Control of rhizopus rot of peaches with postharvest treatments of tebuconazole, fludioxonil and *Pseudomonas syringae*

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Accepted: 29 January 2002 for publication in the Canadian Journal of Plant Pathology.

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Abstract: Rhizopus rot caused by *Rhizopus stolonifer* is a potentially serious postharvest disease of Canadian-grown peaches. Several new fungicides are effective against brown rot (*Monilinia fructicola*) of peaches, but little is known of their postharvest efficacies against *R. stolonifer*. Harvested peaches were arranged in trays, individually punctured once and co-treated with a suspension of both *R. stolonifer* sporangiospores and cells of an isolate of *Pseudomonas syringae* or one of 13 fungicides at 0.5-1.0 times the dilute concentration used to control brown rot in orchard programs. In subsequent experiments, the peaches were inoculated with *R. stolonifer*, incubated for 6 h, chilled, and a day later the inoculated sites were brushed with a suspension of fludioxonil, tebuconazole or *P. syringae*. In the co-treatment studies, azoxystrobin, fenhexamid, fenbuconazole, myclobutanil, benomyl and sulfur reduced rhizopus rot by 0-22%. Cyprodinil and propiconazole gave 20-60% reduction, while fludioxonil, tebuconazole and two *P. syringae* isolates gave 75-100% reduction, comparable to that of dicloran and iprodione. Post-inoculation brushing treatments with *P. syringae* at 10,000µg ESC 10/mL gave less than 20% control while fludioxonil and tebuconazole gave 90% control (EC_{90}) at 472 µg/mL and 718 µg/mL, respectively.

Keywords: biocontrol, Bio-Save™, black whiskers, Elite™, leak, Medallion™, Scholar™, transit rot.

Introduction

In southern Ontario, Canada, rhizopus rot, caused by *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. (*R. nigricans* Ehrenb.), is a potentially serious postharvest disease of stone fruits, particularly peaches. Brown rot caused by *Monilinia fructicola* (Wint.) Honey, is currently the more damaging disease (Biggs and Northover 1985) and fungicides are used for its control with no regard for their effects on the incidence of rhizopus rot. In 1999, *R. stolonifer* and *R. oryzae* Went & Prinsen Geerligs (*R. arrhizus* A. Fischer) were recovered from rotting, fallen peaches on the Niagara peninsula (Northover, unpublished), and the latter also might become a locally important postharvest pathogen. Besides affecting stone fruits (Luepschen et al. 1971, Ogawa 1995, Ogawa et al. 1963, Sholberg and Ogawa 1983), species of *Rhizopus* also cause serious production losses of strawberries and raspberries (Dennis 1983), sweet potatoes (Martin 1964), cantaloupes (Wade and Morris 1982) and grapes (Ben-Arie et al. 1991).

Rhizopus rot is seldom if ever seen on peaches prior to commercial (firm) ripeness except on fruits with tissue separation from split pits (Luepschen et al. 1971). The disease normally develops after harvest, during transportation (transit rot) and particularly as the fruits ripen prior to consumption. Infections occur at stem-end skin tears, at bruises and at wounds created during harvesting and possibly during grading and packing. Most probably these infections arise from sporangiospores on the fruit surface that germinate in juice released from bruises and on the freshly exposed tissues at mechanical injuries. Prior to harvest, these spores are blown or splash-dispersed primarily from rotting fruits with mature sporangia on the soil surfaces (Ogawa 1995) of adjacent orchards where the fruits were harvested earlier. The postharvest losses from rhizopus rot are greatly increased by the rapid spread of the fungus to adjacent fruits during ripening, resulting in nests of soft, rotten peaches covered with mycelium and sporangia.

Three principal practices have been employed for the control of rhizopus rot. These are firstly a preharvest spray, secondly, an early post-pick or "pre-storage" treatment possibly combined with hydrocooling prior to cold storage, and thirdly, a post-cold storage treatment by dipping or spraying, often in combination with wax, immediately prior to grading and packing. The preharvest fungicide application is important because it might kill sporangiospores on the fruit surface, and would provide some protection during harvesting. "Pre-storage" hydrocooling usually includes chlorination of 50-100 µg chlorine/mL but this may not control rhizopus rot (Ogawa et al. 1963). At this early postharvest stage, the sporangiospores will not have germinated, so that this would be the preferred time for treatment with a biocontrol agent or fungicide. The third time of treatment occurs one to several days after harvest with peaches that have been stored at 0-1EC with or without hydrocooling. Hydrocooling and cold storage are used to slow fruit maturity, to increase flesh firmness, and to reduce bruising during washing, defuzzing, chemical treatment (waxing), grading and packing.

