Study of Fitness, Virulence, and Fungicide Sensitivity of *Lambertella corni-maris* Causing Yellow Rot on Apple

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Abstract

Very little is known about the biology, epidemiology, and best practices to manage *Lambertella* rot, a newly identified postharvest disease caused by *Lambertella corni-maris* on apples in the United States. In this study, we investigated the prevalence of *L. corni-maris* in 92 grower lots throughout Washington State in 2016, evaluated the effect of nutrient availability on *L. corni-maris* growth, and *L. corni-maris* sensitivity to pH, osmotic stress, and fungicides in vitro. We assessed pathogen aggressiveness on major apple cultivars and the efficacy of pre- and postharvest fungicides to control *L. corni-maris* on detached fruit. *L. corni-maris* was widespread and was found in 40% of the growers lots surveyed at frequencies ranging from 2 to 40% of the total decay. The fungus grew faster on acidic media such as apple juice agar and V8 agar media and was able to grow equally at pH values ranging from 3 to 7. *L. corni-maris* isolates showed relatively low sensitivity to osmotic stress and grew

evenly at 1M KCl. All nine apple cultivars tested were susceptible to *L. corni-maris*, but the disease severity was significantly higher on some cultivars such as Honeycrisp, Pīnata, and Gala. The baseline sensitivity of 100 *L. corni-maris* isolates was determined based on the effective concentration necessary to inhibit 50% mycelial growth (EC₅₀) for fludioxonil, boscalid, pyraclostrobin + SHAM, pyrimethanil, and thiabendazole. The respective mean EC₅₀ values were 0.07, 0.84, 1.32, 2.45, and 3.68 µg/ml. Fludioxonil and pyrimethanil applied at label rates were the only fungicides able to control *L. corni-maris* effectively on detached apple fruit. This study is a first step toward understanding the eco-physiological requirements for *L. corni-maris* to survive and cause decay on apple, assessing potential shifts in fungicide sensitivity, and enhancing *Lambertella* rot management. We suggest “yellow rot” as the new common name for *Lambertella* rot.

Pome fruit, i.e., apple and pear, are prone to postharvest diseases caused by more than 90 fungal species (Heald and Ruehle 1931; Snowden 1990). Blue mold, gray mold, and bull’s-eye rot caused by *Penicillium* sp., *Botrytis cinerea*, and *Neofabraea* sp., respectively, are the major postharvest diseases in many apple growing regions. Others, such as *Sphaeropsis* rot, speck rot, and mucor rot caused by *Sphaeropsis pyriputrescens* (Kim and Xiao 2008), *Phacidium* sp. (Kim and Xiao 2006; Xiao et al. 2005), and *Mucor piriformis* (Michailides and Spotts 1990), respectively, appear less consistently than the three aforementioned ones. *Penicillium* sp. and *M. piriformis* infect fruit at harvest and mainly during storage (Amiri and Bompeix 2005; Michailides and Spotts 1990; Sanderson and Spotts 1995). Infections by other fungal species may be initiated in the orchard, remain latent, and develop slowly even at low temperatures (–1 to 4°C), low O₂ (0.7 to 2%), and high CO₂ (0.5 to 3%) concentrations.

Lambertella corni-maris von Höhnelt is a new fungal pathogen reported to cause postharvest *Lambertella* rot on apple in Washington State (Wiseman et al. 2015). The disease was found in 36% of packinghouses surveyed between 2003 and 2005 in central Washington at frequencies ranging from 1 to 3% of total decay (Wiseman et al. 2015). *L. corni-maris* was previously reported on mummified apples in Switzerland and pears in Germany (Harrison and El Helaly 1935). The fungus causes brown spongy lesions that may exhibit sporadic yellow-tan and thick mycelia. Under laboratory conditions, *L. corni-maris* has been reported to cause disease on other fruit crops such as pear, plum, quince, orange, and lemon (Harrison and El Helaly 1935). Interestingly, *L. corni-maris* exhibited a strong antagonistic in vitro activity against mycelium of *B. cinerea* and several other fungi, whereas the supernatant from liquid cultures inhibited spore

germination of several fungi but not *B. cinerea* (Wood 1953). More recently, *L. corni-maris* was found to have allelopathic activity against the brown rot fungus *Monilinia fructigena* (Murakami et al. 2007). *L. corni-maris* produces the antibiotics lambertellos 1, 2, and 3, which have been suggested as the main mechanism of action against *M. fructigena* (Hirose et al. 2014).

