Effect of Preharvest Application of Cyprodinil on Postharvest Decay of Apples Caused by *Botrytis cinerea*

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ABSTRACT

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Dry-eye rot and gray mold of apple are important diseases caused by *Botrytis cinerea*. Fungicides available for their control are lacking, and this study was conducted to determine if cyprodinil (Vangard) could be used for this purpose. The mean EC_{50} value of cyprodinil for 32 *Botrytis* spp. isolates (27 from apple) was $0.02 \mu\text{g ml}^{-1}$, indicating that apple isolates are generally very sensitive. Some of the isolates (19%) were less sensitive and had EC_{50} values greater than $0.03 \mu\text{g ml}^{-1}$, and one isolate from 'Gala' apple was considerably less sensitive at $0.095 \mu\text{g ml}^{-1}$. Bloom sprays of cyprodinil alone in 1998 and 1999 or in combination with myclobutanil or metiram in 1998 reduced *Botrytis* spp. infection on developing fruit. Postharvest application of cyprodinil in 1998 indicated that cyprodinil protected apples from gray mold for 3 months. Cyprodinil applied 2 to 3 weeks before harvest in 1999 reduced lesion diameters 68 and 62% on 'Jonagold' and 'Gala' apples, respectively, that had been wounded and inoculated with *B. cinerea* after storage at 1°C for 6 months. In similar trials on 'Gala' apples in 2000 and 2001, preharvest applications of cyprodinil consistently reduced gray mold incidence and lesion diameter on inoculated apples stored for 6 months. New preharvest use patterns for cyprodinil are discussed for control of postharvest diseases caused by *B. cinerea*.

Additional keywords: fruit quality, *Penicillium*, resistance, thiabendazole

Botrytis cinerea Pers.:F causes two diseases of pome fruit. Dry-eye rot, also known as blossom-end rot, has been reported in North America, Europe, and New Zealand (16). The disease in North America is characterized by a dry and shallow red discoloration at the base of one or more sepals on the calyx end of the fruit (9). Immature 'Gala' apples from six orchards in British Columbia, Canada were recently surveyed with these symptoms, and *Botrytis* spp. were isolated from most of the fruit (20). The infected fruit dropped to the ground before harvest and did not appear to lead to any infection in storage. This is in contrast to what occurs in Norway on apples (27) and in California (22) and South Africa (3) on pears. Infection occurs during bloom and remains latent until the fruit matures just before harvest or in storage, when the fungus becomes active again and rots the fruit. In general, dry-eye rot occurs sporadically and seldom causes serious economic loss on apples (9).

B. cinerea also causes the postharvest disease known as gray mold. Gray mold is the most important postharvest disease of

pears and is second to blue mold in importance on apple (15). Conidia of *B. cinerea* have been found to occur in pome fruit orchards, and concentrations up to 3.1 conidia per liter of air have been reported (25). In the packinghouse, *Botrytis* spp. populations in the air are relatively low, ranging from 0 to 0.2 colonies per 10-cm-diameter petri dish and, in the dump tank, the numbers ranged from 0 to 100 propagules per milliliter of dump tank water (24). Wounds or injuries are the primary infection courts for the initiation of gray mold (17). The fungus forms a nest rot by growing from infected to healthy fruit at storage temperatures as low as -0.6°C (14).

In the early 1990s, fungicides belonging to two chemical families, the benzimidazoles and dicarboximides, were the most important for control of *B. cinerea* (5). Dicarboximides are only used on domestic pome fruit crops in the Pacific Northwest of the United States and are not used in British Columbia (BC), Canada. However, benzimidazoles are used, and resistance has become widespread. It was first identified by Bertrand and Saulie-Carter (1), who found a small number (3%) of *B. cinerea* isolates in Oregon and Washington pome fruit packing houses that were resistant to benomyl in both the 1975-1976 and 1976-1977 seasons. By 1984 the number of resistant isolates had risen to 35% (24). More recently, Sholberg and Haag (19) found an average of 22% of the isolates

from one packinghouse in British Columbia were resistant to benomyl.

