Taxonomy / Taxonomie

Neofabraea species associated with bull's-eye rot and cankers of apple and pear in the Pacific Northwest

T.D. Gariepy, J.E. Rahe, C.A. Lévesque, R.A. Spotts, D.L. Sugar, and J.L. Henriquez

Abstract: Species-specific primers for four species of *Neofabraea* associated with apple and pear fruit diseases were used in multiplex PCR assays to identify 29 putative *Neofabraea* isolates, primarily isolated from bull's-eye rot on pears from packing houses in Washington and Oregon. Apples were inoculated with these isolates, and tentative identifications based on morphology of conidia forming on wound-inoculated fruits were compared with identifications made using species-specific primers for different *Neofabraea* species. PCR-based identifications were successful for 26 of 29 isolates, and in all cases were consistent with tentative identifications based on spore morphologies. The results revealed that in addition to the two species of *Neofabraea* already known to occur in the Pacific Northwest, *N. malicorticis* and *N. perennans*, a third species, *N. alba*, is also prevalent.

Key words: Neofabraea, bull's eye rot, molecular diagnostics, multiplex PCR, fungi.

Résumé : Des amorces spécifiques à l'espèce pour quatre espèces de *Neofabraea* associées à des maladies de la pomme et de la poire furent utilisées dans des tests de PCR multiplex afin d'identifier 29 isolats présumés de *Neofabraea* originalement isolés de lésions d'anthracnose sur des poires provenant de conserveries des états de Washington et d'Oregon. Ces isolats furent inoculés à des pommes et des identifications préliminaires, basées sur la morphologie des conidies se formant sur des fruits inoculés après blessure, furent comparées à celles obtenues en utilisant les amorces spécifiques à l'espèce pour les diverses espèces de *Neofabraea*. Des identifications basées sur la PCR ont été obtenues pour 26 des 29 isolats et, dans tous les cas, correspondirent aux identifications préliminaires basées sur la morphologie des spores. Les résultats ont montré qu'une troisième espèce, le *N. alba*, est également fréquente dans la région de la côte nord-ouest du Pacifique en plus des deux espèces de *Neofabraea,* les *N. malicorticis* et *N. perennans*, déjà connues pour y être présentes.

Mots clés : Neofabraea, anthracnose, diagnostic moléculaire, PCR multiplex, champignons microscopiques.

Introduction

Several species of the genus *Neofabraea* can cause bark cankers and post harvest bull's-eye rot of pome fruits (Kienholz 1939; Lockhart & Ross 1961; Farr et al. 1989; Jones and Aldwinkle 1990; de Jong et al. 2001). Bull's-eye rot and bark cankers on pome fruit species in the Pacific Northwest historically have been attributed to *Neofabraea perennans* Kienholz and *Neofabraea malicorticis* Jacks (Kienholz 1939; de Jong 1998). While some geographic overlap of the two species occurs, perennial canker and bull's-eye rot occurring east of the Cascade Mountains have generally been attributed to *N. perennans*, whereas an-

Accepted 16 February 2005.

T.D. Gariepy[1,2] and J.E. Rahe. Center for Environmental Biology, Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
C.A. Lévesque. Eastern Cereal and Oilseed Research Centre, Research Branch, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada.
R.A. Spotts. Oregon State University, Mid-Columbia Research and Extension Center, 3005 Experiment Station Drive, Hood River, OR 97031, USA.
D.L. Sugar and J.L. Henriquez. Oregon State University, Southern Oregon Research and Extension Center, 569 Hanley Road, Medford, OR 97502, USA.

[1]Corresponding author (e-mail: gariepyt@agr.gc.ca).
[2]Present address: Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK S7N 0X2, Canada.

thracnose canker and bull's-eye rot occurring west of the Cascades have been attributed to *N. malicorticis* (Kienholz 1939; Kienholz 1956). Cankers caused by *N. malicorticis* become delimited in size in the year of appearance, whereas perennial cankers can enlarge annually for several years. Cankers are believed to be the source of inoculum for bull's-eye rot of fruits in storage. Both asexual and sexual spores released from cankers contact the fruit surface, infecting the fruit via wounds and lenticels (Kennel 1986; Leibinger et al. 1997). Fruit rots caused by the two species are indistinguishable. According to Dugan (1993), bull's-eye rot does not spread from fruit to fruit after it develops in storage.