The rare studies on *L. corni-maris* mostly investigated its antagonistic activities and it was seldom considered as a plant pathogen. Besides causing decays on fruit and therefore additional loss to packers, the economic impacts can be important if the fungus is considered as a quarantine pathogen in many countries where apples are exported. The reasons behind its emergence as a postharvest pathogen on apple fruit are still unknown. Although Wiseman et al. (2015) suggested *L. corni-maris* to infect fruit through wounds caused at harvest and handling, very little is known about its etiology and epidemiology. Understanding the growth and reproduction requirements, fitness ability, pathogenicity, and sensitivity to fungicides will improve our knowledge about the eco-physiological requirements of the fungus and help in developing efficient management strategies.

Therefore, this study was conducted to (i) investigate the prevalence and distribution of *L. corni-maris* on apple in Washington State, (ii) evaluate the fitness ability of *L. corni-maris* under various environmental conditions, (iii) evaluate *L. corni-maris* virulence to major apple cultivars, and (iv) determine in vitro and in vivo sensitivity to five pre- and postharvest fungicides.

Materials and Methods

Prevalence of *L. corni-maris* during the 2015–16 season. A total of 32 packinghouses and 92 grower lots (orchards) from 10 counties representing major apple production areas in Washington State were surveyed for *L. corni-maris* from January to April of 2016 on fruit harvested from mid-August to November of 2015 (Fig. 1). Fifty decayed fruit originating from each orchard were randomly collected on the packing line, laid on clamshells to avoid contacts between fruit, and transferred to the laboratory for further disease characterization. Following a first visual symptom assessment, isolations were made from all decayed fruit. Fruit were half-split with an ethanol-sterilized knife and then fully split by hand, and a small piece of the flesh taken at the margin of decayed and healthy tissue inside the fruit was transferred to the center of a potato dextrose agar

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure is available online.

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(PDA) plate. Plates were incubated in the dark at 22°C for 6 days and a second characterization of *L. corni-maris* was conducted as described by Wiseman et al. (2015).

***L. corni-maris* isolates.** One hundred (100) *L. corni-maris* isolates were used to evaluate fungicide sensitivity in vitro. Six, 39, 33, and 22 isolates were collected in 2003, 2004, 2005, and 2016 respectively, from decayed apples sampled from multiple Washington packinghouses. These isolates were never exposed to four of the five fungicides tested below. Thiabendazole and thiophanate-methyl had been used years before these isolates were collected. However, these two fungicides were not used in the years of survey. Three isolates were used to study fitness abilities (Tables 1 and 2) and four isolates were used to evaluate fungicide sensitivity on detached fruit. Isolates were stored as mycelial plugs in 40% glycerol at –80°C and grown on PDA in continuous darkness for several weeks until apothecia were observed. Ascospores from mature apothecia were recovered in 1 ml of sterile distilled water (SDW), plated onto PDA plates, and incubated at 20°C for 24 to 36 h. Germinating single

spores observed using a stereoscope were transferred to fresh PDA plates. Isolates were stored at –80°C as mycelial plugs in 40% glycerol.

Fitness abilities in vitro and virulence to different apple cultivars.

