Influence of Harvest Date on Bull's Eye Rot of 'Cripps Pink' Apple and Control Chemical Strategies

Irene Cameldi and Fiorella Neri, CRIOF–Department of Agricultural Science, University of Bologna, Bologna, Italy; Daria Ventrucci and Gianni Ceredi, Apofruit, Cesena, Italy; Enrico Muzzi, Department of Agricultural Science, University of Bologna; and Marta Mari, CRIOF–Department of Agricultural Science, University of Bologna

Abstract

Cameldi, I., Neri, F., Ventrucci, D., Ceredi, G., Muzzi, E., and Mari, M. 2016. Influence of harvest date on bull's eye rot of 'Cripps Pink' apple and control chemical strategies. *Plant Dis.* 100:2287-2293.

The influence of four different harvest times on the bull's eye rot of 'Cripps Pink' apple caused by *Neofabrea* spp. was investigated in two orchards harvested at four different times. In addition, a control strategy based on chemical treatments performed in preharvest or postharvest was evaluated. Regression analysis between harvest time and disease incidence revealed high r^2 values (>0.75). All preharvest fungicide treatments significantly ($P < 0.0085$) reduced the bull's eye rot incidence; however, thiophanate-methyl (achieving $>87\%$ control) was more effective than a mixture of pyraclostrobin and boscalid

($<80.7\%$) or fludioxonil ($<57.6\%$), in all trials. Compared with non-treated control fruit, a postharvest treatment with the ethylene inhibitor 1-methylcyclopropene (1-MCP) halved the incidence of infection in three of four experiments. However, a combination of two preharvest treatments with a mixture of pyraclostrobin plus boscalid and one postharvest 1-MCP treatment suppressed bull's eye rot to a significantly ($P < 0.00001$) greater degree (achieving $>87.5\%$ control) than the single treatments with pyraclostrobin and boscalid ($<65\%$) and 1-MCP ($<80\%$) tested alone.

'Cripps Pink' apple (*Malus domestica* Borkh.), which originated from a cross between 'Lady Williams' and 'Golden Delicious', is a late-maturing and long-storing cultivar, superior in size, appearance, and flavor relative to those of other late-maturing cultivars (Cripps et al. 1993). Since 1990, this cultivar has been cultivated in major apple-producing areas of the world (IPLA 2015). Although Cripps Pink apple represents a small portion of world apple production (1%), it frequently commands a premium value. In Europe, to achieve the best fruit color for marketing, Cripps Pink apple is generally harvested in a period that extends from the end of October to the end of November. Cripps Pink apple is fairly resistant to bitter pit and, if harvested at the correct maturity stage and stored in a controlled atmosphere, it also shows a low susceptibility to superficial scald (Cripps et al. 1993).

After storage for 3 to 4 months at 0°C , Cripps Pink fruit frequently develops bull's eye rot, which is caused by several species of *Neofabrea* (Soto-Alvarez et al. 2013; Spotts et al. 2009; Verkley 1999). Previous investigations (Edney 1964; Spotts 1990; Verhoeff 1974; White and Wilkinson 1962) have well documented that the pathogen infects apple fruit in the orchard prior to harvest and remains latent in the fruit for several months. Although *Neofabrea* spp. can produce cankers in the twigs (Henriquez et al. 2006), fruit remains symptomless in the field, developing lesions only during storage. Initially, the symptoms are restricted to lenticels as circular necrotic areas, usually flat or slightly concave, dark, and firm. The incidence of disease varies considerably, depending on weather conditions; however, in humid areas with abundant rain and persistent fog close to harvest, bull's eye rot can affect over 50% of the fruit (Spotts et al. 2009).

Partial control of bull's eye rot can be achieved by repeated applications of fungicides such as captan, phenyl-pirroles, and strobilurines during the 2 months preceding harvest (Giraud and Bompeix 2012; Henriquez et al. 2006; Minář 2006). Late-season and postharvest use of fungicides on apple, however, is under scrutiny by the

European Commission, because of the increasing demand for fruit without pesticide residues (Poulsen et al. 2009). For a decade, 1-methylcyclopropene (1-MCP), a synthetic cyclic olefin that blocks an ethylene-binding receptor (Sisler and Serek 1997), has been used on the stored apple to extend fruit firmness in storage and marketing (Saftner et al. 2003). 1-MCP has also been shown to delay onset of storage rots on different hosts (Cao and Zheng 2010; McArtney et al. 2011; Su and Gubler 2012; Zhang et al. 2012). However, few data are available on its effect on bull's eye rot management in Cripps Pink apple.

The aims of this work were to (i) determine the influence of harvest time on susceptibility of apple to bull's eye rot, (ii) evaluate the influence of pre- and postharvest chemical treatments on disease incidence, and (iii) assess an integrated control strategy of *Neofabrea* spp. based on field fungicides treatments followed by a postharvest 1-MCP treatment.