Dicloran is very effective against rhizopus rot (Luepschen et al. 1971, Ogawa et al. 1964) and has been recommended in Ontario since 1967 (Anon. 1967, 2000). It may be used as a preharvest spray or as a postharvest dip treatment. It used to be applied as a postharvest spray, with or without wax, immediately before grading and packing. However, very little if any dicloran is used now, because of consumer resistance to the occasionally unsightly yellow residues remaining on treated fruits. Iprodione is also very effective against rhizopus rot in a postharvest treatment (Szkolnik et al. 1975, 1976), and its postharvest use against brown rot and rhizopus rot was registered in the USA until 1996 (Förster and Adaskaveg 1999). Canadian registration for the postharvest use of iprodione was never obtained. The immediate preharvest (pre-pick) application of iprodione is moderately effective against postharvest rhizopus rot

(Yoder et al. 1992). In Canada, iprodione was the preferred preharvest treatment for the control of brown rot between 1983 and 1999, and during this period rhizopus rot was of negligible concern. Growers have not associated the lower incidence of rhizopus rot with the use of iprodione.

The recent introduction of several new fungicides and the potential registration of others for the improved control of brown rot, could reduce the preharvest use of iprodione, possibly leading to an increase in the incidence of rhizopus rot. Yoder et al. (1995) reported on the efficacy of four demethylation inhibiting (DMI) fungicides used in preharvest programs against postharvest rhizopus rot. Tebuconazole and propiconazole were as suppressive as iprodione and dicloran for 6 days, but their control was inferior after 8 days incubation at 16-26EC. In the same experiment, fenbuconazole and myclobutanil were ineffective against rhizopus rot. Of greater concern were the results obtained by Hickey et al. (1999) which showed that preharvest applications of fenbuconazole and tebuconazole resulted in a numerical increase in rhizopus rot of two peach varieties, and a significant increase from 16% to 43% of rhizopus rot of fenbuconazole-treated nectarines after 10 days of incubation. In blackberry, preharvest programs of fenhexamid increased the incidence of rhizopus rot (De Francesco and Koskela 2000, De Francesco et al. 1999), but a formulated combination of fludioxonil and cyprodinil (Switch) reduced the disease significantly (De Francesco et al. 1999). Förster and Adaskaveg (1999) have also reported the efficacy of fludioxonil against rhizopus rot.

Several microorganisms have been effective for the postharvest biocontrol of *Rhizopus* spp. (Droby and Chalutz 1994). Zhou et al. (1999) showed that *Pseudomonas syringae* at a concentration of 1×10^7 CFU/mL gave good control when applied with *R. stolonifer*. In contrast a concentration of 1.5×10^{12} CFU/mL of *Enterobacter cloacae* was required for good control of peach rhizopus rot (Wilson et al. 1987). The suspension of *P. syringae* in calcium chloride ($CaCl_2$) solution improved the control of *M. fructicola* (Zhou et al. 1999) and also increased the recovery of *P. syringae* in serial dilutions (Zhou unpublished). Certain saprophytic yeasts have also shown antagonistic activity against *Rhizopus* spp. These include *Pichia guilliermondii* (syn. *Candida guilliermondii* (Ben-Arie et al. 1991), used at a high concentration of 1×10^9 CFU/mL (Chalutz et al. 1991) and *Hanseniaspora uvarum* (syn. *Kloeckera apiculata*) (Ben-Arie et al. 1991, McLaughlin et al. 1992). *Candida sake* and particularly *Aureobasidium pullulans* (syn. *Pullularia pullulans*), which is widely distributed on temperate fruits, have shown strong antagonism towards *Rhizopus* spp. (Lima et al. 1997, 1999, Vinas et al. 1996).

The objectives of this study were to integrate the control of rhizopus rot with that of brown rot of peaches. Firstly, by determining for 13 fungicides and two isolates of *P. syringae* with activity against brown rot, their efficacy against rhizopus rot when applied simultaneously with inoculum of *R. stolonifer* to wounded peaches. Secondly, to evaluate two fungicides in post inoculation, post cold storage treatments. Thirdly, to determine the efficacy of *P. syringae* applied either simultaneously with *R. stolonifer* inoculum, or in post inoculation, post cold storage treatment, either with or without $CaCl_2$.

A brief preliminary report was published (Northover and Zhou 2000).

Materials and methods

Fruits from several varieties of peach (*Prunus persica* (L) Batsch) were harvested at commercial ripeness, with a yellow ground color over half the fruit surface. Whole orchards of a single peach variety had received a single commercial fungicide application 2-3 days before harvest, of captan, cyprodinil or propiconazole to control brown rot. The peaches were stored at 1EC within 3 h of harvest and were withdrawn as needed for experimental use.

***Rhizopus stolonifer* cultures**

A single isolate of *R. stolonifer* (DAOM 225707) supplied by Fungal Collections, Agriculture and Agri-Food Canada, ECORC, Ottawa, Ont., was used in the initial "co-treatment" experiments. In the later "post-inoculation" treatment experiments, four single sporangiospore cultures were used. These had been isolated in 1999, from infected peaches collected on the Niagara peninsula, Ontario, and were numbered: 99-W7, 12P-36, 7C-38 and 7E-20.