Three different parameters including growth on different media, sensitivity to pH, and osmotic stress were evaluated in vitro using three isolates LCM-2776, LCM-3441, and LCM-4050. Growth ability was evaluated on five different media, i.e., PDA, apple juice agar (AJA; 800 ml H₂O, 200 ml of fresh apple juice made from Red Delicious apples, 15 g agar), V8 agar (V8A; 800 ml H₂O, 200 ml low-sodium V8 juice [Campbell's, Camden, NJ], 2 g CaCO₃, 15 g agar), malt yeast extract agar (MYA; 5 g malt extract, 5 g yeast extract, 10 g dextrose, 15 g agar), and intermediate medium (IM) reported previously to mimic plant substrates (Amiri et al. 2014; Manteau et al. 2003). For each isolate, two 6-mm plugs taken from the edge of a 10-day-old colony were transferred upside down to a plate of each of the five aforementioned media. Plates were incubated for 7 days at 20°C in the dark or a 12-h photoperiod before radial mycelial growth was measured. Four plates of each medium were used for each isolate and photoperiod and the experiment was conducted twice. The ability of the three isolates to grow at different pH levels was evaluated on PDA. The pH of the medium was modified to values ranging between 3 and 5 with different volumes of 2 M citric acid buffer and to values ranging from 6 to 8 with a 2 M Tris (hydroxymethyl) aminomethane buffer. The experiments were conducted and evaluated as described above for growth on different media except that plates were incubated in the dark and the growth was evaluated after 5 days incubation. Sensitivity to osmotic stress was evaluated on PDA amended with 0.25, 0.5, or 1.0 M KCl as described for the pH study but at two different temperatures, 5 and 20°C. Mycelial growth was measured after 20 and 5 days incubation, respectively. For the pH and osmotic stress studies, four plates were used for each pH values or KCl concentration at each temperature and the trials were conducted twice.

The susceptibility to *L. corni-maris* of nine apple cultivars, i.e., Ambrosia, Braeburn, Cameo, Fuji, Gala, Granny Smith, Honeycrisp, Pīñata, and Red Delicious, was evaluated on detached apple fruit. Organic fruit were washed in 0.8% sodium hypochlorite for 2 min, rinsed abundantly with tap water, air-dried, and wounded twice near the stem end area. A 5-mm diameter mycelial plug taken from the edge of 7-day-old colony of isolate LCM-2776 was placed on each wound. To evaluate the ability of *L. corni-maris* to cause infections on unwounded fruit, four Fuji and Honeycrisp apples were inoculated by placing two mycelial plugs directly on the cuticle. Fruit

Fig. 1. Frequency distribution of *Lambertella corni-maris* in Washington apple packinghouses in the 2015–16 season. Black and dashed red portions correspond to grower lots without and with yellow rot, respectively. Bold letters A, B, C, D, F, G, K, O, W, and Y indicate Adams, Benton, Chelan, Douglas, Franklin, Grant, Kittitas, Okanogan, Walla Walla, and Yakima counties, respectively. The letter *n* indicates the number of grower lots survey for each county.

Table 1. Growth of *Lambertella corni-maris* isolates on different media under various photoperiod regimes

Medium	Radial growth (mm) ± SD ^z					
	LCM-2776		LCM-3441		LCM-4050	
	Dark	12 h	Dark	12 h	Dark	12 h
AJA	36.6 ± 2.4 a	33.1 ± 1.6 a	31.9 ± 3.1 a	33.1 ± 3.6 a	32.3 ± 2.4 a	34.0 ± 3.5 a
V8	30.6 ± 3.2 b	29.4 ± 2.5 b	30.1 ± 1.8 ab	27.8 ± 1.3 bc	30.5 ± 0.6 b	30.3 ± 2.0 bc
MYA	31.3 ± 1.4 b	29.0 ± 1.4 b	28.1 ± 3.7 bc	28.5 ± 4.4 bc	30.2 ± 2.1 b	30.9 ± 0.9 b
IM	26.8 ± 2.1 c	27.8 ± 1.0 bc	26.1 ± 1.6 c	28.8 ± 1.1 b	30.1 ± 1.0 b	28.9 ± 2.5 c
PDA	27.3 ± 2.1 c	26.5 ± 3.7 c	27.4 ± 3.4 c	26.1 ± 3.2 c	26.8 ± 4.0 c	26.8 ± 1.4 d

^z Radial growth of *L. corni-maris* isolates after 7 days incubation at 22°C in continuous darkness or a 12 h photoperiod. Data are the means of 16 measurements from 8 replicate-plates across two experimental runs. Values, within each column, followed by different letters are significantly different based on the Student's *t*-test (*P* = 0.05).

