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New or Unusual Disease Reports

First report of *Leucostoma cinctum* on sweet cherry and European plum in Italy

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Summary. *Leucostoma cinctum* and *L. persoonii* are the two species involved in Leucostoma canker, a disease that causes dieback of twigs and branches, bark cankers, gummosis, and tree decline of stone fruit. The aim of this study was to identify the causal agent of Leucostoma canker in Italian stone fruit orchards. More than 200 isolates of *Leucostoma* spp. were obtained from branches and twigs of sweet and sour cherry, apricot, and European plum trees that showed typical symptoms of Leucostoma canker. These trees were in commercial orchards of two Italian regions, Marche and Apulia, in central-eastern and south-eastern Italy, respectively. Soon after isolation, all of the colonies that grew on potato dextrose agar were white in colour, and after about 10 d they became olive-green. Growth was not observed at 33°C, and the pycnidia were larger than 1 mm diam. This information led to the identification of *L. cinctum* as the causal agent of these Leucostoma cankers. To our knowledge, this is the first report of *L. cinctum* on sweet cherry and European plum in Italy.

Key words: Leucostoma canker, stone fruit, *Prunus avium*, *Prunus domestica.*

Introduction

Stone fruit trees can be affected by Leucostoma canker, which is caused by *Leucostoma cinctum* Höhn. (anamorph *Leucocytospora cincta* (Sacc.) Höhn.) and *Leucostoma persoonii* Höhn. (anamorph *Leucocytospora leucostoma* (Pers.) Höhn.). *Leucostoma persoonii* is usually found in warmer climates, whereas *L. cinctum* occurs in cooler areas (Biggs, 1989). These ascomycetes belong to the order Diaporthales and the family Valsaceae. They are frequently found in stone fruit orchards as the imperfect form. The main symptoms include dieback of twigs and branches, bark cankers, gummosis and tree decline. The pathogen cannot infect healthy plants; it can enter plants only through pruning wounds or injuries caused by frost or insects. If the disease progresses, it can cause the death of plants after few years and lead to significant crop losses (Barakat and Johnson, 1997).

Leucostoma canker has been studied for more than a century in North America. The first observations were at the beginning of the twentieth century in western New York and in Missouri, on peach trees (Stewart *et al.*, 1900; Rolfs, 1909). In these areas, the disease has a significant economic impact in stonefruit orchards, and especially with peach trees. Trees with multiple infections show reduced productivity and their condition is conducive to infection by other pathogens, which reduce tree longevity and cause considerable losses (Biggs, 1989). There have been several reports of Leucostoma canker caused both by *L. cinctum* and *L. persoonii* in different areas of North America, especially on peach. In the United States, *L. cinctum* infections on peach have been reported for North Carolina (Endert-Kirkpatrick and Ritchie, 1988) and Michigan (Wang *et al.*, 1998), while in Canada it has been detected in Ontario (James and Davidson, 1971; Tekauz and Patrick, 1974; Biggs,

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1989). For sweet cherry orchards, Leucostoma canker was reported in Washington State to be caused by both of these species (Regner *et al*., 1990) or by only *L. cinctum* (Barakat and Johnson, 1997), in Oregon by *L. cinctum* (Spotts *et al*., 1990), and in Ontario by both species (Biggs, 1989). On European plum, Leucostoma canker has been reported in Idaho (Helton and Mosiey, 1955). Besides being widespread in North America, this disease is also present in Europe, Japan and South America (Biggs, 1989).

In Italy, *L. cinctum* has been reported from several peach orchards in the Province of Verona (Quaroni *et al*., 1983), but not yet on other stone fruit species.

We have identified the causal agent of Leucostoma canker in some Italian orchards of sweet and sour cherry, apricot and European plum.

Materials and methods

The investigated commercial stonefruit orchards were located in the Italian regions Marche and Apulia, in central-eastern and south-eastern Italy, respectively. Samples were taken from late winter to early spring of 2006–2011. Four to 20 mm thick branches with symptoms suggesting Leucostoma canker were cut from 28 sweet cherry (*Prunus avium*), two sour cherry (*Prunus cerasus*), two apricot (*Prunus armeniaca*) and three European plum (*Prunus domestica*) trees. Branches were surface-sterilized with 1% sodium hypochlorite for about 40 s, and then rinsed in sterile distilled water. The bark was removed and five pieces of wood at the edges of lesions between infected and healthy tissue were placed into 90 mm Petri dishes containing potato dextrose agar (PDA; Difco, Detroit, MI, USA), supplemented with 100 mg streptomycin and 100 mg ampicillin, and incubated at 20 ± 1°C. After 5 d, mycelium plugs were excised, placed into Petri dishes containing PDA, and incubated in the light at room temperature to stimulate the formation of pycnidia.