Fungicides effective for control of *B. cinerea* on apple are needed because only thiabendazole, a benzimidazole, is presently used, although dry eye rot (blossom end rot) and gray mold are important diseases. A new chemical class of fungicides, the anilinopyrimidines, have recently been shown to have efficacy against species of *Botrytis* (4). Cyprodinil, an important member of this class, was recently registered on apple for the control of apple scab and could be an effective material for control of apple diseases caused by *Botrytis cinerea*.

The objectives of this study were to: (i) determine the sensitivity of local isolates of *B. cinerea* from apple to cyprodinil by comparing their EC_{50} values to those in published reports, (ii) evaluate cyprodinil applied at bloom for prevention of fruit infection by *Botrytis* spp., (iii) evaluate cyprodinil applied preharvest and postharvest for control of gray mold on fruit stored up to 6 months, and (iv) determine if cyprodinil impacts fruit quality of commercially mature apples. A preliminary report of this research has been published (21).

MATERIALS AND METHODS

EC_{50} values for *Botrytis* spp. isolates.

Tissue suspected to be infected with *Botrytis* spp. were sampled from several orchard locations throughout the Okanagan Valley of BC (Table 1). Thirty-two of the isolates were from immature or mature apples and the remaining five isolates were from other hosts that were included for comparison. The isolates were all collected in 1999, with the exception of one apple isolate collected in 1991. All the isolates, except one from tulip, are considered to be *Botrytis cinerea* Pers.:Fr. based on morphological characteristics. The isolate from tulip is also likely *B. cinerea* but could also be *B. tulipae* (Lib.) Lind and will require further study for positive identification. Isolates were maintained on potato dextrose agar (PDA) (Difco, Detroit, MI) at 2°C , until needed. A special synthetic medium termed Asp agar (7) was used for testing sensitivity to cyprodinil because "rich" media reduce the effect of this fungicide. Asp agar was prepared as follows: 1 g of K_2HPO_4 and 1 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were each dissolved in 30 ml of water (stocks I and II), 0.5 g of KCl and 0.01 g of

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FeSO₄·7H₂O were dissolved in 40 ml of distilled water (stock III), 2 g of L-asparagine and 15 g of agar were dissolved in 400 ml of water (stock IV), and 22 g of glucose-1 H₂O were dissolved in 490 ml of water (stock V). Stocks I and II were pooled. The precipitate that was formed was dissolved by adding 10 M hydrochloric acid dropwise, then stock III was added. Again, the formation of a precipitate was observed, which dissolved after adding stock IV and autoclaving for 20 min. Stock V was autoclaved separately and was pooled with the rest. Commercial grade cyprodinil (Vanguard 75 WG, Novartis Crop Protection Canada Inc., Guelph, Ontario), suspended in sterile distilled water (SDW) to give known stock solutions of active ingredient (a.i.), was added to 250 ml of cooled agar (45°C) while stirring to give the appropriate concentrations. The agar was poured into 60-mm-diameter polystyrene petri dishes. A 5-mm diameter disk from the margin of a 5- to 7-day-old *Botrytis* spp. culture on PDA was placed in the center of the plate containing approximately 10 ml of 0, 0.01, 0.025, 0.05, 0.075, 0.1, and 1.0 µg of amended Asp agar medium per ml. Each concentration was replicated three times. Plates were incubated at 20°C for 6 days, when mean colony diameter of two orthogonal measurements were

recorded. EC₅₀ values were determined by two different methods. The first method, adopted from Hilber and Schüepp (7), involved regressing the relative growth (colony diameter on cyprodinil-amended agar divided by the diameter on unamended agar × 100) against the log of the fungicide concentration using the GLM procedure (SAS Institute, Cary, NC). The second method involved nonlinear regression based on the following one-phase exponential model: $y = ae^{-bx}$, where y = relative growth, x = fungicide concentration, and a and b are parameters (GraphPad Software, San Diego, CA). In this method, $x = EC_{50}$ when $y = 0.5$. Also note that for both linear and nonlinear methods, $x = EC_{50}$ when $y = 0.5$. Following calculation of EC₅₀ and R^2 values, a paired t test was conducted on the paired R^2 and EC₅₀ µg ml⁻¹ values for each isolate.