Table 2. Effect of osmotic stress and temperature on growth of *Lambertella corni-maris* in vitro

KCl concentration	Radial growth (mm) ± SD ^z					
	5°C			22°C		
	LCM-2776	LCM-3441	LCM-4050	LCM-2776	LCM-3441	LCM-4050
0.25 M	17.3 ± 2.2 a	14.2 ± 2.0 a	15.4 ± 1.7 a	29.5 ± 3.1 a	25.6 ± 3.8 a	26.5 ± 3.5 a
0.5 M	16.8 ± 1.2 a	13.1 ± 1.7 a	14.6 ± 1.9 a	27.3 ± 3.8 ab	24.4 ± 1.7 a	23.4 ± 3.5 b
1.0 M	7.5 ± 0.8 b	6.5 ± 0.9 b	5.8 ± 0.6 b	25.5 ± 1.2 b	21.4 ± 1.5 b	23.2 ± 1.2 b

^z Radial growth of *L. corni-maris* isolates after 20 and 5 days incubation at 5 and 22°C, respectively. Data are the means of 16 measurements from 8 replicate-plates across two experimental runs. Values, within each column, followed by different letters are significantly different based on the Student's *t*-test (*P* = 0.05).

were placed in sealed boxes containing 200 ml of sterile water and incubated at 1 or 20°C in continuous darkness. Disease incidence and lesion diameter were evaluated after 45 and 10 days incubation, respectively. Eight fruit were used for each cultivar/temperature combination and the trial was repeated twice.

Baseline fungicide sensitivity in vitro. A total of 100 *L. corni-maris* isolates collected and maintained as described above were evaluated for their sensitivity to five fungicides using a mycelial growth inhibition assay. Sensitivity was evaluated to formulated preharvest boscalid (Endura, BASF Corporation) and pyraclostrobin (Cabrio, BASF) and the postharvest thiabendazole (TBZ, Mertect340-F, Syngenta Crop Protection), pyrimethanil (Penbotec 400 SC, Janssen PMP, Beerse, Belgium), and fludioxonil (Scholar SC, Syngenta Crop Protection). The sensitivity to fludioxonil and boscalid was evaluated on PDA and a minimal medium (Hu et al. 2011), respectively, at final concentrations of 0.001, 0.01, 0.1, and 1.0 µg/ml, and on PDA for TBZ at final concentrations of 0.01, 0.1, 1.0, and 10 µg/ml, and pyraclostrobin at final concentrations of 0.005, 0.05, 0.5, and 5.0 µg/ml. Salicylhydroxamic acid (SHAM) was added to PDA at a final concentration of 100 µg/ml to test for pyraclostrobin. Sensitivity to pyrimethanil was evaluated on asparagine-agar (Hilber and Schüepp 1996) amended with 0.002, 0.02, 0.2, and 2.0 µg/ml active ingredient. For each isolate, mycelial plugs (6-mm diameter), taken from the edge of an actively growing colony, were transferred upside down onto fungicide-amended and nonamended plates (two plugs/plate). Four plates were used for each fungicide concentration/isolate combination and the plates were incubated in continuous dark for 5 days at 20°C. The diameter of each colony was measured in two perpendicular directions and the two values were averaged to calculate effective concentration for 50% inhibition (EC₅₀) values.

Efficacy of pre and postharvest fungicides for *L. corni-maris* control on detached fruit. Four isolates LCM-2876, LCM-2873, LCM-3852, and LCM-3872 were used to evaluate the efficacy of five fungicides on detached fruit. These isolates were selected because they exhibited different levels of sensitivity in vitro. Fruit were surface-sterilized and wounded as described above and dipped for 30 s in fungicide suspensions of formulated boscalid (Endura), pyraclostrobin (Cabrio), thiabendazole (Mertect340-F), pyrimethanil (Penbotec400 SC), and fludioxonil (Scholar SC) at label rates of 0.6 g/liter, 0.72 g/liter, 0.5 ml/liter, 2.5 ml/liter, and 0.78 ml/liter, respectively. Control fruit were dipped in sterile water. Fruit were incubated overnight at room temperature and inoculated with a 5-mm diameter mycelium plug placed upside down on each wound. The fungicides were applied preventively because our main objective is to evaluate the actual efficacy of the fungicides in relation to the sensitivity level of the isolates in vitro. Fruit were incubated as described above and disease incidence and lesion diameter were evaluated after 7 days. Six fruit were used for each treatment and the trial was conducted twice.