Most apple- and pear-growing regions in the Pacific Northwest experience problems with bull's-eye rot of apples and pears. A study of the incidence of post-harvest pathogens in packing houses in British Columbia revealed that bull's-eye rot accounted for 40% of diseased Golden Delicious apples and 9% of diseased McIntosh apples (Sholberg and Haag 1996). In the United States (US), pear-growing regions of Washington and Oregon generally experience less than 5% annual crop loss resulting from bull's eye rot; however, in some years over 50% of the Bosc pear crop is lost because of this post-harvest disease (Lennox et al. 2004).

Washington, Oregon, and California produce more than 97% of the US pear crop, with approximately 44%, 23%, and 30% of the US production from each of these states, respectively, (USDA National Agricultural Statistics Service 2003). The main pear-growing regions in these states are Sacramento, Mendocino, and Lake counties in California, Hood River and Medford, Oregon, and Wenatchee, Washington; 78% of the winter pears grown in the Northwest are from the Hood River and Wenatchee areas (Seavert 2002). Bull's-eye rot on pears occurs sporadically in these centers of pear production, primarily on winter pears or pears that are stored for 3 months or more, and reaches substantial levels in some years despite the fact that the bark cankers that are the putative source of inoculum for bull's eye rot seem to be absent or infrequent on pear trees in these areas.

Morphologically, macroconidia of *N. malicorticis* are curved or comma shaped, whereas those of *N. perennans* are typically straight or slightly curved (Kienholz 1939; Dugan 1992; Dugan et al. 1993). Despite the distinctive macrocondium morphology that distinguishes these two species, variation among isolates within a species is nevertheless considerable. In areas where the two species occur together, isolates with conidia of intermediate size and morphology between the two species are occasionally found (Kienholz 1939). *Neofabraea malicorticis* and *N. perennans* have been considered by some authorities to be a single species (von Arx 1970; Boerema and Gremmen 1959; Sutton 1980). However, this debate has recently been resolved by molecular evidence that clearly demonstrates that isolates of *N. perennans* and *N. malicorticis* are genetically distinct, although closely related (de Jong et al. 2001).

Two other fungi belonging to the genus *Neofabraea* are also capable of causing bull's-eye rot symptoms: *N. alba* Guthrie (Guthrie 1959) and *Neofabraea* sp.nov., a putative new species determined by de Jong et al. 2001. The presence of *N. alba* has been documented in Eastern North America and Europe, and *Neofabraea* sp.nov., sensu de Jong et al. 2001, has only been isolated from Nova Scotia and Portugal (de Jong et al. 2001).

Morphological variation among members of the genus *Neofabraea* is slight, and identification using conventional morphological criteria is challenging. Similar life history patterns coupled with an overlap in geographical range further complicate distinction between these pathogens. Furthermore, there has been a lack of agreement over the years regarding the genus to which these fungi belong. This taxonomic debate fueled a recent revision of members of *Pezicula*, *Dermea*, and *Neofabraea* (Verkley 1999; Abeln et al. 2000) and the phylogenetic analysis of members of *Neofabraea* (de Jong et al. 2001). The molecular data generated from the latter study was used by Gariépy et al. (2003) to design a multiplex PCR assay for *Neofabraea* species. The present study was undertaken to test the utility of the multiplex assay in identifying unknown *Neofabraea* isolates obtained from pear-growing regions in Oregon and Washington. Conidial morphology was used concurrently to characterize the isolates and to compare identifications based on conventional morphological criteria with those obtained using PCR-based techniques.

Materials and methods

Culture acquisition and maintenance

Twenty-nine putative *Neofabraea* isolates were obtained for identification in 2001. Twenty-eight originated from pear and apple fruits, primarily the Bosc pear variety, sampled from fruits that were in cold storage for at least 3 months in commercial packing houses in Oregon and Washington, and one was obtained from an apple bark canker collected near Corvallis, Oregon (Table 1). The fungi were isolated from fruits displaying bull's-eye rot symptoms following surface sterilization for 30 s with 0.5% sodium hypochlorite and rinsing with sterile distilled water. Small sections from the edges of the rot were plated on acidified potato-dextrose agar.