Isolates of *R. stolonifer* were grown on potato dextrose agar (PDA, Difco, Detroit, Mich.), initially on slants in glass tubes, (200 mm long, 24 mm wide) and later in Petri plates sealed with Parafilm. Cultures were grown in diffuse light at 23EC for 10 days. Sporangiospore suspensions were prepared by flooding slant cultures with 15 mL of water or by agitating clusters of sporangia from the margins of Petri plates in 25 mL of water. Sporangiospores from the four isolates were combined in approximately equal proportions. An hemocytometer was used to measure the concentration of the stock suspension which was diluted to give a final concentration of 3×10^3 sporangiospores/mL, after combination with suspensions of fungicides or *P. syringae*.

Fungicides, adjuvant and surfactant

Thirteen fungicides with the following formulations, product names, manufacturers and addresses, were compared against water controls: azoxystrobin (250 g/L, SC, Quadris or Abound, Syngenta Crop Protection, Guelph, Ont.); benomyl (50%, WP, Benlate, DuPont Canada Inc., Mississauga, Ont.); captan (75%, DF, Maestro, Tomen Agro Inc., Guelph, Ont.); cyprodinil (75%, WG, Vangard, Syngenta Crop Protection, Guelph, Ont.); dicloran (75%, WP, Botran, Gowan Co., Yuma, Ariz.); fenbuconazole (75%, WSP, Indar, Rohm and Haas Canada Inc., West Hill, Ont.); fenhexamid (50%, WG, Elevate, Tomen Agro Inc., Guelph, Ont.); fludioxonil (50% WP, Medallion, Syngenta Crop Protection, Guelph, Ont.); fludioxonil (25%) plus cyprodinil (37.5%)(62.5%, WG, Switch, Syngenta Crop Protection, Guelph, Ont.); iprodione (50%, WP, Rovral, Aventis Crop Science Canada Co., Mississauga, Ont.); myclobutanil (40%, WP, Nova, Rohm and Haas Canada Inc., West Hill, Ont.); propiconazole (250 g/L, EC, Topas or Orbit, Engage Agro Corporation, Cambridge, Ont.); sulfur (80% Kumulus, BASF Canada Inc., Toronto, Ont.) and tebuconazole (45%, WP, Elite, Bayer Corporation, Kansas City, Mo.).

Fenbuconazole was suspended in a solution of 1 mL of Companion, agricultural adjuvant (70% solution, octylphenoxypolyethoxy-(9)-ethanol, Rohm and Haas Canada Inc., West Hill, Ont.) dissolved in 1L of water. To improve fungicide drop adhesion to the peach surface immediately prior to post-inoculation brushing, tebuconazole and fludioxonil were suspended in a solution containing 0.1 g Tween 20 (100%, polyoxyethylene (20) sorbitan monolaurate, Fisher Scientific, Ottawa, Ont.) dissolved in 1L water.

Biocontrol agents

P. syringae van Hall MA-4 was stored in 30 % glycerol at -70 EC, and cultured in potato dextrose broth (PDB, Difco, Detroit, Mich.) shaken at 150 rpm at 22 EC in darkness for 3 days (Zhou et al. 1999). The cells were harvested by centrifugation at 6500 g for 5 min at 4 EC. The pellet was resuspended in sterile water. The bacterial concentration was determined with a spectrophotometer (DU 640, Beckman Instruments, Fullerton, Calif.) at 420 nm. The bacterial cell concentration was determined from a calibration curve that related absorbance to the number

of colony forming units (CFU) per mL, from a dilution plating series on PDA.

P. syringae strain ESC-10 was provided by EcoScience Produce Systems Corp., (Orlando, Fla.), as a lyophilized powder (ESC 10) in a sealed container, that was stored at -20 EC. In the initial experiments, a fresh culture of ESC-10 was prepared in the same manner as used for MA-4. In later experiments, samples of a freshly received lyophilized powder of ESC 10 (guaranteed minimum of 9 x 10^{10} CFU/g) were suspended in sterile water at 23EC for 40 min with occasional agitation before being used.

Inoculation and treatment protocols

Peaches were removed from 1EC storage, graded, and placed cheek-side uppermost in 18-or 20-cell plastic trays (Panta-Pak, Richter Mfg., Visalia, Calif.) within plastic boxes (52 x 36 x 14 cm) that could be closed with lids. Peaches at 25EC were wounded once with a flame-sterilized, nail-like, tapered probe 5 mm deep and 4 mm wide at its base, where a collar 12 mm in diameter limited the depth of the wound. Within 2 min of wounding, each site was inoculated with a single 24 µL drop containing 3 x 10^3 sporangiospores of *R. stolonifer* /mL.

Co-treatment experiments

In the co-treatment experiments, the treatment "drop" contained sporangiospores from one isolate of *R. stolonifer* (DAOM 225707) combined with a fungicide or cells of *P. syringae*. This method of treatment was intended to simulate a commercial "post-pick" or hydrocooling treatment applied to peaches a few hours after being wounded and naturally inoculated during harvesting operations. Immediately after co-treatment, the boxes of fruits were closed with tightly-fitting lids and incubated at 25EC for 4 days (7 days for Babygold 7 peaches) prior to an evaluation of the incidence of rhizopus rot. Four replicates each of 10 peaches/treatment were used in these experiments.