Fig 2. Effect of pH on mycelial growth of *Lambertella corni-maris* isolates after 5 days incubation at 22°C in continuous dark. Data point are means of 16 measurements across two experimental runs.

Statistical analysis. Data from the two replications were combined for statistical analysis after no significant difference was observed based on the Student's *t*-test. Logarithmically transformed fungicide concentrations and the probit values (Finney 1952) transformed from the respective percentage inhibition were used to calculate EC₅₀ values. Disease severity relative to untreated control and disease incidence were arcsin transformed for ANOVA analysis and mean separation using Student's *t*-test. All statistical analyses were performed using JMP statistical software version 12.1.0 (SAS Institute Inc. Cary, NC).

Results

Prevalence of yellow rot in the 2015-16 season. Most decayed fruit identified to be caused by *L. corni-maris* showed strong yellow compact mycelium at late stages of disease development (Supplementary Fig. S1). Wiseman et al. (2015) described lesions caused by *L. corni-maris* on artificially inoculated fruit to show yellow-tan mycelia. Therefore, we propose "yellow rot" as a common name for Lambertella rot. Yellow rot was widespread across Washington State and occurred in 37 of 92 grower lots (40.2%) surveyed in 2016 (Fig. 1), accounting for 6.25% of the total decayed fruit. The frequency of grower lots showing yellow rot was the highest in Chelan County (100%) followed by Yakima (68.8%), Grant (43.3%), and Douglas (37.5%) counties (Fig. 1). Yellow rot was not detected in packinghouses from Adams and Kittitas counties in central Washington and Benton County in south Washington (Fig. 1). Yellow rot incidence ranged from 2.0 to 38.0% of decayed fruit per grower lot. Moreover, about 40% of surveyed grower lots had yellow rot incidence higher than 5% (data not shown).

Fitness parameters and cultivar susceptibility. All isolates grew significantly ($P = 0.0092$) faster on apple juice agar compared with all other media, whereas PDA supported growth the least (Table 1). *L. corni-maris* isolates grew equally on V8 and malt extract, slightly faster compared with IM medium. Yellowish colonies were observed on all media for the first 4 days, then turned dark brown except on V8 agar, on which light white colonies were observed. Overall, the lighting conditions did not significantly ($P = 0.6257$) affect *L. corni-maris* growth. *L. corni-maris* isolates were moderately sensitive to osmotic stress as all isolates grew equally at 0.25 and 0.5 M KCl and was significantly reduced at 1 M KCl at 1°C (Table 2). All three *L. corni-maris* isolates grew equality a pH values ranging between 3 and 6, whereas the growth increased slightly at pH 7 but was completely inhibited at pH 8 (Fig. 2). Growth decreased from about 28 to 32 mm at pH 3 to 26 mm at pH values between 4 and 6, and increased to 30 and 34 mm for LCM-4050 and LCM-2776, respectively, at pH 7 (Fig. 2).

Although the disease incidence was similar among all nine apple cultivars tested (data not shown), significant differences were observed in *L. corni-maris* severity among the cultivars. After 45 days of storage at 1°C in regular atmosphere, Red Delicious, Cameo, and Granny Smith had significantly lower *L. corni-maris* severity whereas the severity on Honeycrisp, Fuji, Gala, and Piñata was significantly higher (Fig. 3A). Similar trend was observed after 10 days storage at 20°C where Red Delicious, Granny Smith, and Ambrosia had significantly lower severity compared with Honeycrisp and Piñata (Fig. 3B). Interestingly, apples inoculated on nonwounded cuticle did not develop yellow rot.

During the survey conducted in 2016 in multiple packinghouses, yellow rot was observed on 10 different apple cultivars. However, incidence was higher on Fuji (10%), Cripps Pink (6.4%), Honeycrisp (6.1%), and Piñata and Gala (5%), whereas yellow rot incidence was low (2%) on cultivars such as Breaburn, Red, and Golden Delicious (data not shown).