Materials and Methods

Experimental orchards. Experiments were conducted in two orchards (50 km apart) of Cripps Pink apple on M9 rootstock planted in 1999 in Emilia Romagna Region (Italy). Trees were under-tree irrigated; insects and weeds were controlled following the recommendations commonly used for Italian integrated production applied after planting. The intrarow spacing was 1 m and between-row spacing was 4 m. No fungicides were used in these orchards after August, except those used for the experiments.

Date of harvest experiment. To study the influence of harvest time on bull's eye rot incidence, fruit were harvested on four dates in a period from 19 October to 5 December. After harvest, fruit were stored in air at 0°C . Bull's eye rot was evaluated after storing fruit for 150 days + 10 days of shelf life. The sample unit was represented by four replicates of 75 fruit for each harvest time and orchard.

At each harvest time (except the first harvest), the following fruit quality analyses were performed on a sample of 20 apple fruit (5 per replication): flesh firmness, solid soluble content, titratable acidity, and starch degradation. Firmness was measured using a Chatillon digital penetrometer fitted with an 11-mm probe after removing the skin on opposite sides of each fruit. Solid soluble content (%) was determined with a digital refractometer (Model Atago PR 101) in a portion of filtrate obtained by homogenizing a slice of each fruit. Titratable acidity (percentage of malic acid) was determined using a compact

Corresponding author: M. Mari; E-mail: marta.mari@unibo.it

Accepted for publication 22 June 2016.

titrator (G20; Crison). Starch degradation index was scored on apple fruit cut in half and dyed for 2 min in a solution of iodine-potassium iodide (10 g of KI + 2.5 g of I₂ per liter of water) using a 1-to-5 scale, where 1 = 0% starch loss and 5 = 100% starch loss (Foschi 2013).

Preharvest fungicide treatments. The influence of preharvest fungicide treatments on bull's eye rot was evaluated for 3 years in the same orchard. Materials applied were a premixed formulation of pyraclostrobin and boscalid (Bellis; Basf Italia), fludioxonil (Geoxe Syngenta), and thiophanate-methyl (Enovit-methyl; Sipcam). Fungicide treatments were applied with a handheld spray gun (Sthil SR430; Sthil Co.). Trees with maturing fruit were treated once or twice, at 14 or 7 days before the first harvest. However, in the first year, only a premixed formulation of pyraclostrobin and boscalid was applied as fungicide twice at 14 and 7 days before harvest whereas, in the second year, the mixture of pyraclostrobin and boscalid was applied twice as described above, as were fludioxonil and thiophanate-methyl. In the third year, the complete experimental design was performed (Table 1). Each treatment was applied to a set of seven trees replicated four times. After fungicide application, no rain occurred before harvest in any year. Fruit were harvested in two sessions (in the first week of November and 15 days later). For each treatment and harvest, 120 fruit from each set were harvested, for a total of 480 fruit per treatment. Fruit were transported to a packinghouse on the day of harvest or the following day and stored in air at 0°C. Bull's eye rot incidence was recorded after 150 days of storage plus 10 days of shelf life at 20°C.

Postharvest fungicide treatment. Apple fruit were selected from untreated trees in the same orchard used for the fungicide trials. Two sets of 480 fruit (four replicates of 120 fruit each) were harvested on each of two harvest dates (15 days apart). The first set was fumigated with 1-MCP at 600 µl liter⁻¹ according to the manufacturer's recommendation (Smartfresh; AgroFresh Inc.). Fruit were treated inside a sealed cold-storage room at 15°C for 24 h. The atmosphere inside the room was circulated constantly with a fan. The second set, considered as the control, was untreated. After treatment, all fruit were stored in plastic bins at 0°C and evaluated for bull's eye rot incidence after 150 days supplemented with a 10-day period of shelf life storage at 20°C. The experiment was repeated for two seasons.

Integrated fungicide and 1-MCP treatments. For 2 years, one set of fruit derived from trees treated in the field with pyraclostrobin and boscalid twice (1 and 2 weeks before harvest) was also postharvest treated with 1-MCP, as reported above. Moreover, the experimental design included fruit treated only in the field with the fungicide mixture and fruit treated only in postharvest with 1-MCP. Fruit untreated in both the field and postharvest phase were considered to be the control. Fruit were harvested on two dates 15 days apart. For each treatment and harvest, the sample unit was represented by four

replicates of 120 fruit each. The 1-MCP treatment was performed the same day of harvest; then, all fruit were stored in plastic bins at 0°C and evaluated after 120 and 150 days, supplemented with a 10-day period of shelf life storage at 20°C. The experiment was repeated twice.