The first experiment examined the effect of the sporangiospore suspension containing one of 10 fungicides at concentrations equivalent to half the registered or suggested rate/ha for the control of brown rot, suspended or emulsified in 3000 L of water ("dilute" spray concentration). Fenbuconazole was suspended in 1 mL Companion/L water. Cultures of *P. syringae* ESC-10

and MA-4 at concentrations of 1 x 10^8 CFU/mL water, were co-applied with *R. stolonifer* sporangiospores. The experiment was conducted on Redhaven peaches and repeated later with Harrow Beauty peaches, both of which had been stored at 1EC for 3 days after harvest.

The second experiment was conducted twice (2A and 2B) with Babygold 7 peaches and evaluated 4 fungicides suspended in water at concentrations equivalent to their full registered rate/ha in 3000 L of water. The peaches had been stored at 1EC for 8 days (2A) and 16 days (2B) respectively.

The third experiment was conducted twice with Babygold 7 peaches to compare, in the same experiment, the efficacy against rhizopus rot, of fludioxonil at concentrations of 6.2 - 50.0 µg/mL when applied as either a 50% formulation (Medallion) or a 25% formulation combined with 37.5% cyprodinil (Switch). The peaches used in these two experiments had been stored at 1EC for 8 and 16 days, respectively.

Post-inoculation fungicide experiments

The post-inoculation experiments were intended to simulate commercial situations where peaches are treated 1 day after harvest. Prior to experimental use, harvested peaches of 3 varieties were stored at 1EC for up to 5 days, as reported in a footnote to Table 4. The peaches were wounded and inoculated with a suspension of 3 x 10^3 sporangiospores/mL derived from four isolates. Inoculated peaches were incubated in closed boxes at 25EC for 6 h resembling the method used by Kim et al. (1997). The closed boxes were then stacked in a 1EC, forced-air, cold storage room for 11 h, overnight during which time the surface temperature of the peaches declined to 2EC as measured by 2 Campbell Scientific 107 Temperature Probes connected to separate 21X Microloggers (Campbell Scientific, Edmonton, Alta.). The boxes were returned to 25 EC, the lids were removed and the peaches were warmed to 25 EC with fan-driven air over a 4-h period, allowing newly condensed moisture on the fruit surface to evaporate.

The previously inoculated peaches were treated with tebuconazole or fludioxonil (50% formulation) suspended in 0.1 g Tween 20/L water, to improve wetting of the peach surface. Both fungicides were used at concentrations of 1-400 µg/mL. In the first of two treatment methods, a

single 24 µL drop was applied to the inoculated site and covered an area approximately 3 mm in radius. The second method consisted of applying 3 drops each of 24 µL spaced equidistantly around the inoculation site, and 1 cm from it. The 3 drops were spread with 5 brush strokes, in different directions, covering an area approximately 19 mm in radius, centered on the inoculation site. The brush was moderately stiff, and the bristles were trimmed to be 25 mm long, cumulatively 30 mm wide and 8 mm thick. The brushing actions were intended to simulate the brushing or spreading devices used on conveyors to spread wax or fungicides applied to fruits, immediately prior to grading and packing. Before each brushing treatment, the brush was dried with paper toweling and conditioned by spreading three drops of the new fungicide treatment on each of three peaches that were not included in the experiment.

These "drop" and "brushing" treatments were conducted 2-4 times with fruits of 3 peach varieties using ranges of concentrations of tebuconazole and fludioxonil to obtain a range of disease reduction data. In each experiment, the treatments were replicated 3 times, each with 18-20 fruits that had been stored at 1EC for 0-5 days as given in a footnote to Table 4.

Co-treatment and post-inoculation treatment with Pseudomonas syringae ESC 10 suspended in water or calcium chloride solution.

Two sources of Madison peaches were stored at 1EC for 2-3 days, warmed to 24EC as needed for an experiment. The co-treated peaches were wounded and received a drop containing 3 x 10^3 sporangiospores combined with 100-10,000 µg of ESC 10 product/mL suspended in either sterile water or a sterile solution of 5g $CaCl_2.2H_2O$/L. For the post-inoculation treatments, the peaches were wounded and inoculated with a drop of 3 x 10^3 sporangiospores/mL of sterile water, incubated at 25EC for 6 h, cooled slowly to 2EC overnight and warmed to 25EC the next morning. The peaches were treated by brushing with suspensions of 100-10,000 µg ESC 10 /mL in either sterile water or a sterile solution of 5 g $CaCl_2.2H_2O$/L water. The treated peaches were incubated in closed boxes at 25EC for 4 days prior to evaluation for the development of rhizopus rot at the treatment site. The experiment was conducted twice with each treatment being replicated 3 times with 20 peaches/replicate. Similar data from the two experiments were

averaged and examined by probit analysis.