Fungicide sensitivity in vitro. The lowest mean EC₅₀ value was registered for fludioxonil (0.07 µg/ml), for which values ranged from 0.02 to 0.14 µg/ml, whereas the highest mean EC₅₀ was observed for TBZ (3.68 µg/ml) with values ranging from 0.64 to 10.9 µg/ml (Table 3). The mean EC₅₀ values for boscalid, pyraclostrobin, and pyrimethanil were 0.84, 1.32, and 2.45 µg/ml, respectively. Variation factors were all below 100 except for pyrimethanil (VF = 134). The

frequency distribution of EC₅₀ values fitted a unimodal curve with a normal distribution for fludioxonil and had a strong skew to the left compared with that of pyraclostrobin and pyrimethanil (Fig. 4).

Control of *L. corni-maris* with pre- and postharvest fungicides on detached fruit. Fludioxonil (Scholar) applied at label rate completely controlled *L. corni-maris* isolates regardless of their EC₅₀ values (0.07 to 0.14 µg/ml) on detached fruit after 10 days incubation at 20°C (Table 4). Pyrimethanil (Penbotec 400SC) provided a significantly greater efficacy compared with the other fungicides except fludioxonil and *L. corni-maris* severity was not significantly different from that of fludioxonil, i.e., for the isolate with an EC₅₀ value of 13.5 µg/ml. Although the disease incidence was numerically lower when pyraclostrobin was used at label rate compared with boscalid, significant differences were not observed between the two fungicides in incidence and severity, which were correlated to the sensitivity

levels of isolates. Thiabendazole failed to control *L. corni-maris* and resulted in a higher severity compared with the untreated control fruit (Table 4).

Discussion

Yellow rot caused by *L. corni-maris* continues to cause postharvest losses in multiple packinghouses in Washington State. Hence, 40% of grower lots surveyed in the 2015–16 season had yellow rot incidences higher than 5%. Even though the overall disease incidence may seem low to cause significant economic losses for apple fruit packers, other fungal pathogens, such as *Phacidiopycnis washingtonensis* (Xiao et al. 2005) and *Sphaeropsis pyriputrescens* (Xiao and Rogers 2004), reported to cause postharvest decay at relatively similar frequencies in the Pacific Northwest, resulted in nearly \$20 million losses a year due to rejection of fruit lots from many countries where these pathogens are considered quarantine pathogens. It is still unclear whether the presence of *L. corni-maris* in many packinghouses, nearly 10 years after its first characterization in 2005, only reported recently (Wiseman et al. 2015), is due to a change in climatic conditions that affected the region or by a shift in pre- and postharvest practices undergone by the apple fruit industry in the recent years, including the introduction of new cultivars and reliance on new fungicides.

Nutrients, water, space, and oxygen are among various conditions required by fungi to grow and reproduce. Knowing that a fungus often has to compete with other fungi and microorganisms for its survival, it becomes important to understand the specific needs of each species and conditions that may favor infections. In a recent study, growth and germination of *L. corni-maris* was optimal at 20°C, whereas its growth was reduced at 0°C and completely inhibited at temperatures ≥30°C (Wiseman et al. 2015). Findings from our investigation suggest that *L. corni-maris* can grow under a wide range of conditions including some that may be less favorable for other fungal species. Thus, *L. corni-maris* grew slightly faster under more acidic conditions (pH 3) compared with pH values ranging from 4 to 6 and grew significantly faster on apple juice agar (AJA) compared with other media, such as PDA, commonly used to grow fungi in vitro. Harrison and El-Helaly (1935) reported that *L. corni-maris* was able to grow over a wide range of pH ranging from 1.6 to 8.3 on different media and that growth was optimal at pH 4.4. Herein, *L. corni-maris* was completely inhibited at pH 8 and grew optimally at pH 7. Although environmental H⁺ concentration (pH) impact on fungi has been suggested to be minimized by the buffering system in hyphae and conidia (Dix and Webster 1995), extreme pH conditions may influence enzymatic activity of fungi (Aleandri et al. 2007; Enokibara et al. 1993). Thus, *L. corni-maris* was reported to be very active in excreting oxidizing enzymes and pectinase (Harrison and El-Helaly 1935) and antibiotics known as lambertellin or lambertellols 1, 2, and 3 (Armstrong and Turner 1965; Murakami et al. 2004). Very interestingly, lambertellols were produced by *L. corni-maris* at substantial amounts under acidic conditions (pH 3) but very scarcely at pH 6.3 (Murakami et al. 2007). Consequently, antagonistic activity of *L. corni-maris* was only observed at low pH against a range of other fungal pathogens (Murakami et al.

