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## A Review of *Neofabraea malicorticis* Biology and Management of Anthracnose Canker in Apple Orchards in the Maritime Pacific Northwest

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## Abstract

Cider apple (Malus ×domestica Borkh.) is an emerging crop in western Washington and the maritime Pacific Northwest (PNW) region in general, but the planting of new orchards and orchard productivity are limited by the widespread occurrence of anthracnose canker, caused by the fungal pathogen Neofabraea malicorticis (H.S. Jacks). In the maritime PNW region, the pathogen induces tree cankers that can kill newly planted trees and structurally weaken established trees. Current management practices include excising cankers during the dormant season and applying fungicides prior to autumn rains. Yet these management practices have not provided adequate disease control in the region, as new N. malicorticis infections of susceptible hosts occur even after applying the recommended controls. Poor management of anthracnose canker in the region is likely due to the lack of effective treatments, treatments being applied at the wrong time, or treatments not being applied over an adequate period of time. High inoculum levels and favorable environmental conditions for pathogen infection in the region also contribute to disease severity. Research on disease development and the management of N. malicorticis in an orchard environment is limited to dessert apples and is contradicting, which further exacerbates the difficulty in developing an effective disease management plan for cider apples. If cider apple production is to be successful in the maritime PNW, it is necessary to have a more comprehensive understanding of the pathogen, and to incorporate this knowledge into the development of an effective plan to manage anthracnose canker on apple. The objective of this review is to provide an overview of the existing literature on *Neofabraea* spp. in apple orchards, address factors that may explain why managing anthracnose canker has been difficult, and to identify topics for future research that will lead to more effective disease management.

Washington State is the leading producer of apple (*Malus* × *domestica* Borkh.) in the U.S. and also is playing a leading national role in the expansion of cider apple production (Miles et al., 2017). In Washington, the cider apple industry was first established in the western half of the state where the climate is similar to regions of Europe in which cider apple trees have thrived for centuries. Production of cider apples is increasing in western Washington, where yield from a cider apple orchard is about 40,350 kg·ha<sup>-1</sup> with a net value of \$35,508 per ha (\$0.88 per

kg) (Galinato et al., 2014). As the production of cider continues to expand, the demand for specialty cider apples will increase, and already the demand greatly exceeds the supply (Galinato et al., 2014). In western Washington, the widespread occurrence of anthracnose canker, a fungal disease caused by *Neofabraea* species, is the major limitation to planting new cider apple orchards and is a constraint to long-term orchard productivity. In other apple production regions, *Neofabraea* species also induce a postharvest rot of pome fruit (known as bull's-eye rot); while bull's

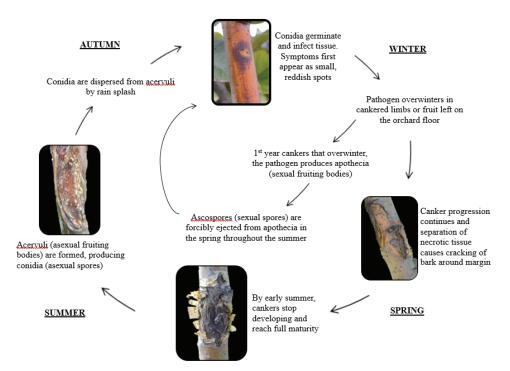
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eye rot can reduce marketable yield, it does not kill apple trees like anthracnose canker can do in western Washington. Although several species can incite anthracnose canker on apple, including Phlyctema vagabunda (Desm.) [synonym N. alba (E.J. Guthrie) Verkley] and N. kienholzii (Seifert, Spotts, & Lévesque, sp. nov.), N. malicorticis (synonyms Cryptosporiopsis curvispora, Cryptosporiopsis malicorticis. Pezicula malicorticis. Gloeosporium malicorticis, Macrophoma curvispora) is the primary causal agent of this disease (Zang et al., 2011). Developing a more thorough understanding of the biology of *N. malicorticis* and current management practices for anthracnose canker will help improve management strategies and protect cider apple production in western Washington.