Cultures were maintained on 10% V8 agar and subcultured every 4–6 weeks. Subcultures destined for DNA extraction were made by aseptically transferring mycelial fragments from agar culture into 20% V8 broth (Lévesque et al. 1993). The broth cultures were maintained at room temperature for approximately 4 weeks prior to DNA extraction.

Morphology of acervuli and macroconidia

To examine morphological features of the acervuli and macroconidia, the isolates in question were inoculated into apple fruits. This was done by inserting inoculum consisting of pieces of mycelium and agar (nominal 10-mm^3 volumes) beneath three V-shaped flaps cut into the surfaces of Jonagold apples (Senula and Ficke 1985). Inoculum was taken from the margins of 14-day-old V-8 agar subcultures of the isolates. Wound-inoculated apples were placed on moist seed germination paper in trays, loosely covered with plastic food wrap, and kept at 13 °C. Moisture was adjusted periodically as required to maintain high relative humidity without visible free water. Information regarding symptoms, fructifications, sporulation, and spore morphology were recorded 6 weeks after inoculation. All inoculum-induced rots

Table 1. Unidentified *Neofabraea* isolates from Washington and Oregon, including information on the location and host from which each was obtained.

Identification No.	Location	Host	Variety	Host tissue
CA0101	Corvallis, Oregon	Apple	Unknown	Branch
CW0101	Cashmere, Washington	Pear	Anjou	Fruit
HR236	Oregon (Hood River)	Unknown	Unknown	Fruit
HR238	Oregon (Hood River)	Pear	Bosc	Fruit
HR240	Odell, Oregon (Hood River)	Pear	Anjou	Fruit
HR241	Odell, Oregon (Hood River)	Unknown	Unknown	Fruit
HR242	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
HR243	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
HR244	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
HR245	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
HR246	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
HR247	Bingen, Washington (Hood River)	Pear	Bosc	Fruit
HR248	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
HR249	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
HR250	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
HR251	Odell, Oregon (Hood River	Pear	Bosc	Fruit
HR252	Odell, Oregon (Hood River)	Unknown	Unknown	Fruit
HR253	Odell, Oregon (Hood River)	Pear	Bosc	Fruit
MA0001	Medford, Oregon	Pear	Asian	Fruit
MB0102	Medford, Oregon	Pear	Bosc	Fruit
MB0103	Medford, Oregon	Pear	Bosc	Fruit
MB0120	Medford, Oregon	Pear	Bosc	Fruit
MB0128	Medford, Oregon	Pear	Bosc	Fruit
MB0140	Medford, Oregon	Pear	Bosc	Fruit
MB0144	Medford, Oregon	Pear	Bosc	Fruit
MB9901	Medford, Oregon	Pear	Bosc	Fruit
MC0106	Medford, Oregon	Pear	Comice	Fruit
MM0113	Medford, Oregon	Apple	Unknown	Fruit
MM0114	Medford, Oregon	Apple	Unknown	Fruit

produced were examined at magnifications ranging from 20× to 40× for evidence of sporulation, and representative fresh mounts of spores from each rot were examined at 400×.

Molecular diagnostics of *Neofabraea* spp.

DNA was extracted from mycelium using a FastDNA kit (Q-Biogene/Bio101, Vista, California) following the manufacturer's instructions for DNA extraction from fungi. Multiplex PCR reactions were performed in a PE GeneAmp 2400 PCR machine (Perkin Elmer, Foster City, California) using the protocol established for primer sets for *N. malicorticis*, *N. perennans*, *N. alba*, *Neofabraea populi* Thompson, and *Neofabraea* sp.nov., sensu de Jong et al. (2001) (Gariepy et al. 2003), briefly described as follows: 25-µL reaction mixtures contained 0.2 mmol/L dNTP, 0.1 µmol/L of the universal fungal primers UN-UP18S-42 and UN-LO28S-22 (Bakkeren et al. 2000), 0.4 µmol/L of Neofab-upTub-100 (5′-TGATGAGACCTTCTGTATGC-3′), and 0.1 µmol/L each of the species-specific lower primers Neo_mal-loTub-262 (5′-GACAGCCAACTTGCGG-3′), Neo_per-loTub-382 (5′-GGG-TCGAACATCTGTTGT-3′), Neo_alba-loTub-439 (5′-AGC-AGAGCAGGTCAAGTAA-3′), Neo_pop-loTub (5′-AAG-AGTAGCACCATAAGAAAGT-3′), and Neo_spnov-loTub-319 (5′-TGGTGAGAGGAGCGAAC-3′), and 0.5 µL of 50× Titanium *Taq* DNA polymerase (CLONTECH, Palo Alto, California) in a 10× Titanium *Taq* PCR buffer (CLONTECH). Approximately 1–10 ng of total DNA template was used in these reactions. The PCR conditions used a "touch-down" ramping of the annealing temperature from 72–66 °C, as described in Gariepy et al. (2003).