The following criteria were applied for identification of isolated fungi: (i) color of the mycelium (*L. cinctum*: white turning to buff or olive-buff; *L. persoonii*: white turning to brown or darker brown); (ii) size and properties of the pycnidia (*L. cinctum*: large, 1–3 mm diam, white, felty, rarely if ever exuding cirri; *L. persoonii*: small, 1 mm or less diam, with beaks, usually dark, exuding cirri when mature); and (iii) presence or absence of growth at 33°C (Surveiyer *et al*., 1995) (*L. persoonii*: grows best at 25–30°C, with a maximum at 32°C; *L. cinctum*: has an optimum of 18–20 °C, with a maximum at 30°C).

Results and discussion

In the infected plants with dead branches the bark appeared darker and depressed (Figure 1). In spring, reddish gummy exudates were often seen oozing from the bark near the infected portions (Figure 2). Colonies of *Leucostoma* spp. were isolated from 21 out of 28 samples of sweet cherry trees, and from two of three European plum trees. These pathogens were not isolated from the samples of sour cherry and apricot. A few days after isolation on PDA, the resulting colonies showed irregular margins and whitish mycelia; they became olive-green after about 10 d

Figure 1. Branches of sweet cherry with portions of dark wood (arrow) infected by *Leucostoma cinctum.*

(Figure 3). Pycnidia were produced in PDA cultures after at least 3–4 weeks, and these were white and felty, with diameters >1 mm. On PDA incubated for 2 weeks at 33°C, no growth was observed. Therefore the isolates from sweet cherries (cv. Ferrovia, Bing, Moreau, Forlì, Montagnola, Durone di Vignola, Burlat, Lapins, and Chiusa di San Michele) and European plum (unknown cultivar) were confirmed as *L. cinctum*.

Figure 2. Gummy exudates oozing from the trunk of a sweet cherry tree affected by Leucostoma canker.

In Italy, Leucostoma canker has been reported in particular for peach orchards, where it can cause considerable damage (Quaroni *et al*., 1983; Mancini and Cotroneo, 1996), and it is known to be among the diseases that affect sweet cherry trees (Faretra, 1997). With sweet cherry, Leucostoma canker is an important disease, as has been shown in investigations carried out in some Italian regions, particularly in Apulia (Frisullo and Ferrara, 1997; Romanazzi *et al*., 2011) and Marche (Romanazzi *et al*., 2011). Moreover, in our investigations, Leucostoma canker was also found in some European plum orchards, from two localities of Marche, as previously shown in Basilicata (Caponero *et al*., 1999).

In Italy, for peach trees, both *Leucostoma* species have been reported (Quaroni *et al*., 1983; Mancini and Cotroneo, 1996), while on sweet cherry and European plum trees, only *L. persoonii* has so far been found in Apulia (Frisullo and Ferrara, 1997) and Basilicata (Caponero *et al*., 1999).

Since Leucostoma canker is considered a secondary disease, at present, no special control measures are taken for it in sweet cherry and European plum orchards, and attention is focused on more damaging diseases. Once it is established, Leucostoma canker is very difficult to manage, and therefore effective disease control strategies should be based largely on avoiding the factors that predispose the trees to this disease. The damage is particularly important if

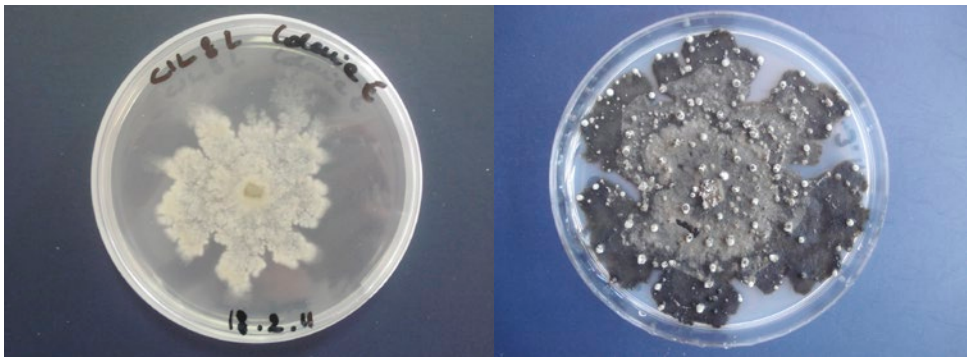

Figure 3. Isolate CIL8L of *Leucostoma cinctum* on potato dextrose agar, a 7-d-old colony (left) and a 2-month-old colony with pycnidia (right).

the pathogen affects the main branches or the trunks, because then the plants die within a few years due to occlusion of the vessels. Disease control methods would comprise removal of infected branches and prevention of insect, rodent and winter injuries, regular monitoring of susceptible plants, and correct pruning procedures. With regard to the economic importance of sweet cherry and European plum in Italy, and considering the heavy damage that can be caused by this disease, we propose the inclusion of *L. cinctum* in the list of pathogens known to affect the quality of stone fruit propagating material. Further studies are required to develop the necessary protocols for molecular detection of these pathogens, as these would assist rapid diagnosis of potential problems caused by Leucostoma canker.

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