Life Cycle of Neofabraea malicorticis. Neofabraea malicorticis is considered an aggressive fungal plant pathogen that is able to infect intact bark tissue, with most infections occurring through the lenticels (Cordley, 1900; Kienholz, 1939). Stem and trunk infections by N. malicorticis appear to occur primarily in the autumn but can take place throughout the winter and early spring during mild, moist weather (Davidson and Byther, 1992; Rahe, 2010). Infections first appear on the bark surface as small, circular spots that are red or purple when moist (Fig. 1). Mycelial growth occurs in the cambium beneath the bark for a period of time before killing the bark itself to form a visible canker. In inoculation studies, visible canker symptoms developed two to six months after inoculation (Dugan et al., 1993; Rahe, 1997a; Zang et al., 2011); however, the period of time required for symptom development to occur in response to natural infections is unknown. During the winter months,



**Figure 1.** Disease cycle of anthracnose canker caused by *Neofabraea malicorticis*.

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canker progression ceases but rapidly resumes development upon spring sap flow (Creemers, 2014). As the canker enlarges, infected bark tissues begin to peel away and the canker becomes elongated, sunken, and turns orange to brown. Following this, a distinct margin develops between healthy and necrotic tissue causing the bark to crack around the infected area. The necrotic bark tissue over the canker separates into small pieces and curls upwards from the lesion, and eventually sloughs off leaving bast fibers behind, giving the appearance often referred to as "fiddle-string" (Turechek, 2004). Larger cankers on main branches or trunks may not display the "fiddle-string" appearance. Cankers become fully developed by early summer, attaining full size ranging from 30 to 250 mm in length (Davidson and Byther, 1992).

By midsummer to late-autumn, acervuli (asexual fruiting bodies) form on mature cankers, producing conidia (asexual spores) that are disseminated by rain and wind to other parts of the tree, as well as surrounding trees and fruit, causing new infections (Creemers, 2014). The acervuli first appear as cream-colored pustules on the center of the canker surface, and later on the canker margin. As acervuli age, they become dark in color (Turechek, 2004). On cankers that are allowed to overwinter, anothecia (sexual fruiting bodies) may develop in the old acervuli and forcibly discharge ascospores (sexual spores) in the spring (Powell et al., 1970; Rahe, 1997a). The capacity of ascospores to incite infection in western Washington is uncertain and previous reports are conflicting. Creemers (2014) indicated that the sexual stage is insignificant in the disease epidemiology. In contrast, the British Columbia Ministry of Agriculture (2016) states that ascospores are responsible for inciting new infections on surrounding trees, while dispersal of conidia is responsible for localized intensification of the disease infected trees. In an in-vitro study, ascospores were discharged from mature

ascocarps onto cankered bark tissue from late March through Sept. under high humidity and mild temperature (4 – 13 °C) conditions (Jurkemikova and Rahe, 1998). The pathogen survives as mycelium in cankered limbs or in fruit left lying on the orchard floor, and can produce spores that incite new infections during cool, moist weather at almost any time of the year (Ogawa and English, 1991; Turechek, 2004).

Based on *in-vitro* inoculation studies, additional hosts of N. malicorticis include native PNW crab apple (Malus fusca Raf.), quince (Cydonia oblonga Mill.), flowering quince (Chaenomeles japonica Thunb.), peach (Prunus persica L.), serviceberry (Amelanchier pallida Greene), apricot (P. armeniaca L.), plum (Prunus salicina Lindel.), sweet cherry (*P. avium* L.), hawthorn (Crataegus spp.), and mountain ash (Sorbus spp.) (Kienholz, 1939). However, symptoms produced on stone fruit trees were not similar to those produced on pome fruit trees. Additionally, the pathogen did not produce fruiting bodies or spores on stone fruit trees, and it is presumed that these pathogens are not capable of inciting infection naturally on stone fruit trees (Kienholz, 1939).