Data analysis

Data expressed as the percentage of peaches with rhizopus rot were transformed (arcsin/%) and examined by ANOVA. Significant (P<0.05) differences between paired means were determined using Fisher's test of least significance difference (LSD) for paired means. For experiments where disease reduction was calculated over a range of concentrations of fungicides or ESC 10 product, the data were examined using a probit analysis procedure (LeOra Software 1987). The effective concentrations giving 50% reduction (EC_{50}) and 90% reduction (EC_{90}) relative to the disease incidence in the inoculated control, were calculated with their 95% fiducial limits (Finney 1971). Paired EC values with non-overlapping 95% fiducial limits differed significantly (P<0.05).

Results

Efficacy of fungicides and biocontrol agents when co-applied with *R. stolonifer*

The cultures of ESC 10 and MA-4 and the fungicides fludioxonil + cyprodinil and tebuconazole were as efficacious as dicloran giving a 85-100% reduction of rhizopus rot (Table 1). Iprodione at 125 µg/mL appeared more effective on Harrow Beauty (97%) than on Redhaven (55%). Propiconazole and cyprodinil alone gave 20-60% control, whereas azoxystrobin, captan, fenbuconazole and fenhexamid reduced rhizopus rot by 0-22%.

In the second experiment, iprodione at 250 µg/mL gave 77-95% control of rhizopus rot, but benomyl, myclobutanil and sulfur were ineffective (Table 2).

In the comparison of the efficacies of the two formulations of fludioxonil, the 50% formulation (Medallion) was significantly more effective with a lower EC_{50} value of 6.0 µg/mL than the 25% formulation which included cyprodinil (Switch) (EC_{50} 9.9 µg/mL) (Table 3). However, the respective EC_{90} values were not significantly different (16.6-17.1 µg/mL).

Efficacy of post-inoculation treatment with tebuconazole and fludioxonil

For the "drop" and "brushing" treatments with tebuconazole and fludioxonil of peaches inoculated one day earlier, the replicate and mean EC_{50} and EC_{90} values are shown in Table 4.

The "brushing" treatment with tebuconazole gave mean EC_{50} and EC_{90} values of 161 and 718 µg/mL respectively, that were 13.4 times and 21.2 times greater than the respective mean values for the drop treatments. These values were referred to as "spreading factors". Likewise, for fludioxonil the mean EC_{50} and EC_{90} values from brushing were 30.6 and 472 µg/mL, respectively, and were 9.0 times and 40.3 times greater than the respective mean values for the "drop" treatments.

A slight peach varietal response was apparent in some of the determinations. The EC_{50} values for tebuconazole were lower for Redhaven than for Harrow Beauty in both the "drop" and "brushing" methods, but the respective EC_{90} values were similar. With the fludioxonil "drop" treatment, the EC_{50} values for Loring were significantly greater than the value for Harrow Beauty. There were no differences between the EC_{50} and EC_{90} values for fludioxonil spread by "brushing" on Redhaven and Harrow Beauty peaches. These apparent varietal differences were not correlated with slight differences in the duration of storage of these peaches at 1EC prior to the start of each experiment.

Co-treatment and post-inoculation treatment with *P. syringae* ESC 10, with and without calcium chloride

The co-treatment of peaches with *R. stolonifer* and ESC 10 at 1000µg/mL suspended in water provided 88% disease reduction, but higher concentrations were not more effective (Table 5). The calculated EC_{50} and EC_{90} values were 113 and 8,219 µg/mL, but were without 95% fiducial limits. The use of $CaCl_2$ solution resulted in numerically lower responses and correspondingly higher EC_{50} and EC_{90} values of 843 and 11, 700 µg/mL, respectively. The post-inoculation "brushing" treatments provided poor control with less than a 20% reduction of rhizopus rot at 10,000 µg ESC 10/mL of either water or $CaCl_2$ solution.

Discussion

In the co-treatment studies, the weakness and inactivity of captan and benomyl relative to the efficacy of dicloran and iprodione confirmed earlier studies of these postharvest treatments (Szkolnik et al. 1975, 1976). However, we found very few publications of the efficacies of

postharvest treatments with new fungicides against rhizopus rot. Förster and Adaskaveg (1999) reported the superior activity of fludioxonil against several postharvest diseases including rhizopus rot. There were several reports of the effects of preharvest applications of fungicides on the incidence of postharvest rhizopus rot but the results were variable and sometimes contradictory. Field applications of fludioxonil combined with cyprodinil (Switch) were very effective in 1998, but in 1999 they were ineffective against rhizopus rot of blackberries (De Francesco and Koskela 2000, De Francesco et al. 1999). At certain postharvest intervals, tebuconazole and propiconazole reduced rhizopus rot of peaches, but were often inferior to iprodione (Yoder et al. 1994, 1995, 1996) These results contrast with the excellent activities of fludioxonil and tebuconazole and the moderate activity of propiconazole in our postharvest experiments. The relative efficacies of fungicides applied as preharvest or as postharvest treatments against rhizopus rot, were not consistent and could not be extrapolated reliably from one use to the other.