Cyprodinil treatments applied at bloom and their effect on immature and mature apples. The trials were conducted in 'Gala' and 'Spartan' orchard blocks at the research orchard (Pacific Agri-Food Research Centre (PARC), Summerland, BC, Canada). Treatments on single-tree replicates were arranged according to the randomized complete block design in four 'Gala' and five 'Spartan' blocks. Fungicide treatments consisted of cyprodinil alone

(0.16 g a.i. per liter) or cyprodinil in combination with myclobutanil (0.16 + 0.09 g a.i. per liter) (Nova 40 W, Rohm and Haas Canada, Inc., Westhill, ON) or metiram (0.16 + 2.92 g a.i. per liter) (Polyram 80 DF, BASF Canada Inc., Toronto, ON). The bloom sprays were applied to 'Gala' at pink (29 April), full bloom (6 May), and petal fall (11 May). Similarly bloom sprays were applied to 'Spartan' on 27 April, 5 May, and 8 May. The fungicides were applied by handgun or backpack sprayer until run-off. Infection of fruit by *Botrytis* spp. was monitored on developing immature fruit of both 'Gala' and 'Spartan'. Samples of 50 or 25 fruit, randomly chosen from each treatment, were returned to the laboratory and tested for infection by *Botrytis* spp., according to the method of Northover and Cerkauskas (13), after each sampling date on 7 May, 15 May, 10 June, and 24 June. Commercially mature apples were harvested on 8 September (Gala) and 16 September (Spartan), and fruit weight, firmness, starch index, titratable acidity, and soluble solids were determined for 10 fruit from each treatment replicate, according to the method of Meheriuk et al. (11). Harvested fruit from each treatment replicate were stored for 3 months in air at 1.0 ± 0.2°C. Upon removal from storage, subsamples of fruit from one replicate of each treatment were collected, were swabbed with a sterile Q-tip, and were streaked on PDA to quantify *Botrytis* spp. and identify other fungi present on the fruit. Replicate samples of stored treated apples were wounded in triplicate with a 2.5-mm diameter nail to a depth of 3-mm. The wounds were inoculated with 20 µl of a *B. cinerea* conidial suspension (5 × 10⁵ CFU/ml) or 20 µl of SDW. The inoculated apples were incubated at 20°C for 7 days. Decay diameters indicated by reddish-brown discoloration were measured in two directions, were averaged, and were statistically analyzed using the GLM procedure (SAS, Cary, NC). Means for each treatment were separated by the LSMeans comparative test or the least significant difference mean separation test.

Postharvest cyprodinil treatments and their effect on *Botrytis* spp. infection. 'Gala' and 'Spartan' apples from control trees were grouped into five replicate samples of 10 apples per sample and were treated with thiabendazole (TBZ) (0.22 ml a.i. per liter) (Merck Frosst Canada, Inc., Kirkland, QU) or cyprodinil (0.31 g a.i. per liter) by dipping in a water suspension of these fungicides for 3 min 10 days after harvest. The apples were also evaluated for the effect of TBZ and cyprodinil on natural contamination of the fruit. The apples were stored for 3 months, were inoculated with *B. cinerea*, and were analyzed for decay as above.