In addition to using the multiplex system, each of the unknown *Neofabraea* isolates was amplified using individual species-specific primer sets using the same PCR conditions to confirm the results obtained using the multiplex approach. However, 0.4 µmol/L of the appropriate species-specific lower primer was used in combination with Neofab-upTub-100 instead of the 0.1 µmol/L used in the multiplex system. The β-tubulin gene was partially sequenced for selected isolates following the protocol of de Jong et al. (2001).

Results

Morphology of acervuli and macroconidia

Rot symptoms began to appear around sites of wound inoculation within 2–3 weeks, and 25 of the 29 isolates caused rot at sites of wound inoculation. Blue mold rot, caused by *Penicillium* spp., that consumed entire fruits precluded obtaining data for three of the putative *Neofabraea* isolates (HR244, MB0103, MM0114), and one of the isolates (HR241) was inadvertently not inoculated into apple fruits. Rots caused by the isolates were firm, roughly circular in shape, and tan to brown in color. Concentric zonation

Table 2. Identification of *Neofabraea* isolates from Oregon and Washington based on multiplex PCR and pathogenicity testing, including details pertaining to spore morphology.

	Species identification		
Isolate code	PCR-based	Spore type[a]	Spore dimensions (μm)
HR236	*N. alba*	None found	No data
HR241	*N. alba*	No data	No data
HR242	*N. alba*	*N. alba*	17–20 × 3–4
HR244	*N. alba*	No data	No data
HR245	*N. alba*[b]	None found	No data
HR248	*N. alba*	*N. alba*	18–25 × 3–4
HR249	*N. alba*	*N. alba*	17.5–21 × 2.5–3
HR251	Did not amplify[c]	*N. alba*[c]	17–21 × 4.5–5.8
MB0102	*N. alba*	None found	No data
MB0103	*N. alba*	No data	No data
MB0120	Did not amplify[c]	*N. alba*	20–25 × 3–4
MB0128	*N. alba*	None found	No data
MB0140	*N. alba*	*N. alba*[c,d]	15–19 × 4.4–5
MB9901	*N. alba*	None found	No data
MBO144	*N. alba*	*N. alba*[c]	23–28 × 3–5
MC0106	*N. alba*	*N. alba*[c,d]	15–19 × 4.4–5
MM0113	*N. alba*	None found	No data
MM0114	*N. alba*	No data	No data
CW0101	*N. perennans*	*N. perennans*	15–19 × 6–6.5
HR238	*N. perennans*	*N. perennans*	14–25 × 5–6
HR240	*N. perennans*	*N. perennans*	14–21 × 5–6
HR243	*N. perennans*	*N. perennans*	14–25 × 5–6
HR246	*N. perennans*	*N. perennans*	14–25 × 5–6
HR247	*N. perennans*	*N. perennans*[c]	14–21 × 5–6
HR250	*N. perennans*	*N. perennans*	14–17 × 4–5
HR253	*N. perennans*	*N. perennans*	14–21 × 5–6
MA0001	*N. perennans*	None found	No data
CA0101	*N. malicorticis*	*N. malicorticis*[c]	15–18 × 5.5–6.8
HR252	Did not amplify[e]	*N. malicorticis*[e]	19–22 × 5

[a]Species identification made prior to obtaining molecular results.
[b]Faint band produced in multiplex PCR.
[c]Identification of this isolate performed by partial sequencing of β-tubulin gene to confirm PCR and (or) spore type results.
[d]Possibly *N. malicorticis* based on spore morphology.
[e]Sequencing of DNA identified this isolate as *Pestalotia* or *Pestalotiopsis* sp.