Fungicides that are ineffective against *R. stolonifer* may sometimes increase the incidence of rhizopus rot. Fenbuconazole, which had no activity against rhizopus rot in our co-treatment studies, increased rhizopus rot of nectarine (Hickey et al. 1999). Preharvest programs of fenhexamid worsened rhizopus rot of blackberries (De Francesco and Koskela 2000, De Francesco et al. 1999). Also in earlier studies, benzimidazole fungicides increased rhizopus rot of peaches (Northover and Howard 1976) and of late-harvested strawberries (Dennis 1975, Jordan 1973). This effect may be explained by the fungicidal suppression of naturally occurring biocontrol agents. *Aureobasidium pullulans* has been shown to control *R. stolonifer* (Lima et al. 1997, 1999), and the incidence of *A. pullulans* on peach trees has been reduced by a full-season program of benomyl (Northover 1992). Similarly, on strawberry fruits, benomyl and dichlofluanid reduced the incidence of *A. pullulans* and simultaneously increased the incidence of fruits infected by *R. stolonifer* (Dennis 1975). Slight differences in the ease of controlling rhizopus rot were noticed between peach varieties in the post-inoculation drop and brushing treatment comparison (Table 4). Tebuconazole was more effective with lower EC_{50} values on

Redhaven than on Harrow Beauty peaches, but this difference was not shown with fludioxonil. However, with fludioxonil, the EC_{50} values for drop application were slightly higher for Loring than for Harrow Beauty. These differences were tentatively attributed to the varieties involved, but a slight unnoticed difference in fruit maturity might have been a contributory factor. In other experiments, twice the concentration of fludioxonil was needed to control rhizopus rot of peaches stored for 16 days at 1EC compared with peaches stored for only 8 days. No such correlation was apparent between the length of pretreatment storage and EC_{50} values of tebuconazole and fludioxonil for the 3 peach varieties included in the main experiment (Table 4).

The treatment of freshly wounded peaches with a drop containing both *R. stolonifer* sporangiospores and a fungicide or biocontrol agent, was a convenient laboratory method for the initial phase of the study. However, the "drop" method had little relevance to the current methods of applying fungicides to harvested peaches on a large commercial scale. Growers' practices generally involve either a drench or hydrocooling treatment before storage, or else a drip or spray application followed by spreading of the deposit with brushes, rollers or fruit-against-fruit contact on conveyor lines prior to fruit packing. In both of these processes, the resulting deposit of treatment fluid would be less than that from a drop applied in the experimental method.

Our comparison of the "drop" and "brushing" methods, on previously-inoculated peaches, allowed the derivation of a "spreading factor" to relate the differing fungicidal efficacies of these two methods of treatment. The single undisturbed 24 µL drop covered an area with a radius of 3 mm, whereas 3 similarly-sized drops were brushed over a circular area of 19 mm radius. The ratio of the theoretical deposits per unit area for the "drop" : "brushing" methods was 13:1. Hence the concentration of fungicide for the "brushing" method would need to be 13 times greater than that for the "drop" method, to give the a similar deposit and fungicidal effect. The observed "spreading factors" for EC_{50} values for tebuconazole and fludioxonil of 13.4 and 9.0 respectively, were close to the theoretical value of 13.0. However, the respective "spreading factors" of the EC_{90} values of 21.2 and 40.3 were appreciably higher than the theoretical value,

for unknown reasons. The mean EC_{90} value for the "brushed" application of fludioxonil was 472 µg/mL which compared favorably with a value of 603 µg/mL that was effective against several postharvest diseases (Förster and Adaskaveg 1999).

Possibly it is inappropriate to use the same "spreading factor" to adjust the fungicide concentrations used in the co-treatment experiments, to the concentrations anticipated for a drench or hydrocooling treatment. This is because in the drop method, the *R. stolonifer* inoculum was mixed with each fungicide 1-2 h before it was applied to the freshly-created wound. During this time, the fungicide could have had a greater effect on sporangiospore viability than a brief exposure to fungicide during a drench treatment, followed by its residual effects. An additional consideration is that the co-treatment "drop" method would have resulted in relatively high fungicidal deposits at the treatment site. Applying the "spreading factor" of 13.0, the deposits were probably 6.5-13.0 times greater than those resulting from an orchard spray application, because the fungicides in the first and second experiments were used respectively at 0.5-1.0 times their recommended or suggested orchard application rate as a dilute concentration, (rate/ha/3000 L). Despite these reservations, the relative efficacies of many of these fungicides and *P. syringae* agreed well with published data (Förster and Adaskaveg 1999, Szkolnik et al. 1975, 1976, Zhou et al. 1999).

In our post-inoculation studies, treatments with tebuconazole and fludioxonil were effective when applied to fruit after a sequence of 6 h of post-inoculation incubation, cooling for 11 h to 2EC and subsequent warming over 4 h to 25 EC. By comparison, Kim et al. (1997) treated fruit with fungicides 6 h after inoculation, to simulate a "pre-storage" treatment interval. In earlier studies, dicloran was effective when peaches were treated 10-12 h after fruit inoculation (Cappellini and Stretch 1962, Ogawa et al. 1963).