Preharvest cyprodinil treatments and their effect on stored fruit. The trials were conducted in 'Gala' and 'Jonagold'

Table 1. Sensitivity (EC₅₀) to cyprodinil and sampling information for *Botrytis* spp. isolates from British Columbia

Isolate	Sampling date	Host	Plant part	Location ^y	EC ₅₀ (µg ml ⁻¹) ^z
B5	04/99	Apple, 'Braeburn'	Stored Fruit	Summerland	0.006
B7	04/99	Apple, 'Braeburn'	Stored Fruit	Summerland	0.021
B9	04/99	Apple, 'Braeburn'	Stored Fruit	Vernon	0.006
B11	04/99	Apple, 'Braeburn'	Stored Fruit	Vernon	0.005
B26	05/99	Apple, 'Braeburn'	Immature Fruit	Oliver	0.039
B27	05/99	Apple, 'Braeburn'	Immature Fruit	Summerland	0.040
B60	07/99	Apple, 'Braeburn'	Immature Fruit	Oliver	0.023
B72	07/99	Apple, 'Braeburn'	Immature Fruit	Kelowna	0.023
B116	09/99	Apple, 'Braeburn'	Immature Fruit	Kelowna	0.010
B124	09/99	Apple, 'Gala'	Mature Fruit	Summerland	0.021
B127	09/99	Apple, 'Gala'	Mature Fruit	Summerland	0.020
B3339	12/99	Apple, 'Gala'	Stored Fruit	Vernon	0.008
B3349	12/99	Apple, 'Gala'	Stored Fruit	Westbank	0.005
B3367	12/99	Apple, 'Gala'	Stored Fruit	Cawston	0.095
B43	05/99	Apple, 'Jonagold'	Immature Fruit	Summerland	0.024
B102	08/99	Apple, 'Jonagold'	Immature Fruit	Summerland	0.014
B104	08/99	Apple, 'Jonagold'	Immature Fruit	Summerland	0.013
B1644	05/91	Apple, 'McIntosh'	Stored Fruit	Winfield	0.041
B3337	12/99	Apple, 'McIntosh'	Stored Fruit	Summerland	0.012
B90	07/99	Apple, 'Red Del.'	Immature Fruit	Kelowna	0.009
B91	07/99	Apple, 'Red Del.'	Immature Fruit	Kelowna	0.012
B1766	----	Apple, 'Red Del.'	Mature Fruit	Kelowna	0.008
B1767	----	Apple, 'Red Del.'	Mature Fruit	Kelowna	0.020
B87	07/99	Apple, 'Spartan'	Immature Fruit	Westbank	0.008
B153	09/99	Apple, 'Spartan'	Mature Fruit	Westbank	0.050
B3294	12/99	Apple, 'Spartan'	Stored Fruit	Oliver	0.018
B3297	12/99	Apple, 'Sunrise'	Stored Fruit	Penticton	0.011
B1728	10/99	Sea buckthorn	Mature Fruit	Summerland	0.024
B1710	08/99	Strawberry	Mature Fruit	Summerland	0.008
B1684	06/99	Cherry	Mature Fruit	Unknown	0.010
B1709	08/99	Peach	Mature Fruit	Unknown	0.010
B1663	04/99	Tulip	Flower Stalk	Summerland	0.030

^y Location refers to town in British Columbia, Canada nearest to where the sample was obtained.

^z EC₅₀ value was determined based on a mycelial growth assay on Asp agar amended with cyprodinil.

orchard blocks at PARC. The 'Gala' orchard block used in 1998 was used again in 1999, and treatments were applied as in 1998. The 11-year-old 'Jonagold' trees were on Malling 7 rootstocks. Single-tree replicates (4 blocks for 'Jonagold') were treated with cyprodinil applied at different times. Bloom sprays were applied at pink (30 April, 'Jonagold'; 4 May, 'Gala') and full bloom (13 May, 'Jonagold'; 14 May, 'Gala'). Preharvest treatments were applied (26 August, 'Gala'; 9 September, 'Jonagold') and fruit were harvested (29 September, 'Gala'; 1 October, 'Jonagold'). Fruit quality was assessed as in 1998. Harvested fruit from each treatment replicate were stored for 3 to 6 months in air at $1.0 \pm 0.2^\circ\text{C}$, and decay was evaluated as described above.

