medford - RoxyAnn	*N. alba*
MB 02204	Bosc	Medford - RoxyAnn	*N. alba*
MB 02205	Bosc	Medford - RoxyAnn	*N. alba*
MB 02206	Bosc	Medford - RoxyAnn	*N. alba*
MB 02207	Bosc	Medford - RoxyAnn	nd[5]
MB 02208	Bosc	Medford - RoxyAnn	*N. alba*
MB 02209	Bosc	Medford - RoxyAnn	*N. alba*
MB 02210	Bosc	Medford - RoxyAnn	*N. alba*
MB 02211	Bosc	Medford - RoxyAnn	*N. alba*
MB 02212	Bosc	Medford - RoxyAnn	*N. alba*
MB 02213	Bosc	Medford - RoxyAnn	*N. alba*
MB 02214	Bosc	Medford - RoxyAnn	*N. alba*
MB 02215	Bosc	Medford - RoxyAnn	nd[5]
MB 02216	Bosc	Medford - RoxyAnn	*N. alba*
MB 02217	Bosc	Medford - RoxyAnn	*N. alba*
MB 02218	Bosc	Medford - RoxyAnn	*N. alba*
MB 02219	Bosc	Medford - RoxyAnn	*N. alba*
MB 02220	Bosc	Medford - RoxyAnn	*N. alba*
MB 02221	Bosc	Medford - RoxyAnn	*N. alba*
MB 02222	Bosc	Medford - RoxyAnn	*N. alba*
MB 02223	Bosc	Medford - Hanley	*N. alba*
MB 02224	Bosc	Medford - Hanley	*N. alba*
MB 02225	Bosc	Medford - Hanley	*N. alba*
MB 02226	Bosc	Medford - Hanley	*N. perennans*
MB 02227	Bosc	Medford - Hanley	*N. alba*
MB 02228	Bosc	Medford - Hanley	*N. alba*

MB 02229	Bosc	Medford - Hanley	*N. alba*
MB 02230	Bosc	Medford - Hanley	*N. perennans*
MB 02231	Bosc	Medford - Hanley	*N. alba*
MB 02232	Bosc	Medford - Hanley	*N. alba*
MB 02233	Bosc	Medford - Hanley	*N. alba*
MB 02234	Bosc	Medford - Hanley	*N. alba*
MB 02235	Bosc	Medford - Hanley	*N. alba*
MB 02236	Bosc	Medford - Hanley	*N. alba*
MB 02237	Bosc	Medford - Hanley	*N. alba*
MB 02238	Bosc	Medford - Hanley	*N. alba*
MB 02239	Bosc	Medford - Hanley	mix[4]
MB 02240	Bosc	Medford - Hanley	mix[4]
MB 02241	Bosc	Medford - Hanley	*N. alba*
MB 02242	Bosc	Medford - Hanley	*N. perennans*
MB 02243	Bosc	Medford - Hanley	*N. alba*
MB 02244	Bosc	Medford - Hanley	*N. alba*
MB 02245	Bosc	Medford - Hanley	*N. alba*
MB 02246	Bosc	Medford - Hanley	*N. alba*
MB 02247	Bosc	Medford - Hanley	*N. alba*
MB 02248	Bosc	Medford - Hanley	*N. alba*
MB 02249	Bosc	Medford - Hanley	*N. alba*
MB 02250	Bosc	Medford - Hanley	*N. alba*
MB 02251	Bosc	Medford - Hanley	*N. alba*
MB 02252	Bosc	Medford - Hanley	nd[5]
MB 02253	Bosc	Medford - Hanley	*N. perennans*
MB 02254	Bosc	Medford - Hanley	*N. perennans*
MB 02255	Bosc	Medford - Hanley	*N. perennans*
MB 02256	Bosc	Medford - Hanley	*N. alba*
MB 02257	Bosc	Medford - Hanley	*N. perennans*
MB 02258	Bosc	Medford - Hanley	*N. alba*
MB 0301	Bosc	Medford - Hanley	*N. alba*
MB 0302	Bosc	Medford - Hanley Spray Bl	mix [4]
MB 0303	Bosc	Medford - Hanley Spray Bl	mix [4]
MB 0304	Bosc	Medford - Hanley Spray Bl	mix [4]
MB 0305	Bosc	Medford - Hanley	*N. alba*
MB 0306	Bosc	Medford - Hanley	*N. alba*
MB 0307	Bosc	Medford - Hanley	*N. perennans*
MB 0308	Bosc	Medford - Hanley	*N. alba*
MB 0309	Bosc	Medford - Old Station	*N. alba*
MB 0310	Bosc	Medford - Hanley	*N. sp nova*
MB 0311	Bosc	Medford - Hanley	mix[4]
MB 0312	Bosc	Medford - Hanley	mix[4]
WPMO01		Wenatchee, WA	mix[4]
WPMO03		Wenatchee, WA	mix[4]
WPMO04		Wenatchee, WA	mix[4]

WPMO05		Wenatchee, WA	nd[5]
WPMO06		Wenatchee, WA	mix[4]
WPMO07		Wenatchee, WA	*Cylindrocarpon*[3]
WPMO011		Wenatchee, WA	*N. perennans*
WPMO013		Wenatchee, WA	*Cylindrocarpon*[3]
WPMO016		Wenatchee, WA	*Cylindrocarpon*[3]
WPMO016b		Wenatchee, WA	nd[5]
MM-0114[2]	Apple	Medford - Hanley	*N.alba*
MM 0201	Apple	Medford - Hanley	*N.alba*
MM 0202	Apple	Medford - Hanley	*N.alba*
MM 0203	Apple	Medford - Hanley	*N.alba*
MM 0204	Apple	Medford - Hanley	*N.alba*
MM 0205	Apple	Medford - Hanley	*N.alba*
MM 0206	Apple	Medford - Hanley	*N.alba*
MM 0207	Apple	Medford - Hanley	*N.alba*
MM 0208	Apple	Medford - Hanley	*N. sp nova*
MM 0209	Apple	Medford - Hanley	*N.alba*
CA-0101[2]	Apple-canker	Corvallis - OR	*N.malicorticis*

[1] Pear cultivars; Bosc, Comice, d'Anjou, Red d'Anjou, Packam's Triumph, Red Comice, Rogue Red, Seckel, Winter Nelis.
[2] Identification performed by Gariepy (2002).
[3] *Cylindrocarpon magnusianum* Wollenweber
[4] Mixed reaction; isolates that reacted to primers of *N. alba* and *N. perennans* simultaneously.
[5] No identification was obtained.