Distribution and Impact of Anthracnose Canker. Populations of N. malicorticis have been reported throughout North America including British Columbia, California, Illinois, Maine, Massachusetts. Michigan, and Nebraska, as well as in Africa (Zimbabwe), Oceania (Australia and New Zealand), and Europe (Denmark, Netherlands, and Portugal) (EPPO Global Database, 2017; Turechek, 2004). Looking at the historical reports of anthracnose canker on apple, Heald (1926) found a single occurrence of anthracnose canker in Nebraska. In southwestern and central Maine, anthracnose canker was reported on more than one thousand 'McIntosh' apple trees (Hilborn, 1938). In Massachusetts, anthracnose canker was first observed on one tree each of 'McIntosh' and 'Cortland', but very few new cankers were observed the following year (Boyd, 1939). In Santa Cruz County, California, several anthracnose cankers were detected in three apple orchards that were eight to 10 years old (Barnett, 1944; Kienholz, 1939). In the Fraser Valley of British Columbia, five out of six apple orchards that were surveyed were heavily infested with anthracnose canker, and disease incidence was 50% to 80% on a per tree basis (Rahe, 1997a). There have been recent reports of anthracnose canker killing 'McIntosh' apple trees in Michigan, though specific incidence and severity reports were not provided (Rahe, 2010).

A recent informal survey of growers in western Washington by Garton et al. (2016) found that a grower in Vashon Island removed 2% to 5% of their cider apple trees each year due to anthracnose canker. Additionally, a cider apple grower on San Juan Island reported that 80% of the trees in a 1 ha orchard were infected with anthracnose cankers. In Bellingham, a grower reported that 100% of the trees in his 4 ha apple orchard possessed anthracnose cankers, while a grower in Port Angeles reported that 66% of trees in a 1 ha established cider apple orchard and 16% of trees in a newly planted 1 ha cider apple orchard exhibited anthracnose cankers. In Everson, a grower reported removing 5 out of 6 ha of apple trees due to anthracnose canker.

Although multiple *Neofabraea* spp. may coexist with each other, the geographical distribution and relative importance of any single species may vary at each location (Gariépy et al., 2003; Henriquez et al., 2004; Kienholz, 1939). For example, Kienholz (1939) found that *N. perennans* [(Kienholz) Dugan, R.G. Roberts & G.G. Grove] was dominant in the Kootenay Valley and Okanagan Valley of British Columbia. In Nova Scotia, P. vagabunda and N. malicorticis were isolated from anthracnose cankers on the apple cultivars 'Cortland', 'McIntosh', 'Russett', and 'Spy' (Lockhart and Ross, 1961). In Australia, P. vagabunda and N. perennans were found to be the causal

species of tree cankers and fruit rot, while *N. malicorticis* was reported as an exotic (rare) species (Cunnington, 2004). In contrast, Verkley (1999) reported populations of *N. malicorticis* in New Zealand. Verkley (1999) also reported *N. malicorticis* in parts of Europe (Denmark, Netherlands, and Portugal). In past studies in Europe, *N. malicorticis* and *N. perennans* were considered to be a single species (Boerema and Gremmen 1959; Sutton, 1980; von Arx, 1970), but this has since been resolved by molecular evidence demonstrating they are genetically distinct, although closely related (de Jong et al., 2001).

Environmental Conditions and Pathogen Virulence. While N. malicorticis occurs worldwide (EPPO Global Database, 2017; Turechek, 2004), the disease appears to be most damaging in areas where the climatic conditions include mild year-round temperatures, cool-humid summers, and abundant winter rains. In areas of cider apple production in western Washington where anthracnose canker is most prevalent, the temperature averages approximately 14 °C during the growing season (April-Oct.) and 6 °C during the dormant season (Nov. – Mar.) (WSU AgWeatherNet, 2017). The average relative humidity ranges from 73% to 82% during the growing season and 82% to 91% during the dormant season. The average amount of precipitation received is 76 mm during the growing season on average, and 101 mm during the dormant season. The amount of solar radiation received during the growing season is 506 MJ/m<sup>2</sup> on average and 157 MJ/m<sup>2</sup> during the dormant season.

The influence of temperature and moisture on *Neofabraea* spp. mycelial growth, sporulation, and germination has been evaluated mainly *in-vitro* or in studies addressing the development of bull's-eye rot on pome fruit. Mycelial growth of *Neofabraea* spp. in culture was observed in the range of 0 to 22 °C with optimal growth around 15 °C (Hortová et al., 2014; Kienholz, 1939; Miller, 1932; Senula, 1985). Kienholz (1939)

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observed an increase in mycelial growth by *N. malicorticis* in the range of 0 to 20 °C. Similarly, Miller (1932) found mycelial growth by *N. malicorticis* was greater at 15 °C than at 20 °C but noted that the pathogen was capable of growth at 0 °C. Senula (1985) found the optimum temperature range for *N. malicorticis* was 18 to 22 °C, while Hortová et al. (2014) found the optimum temperature range was 18 and 20 °C. Aguilar et al. (2017) indicated that growth of *Neofabraea* spp. may be inhibited when temperature approaches or exceeds 30 °C.