Fig. 3. Distribution and mean diameter of lesions caused by *Lambertella corni-maris* on several apple cultivars after 45 days of storage at 1°C (A) and 10 days of storage at 22°C (B). Points distributed vertically represent 16 specific measurements for each cultivar across two experimental runs and the mean diameter values are shown at the top-end distribution for each cultivar. Mean lesion diameter values followed by different letters are significantly different based on the Student's t-test ($P = 0.05$).

Table 3. Effective concentrations to inhibit 50% mycelial growth of *Lambertella corni-maris* by five pre- and postharvest fungicides

Fungicide	EC ₅₀ (µg/ml) ^y		CV ^z
	Mean	Range	
Fludioxonil	0.07	0.02–0.14	7
Boscalid	0.84	0.11–2.55	23
Pyraclostrobin	1.32	0.10–9.91	99
Pyrimethanil	2.45	0.10–13.4	134
Thiabendazole	3.68	0.64–10.9	17

^y Mean and range of effective concentrations to inhibit 50% mycelial growth (EC₅₀) calculated using 100 *L. corni-maris* isolates from multiple packinghouses in Washington between 2003 and 2005.

^z Coefficient of variation was calculated by dividing the highest EC₅₀ value by the lowest EC₅₀ value for each fungicide within the population.

2007; Wood 1953). In addition to its ability to grow in acidic environments, *L. corni-maris* was not susceptible to osmotic stress as it grew at 1M KCl even at low temperatures. While water availability may not be an issue in postharvest storage rooms where ambient humidity is usually high enough for fungi to thrive, apple growing regions in the Pacific Northwest are subaridic and can be unfavorable for many fungal pathogens in such orchards. However, it may be possible that *L. corni-maris* can survive effectively in relatively dry conditions and continue to cause latent infections that result in postharvest diseases.

Mean EC₅₀ values for fludioxonil in vitro correlated well with in vivo efficacy of the fungicide applied at label rate. A variation factor (7) for this fungicide was the lowest among all fungicides tested, which may reflect a low intrinsic variability in sensitivity to fludioxonil among *L. corni-maris* isolates. The latter was the only postharvest fungicide applied at low label rate able to completely control *L. corni-maris* isolates, i.e., those with EC₅₀ values as high as 0.14 µg/ml. Correlation between in vitro EC₅₀ values for pyrimethanil and in vivo efficacy were not always high since this fungicide had one of the highest mean EC₅₀ (2.45 µg/ml) in vitro. However, pyrimethanil applied at medium label rate successfully controlled *L. corni-maris* isolates, i.e., those with an EC₅₀ value as high as 13 µg/ml. Although not tested in this study, the higher label rate of pyrimethanil (Penbotec) should fully control isolates with high EC₅₀ values equivalent to the ones found in this study. The wide range of EC₅₀ values for pyrimethanil, seen in this study, may denote a wider inherent variability in sensitivity to this fungicide and potentially a higher risk, compared with fludioxonil, to shift toward reduced sensitivity. The two preharvest fungicides, pyraclostrobin and boscalid, are premixed as Pristine, which is widely used by apple growers as a preharvest fungicide. The two fungicides applied separately provided a moderate efficacy only against *L. corni-maris* isolates regardless of their EC₅₀ value.