The 2000 and 2001 trials were conducted in the same 'Gala' orchard block as used previously in 1998 and 1999. Replicates of four 'Gala' trees with 'Spartan' guard trees were arranged in four (2000) or five (2001) blocks. Preharvest treatment with cyprodinil (0.31 g a.i./liter) in 2000 was on 5 September, and fruit was harvested 20 days later. In 2001, cyprodinil (0.31 g a.i. per liter) was applied on 4 September, and fruit was harvested 10 days later (14 September). Harvested fruit was tested for quality as in previous years. Fruit from each treatment replicate were stored for 3 to 6 months in air at $1.0 \pm 0.2^\circ\text{C}$, and decay was evaluated as above.

RESULTS AND DISCUSSION

EC₅₀ values for *Botrytis* spp. isolates.

The nonlinear regression model fit the data better than the linear regression model based on the mean R^2 values (Table 2). Transforming data was not necessary with the nonlinear regression model, which is preferable according to Motulsky (12). The EC₅₀ values did not differ significantly between models and came to the same numerical mean; therefore, previously published EC₅₀ values based on the linear regression model remain valid. In this study, all EC₅₀ values were calculated with the nonlinear regression model, because the values for specific isolates always had higher R^2 values than those found with the linear regression model. The EC₅₀ values of the isolates ranged from 0.005 to 0.095 $\mu\text{g ml}^{-1}$. Most of the isolates had EC₅₀ values less than 0.05 $\mu\text{g ml}^{-1}$, and the largest number of values were between 0.005 and 0.03 $\mu\text{g ml}^{-1}$.

Six isolates were less sensitive than the rest, with EC₅₀ values ranging from 0.03 to 0.095, and would be considered candidates for possible field resistance according to Hilber and Hilber-Bodmer (8), who chose 0.03 $\mu\text{g ml}^{-1}$ as the discriminatory dose. One of these isolates grew on 0.095 $\mu\text{g ml}^{-1}$ cyprodinil, indicating that it was able to grow at three times the discriminatory dose. In Swiss vineyards, at least nine cyprodinil applications are required before

field resistance can be expected (8). The isolates tested in this study had been exposed to at most one application of cyprodinil and would not likely have been selected for resistance. Thus some isolates from BC are naturally less sensitive to cyprodinil than Swiss isolates, but the main distinction between the two groups is the wide range in sensitivity for British Columbian isolates. Fortunately, the mean EC₅₀ value for all 32 isolates was only 0.02 $\mu\text{g ml}^{-1}$, so a baseline value of 0.03 $\mu\text{g ml}^{-1}$ can still be used to indicate the development of resistance in the BC population of *B. cinerea* isolates from apple.

Cyprodinil treatments applied at bloom and their effect on immature and mature apples. Bloom sprays of cyprodinil alone in 1998 and 1999 or in combination with myclobutanil or metiram in 1998 significantly reduced the incidence of infection by *Botrytis* spp. on developing

'Gala' fruit (Fig. 1). The results for 'Spartan' in 1998 were similar, except that bloom sprays of cyprodinil appeared to be more effective on 'Spartan' than on 'Gala', especially when combined with metiram. The results for 'Jonagold' in 1999 were more variable than for 'Gala' or 'Spartan' but followed a similar trend; application of cyprodinil reduced the percentage of *Botrytis*-infected fruit. In all cases, the effect was most pronounced within 1 month of the last application of cyprodinil or cyprodinil in combination with another fungicide. Beyond 1 month, there was little difference in *Botrytis* infection between the control and treated apples (data not shown).