Statistics. Regression analysis was used to evaluate the influence of harvest time on bull's eye rot incidence. For the experiment on preharvest fungicide treatment, a multifactorial analysis with two factors (fungicide and harvest time) was performed for each year. For the experiment on postharvest 1-MCP treatment, a multifactorial analysis with three factors (harvest, year and treatment) was carried out. While for the experiment on integration between pre- and postharvest chemical treatments a multifactorial analysis with four factors (preharvest and postharvest treatment, harvest time and their combination) was performed. Statistical package STATISTICA, version 10.0 software (StatSoft 2011) was used. The least significance difference test at $P < 0.05$ for means separation of means was used. Experiments were carried out in a completely randomized block design.

Results

Influence of harvest dates on bull's eye rot in storage. The harvest dates influenced the physical-chemical parameters of the fruit (Table 2) and the appearance of bull's eye rot during storage (Fig. 1). Because the maturity indices were similar among orchards, only values of orchard 1 were reported. Firmness decreased to 80 Newton in the last harvest and titratable acidity was reduced by 28% from the second to the fourth harvest. Conversely, delaying the harvest time increased the soluble solid content and starch index. In both orchards, fruit stored for 150 days plus shelf life showed an increase of bull's eye rot incidence in the latter two harvests compared with the earlier two harvests. Regression analysis between harvest time and bull's eye rot incidence showed high r^2 values (0.87 and 0.77 for the first and second orchard, respectively), revealing a significant relationship among harvest time and fruit infection. Above all, apple harvested on the two later dates (21 November and 5 December) reached the highest incidence of bull's eye rot in both orchards, over 60% in orchard 1 and over 25% in orchard 2. On the other hand, the lowest levels of disease (<14% of infected fruit) were found in fruit harvested on the two earlier dates (19 October and 6 November) in both orchards. Meteorological data related to orchard 1 recorded the highest precipitations between the second and third harvest (43.9 mm), corresponding to 82.4% of all rain occurring in the considered period, while the temperatures (minimum and maximum) were over 14°C until the second harvest (Fig. 2).

Preharvest fungicide treatments. In untreated fruit, after 150 days of storage plus 10 days of shelf life, the percentage of infections caused

Table 1. Application timing (days before harvest), fungicides, and active ingredients evaluated for the control of bull's eye rot caused by *Neofabraea* spp. in a commercial orchard of Cripps Pink apple^z

Treatment	Fungicide	Active ingredient (%)	Days before harvest	Rates
Control	...	...	...	...
Bellis 1	BellisWG	Pyraclostrobin + boscalid (12.8 + 25.2)	7	0.8 kg ha ⁻¹
Bellis 2	BellisWG	Pyraclostrobin + boscalid (12.8 + 25.2)	14–7	0.8 kg ha ⁻¹
Geoxe	GeoxeWG	Fludioxonil (50)	14–7	0.45 kg ha ⁻¹
Enovit-metil	Enovit-metil FL	Thiophanate methyl (58.3)	14–7	100 ml hl ⁻¹

^z The experiment was performed in the same orchard over three consecutive years; in the third year, the complete control program was evaluated.

Table 2. Influence of harvest time on quality characteristics of Cripps Pink apple^x

Harvest	Dates	Firmness (N)	Soluble solid content (%)	Titratable acidity (mg 100 g ⁻¹ FW) ^y	Starch index ^z
Early	19 October	nd	nd	nd	nd
First	6 November	89.1 ± 0.57	12.2 ± 0.23	0.71 ± 0.03	3.4 ± 0.10
Second	21 November	81.0 ± 0.33	13.1 ± 0.41	0.64 ± 0.03	4.0 ± 0.32
Late	5 December	80.0 ± 0.38	14.2 ± 0.46	0.51 ± 0.10	4.4 ± 0.08

^x Abbreviation: nd = not determined.

^y Calculated as mg of malic acid.

^z On a scale of 1 to 5.

by *Neofabraea* spp. were higher in fruit of the second harvest than in fruit of the first harvest (Table 3). The incidence of bull's eye rot in control fruit of the first harvest changed in the 3 years of experiments, ranging from 9.4% (first year) to 89.8% (third year), while the incidence of disease in fruit harvested 15 days later exceeded 49% in all years tested. All chemical treatments significantly reduced the disease

incidence ($P < 0.0085$); however, some differences among fungicide activity were recorded. The pyraclostrobin and boscalid mixture and fludioxonil were generally less active than thiophanate-methyl, because its efficacy was higher than 87% in all experiments performed. No significant differences were observed between one or two treatments of pyraclostrobin and boscalid mixture, except for the first

Fig. 1. Influence of harvest dates on bull's eye rot of Cripps Pink apple fruit stored at 0°C for 150 days plus 10 days of shelf life. The dates 6 and 21 November correspond to the first and second commercial harvest, respectively. Data represent the mean of 300 fruit (four replicates of 75) for each harvest time and orchard. Vertical bars represent standard errors calculated for each orchard and harvest time.