In our biocontrol studies, co-treatment of *R. stolonifer* with fresh cultures of *P. syringae* MA-4 and ESC-10 at concentrations of 1×10^8 CFU/mL, gave 85-97% control of rhizopus rot. This confirmed the efficacy of *P. syringae* shown in our earlier study (Zhou et al. 1999). In the current co-treatment experiments, the ESC-10 lyophilized product, gave only 82% control at

10,000 µg/mL, equivalent to approximately 1 x 10^{10} CFU/mL. The application of ESC 10 suspended in $CaCl_2$ solution, resulted in numerically lower levels of control, than a suspension in water. In contrast, a fresh culture of *P. syringae* suspended in $CaCl_2$ solution gave a slightly improved control of a high incidence of latent brown rot infections of peach fruits, in comparison with its suspension in water (Zhou et al. 1999). The post-inoculation treatment of peaches with ESC 10 reduced rhizopus rot by less than 20%, and would be of no commercial interest. However, a pre-storage drench treatment of fruits with *P. syringae* might be effective for reducing relatively low levels of contamination by *R. stolonifer* and recent infections by *M. fructicola*.

Besides their activity against rhizopus rot, tebuconazole and fludioxonil were very efficaceous against symptomless brown rot infections of plum and peach. A 4-min soak of firm-ripe Stanley plums in tebuconazole at 100 µg/mL reduced brown rot from 90% to 14% (Northover and Cerkauskas 1998). A 2-min soak of peaches in tebuconazole at 25 µg/mL gave an 87-99% reduction of brown rot and 85% control was obtained with concentrations of 3-6 µg/mL (Northover and Homeyer 2001a). In the same study, a 2-min soak of peaches in fludioxonil reduced brown rot with an EC_{90} concentration of 107-136 µg/mL, comparable to that of iprodione (EC_{90} = 120 µg/mL) (Northover and Homeyer 2001a). By comparison, the calculated EC_{90} concentrations for post-inoculation treatments to control rhizopus rot with tebuconazole and fludioxonil were respectively, 718 µg/mL and 472 µg/mL. These latter concentrations appear sufficient to give simultaneously a high degree of control of both rhizopus rot and brown rot..

The choice of fungicides for postharvest use might affect the choice of fungicides and their pattern of use in the extended bloom to preharvest period. The frequent use of propiconazole resulted in the selection of *M. fructicola* populations with reduced sensitivity to propiconazole in South Carolina (Zehr et al.1999). However, propiconazole was still effective for crop protection, with no evidence of "practical resistance". Similarly, the frequent use of several DMI fungicides against grapevine powdery mildew (*Uncinula necator)* resulted in a fairly rapid change to "practical resistance" (Erickson and Wilcox 1997, Gubler et al. 1996, Northover and Homeyer

2001b). To avoid the possible development of resistance to tebuconazole, also a DMI material, in *R. stolonifer* and *M. fructicola*, the use of tebuconazole and other DMI fungicides including propiconazole, may need to be reduced during the preharvest period. This could be achieved by alternating with fungicides in other chemical groups such as cyprodinil (anilinopyrimidine group) and fludioxonil (phenylpyrrole group), that are also effective against *M. fructicola*. For economic reasons, fludioxonil and *P. syringae* may be better suited to postharvest use, furthermore their efficacy would not be affected by any loss of sensitivity of either *R. stolonifer* or *M. fructicola* to DMI or anilinopyrimidine fungicides applied prior to harvest.

Acknowledgments

We are grateful to Barry Kemp for his management of peach orchards and supply of fruit, to Gerry Walker for technical discussions, and for the technical support of Cheryl Homeyer, Karin Schneider, Noella Gray, Lisa Trang and Todd Larmet. We also extend our thanks to Tara McCaughley (Syngenta Crop Protection), Leo Blydorp (Bayer) and Lucie Grant (EcoScience Produce Systems Corp.) for the generous and timely supply of research samples.

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Table 1. Reduction of postharvest rhizopus rot of wounded Redhaven and Harrow Beauty peaches, co-treated with sporangiospores of *Rhizopus stolonifer* combined with *Pseudomonas syringae* or a fungicide.

Bacteria and fungicides	CFU/ml or Fg a.i./mL	Reduction of rhizopus rot relative to control (%)	
		Redhaven	Harrow Beauty
P. syringae, ESC-10 culture	1 x 10^8	97 a*	90 a
P. syringae, MA-4 culture	1 x 10^8	85 bc	92 a
Fludioxonil + cyprodinil	42 + 63	95 ab	100 a
Dicloran	406	87 ab	100 a
Tebuconazole	23	85 bc	97 a
Iprodione	125	55 d	97 a
Propiconazole	21	60 cd	44 b
Cyprodinil	93	45 d	20 bc
Azoxystrobin	25	22 ef	0 d
Captan	500	15 e	3 cd
Fenbuconazole†	18	2 fg	0 d
Fenhexamid	142	0 g	0 d
Inoculated control‡	-	0 g	0 d

* Arithmetic means in the same column with no letter in common are significantly (P<0.05) different by Fisher's LSD test for paired means, using transformed (arcsin/%) data.