Dry-eye rot of apple is controlled by vinclozolin or iprodione sprays applied at bloom in Norway (28). It is likely that cyprodinil would have reduced apple dry-eye rot if conditions had been conducive

Table 2. Comparison of nonlinear and linear regression models for calculating EC₅₀ $\mu\text{g ml}^{-1}$ values as measures of sensitivity to cyprodinil for 32 isolates of *Botrytis* spp.

Regression model	R^2 values		EC ₅₀ $\mu\text{g ml}^{-1}$ values	
	Mean	SD	Mean	SD
Nonlinear ^x	0.9441	0.054	0.01997	0.020
Linear ^y	0.8063	0.117	0.01990	0.020
Paired <i>t</i> test ^z	$P < 0.0001$		$P = 0.9394$	

^x Based on the one-phase exponential model $y = ae^{-bx}$, where y = relative growth, x = fungicide concentration, and a and b are parameters.

^y Based on linear regression of the relative growth, in percent of the control, on log₁₀-transformed fungicide concentration.

^z Paired *t* test was conducted on the paired R^2 and EC₅₀ $\mu\text{g ml}^{-1}$ values for each isolate. The correlation coefficient between the R^2 values for the linear and nonlinear models was 0.9638 ($P < 0.0001$).

Fig. 1. Mean percent of developing *A*, 'Gala' and *B*, 'Spartan' apples infected with *Botrytis* spp. in 1998 between 7 May and 24 June. Trees were sprayed to runoff with cyprodinil (0.31 g a.i./liter) or cyprodinil in combination with myclobutanil (0.16 + 0.09 g a.i. per liter) or metiram (0.16 + 2.92 g a.i. per liter) on 29 April, 6 May, and 11 May (Gala) or 27 April, 5 May, and 8 May (Spartan). Mean percent of developing immature *C*, 'Gala' and *D*, 'Jonagold' apples infected with *Botrytis* spp. in 1999 between 13 May and 14 June. Trees were sprayed to runoff with cyprodinil (0.31 g a.i./liter) on 4 May and 14 May (Gala) (cyprodinil B and cyprodinil B + PH) or 30 April and 13 May (Jonagold) (cyprodinil B and cyprodinil B + PH). The trees used for the cyprodinil B + PH treatment were scheduled to receive a later preharvest spray as well.

for its development. Sommer et al. (22) found that benomyl application in pear near the time of petal fall dramatically reduced the incidence of infected stamens and styles, as we found with 'Spartan' in 1998 when combined with metiram. The data on 'Gala' in 1999 supports the application of two sprays as recommended by De Kock and Holz (3), who applied sprays at late bloom and 1 month before harvest.

The statistical analysis of fruit quality data collected from 'Gala' and 'Spartan' apples (in 1998) and 'Gala' and 'Jonagold' apples (in 1999) treated with cyprodinil indicated no significant difference ($P > 0.05$) from the untreated apples in weight, firmness, starch index, soluble solids, and titratable acidity (data not shown).

No *Botrytis* spp. were recovered from any of the swab samples that were taken after the fruit had been in cold storage for 3 months. Total microbial counts were low, and organisms recovered from the fruit were predominantly saprophytes such as *Trichoderma* and *Aureobasidium* spp. Bloom sprays with cyprodinil did not reduce decay in 'Gala' or 'Spartan' apples inoculated with *B. cinerea* after storage for 3 months in 1998 nor on 'Jonagold' apples after storage for 6 months in 1999 (Table 3). 'Gala' apples tested after 3 months storage developed virtually no *B. cinerea* decay regardless of bloom spray treatment. No effect of bloom sprays on incidence of *B. cinerea* decay of 'Gala' fruit was evident after 6 months (Table 4). It is not surprising that treatment of immature apples with cyprodinil failed to prevent growth of *B. cinerea* on the fruit 3 or 6 months after harvest. A duplicate set of fruit wounded but not inoculated with *B. cinerea* developed *Penicillium* rot in some of the wounds. Analysis of these data showed that there were no differences between the percentage of wounded apples that developed *Penicillium* rot. It can be concluded from these trials that cyprodinil blossom sprays alone are not likely to con-

trol postharvest decay caused by *Botrytis* or *Penicillium* spp. in commercially stored apples.