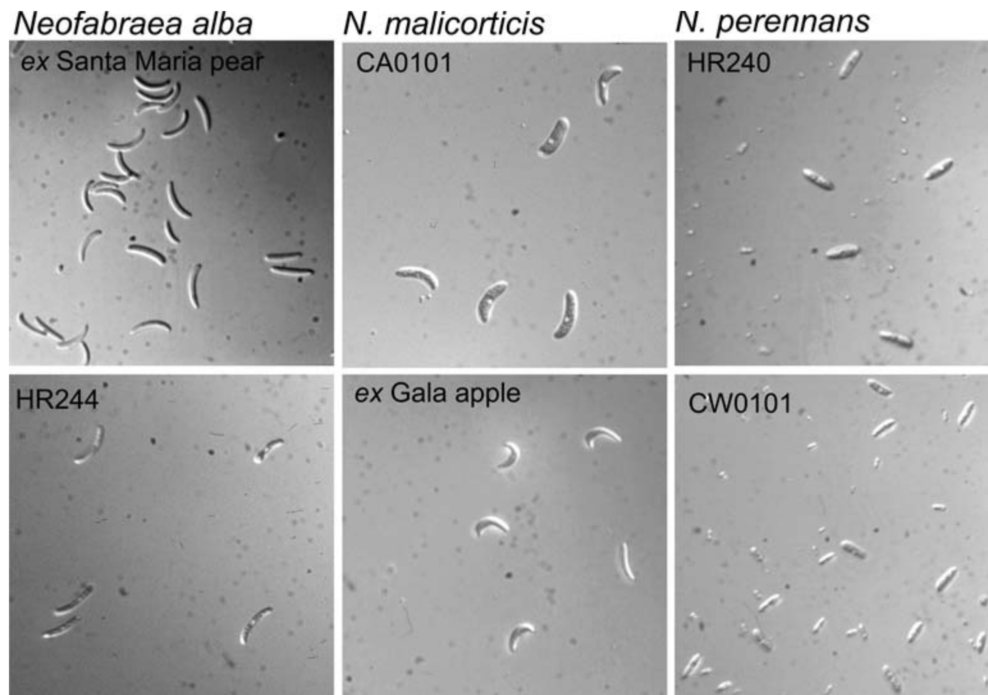
was a prominent feature of rots caused by some of the isolates and was lacking in the case of other isolates.

At 6 weeks after inoculation, acervulus-like fructifications had developed on the surfaces of rots produced by 22 of the 25 isolates (Table 2). Acervuli produced by most of the isolates were circular in shape and either covered or erupted, but those produced by HR245, MB0113, MB0120, MB0140, and MC0106 appeared as undulating ridges. Eruptive mycelial tufts grew from acervuli produced by some of the isolates (HR242, MB0120). Macroconidia were obtained from acervuli of 18 of 25 isolates and produced macroconidia characteristic of either *N. perennans*, *N. malicorticis*, or *N. alba*. Macroconidia with morphology characteristic of *N. perennans* were produced by eight of the isolates. Perennans-type macroconidia were hyaline, aseptate, 14–25 μm × 4–6 μm in size and generally lacked curvature. Some of the macroconidia produced by HR247 had malicorticis-type curvature. Macroconidia with morphology characteristic of *N. malicorticis* were produced by two of the isolates, HR252 and CA0101. Malicorticis-type macroconidia were hyaline, aseptate, typically 15–22 μm × 4–7 μm in size and distinctly curved or comma shaped. *Neofabraea alba* also produces hyaline, aseptate, curved macroconidia. Macroconidia produced by isolates of *N. alba* were generally longer and narrower (typically 17–25 μm × 3–5 μm) than those produced by *N. malicorticis*, but the distinction was subtle and sometimes ambiguous. Figure 1 shows macroconidia of two isolates of each of the three species of *Neofabraea* that are representative examples of the inter- and intra-specific variation. The species assignments based on morphology of macroconidia and shown in Table 2 were made prior to obtaining identifications using species-specific primers. In all instances the morphology-based assignments were consistent with the PCR identifications.