†Fenbuconazole was suspended in Companion surfactant (1 ml/L water). The other fungicides were suspended or emulsified in water.

‡Rhizopus rot developed in 98-100% of the inoculated control peaches.

Table 2. Efficacy of four fungicides for reducing rhizopus rot of Babygold 7 peaches wounded and co-treated with sporangiospores of *Rhizopus stolonifer* and a fungicide.

Fungicide	Concentration Fg a.i./mL	Reduction of rhizopus rot relative to control (%)	
		Experiment 2A	Experiment 2B
Iprodione	250	95 a*	77 a
Benomyl	283	0 b	0 b
Myclobutanil	45	0 b	5 b
Sulfur	5333	0 b	0 b
Inoculated control†	-	0 b	0 b

*Means in the same column followed by a different letter are significantly (P<0.05) different using Fisher's LSD test for paired means.

†The incidence of peaches with rhizopus rot in the controls of both experiments was 100%.

Table 3. Efficacy of two formulations of fludioxonil for controlling rhizopus rot of Babygold 7 peaches, wounded and co-treated with *Rhizopus stolonifer* and a fludioxonil formulation.

Fludioxonil concentration (µg/mL)	Reduction of rhizopus rot relative to control (%)	
	Fludioxonil formulation	
	Medallion	Switch
0	0*	0
6.2	51	14
12.5	84	69
25.0	95	99
50.0	100	100
EC_{50} (µg/mL) (95% fiducial limits)	6.0 (4.8-7.1)	9.9 (9.1-10.8)
EC_{90} (µg/mL) (95% fiducial limits)	16.6 (14.2-20.6)	17.1 (15.3-19.8)

*The incidences of peaches with rhizopus rot in the controls were 100%.

Table 4. Efficacy of "drop" and "brushed" applications of tebuconazole and fludioxonil against rhizopus rot* of peaches inoculated with *Rhizopus stolonifer* and chilled overnight before treatment, expressed as concentrations giving 50% (EC_{50}) and 90% (EC_{90}) disease reduction, with their respective 95% fiducial limits.

Fungicide	Treatment method	Peach variety	EC_{50} values with fiducial limits (µg/mL).	
			EC_{50}	EC_{90}
Tebuconazole	drop	Redhaven	7.2 (4.9-10.7)†	20.5 (13.2-48.7)
	drop	Harrow Beauty	16.8 (15.0-18.8)	47.0 (40.1-56.9)
		mean:	12.0	33.8
	brush	Redhaven	90.6 (49.3-160)	734 (345-3924)
	brush	Harrow Beauty	231 (188-288)	701 (518-1099)
		mean:	161	718
	Spreading factor:		13.4	21.2
Fludioxonil	drop	Redhaven	2.1 (-)‡	7.4 (-)‡
	drop	Harrow Beauty	1.9 (1.0-2.8)	4.0 (2.8-10.7)
	drop	Loring	4.5 (3.2-6.3)	18.1 (11.3-45.6)
	drop	Loring	4.9 (3.4-7.2)	17.2 (10.6-48.8)
		mean:	3.4	11.7
	brush	Redhaven	44.7 (36.0-53.4)	234 (175-366)
	brush	Harrow Beauty	16.5 (7.8-293)	710 (88.5->5000)
		mean:	30.6	472
	Spreading factor:		9.0	40.3

* Mean incidence of rhizopus rot in the inoculated controls was 92% with a range of 83%-98%. Prior to inoculation, peaches were stored at 1EC for 0 - 5 days: tebuconazole; Redhaven 5days and Harrow Beauty 0 days; fludioxonil; Redhaven 0 days, Harrow Beauty 2 days, Loring 1 and 3 days. † Paired means with non-overlapping 95% fiducial limits are significantly (P<0.05) different.

‡ Due to an abrupt response, the fiducial limits could not be calculated.

Table 5. Reduction of rhizopus rot of Madison peaches with *Pseudomonas syringae* ESC 10 suspended in water or $CaCl_2$ solution, and co-treated with *Rhizopus stolonifer* sporangiospores immediately after wounding, or applied as a post-inoculation treatment by brush one day after inoculation with *R. stolonifer* sporangiospores.

Concentration of ESC 10 (µg/mL)	Reduction of rhizopus rot relative to controls* (%)			
	Co-treatment		Post-inoculation treatment	
	Water	$CaCl_2$ solution	Water	$CaCl_2$ solution
100	31.4	9.5	0	1.0
300	72.9	33.6	9.4	4.5
1, 000	88.1	59.4	11.1	3.6
3, 000	85.4	74.1	17.9	11.7
10, 000	82.2	84.5	19.6	18.7
EC_{50} (µg/mL):	113	843	>10,000	>10,000
95% fiducial limits:	none	470-1,463	none	none
EC_{90} (µg/mL)	8,219	11,700	>10,000	>10,000
95% fiducial limits	none	5,235-53,841	none	none

* The mean incidence of rhizopus rot in the controls was 96.5% (range 93.4% to 98.4%).