The climate conditions in the maritime PNW are conducive for N. malicorticis spore germination and growth all year long. Cordley (1900) reported that conidia of N. malicorticis germinated at 22 °C within 12 h, and germination was slowed at 29 °C. Spotts and Peters (1982) reported conidial germination of N. malicorticis at 10 and 20 °C but was greater at -1.1 °C with a relative humidity of 97% to 100%. Spotts (1985) also reported the viability of N. malicorticis conidia was greater at 10 and 20°C than at 30 °C when relative humidity ranged between 40% and 90%. In studies with the closely related pathogen N. perennans, Henriquez et al. (2008) found that fruit infection occurred when the temperature was between 10 and 30 °C and the period of wetness was  $\ge 0.5$ h, and suggested that moisture may have a greater impact on conidial dispersal than on infection itself.

Cultivar Susceptibility and Resistance. Information on cultivar susceptibility and resistance toward anthracnose canker is limited and contradictory. Braun (1997) surveyed 25 apple orchards throughout Nova Scotia for the presence of anthracnose canker, and found a greater incidence of anthracnose canker on 'McIntosh', 'Idared', and 'Golden Russet' than on 'Northern Spy', 'Gloster', or 'Red Delicious'. In two of the orchards where 'McIntosh' and 'Idared' were planted on M.26 and M.111 rootstocks, the authors found no difference in canker incidence due to rootstock but suggested that 'McIntosh'

was less susceptible to anthracnose canker than 'Idared'. In addition, the authors observed that on 'Idared' and 'Golden Russett' the anthracnose cankers occurred on small twigs and spurs, whereas on 'McIntosh' the cankers appeared on the trunk, central axis, and scaffold limbs. These results regarding cultivar susceptibility contradict those of Borecki and Czynczyk (1985) in Poland, where the authors inoculated 26 cultivars with N. malicorticis; 'McIntosh', 'Melrose', 'Delikates', and 'Spartan' were rated highly susceptible, and 'Idared', 'NY 58-553-1', and 'Golden Delicious' as least susceptible. The authors further noted that none of the cultivars evaluated in the study were completely resistant to N. malicorticis. Currently, all apple cultivars, including cider cultivars, are considered to be susceptible to N. malicorticis, and 'Akane', 'Baldwin', 'Chehalis', 'Elstar', 'Empire', 'Gravenstein', 'Melrose', 'Spartan', and 'Sinta' were reported to be very susceptible (British Columbia Ministry of Agriculture, 2016; Creemers, 2014; Pscheidt and Ocamb, 2017).

Management Strategies for Anthracnose Neofabraea malicorticis induce cankers that girdle young wood and structurally weaken established trees, resulting in severe damage or tree death (Davidson and Byther, 1992). Because these cankers serve as a source of inoculum infecting adjacent trees and capable of fruit, disease management relies heavily on excising cankers from infected trees in dry weather to minimize disease spread, and also application of fungicides prior to autumn rains (Pscheidt and Ocamb, 2017). Canker excision is a common cultural practice that is effective at managing canker diseases on fruit trees (Horner et al., 2015; Pscheidt and Ocamb, 2017). Excision of anthracnose cankers from apple trees (cultivars not specified) reduced the occurrence of new cankers by 45% (Byther, 1986); however, Rahe (2010) did not observe a reduction in the number of new canker infections on apple trees (cultivars nor specified) the year following canker removal. Excision of cankers or pruning creates wounds that can provide both entry points for pathogen colonization and leakage of contents from disrupted cells that can provide nutrients for pathogens (Bostock and Stermer, 1989). Thus, fungicide applications to wounded areas are recommended to prevent infection (Davidson and Byther, 1992; Zeller and Childs, 1925).