PCR-based identification of *Neofabraea* isolates

The multiplex protocol amplified the majority of the 29 isolates, confirming the identifications based on morphology. Two of the 29 isolates (MB0120 and HR252) failed to

Fig. 1. Appearance of macroconidia of isolates of *Neofabraea alba, Neofabraea malicorticis*, and *Neofabraea perennans* in fresh mounts. Actual sizes are listed in Table 2.



amplify using any of the species-specific primer sets or combinations thereof. One isolate, HR251 failed to amplify using the multiplex protocol but yielded a very faint band upon amplification with the species-specific *N. alba* primers. These three isolates did amplify using the universal fungal primers as internal positive controls (data not shown). The results obtained for the remaining 26 isolates proved consistent using both amplification protocols (primer sets used individually and in multiplex). The identities of these 26 isolates include 16 *N. alba*, 1 *N. malicorticis*, and 9 *N. perennans* (Table 2). The isolates obtained from Hood River, Oregon, consisted of seven *N. alba*, seven *N. perennans*, and two unknowns. Of the 11 isolates from Medford, Oregon, 10 were confirmed as *N. alba* and one as *N. perennans*. Cashmere, Washington, and Corvallis, Oregon, were each represented by one isolate; these were confirmed as *N. perennans* and *N. malicorticis*, respectively. Sequencing the β-tubulin gene of the three isolates that did not amplify with the *Neofabraea* primers in multiplex identified them as *N. alba* (HR251 and MB0120) and either *Pestalotia* sp. or *Pestalotiopsis* sp. (HR252) based on comparison of gene sequences published in GenBank.

Discussion

The diagnostic utility of the molecular techniques described for *Neofabraea* species (Gariepy et al. 2003) is exemplified by the results obtained in the current study on the identification of 29 unknown *Neofabraea* isolates obtained from bull's eye rot fruits from Washington and Oregon packing houses. Although 17 of the 18 identifications that were made using conventional criteria were correct, a low level of confidence would have been associated with at least two of these, MB0140 and MC0106, if spore morphology had been the only basis for identification. Identifications using spore morphology required at least 6 weeks of incubation of wound inoculated fruit, during which spore production occurred for only 18 of the 25 isolates. As such, identification of only 60% of the isolates was possible using conventional criteria; conversely, the identity of 90% of the isolates was achieved using the multiplex PCR assay. One of the identifications (HR252) produced curved macroconidia when wound-inoculated into apple fruits, and was thus identified as *N. malicorticis.* DNA of this isolate was positive with universal primers but did not amplify with the species-specific primers. Subsequently, the HR252 DNA used in the multiplex assays was partially sequenced for the β-tubulin gene, and comparison with published sequences in Genbank suggested it was *Pestalotia* or *Pestalotiopsis* sp. It is possible that isolate HR252 was received as a mixed culture containing both *N. malicorticis* and *Pestalotia* or *Pestalotiopsis* and that liquid medium growth was not conducive to growth of *N. malicorticis* in the presence of the other species.

The identification of these 29 isolates provides new insight concerning the species of *Neofabraea* present in the Pacific Northwest, particularly in Oregon, where the majority of isolates originated. Historically bull's-eye rot in this area has been attributed solely to *N. malicorticis* and *N. perennans*. This research demonstrates that a third species, *N. alba*, is also prevalent. While *N. alba* is known to occur in eastern North America and Europe, it has not been reported previously in the Pacific Northwest as far as we are aware. Nevertheless, it appears that *N. alba* may be both common and widespread in Oregon, and likely in other regions of the Pacific Northwest. Subsequent to confirming the prevalence of *N. alba* in the 28 isolates from bull's eye rot in Oregon and one from bull's-eye rot in Cashmere,

Washington, one of the authors (J.E. Rahe) has found it on bull's-eye rot on apple fruit from Whatcom County, Washington, pear fruit from Langley, British Columbia, Canada, and bark canker on Santa Maria pear from Aldergrove, British Columbia, Canada. It is possible that *N. alba* is a long-standing component of the *Neofabraea* complex affecting pome fruits that had been overlooked in the Pacific Northwest and southwestern coastal British Columbia because of the morphological similarity of its macroconidia to those of *N. malicorticis*, and the overlap in morphologies of macroconidia of *N. malicorticis* and *N. perennans*.