Preharvest cyprodinil treatments and their effect on stored fruit. In 1999, both bloom and preharvest sprays were applied to 'Jonagold' and 'Gala' apples. The fruit were stored for 3 and 6 months before wounding and inoculating with *B. cinerea*. The preharvest cyprodinil spray applied 3 weeks before harvest prevented the mean lesion diameter from expanding beyond the original wound (4-mm) in 'Jonagold' apples and effectively prevented decay (Table 3). After 6 months, the effect of the cyprodinil preharvest spray was still evident, resulting in a 68% smaller mean lesion size than the nontreated control. 'Gala' preharvest cyprodinil spray trials were conducted in three consecutive years, resulting in a significantly lower frequency of infected wounds (Table 4). The reduction in decay diameter in 2000 was not as great as in 1999 and 2001. It is possible that, in 2000, the apples were less resistant after storage because they were overmature at harvest. They had a starch index range of 4.2 to 4.6 compared with 2.8 to 3.8 in 1999 and 2.7 to 4.1 in 2001. Earlier harvest within the range of acceptable maturity reduces decay risk (26).

Decay of pome fruit in North America by *Botrytis* spp. results from infection that occurs during wounding at harvest or during sizing when the fruit are in flumes (17). These repeated preharvest trials with 'Jonagold' and 'Gala' apples show the

powerful effect cyprodinil has in preventing infection of wounds and development of decay. In Canada and the United States, cyprodinil is not registered for control of *Botrytis* spp. on apples or pears, although it is registered for this use on grapes. It can be used on apples for control of scab up to 72 days before harvest, allowing for bloom sprays. This may provide some benefit for preventing dry-eye rot of apples but, as the data in this study shows, it will not consistently reduce *Botrytis* rot in storage. Cyprodinil would be much more useful to North American pome fruit growers who store fruit for 3 or more months if it could be used up to 3 weeks before harvest. Of course, this would be contingent on maintaining acceptable consumer safety with regard to pesticide residues. Preharvest sprays to control postharvest storage decay problems is not a new concept. For example, Colgan (2) found that late-season sprays of captan reduced the incidence of *Nectria*, *Phytophthora*, and *Monilinia* rots of stored Cox's Orange Pippin apples. Preharvest sprays have been used to control Coprinus rot (LTB rot) of pears (23) and apples (18). As far as we know, this is the first report of using a preharvest fungicide treatment for the control of apple *Botrytis* rot (gray mold).

Postharvest cyprodinil treatments and their effect on *Botrytis* spp. infection. Treatment in 1998 of both 'Gala' and 'Spartan' apples 10 days after harvest with cyprodinil reduced decay by *B. cinerea* in fruit stored for 3 months (Table 5).

Table 3. Lesion diameter (mm) on Jonagold apples treated with cyprodinil (0.31 g a.i./liter) in 1999 and stored for 3 and 6 months

Cyprodinil applications	3-month storage ^y	6-month storage
None	45.0 a	25.0 a ^z
2 Bloom	24.7 b	25.5 a
1 Preharvest	4.0 c	8.1 b

^y Apples were stored in air ($1 \pm 0.2^\circ\text{C}$) for 3 or 6 months. After storage, four replicates of five apples per treatment were wounded in triplicate, and each wound was inoculated with 20 μl of a suspension containing 1×10^5 CFU/ml of *B. cinerea*. The fruit were incubated at 20°C for 5 to 7 days, when diameters of rot lesions were measured and recorded. A value of 4 mm indicates no decay.

^z Values in columns followed by the same letter are not significantly different, according to the least significant difference mean separation test ($P > 0.05$).