Research on managing anthracnose canker with fungicide applications on apple trees is limited and also contradictory. Current recommendations in Washington and Oregon include the application of captan, zinc, or copper-based products prior to autumn rains (Pscheidt and Ocamb, 2017). Creemers (2014) reported that anthracnose canker control was possible using fungicides, but listed chemistries (e.g., quinone outside inhibitors and fluodioxinil) that were ineffective in controlling diseases incited by Neofabraea spp. (Aguilar et al., 2015). In an in-vitro study, captan was moderately toxic to N. malicorticis, and copper-based fungicides were non-toxic (Rahe, 1997a). Spotts et al. (2009) investigated fungicide efficacy for controlling bull's-eye rot on apple fruit, and found that thiabendazole, thiophanatemethyl, pyrimethanil, and pyraclostrobin + boscalid controlled all *Neofabraea* spp., whereas zinc did not control N. malicorticis but basic copper sulfate did. In an orchard study, Byther (1986) found that zinc and basic copper sulfate reduced the number of new cankers on apple trees by 50% when applications were made in mid-Oct. and again in mid-Feb. Rahe (1997b) found that thiophanate-methyl and thiram were ineffective against anthracnose canker when applications were made every two weeks from midsummer through autumn (Aug. -Oct.) (British Columbia Government, 2016). Bordeaux mixture (basic copper sulfate and calcium hydroxide) is a traditional copperbased fungicide that has been recommended to manage anthracnose canker (Barss and

Mote, 1931; Childs, 1927; Cordley, 1900; Heald, 1920). Copper-based products are the only materials recommended for control of anthracnose canker that are allowable in organic production, but only certain formulations of copper are registered and the material must be used in a manner that minimizes accumulation in the soil (OMRI, 2017; USDA-AMS, 2011).

It appears that *Neofabraea* spp. is sensitive to individual fungicides. These fungi may develop resistance to particular active ingredients or modes of action, further contributing to the difficulty in controlling anthracnose canker (Spotts et al., 2009). Furthermore, failure of fungicides to control anthracnose canker in the orchard may be due in part to the frequent rains in the region, which limits drying of the spray-applied materials on the bark surfaces (Rahe, 1997b).

## **Conclusions**

Current anthracnose canker management recommendations (canker excision and the application of fungicides during late autumn) have not provided adequate disease control in the maritime PNW region, including western Washington. The lack of effective anthracnose canker control may be due to the application of fungicides at inappropriate times or inadequate duration of treatment application. Currently, fungicide applications are recommended before initiation of autumn rains and one month later (Pscheidt and Ocamb, 2017). Additionally, fungicide applications in late autumn through winter are recommended to prevent the germination of conidia. However, there are no recommendations on how often the fungicides should be applied. Information is needed on disease development and the key vulnerable stages of the pathogen in order to refine selection of effective chemistries, and target timing of fungicide applications to enhance management of N. malicorticis in an orchard environment.

The maritime climate of western Washington is conducive to disease

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development and dissemination of *N. malicorticis* all year long, suggesting the need for a year-round management plan to obtain effective control. Furthermore, the capacity of ascospores to infect trees in the maritime PNW has not been evaluated. Therefore, further investigations examining the disease cycle should be conducted to determine the timing of infections, and the capability of ascospore infection. This information may influence the timing and frequency of treatment applications, which could potentially improve management of this disease in the region.

If cider apple production is to be successful in the maritime PNW, an effective management program for anthracnose canker is required, as currently this disease is killing newly planted trees and limiting productivity of established orchards. While susceptibility of specialty cider apple cultivars to N. malicorticis has not been reported, observations at the Washington State University Northwestern Washington Research and Extension Center in Mount Vernon indicate that of the 70 specialty cider apple cultivars planted at this location, all are susceptible to the disease. Future work should investigate the level of cultivar anthracnose susceptibility to An integrated management program for anthracnose canker should include removal of infected host tissues to reduce inoculum sources in the orchard. Such an approach may help minimize the over-reliance on chemical controls and potential development of fungicide resistance that has already been detected in certain populations of Neofabraea spp. (Weber and Palm, 2010). A better understanding of anthracnose canker management will also have positive implications on managing this disease outside of the maritime PNW in case future changes in climate lead to greater disease incidence in other regions.

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