Pear-growing regions in Oregon and Washington commonly have post-harvest bull's-eye rot problems occurring on fruit harvested from orchards that have no evidence of the tree cankers generally associated with *N. malicorticis* and *N. perennans* (authors' personal observations; Kienholz 1951). The presence of bull's-eye rot problems in areas not displaying canker symptoms on pome fruit trees could be characteristic of *N. alba*, although further research is needed to support this. A thorough collection of isolates from infected fruits from packing houses in these regions, along with details pertaining to the orchards from which they came, would provide further insight into this question and into the occurrence and degree of geographic overlap of these three species of *Neofabraea* in the Pacific Northwest.

In situations where cankers are not present in the field, but bull's-eye rot remains an issue, the source of inoculum of *N. alba* needs to be identified. In addition, the recent detection of species previously not known to occur in Nova Scotia (de Jong et al. 2001) and the Pacific Northwest suggests that the identity of causal agents in other regions with similar bull's eye rot problems attributed to *N. malicorticis* or *N. perennans* may merit confirmation. For example, in New Zealand a post-harvest rot referred to as ripe-spot of apples (synonym of bull's eye rot) has been attributed to *N. malicorticis*; however, cankers were not found in these regions (Cunningham 1942). Future research in areas experiencing bull's eye rot symptoms without visible evidence of cankers should benefit from the molecular techniques implemented in this research to confirm the identity of the pathogens responsible for bull's eye rot.

Literature reports suggest that *N. alba* can survive on dead bark tissue of apple, pear, and olive trees, and as a saprophyte on various woody and herbaceous plants (Zazzerini and Van der Aa 1979; Sutton 1980; Verkley 1999). In addition, *N. alba* has recently been isolated from ash trees in Michigan, where it causes a disease referred to as coin canker, and it is the first time this pathogen has been found on this host (Rossman et al. 2001). This information is important with respect to determining the potential source of inoculum for bull's eye rot caused by *N. alba*. Management of diseases caused by *N. malicorticis* and *N. perennans* relies heavily on removal of inoculum sources by pruning out cankered bark. However, in a situation where the inoculum source is unknown, control by removal is impossible. If indeed *N. alba* is surviving as a saprophyte on plant material other than pear trees in the Pacific Northwest, the identification of potential hosts upon which *N. alba* may survive in these environments is essential. The molecular diagnostic techniques described in this research could be used in such studies, and would be fundamental in identifying the source of inoculum responsible for bull's-eye rot in these regions.

References

Abeln, E.C.A., de Pagter, M.A., and Verkley, G.J.M. 2000. Phylogeny of *Pezicula*, *Dermea* and *Neofabraea* inferred from partial sequences of the nuclear ribosomal RNA gene cluster. Mycologia, 92: 685–693.

Arx, J.A., von. 1970. A revision of the fungi classified as *Gloeosporium*. 2nd Ed. J. Cramer, Lehre.

Bakkeren, G., Kronstad, J.W., and Lévesque, C.A. 2000. Comparison of AFLP fingerprints and ITS sequences as phylogenetic markers in Ustilaginomycetes. Mycologia, 92: 510–521.

Boerema, G.H., and Gremmen, J. 1959. Een oppervlakkige bastkanker bij appel en peer veroorzaakt door *Pezicula corticola*. T. Pl. Ziekten, 65: 165–176. [English summary.]

Cunningham, G.H. 1942. Research work on ripe-spot of apples. Orchardist, 15: 2.

de Jong, S.N. 1998. The development of specific, non-discriminating PCR primers for the detection of *Pezicula malicorticis* and *P. perennans*, agents of anthracnose canker, perennial canker, and bull's-eye rot of apple. MPM thesis, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia.

de Jong, S.N., Lévesque, C.A., Verkley, G.J.M., Abeln, E.C.A., Rahe, J.E., and Braun, P.G. 2001. Phylogenetic relationships among *Neofabraea* species causing tree cankers and bull's eye rot of apple based on DNA sequencing of ITS nuclear rDNA, mitochondrial rDNA, and the β-tubulin gene. Mycol. Res. 105: 658–669.

Dugan, F.M. 1992. Comparative morphological, cytological and behavioral studies of *Cryptosporiopsis curvispora* and *C. perennans*. PhD thesis, Department of Plant Pathology, Washington State University, Pullman, Wash.