Table 4. Percent incidence of decayed wounds and lesion diameter on Gala apples treated with cyprodinil (0.31 g a.i./liter) and stored for 6 months in three consecutive years

Cyprodinil applications	1999 ^y		2000 ^y	2001 ^y	
	% Incidence	Diameter (mm)	Diameter (mm)	% Incidence	Diameter (mm)
None	50.0 a ^z	18.4 a	21.0 a	65.6 a	21.0 a
1 Bloom	45.9 a	17.9 a	...	...	...
1 Preharvest	24.9 b	9.5 b	16.1 b	16.5 b	4.2 b
1 Bloom + 1 Preharvest	10.1 c	7.0 c	...	...	...

^y Apples were stored in air ($1 \pm 0.2^\circ\text{C}$) for 6 months, in 1999 to 2001. After storage, four replicates of five fruit per treatment were wounded in triplicate, and each wound inoculated with 20 μl of a suspension containing 1×10^5 CFU/ml of *B. cinerea*. The fruit were incubated at 20°C for 5 to 7 days when incidence of decay and diameters of rot lesions were measured and recorded. A value of 4 mm indicates no decay.

^z Values in columns followed by the same letter are not significantly different according to the least significant difference mean separation test ($P > 0.05$).

Table 5. Lesion diameter of apples treated with thiabendazole or cyprodinil immediately after harvest and stored for 3 months

Postharvest fungicide application	Rate (g a.i./liter)	Lesion diameter (mm)	
		Gala ^y	Spartan ^y
None	0.00	21.8 b ^z	19.0 a
Thiabendazole	0.45	25.2 a	19.1 a
Cyprodinil	0.31	9.8 c	17.7 b

^y Apples were stored in air ($1 \pm 0.2^\circ\text{C}$) for 3 months. After storage, four replicates of five fruit per treatment were wounded in triplicate, and each wound was inoculated with 20 μl of a suspension containing 1×10^5 CFU of *B. cinerea* per ml. The fruit was incubated at 20°C for 5 to 7 days, when diameters of rot lesions were measured and recorded. A value of 4 mm indicates no decay.

^z Values in columns followed by the same letter are not significantly different according to the LS means comparative test of SAS ($P > 0.05$).

Cyprodinil was more effective than thiabendazole, probably because the *B. cinerea* isolate used in this trial was resistant to thiabendazole. Benomyl resistance in *B. cinerea* is relatively common in BC, where an average of 10% of the population was resistant to benomyl in a 1991 to 1993 packinghouse survey (19). The most interesting discovery from this postharvest trial was that cyprodinil reduced *Botrytis* decay after the fruit had been stored for 3 months. Postharvest treatments applied before harvest are not expected to last this long. This led to preharvest trials in 1999 because of reluctance by the industry to use postharvest fungicides and the difficulties presented by regulatory authorities in registering fungicides for postharvest use.

The data presented in this study show that cyprodinil is very effective against isolates of *B. cinerea* from apples and could be an effective fungicide for the control of dry-eye rot (blossom end rot) and *Botrytis* rot (gray mold) of pome fruit. One preharvest spray approximately 3 weeks before harvest inhibited *B. cinerea* infection for 6 months after storage in 'Gala' apples in three consecutive years. Knauf-Beiter et al. (10) reported that the antifungal activity of cyprodinil is based on its systemic activity, and our results support this conclusion. Heye et al. (6) concluded that cyprodinil is most efficacious when applied before bloom early in the season. Triazoles are applied later to optimize the efficacy potential of both these fungicides and cyprodinil. This use pattern makes sense for apple scab but would not control dry-eye rot or gray mold. Dry-eye rot would be most effectively controlled by one late bloom spray and gray mold with one spray applied 1 to 3 weeks before harvest. The use of only one cyprodinil spray per season, or two when both diseases are a problem, should minimize chances of fungicide resistance.

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