Fig. 2. Meteorological parameters (temperature minimum and maximum and rainfall) registered in orchard 1 from 10 October to 10 December.

Table 3. Effects of field treatments on bull's eye rot incidence of Cripps Pink apple, naturally infected after 150 days of storage at 0°C plus 10 days of shelf life^z

Treatment	First year		Second year		Third year	
	First	Second	First	Second	First	Second
Control	9.4 a	49.7 a	58.9 a	95.7 a	89.8 a	96.8 a
Pyraclostrobin and boscalid 1	2.3 c	32.6 b	nd	nd	56.3 b	59.6 b
Pyraclostrobin and boscalid 2	5.6 b	30.2 b	11.4 bc	21.9 bc	59.4 b	65 b
Fludioxonil	nd	nd	25 b	41.2 b	50.4 b	66.3 b
Enovit methyl	nd	nd	3.2 c	5.9 c	9.7 c	12.4 c

^z First and second harvests are shown for each year. First harvest was performed every year in the first week of November and the second after 14 days. Data are the mean of 480 fruit per treatment, per harvest. In the same column, data followed by different letters are significantly different for least significance difference test ($P < 0.05$); nd = not determined.

harvest of the first year, where the incidence of bull's eye rot in control fruit was low (<10%). Harvest time affected the incidence of disease in all 3 years ($P < 0.0318$) (Table 4), whereas no interaction between chemical treatments and harvest time was found in all three trials ($P > 0.1501$).

Postharvest 1-MCP treatment. At the end of storage, after 150 days plus shelf life, 1-MCP treatment reduced the incidence of bull's eye rot in both years ($P < 0.0001$). The greatest disease reduction was observed in the second harvest of the second year (>70% less incidence compared with control) while the lowest reduction was found in the second harvest of the first year (20.5% less incidence compared with control) (Fig. 3). The multifactor analysis of variances confirmed that postharvest 1-MCP treatment reduced bull's eye rot in both years and harvests; however, a significant interaction was also observed among year, harvest, and treatment (Table 5).

Table 4. Statistical main effects and interactions for the multifactor analysis of variance of field chemical treatments

Year, main effects and interactions	Degrees of freedom	<i>P</i> value ^z
Year 1		
Harvest	18	<0.0001**
Chemical treatments	18	0.0085**
Harvest × chemical treatments	18	0.1501 ns
Year 2		
Harvest	24	0.0017**
Chemical treatments	24	<0.0001**
Harvest × chemical treatments	24	0.2048 ns
Year 3		
Harvest	30	0.0318**
Chemical treatments	30	<0.0001**
Harvest × chemical treatments	30	0.5301 ns

^z Asterisks (**) indicate that effect and interactions are significant at *P* value < 0.05; ns = not significant.

Fig. 3. Influence of a 1-methylcyclopropene (1-MCP) postharvest treatment on bull's eye rot of Cripps Pink apple fruit stored at 0°C for 150 days plus 10 days of shelf life. In the **A**, first and **B**, second year, the first harvest was performed in the first week of November and the second 14 days later. Columns are the mean of 480 fruit/treatment; within each year, harvest, and treatment, different letters represent significant difference for least significance difference test ($P < 0.05$).

Integration of pre- and postharvest chemical treatments. At the end of storage and shelf life, all treatments significantly reduced ($P < 0.00001$) the bull's eye rot incidence. However, the combination of pre- and postharvest treatments produced a disease control greater than that observed with single treatment alone (Fig. 4). A multifactor analysis of variance revealed an interaction between the pre- and postharvest treatments and was consistent throughout harvest times ($P < 0.0246$) (Table 6). In fact, the efficacy of both pre- and postharvest treatments resulted in disease control greater than 77.9 and 79.8% for the fruit of first and second harvests, respectively, the highest reductions observed. Conversely, after 120 days of storage, in both harvests, no significant difference was found in bull's eye rot incidence between 1-MCP postharvest treatment alone and the integration with a pre- and postharvest chemical application. No statistic interaction was found among pre- and postharvest treatments, harvest, and days of storage ($P = 0.5805$).