Dugan, F.M. 1993. Anthracnose, perennial canker and bull's-eye rot of apple and pear. Tree Fruit Postharvest J. 4: 5–6.

Dugan, F.M., Grove, G.G., and Rogers, J.D. 1993. Comparative studies of *Cryptosporiopsis curvispora* and *C. perennans*. I. Morphology and pathogenic behavior. Mycologia, 85: 551–564.

Farr, D.F., Bill. G.F., Chamuris, G.P., and Rossman, A.Y. (*Editors*). 1989. Fungi of plants and plant products in the United States. American Phytopathological Society Press, St Paul, Minn.

Gariepy, T.D., Lévesque, C.A., de Jong, S.N., and Rahe, J.E. 2003. Species specific identification of the *Neofabraea* pathogen complex associated with pome fruits using PCR and multiplex DNA amplification. Mycol. Res. 107: 528–536.

Guthrie, E.J. 1959. The occurrence of *Pezicula alba* sp. nov., and *P. malicorticis*, the perfect states of *Gleosporium album* and *G. perennans*, in England. Trans. Br. Mycol. Soc. 42: 502–506.

Jones, A.L., and Aldwinkle, H.S. (*Editors*). 1990. Compendium of apple and pear diseases. American Phytopathological Society Press, St Paul, Minn.

Kennel, W. 1986. Parasitare lagerkrankheiten beim Apfel. Obst Garten, 3: 151–152.

Kienholz, J.R. 1939. Comparative study of apple anthracnose and perennial canker fungi. J. Agric. Res. 59: 635–665.

Kienholz, J.R. 1951. The bull's eye rot problem of apples and pears. Proc. Oreg. Horticul. Soc. 43: 75–77.

Kienholz, J.R. 1956. Control of bull's-eye rot on apple and pear fruits. Plant Dis. Rep. 40: 872–877.

Leibinger, W., Breuker, B., Hahn, M., and Mendgen, K. 1997. Control of postharvest pathogens and colonization of the apple

surface by antagonistic microorganisms in the field. Phytopathology, 87: 1103–1110.

Lennox, C.L., Spotts, R.A., and Booyse, M. 2004. Postharvest decay of d'Anjou pears from the Pacific Northwest and control with a thiabendazole drench. Plant Dis. 88: 474–478.

Lévesque, C.A., Beckenbach, K., Baillie, D.L., and Rahe, J.E. 1993. Pathogenicity and DNA restriction fragment length polymorphisms of isolates of *Pythium* spp. from glyphosate-treated seedlings. Mycol. Res. 97: 307–312.

Lockhart, C.L., and Ross, R.G. 1961. *Gloeosporium album* and *G. malicorticis* on apples in Nova Scotia. Can. Plant Dis. Survey, 41: 280–285.

Rossman, A.Y., Castlebury, L.A., Putnam, M., and Adams, G. 2002. *Phlyctema vagabunda* causes coin canker of ash trees in Michigan. Plant Dis. 86: 442.

Seavert, C.F. 2002. The economic costs and returns of growing pears on Oregon, Washington, and California, USA. ISHS Acta Hortic. 596: 109–114.

Senula, A., and Ficke, W. 1985. Untersuchungen zur Pathogenese des *Gloeosporium*–Rindenbrandes. Arch. Phytopathol. Pflanzenschutz, 21: 183–198.

Sholberg, P.L., and Haag, P.D. 1996. Incidence of postharvest pathogens of stored apples in British Columbia. Can. J. Plant Pathol. 18: 81–85.

Sutton, B.C. 1980. The Coelomycetes: Fungi Imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute, Surrey, UK.

National Agricultural Statistics Service. 2003. Crop production. Agricultural Statistics Board, US Department of Agriculture, Washington, D.C. Available from http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bb/2003/crop0803.pdf [Updated 12 August 2003; cited 12 January 2004].

Verkley, G.J.M. 1999. A monograph of *Pezicula* and its anamorphs. Stud. Mycol. 44: 1–176.

Zazzerini, A., and Aa, H.A., van der. 1979. Morphology and pathogenicity of *Phlyctaena vagabunda*, a parasite of the olive tree. Phytopathol. Z. 96: 258–262.