Further studies are needed to determine when apples are infected by *L. corni-maris*; however, our findings indicate that Pristine will not protect the fruit from infections in the orchard. Therefore, screening additional preharvest fungicides used in apple orchards would help identify most effective fungicide for yellow rot management if proven that fruit

Table 4. Efficacy of five pre and postharvest fungicides against *Lambertella corni-maris* isolates with different fungicide sensitivity levels on detached apple fruit

Fungicide ^y	Isolate	EC ₅₀ (µg/ml)	Incidence ^z	Severity ^z
Fludioxonil	LCM-2876	0.07	0.0 a	0.0 a
	LCM-2873	0.14	0.0 a	0.0 a
Pyrimethanil	LCM-2876	3.18	6.3 b	6.4 a
	LCM-3852	13.45	21.2 a	7.3 a
Pyraclostrobin	LCM-2876	0.76	46.2 b	50.2 b
	LCM-2887	9.91	68.8 a	87.5 a
Boscalid	LCM-2876	0.66	46.3 b	59.7 a
	LCM-2873	2.55	68.8 a	69.9 a
Thiabendazole	LCM-2876	1.74	100.0 a	120.5 a
	LCM-3872	10.91	100.0 a	121.2

^y Fludioxonil, pyrimethanil, pyraclostrobin, boscalid, and thiabendazole were applied preventively as formulated Scholar_SC, Penbotec, Cabrio, Endura, and Mertect, respectively, at label rates. Treated fruit were incubated overnight at room temperature before inoculation.

^z Disease incidence was calculated based on the number of artificially inoculated wounds showing yellow rot infection whereas severity was calculated as the reduction in lesion diameter on fungicide-treated fruit relative to the control after 7 days incubation at 20°C. Values, within each column and for each fungicide, followed by different letters are significantly different based on the Student's *t*-test (*P* = 0.05).

Fig. 4. Frequency distribution of EC₅₀ values (µg/ml) for pyraclostrobin, thiabendazole pyrimethanil, boscalid, and fludioxonil of a *Lambertella corni-maris* baseline population (*n* = 100) from multiple packinghouses in Washington.

infections occur preharvest. The postharvest fungicide TBZ had the highest mean EC₅₀ value in vitro and completely failed to control *L. corni-maris* isolates in vivo regardless of their EC₅₀ value. Similarly, thiophanate-methyl (Topsin-M) used as a preharvest fungicide in apple orchards will not, most likely, protect apples from *L. corni-maris* infections. Presently, applications of fludioxonil or pyrimethanil at harvest would provide appropriate control of yellow rot.

The ability of *L. corni-maris* to cause disease on multiple fruit and vegetables has been reported previously (Harrison and El Helaly 1935). However, its etiology and epidemiology in apple orchards is completely unknown. Wiseman et al. (2015) suggested that *L. corni-maris* infect the fruit through wounds, which implicates that the infections should occur at harvest when most wounds are caused on fruit. We present clear evidence that *L. corni-maris* requires a wound to infect apples. However, we cannot rule out other ways of pathogen penetration into fruit in the orchard. Whether *L. corni-maris* overwinters on apple trees between seasons or if there are alternative hosts that would be potential sources of *L. corni-maris* inoculum is still unknown. Recently, crabapple fruit and rose hips were found to be susceptible to *L. corni-maris* when wounded and inoculated artificially (Wiseman et al. 2016). Manchurian crabapple trees are common pollinizers in apple orchards and have been shown to be susceptible to other pathogens such as *P. washingtonensis* and *S. pyripitrescens* and suggested to be the main source of inoculum of these two pathogens (Xiao et al. 2009, 2014). Rose hip shrubs can be found around some orchards but not necessarily everywhere where apples are grown. Our present study indicates that *L. corni-maris* is present in all apple growing regions in the state and some surveyed locations, positive to *L. corni-maris*, did not have rose hips or other potential hosts in the vicinity. Further investigation is needed to assess the role of crabapple, and potentially, apple trees themselves as inoculum sources, as well as the infection timings to improve yellow rot management. The pathogen-pathogen interactions between *L. corni-maris* and other apple pathogens is also worth of future investigation to assess potential decay suppression by *L. corni-maris*. Indeed, the later produces antibiotics known as lambertellos shown to be antagonistic to several pathogens such as *B. cinerea* and *M. fructigena* (Hirose et al. 2014; Murakami et al. 2007; Wood 1953). It would be interesting to find some *L. corni-maris* isolates non-virulent on apple that could be potentially used as biocontrol agents.

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