Discussion

Cripps Pink is a highly regarded apple cultivar but it is extremely susceptible to bull's eye rot; in certain years where conditions are favorable to the disease, after extended storage, the incidence of bull's eye rot can exceed 90%. The fruit can be stored for up to 4 months; therefore, an optimum harvest time is essential to maintaining elevated quality levels. In our trials, fruit maturity greatly influenced the susceptibility to bull's eye rot; similar results were found in organic 'Aroma' apple, where the incidence of total fruit rot, including bull's eye rot, increased significantly in fruit harvested 1 week after the commercial date (Børve et al. 2013). In 'Ligol' and 'Lodel' apple cultivated in Lithuania, a high incidence of bull's eye rot was recorded in fruit after 180 days of storage; the percentage of disease increased on delaying the harvest from 1 to 5 weeks after commercial harvest time (Valiuskaitė et al. 2006). Likewise, the timing of fruit harvest affected the incidence of bull's eye rot of 'Bosc' (Henriquez et al. 2008), 'Anjou', and 'Barlett' (Spotts 1985) pear, showing the highest levels of disease in late-harvested fruit. On the other hand, the Association Pink Lady Europe has defined restricted color standards for Cripps Pink commercialization (40% of diffuse pink-red color), forcing a delay of the harvest in many producing areas. In fact, in cooler climates, a brilliant pink color can be obtained at the optimum maturity stage; conversely, in warmer growing areas or in years where the warm temperatures continue in the weeks preceding the harvest, the development of pink color is more difficult (Lin-Wang et al. 2011). The growers, fearing to compromise the pink-red color of the fruit, favor delaying the harvest while awaiting its appearance but also exposing the fruit to a high risk of *Neofabraea* infections.

The factors that influence the fruit's susceptibility to the pathogens are still partially unknown; a higher level of inoculum, as reported by Henriquez et al. (2008), on mycelial-induced cankers during fall could explain the increase of fruit rot when the fruit is harvested late. Although, in our trials, a fungicide treatment performed between the second and the third harvest did not significantly reduce bull's eye rot symptoms (data not reported). This suggests that some changes occurring in the fruit in the late weeks before harvest are probably involved in the increased susceptibility to the disease, as noticed by Xu and Robinson (2010) in apple fruit infected by *Neonectria galligena*.

Table 5. Statistical main effects and interactions for the multifactor analysis of variance of postharvest 1-methylcyclopropene (1-MCP) treatment

Main effects and interactions	Degrees of freedom	<i>P</i> value ^z
1-MCP	24	<0.0001**
Harvest	24	<0.0001**
Year	24	<0.0001**
1-MCP × harvest	24	<0.0001**
1-MCP × year	24	<0.0001**
1-MCP × year × harvest	24	0.0186**

^z Asterisks (**) indicate that effects and interactions are significant at *P* value < 0.05.

However, more investigations are required to better elucidate the pathogenesis of *Neofabraea* spp.

In order to find an effective and sustainable strategy of bull's eye rot reduction, four control strategies based on preharvest fungicide treatments were evaluated over 3 years. All tested fungicides significantly reduced the bull's eye rot infections compared with untreated fruit; however, thiophanate-methyl was the most effective in all trials, with an efficacy over 87%. Thiophanate-methyl belongs to the class of methyl benzimidazole carbamate (MBC) fungicides that were introduced in the early 1960s and were quickly shown to be active against a wide spectrum of pathogens (Baraldi et al. 2003). However, they are typically site-specific inhibitors and quickly induced resistance problems against fruit pathogens (Ma and Michailides 2005), including *Neofabraea* spp. in Northern European apple production areas (Weber and Palm 2010). In our work, thiophanate-methyl was effective against bull's eye rot, minimizing the problem relating to the presence of resistance in the Italian population of *Neofabraea* spp. strains. However, thiophanate-methyl and the other MBC such as carbendazim and thia-bendazole are responsible for chemical residues in stored apple, which is an important issue for food retail chains that are obliged to limit the number of detectable residues in produce (Palm 2009). For these reasons, we cannot recommend the use of thiophanate-methyl against storage apple rot at the present time.

The other two fungicides (pyraclostrobin and boscalid mixture and fludioxonil) controlled bull's eye rot but with a lower efficacy than thiophanate-methyl. The explanation of the different antifungal activity probably lies in the fact that the former are preventive fungicides, acting on the fruit surface; thus, they cannot reach the pathogen if it is already established in the lenticels. Thiophanate-methyl, on the other hand, is a systemic fungicide; after application, it penetrates the fruit

tissue and, after the translocation, it could interact with *Neofabraea* spp. inside the lenticels. However, Spotts et al. (2009) showed that a postharvest-based pyraclostrobin and boscalid treatment had the same efficacy as a treatment with thiophanate-methyl on pear fruit against *Neofabraea* spp. Nevertheless, that work is not comparable with the present study because the methodology was substantially different; the fruit were wounded, treated with fungicides, and then artificially inoculated. The strobilurin fungicides act by inhibiting cellular respiration, preventing spore germination; therefore, their application is generally recommended just prior to or shortly after an infection (Bartlett et al. 2002). This could explain the good control obtained by Spotts

Table 6. Statistical main effects and interactions for the multifactor analysis of variance of integrated fungicide and 1-methylcyclopropene (1-MCP) treatments

Main effects and interactions	Degrees of freedom	P value ^z
Pyraclostrobin and boscalid	112	<0.0001**
1-MCP	112	<0.0001**
Harvest	112	<0.0001**
Days of storage	112	<0.0001**
Pyraclostrobin and boscalid × 1-MCP	112	<0.0001**
Pyraclostrobin and boscalid × 1-MCP × harvest	112	<0.0246**
Pyraclostrobin and boscalid × 1-MCP × harvest × days of storage	112	0.5805 ns

^z Asterisks (**) indicate that effects and interactions are significant at *P* value < 0.05; ns = not significant.

Fig. 4. Influence of pre- or postharvest chemical treatments on bull's eye rot of Cripps Pink apple fruit stored at 0°C for 120 and 150 days plus 10 days of shelf life. The pyraclostrobin and boscalid mixture was applied in the field twice (14 and 7 days before harvest). The first harvest was performed in the first week of November and the second 14 days later. The same day as the harvest, the apple fruit were transported to the packinghouse and the following day the 1-methylcyclopropene (1-MCP) was applied. Columns represent the mean of 400 fruit (four replicates) per treatment ± error bars. For each harvest, values followed by different letters (lowercase = after 120 days of storage and uppercase = after 150 days of storage plus shelf life) were significantly different according to least significance difference test ($P \leq 0.05$).

et al. (2009) on fruit treated before inoculation with Pristine, a commercial formulation of the mixture pyraclostrobin and boscalid. Thus, new registrations of compounds with a high capacity of mobility in the plant and into the fruit are urgently required. However, the tendency toward alternative strategies is encouraged in order to limit the issues derived from the presence of chemical residues on fruit; in this context, the use of an inhibitor of fruit ethylene production, as 1-MCP has been extensively used in the apple industry since 2002. The influence of a 1-MCP treatment on bull's eye rot appearance on naturally infected Cripps Pink apple fruit was evaluated in this study for the first time. The efficacy of the treatment varied depending on year and harvest date, probably as a result of fruit maturity, but remained over 48% in three of four trials. It is well known that postharvest pathogens produce a wide array of cell-wall-degrading enzymes involved in tissue maceration such as polygalacturonase (PG) (Misaghi 1982). The 1-MCP bound to ethylene receptors in apple fruit could inhibit the PG secreted by pathogens, preventing pathogenesis (McArtney et al. 2011; Spotts et al. 2007). Research is needed in this sense to better elucidate the mechanism of action of 1-MCP, although no direct activity on the spore germination of *Neofabrea vagabunda* was observed (data not reported). A reduction of bull's eye rot by 1-MCP was also observed on 'd'Anjou' pear fruit stored at -1°C for 6 months in semicommercial trials (Spotts et al. 2007). The 1-MCP treatment also controlled stem gray mold caused by *Botrytis cinerea* and Phacidiopycnis rot caused by *Phacidiopycnis piri* (Maxin and Weber 2011). The authors confirmed the activity of 1-MCP also on 'Elstar' apple fruit stored for 6 months in a controlled atmosphere, with a 55.3% reduction of the *N. perennans* incidence, while the treatment on apple stored in a dynamically controlled atmosphere failed to produce any significant additive effect on the pathogen. For the first time, an integrated strategy (two preharvest fungicide treatments and one 1-MCP postharvest treatment) to control bull's eye rot was proposed with encouraging results, above all for storage longer than 4 months. Cripps Pink apple fruit are stored for extended periods, up to 180 days under controlled atmosphere; therefore, the risks of disease insurgence have to be carefully evaluated. Our data reported an elevated control of the disease by chemical treatments performed in the field or after harvest; however, their efficacy can be increased by a combination of the chemicals. Following our trials, an integrated strategy could be defined, in particular for fruit that must remain in storage for a long time. 1-MCP treatment positively affects the retention of 'Pink Lady' apple quality; moreover, 1-MCP fumigation is recommended for the reduction of superficial scald incidence on apple fruit (Watkins et al. 2000).

Although fungal pathogens belonging to genus *Neofabrea* have been known for a long time (Burchill and Edney 1963; Lockhart and Ross 1961; White and Wilkinson 1962), they still raise important questions regarding some epidemiological aspects that could influence the control strategies. The coexistence of four different species causing bull's eye rot—*N. vagabunda* (synonym *N. alba*) (Johnston et al. 2014), *N. malicorticis*, *N. perennans*, and *N. kienholzii*—with different levels of sensitivity to fungicides relating to sporulation and conidia germination may affect the disease incidence (Henriquez et al. 2006). The scarce knowledge on the inoculum sources, mainly for *N. vagabunda*, the prevalent *Neofabrea* sp. in South European pome-fruit-producing countries, limits the possibility of reducing the pathogen population in the field, with important consequences for bull's eye rot control (Kohl et al. 2015). Finally, although bull's eye rot can be considered an old disease, it's becoming increasingly important because of the introduction of cultivars such as Cripps Pink that are harvested late, when weather conditions are more favorable to *Neofabrea* sp. infection, and stored for a long time, compromising fruit health.

Acknowledgments

We thank the LIFE financial instrument of the European Union (contract number LIFE13 ENV/HR/000580) for financial support.

Literature Cited

- Baraldi, E., Mari, M., Chierici, E., Pondrelli, M., Bertolini, P., and Pratella, G. C. 2003. Studies on thiabendazole resistance of *Penicillium expansum* of pears: Pathogenic fitness and genetic characterization. *Plant Pathol.* 52:362-370.
- Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., and Parr-Dobrzanski, R. 2002. The strobilurin fungicides. *Pest Manage. Sci.* 58:649-662.
- Børve, J., Roen, D., and Stensvand, A. 2013. Harvest time influences incidence of storage diseases and fruit quality in organically grown 'Aroma' apples. *Eur. J. Hortic. Sci.* 78:232-238.
- Burchill, R. T., and Edney, K. L. 1963. The control of *Gloeosporium album* rot of stored apples by orchard sprays which reduce sporulation of wood infections. *Ann. Appl. Biol.* 51:379-387.
- Cao, S., and Zheng, Y. 2010. Effect of 1-methylcyclopropene on anthracnose rot caused by *Colletotrichum acutatum* and disease resistance in loquat fruit. *J. Sci. Food Agric.* 90:2288-2294.
- Cripps, J. E. L., Richards, L. A., and Mairata, A. M. 1993. 'Pink Lady' apple. *HortScience* 28:1057.
- Edney, K. L. 1964. Some factors affecting the rotting of stored apples by *Gloeosporium* spp. *Ann. Appl. Biol.* 53:119-127.
- Foschi, S. 2013. Come misurare la qualità della frutta in campo. Online publication. http://www.informatoreagricario.it/Dem/2013_Macfrut/19042.pdf
- Giraud, M., and Bompeix, G. 2012. Postharvest diseases of pome fruits in Europe: Perspective for integrated control. *IOBC WPRS Bull.* 84:257-263.
- Henriquez, J. L., Sugar, D., and Spotts, R. A. 2006. Induction of cankers on pear tree branches by *Neofabrea alba* and *N. perennans* and fungicides effects on conidial production on cankers. *Plant Dis.* 90:481-486.
- Henriquez, J. L., Sugar, D., and Spotts, R. A. 2008. Effects of environmental factors and cultural practices on bull's eye rot of pear. *Plant Dis.* 92:421-424.
- IPLA. 2015. Online publication. <http://pinkladyapples.com/login/Files/news/2015/2015%20Vol%209%20Iss%202.pdf>
- Johnston, P., Seifert, K. A., Stone, J., Rossman, A. Y., and Marvanová, L. 2014. Recommendations on generic names competing for use in Leotiomyces (Ascomycota). *IMA Fungus* 5:91-120.
- Kohl, J., Wenneker, M., de Haas, L., Anbergen, R., Lombaers, C., and Kastelein, P. 2015. Inoculum sources of the post-harvest pathogens *Neofabrea* spp. and *Cadophora* spp. in Dutch apple and pear orchards. Page 288 in: *Proc. XVIII Int. Plant Prot. Congr. Berlin*.
- Lin-Wang, K., Micheletti, D., Palmer, J., Volz, R., Lozano, L., Espley, R., Hellens, L. P., Chagné, D., Rowan, D. D., Troglio, M., Iglesias, I., and Allan, A. C. 2011. High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. *Plant Cell Environ.* 34:1176-1190.
- Lockhart, C. L., and Ross, R. G. 1961. *Gloeosporium album* and *G. malicorticis* on apple in Nova Scotia. *Can. Plant Surv.* 41:280-285.
- Ma, Z., and Michailides, T. 2005. Advances in understanding molecular mechanisms of fungicide resistance in *Monilinia fructicola* using real-time PCR. *Pest Manage. Sci.* 63:1178-1184.
- Maxin, P., and Weber, R. W. S. 2011. Control of *Phacidiopycnis washingtonensis* storage rot of apples by hot-water treatments without the ethylene inhibitor 1-MCP. *J. Plant Dis. Prot.* 118:222-224.
- McArtney, S., Parker, M., Obermiller, J., and Hoyt, T. 2011. Effects of 1-methylcyclopropene on firmness loss and the development of rots in apple fruit kept in farm markets or at elevated temperatures. *HortTechnology* 21:494-499.
- Minář, P. 2006. Effect of late summer treatments by strobilurines on storage diseases of apples. *Acta Univ. Agric. Silv. Mendelianae Brun.* 54:39-44.
- Misaghi, I. J. 1982. The role of pathogen-produced cell-wall degrading enzymes in pathogenesis. Pages 17-34 in: *Physiology and Biochemistry of Plant Pathogen Interactions*. I. J. Misaghi, ed. Plenum Press, New York.
- Palm, G. 2009. Untersuchung und Bewertungen der Pflanzenschutzmittel-Rückstände im niederelbischen Obstbau. *Mitt. OVR Alten Landes* 64:220-226.
- Poulsen, M. E., Naef, A., Gasser, S., Christen, D., and Rasmussen, P. H. 2009. Influence of different disease control pesticide strategies on multiple pesticide residue levels in apple. *J. Hortic. Sci. Biotechnol.* 84:58-61.
- Saftner, R. A., Bai, J., Abbott, J. A., and Lee, S. Y. 2003. Sanitary dips with calcium propionate, calcium chloride, or a calcium amino acid chelate maintain quality and shelf stability of fresh-cut honeydew chunks. *Postharvest Biol. Technol.* 29:257-269.
- Sisler, E. C., and Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent development. *Physiol. Plant.* 100:577-582.
- Soto-Alvarez, S., Lolas, M., Rosales, I. M., Chavez, E. R., and Latorre, B. A. 2013. Characterization of bull's eye rot of apple in Chile. *Plant Dis.* 97:485-490.
- Spotts, R. A. 1985. Effect of preharvest pear fruit maturity on decay resistance. *Plant Dis.* 69:388-390.
- Spotts, R. A. 1990. Bull's eye rot. Page 56 in: *Compendium of Apple and Pear Diseases*. A. L. Jones and H. S. Aldwinckle, eds. American Phytopathological Society, St. Paul, MN.
- Spotts, R. A., Seifert, K. A., Wallis, K. M., Sugar, D., Xiao, C. L., Serdani, M., and Henriquez, J. L. 2009. Description of *Cryptosporiopsis kienholzii* and species profiles of *Neofabrea* in major pome fruit growing districts in the Pacific Northwest USA. *Mycol. Res.* 113:1301-1311.
- Spotts, R. A., Sholberg, P. L., Randall, P., Serdani, M., and Chen, P. M. 2007. Effects of 1-MCP and hexanal on decay of d'Anjou pear fruit in long-term cold storage. *Postharvest Biol. Technol.* 44:101-106.
- StatSoft. 2011. STATISTICA (data analysis software system), version 10.0. Online publication. StatSoft, Inc. www.statsoft.com

- Su, H., and Gubler, W. D. 2012. Effect of 1-methylcyclopropene (1-MCP) on reducing postharvest decay in tomatoes (*Solanum lycopersicum* L.). *Postharvest Biol. Technol.* 64:133-137.
- Valiuskaitė, A., Kviklienė, N., Kviklys, D., and Lanuauškas, J. 2006. Post-harvest fruit rot incidence depending on apple maturity. *Agron. Res.* 4:427-431.
- Verhoeff, K. 1974. Latent infections by fungi. *Annu. Rev. Phytopathol.* 12:99-110.
- Verkley, G. J. M. 1999. A Monograph of the Genus *Pezicula* and its Anamorphs. *Studies in Mycology*, Vol. 44. Centraalbureau voor Schimmelcultures.
- Watkins, C. B., Nock, J. F., and Whitaker, B. D. 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biol. Technol.* 19:17-32.
- Weber, R. W. S., and Palm, G. 2010. Resistance of storage rot fungi *Neofabraea perennans*, *N. alba*, *Glomerella acutata* and *Neonectria galligena* against thiophanate-methyl in Northern German apple production. *J. Plant Dis. Prot.* 117:185-191.
- White, L. J., and Wilkinson, E. H. 1962. Pathogenicity of *Gloeosporium album* to maiden shoots of Cox's Orange Pippin. *Plant Pathol.* 11:110-114.
- Xu, X. M., and Robinson, J. D. 2010. Effects of fruit maturity and wetness on the infection of apple fruit by *Neonectria galligena*. *Plant Pathol.* 59:542-547.
- Zhang, Z., Tian, S., Zhu, Z., Xu, Y., and Qin, G. 2012. Effects of 1-methylcyclopropene (1-MCP) on ripening and resistance of jujube (*Zizyphus jujube* cv. Huping) fruit against postharvest disease. *LWT-Food. Sci. Technol.* 45:13-19.