Effects of Wounding, Inoculum Density, and Biological Control Agents on Postharvest Brown Rot of Stone Fruits

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ABSTRACT

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The effects of wounding, inoculum density, and three isolates (New, Ta291, and 23-E-6) of *Trichoderma* spp. and one isolate (BI-54) of *Rhodotorula* sp. on postharvest brown rot of stone fruits were determined at 20°C and 95% relative humidity (RH). Brown rot was observed frequently on wounded nectarine, peach, and plum fruits inoculated with two spores of *Monilinia fructicola* per wound, and occasionally on unwounded nectarine and peach fruits inoculated with the same spore load. Brown rot was observed on wounded plums only. A substantial increase in lesion diameter of brown rot was also recorded on wounded nectarines and peaches inoculated with suspensions of ≤ 20 spores and ≤ 200 spores per wound, respectively, compared with unwounded fruit. At concentrations of 10^7 and 10^8 spores per ml, all *Trichoderma* isolates substantially reduced brown rot on peaches (63 to 98%) and plums (67 to 100%) when fruits were inoculated with *M. fructicola* following the application of a biological control agent. Similarly, at 10^8 spores per ml, the yeast BI-54 also suppressed brown rot on peaches completely and on plums by 54%. Significant brown rot reduction was also achieved with the isolate New at a concentration of 10^8 spores per ml, even when the biocontrol agent was applied 12 h after inoculation with *M. fructicola* and under continuous conditions of 95% RH. The isolates Ta291 and 23-E-6 also reduced brown rot significantly under drier (50% RH) incubation conditions. These isolates provided the best control of brown rot on plums when they were applied 12 h earlier than inoculation with *M. fructicola*. Satisfactory control of brown rot on plums inoculated with *M. fructicola* at 8×10^4 spores per ml was achieved with New at 10^6 spores per ml and with Ta291 at 10^7 spores per ml. Measures taken to avoid injuring fruit will greatly reduce brown rot of stone fruit at any spore load for plum, but only at ≤ 50 spores per mm² for peach, and at ≤ 5 spores per mm² for nectarine. This study identifies two isolates (Ta291 and New) of *Trichoderma atroviride*, one isolate (23-E-6) of *T. viride*, and one of *Rhodotorula* sp. that show potential for further development as biocontrol agents of postharvest brown rot of stone fruits.

Additional keywords: incubation period, infection efficiency, *Prunus*

Brown rot, caused by *Monilinia fructicola* (G. Wint.) Honey, is one of the most destructive diseases of stone fruits (7,20,32). This disease starts with blossom blight, then progresses into a twig blight and canker, which can provide inoculum for latent infection of green fruit. From these latent infections, fruit brown rot develops prior to harvest and during storage and transport to market. Sometimes brown rot even affects the fruit at the processing stage and the processed product (3). In California, blossom blight and preharvest fruit brown rot may differ greatly from season to season, mainly depending on weather conditions (32). In contrast, a

decay of >50% of fruit caused by *M. fructicola* at the postharvest stage is common, regardless of the incidence of preharvest brown rot (44).

A number of factors affecting decay of stone fruit by *M. fructicola* have been studied separately. The higher the inoculum concentration, the more severe and rapid the decay that developed on unwounded peach and cherry fruits (34). Wounds, including bruising, also greatly facilitated decay of apricot (47), peach (20), and plum fruits (46).

Two approaches have been employed to control postharvest brown rot in California. One approach has been the preharvest application of fungicides to prevent the activation of latent infection and/or to protect fruits from further infections (44). The success of this strategy depends largely on the prediction of latent infections. The other approach has been the use of postharvest treatments, primarily with fungicides. Before 1996, fungicides such as benomyl, iprodione, and triforine were registered for postharvest treatment of

stone fruits to control brown rot (11,44). Because of fungicide resistance, benomyl can no longer be used, and iprodione was voluntarily canceled by Rhône-Poulenc Ag. Co. in 1996 (1). Since then, the stone fruit industry has had no fungicide registered for control of postharvest brown rot.

Emerging alternatives to the chemical control of postharvest decay of stone fruits include (i) a treatment with hot water mixed with 10% alcohol (25); (ii) fumigation with carbon dioxide (7), acetic acid (42), and other volatile compounds from peach fruit (18); (iii) plant hormesis induced by ultraviolet light (45); (iv) use of the toxicity of calcium salts (2) and sugar analogs (9); (v) biological control (10,17,43,44); and (vi) combinations of the above (11). Some of these alternative measures, such as biological control, provide great promise for future management of postharvest brown rot and have been investigated intensively.

An isolate of *Bacillus subtilis* partially controlled postharvest peach brown rot experimentally (13,26) and on commercial packing lines (39,40). *Pseudomonas corrugata* and *P. cepacia* also substantially reduced the decay of artificially wounded and inoculated nectarines and peaches (44), and improved peach puree quality (3). Furthermore, some yeasts either reduced brown rot incidence (44) or decreased lesion diameter (27). However, the efficacy of *Trichoderma* spp., one of the most widely demonstrated and used biological agents for control of plant diseases (8,35), has not been documented against *M. fructicola*.

The purpose for the present research was to expand the search for potential biological agents after the cancellation of the registration of iprodione for postharvest brown rot control. The specific objectives of this research were to determine (i) the effects of wounding and inoculum density on brown rot of nectarine, peach, and plum fruits; and (ii) the effectiveness of biological control agents against brown rot of peach and plum fruits.

MATERIALS AND METHODS

Effects of wounding and inoculum density of *M. fructicola* on infection of nectarine, peach, and plum fruits. Mature fruits of peach (*Prunus persica* (L.) Batsch. cv. Elegant Lady), nectarine (*P. persica* var. *nectarina* (Ait.) Maxim. cv.

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Fantasia), and plum (*P. salicina* Lindl. cv. Casselman) were hand-picked from experimental orchards at Kearney Agricultural Center, Parlier, California, during commercial harvest and kept in a cold room at 0.5°C until used (up to 3 weeks). Fruit were surface disinfested by immersing them in a solution containing 160 ml of ethyl alcohol and 160 ml of chlorine (NaOCl) plus 0.5 ml of Tween 20 in 10 liters of water for 4 min, and allowed to air-dry on a laboratory bench. Disinfested fruits were placed on plastic holders (one layer) in commercial cardboard boxes with their equatorial part facing uppermost and were preconditioned for 3 to 6 h prior to inoculation with *M. fructicola* in a walk-in chamber at 20°C and 95% relative humidity (RH).

M. fructicola isolate 5-E-3 was isolated from a nectarine fruit and used throughout this study. The isolate was cultured in petri dishes containing acidified (2.5 ml of a 25% [vol/vol] lactic acid per liter of medium) potato dextrose agar. Inoculum of *M. fructicola* was prepared by flooding the surface of a 9-day-old culture, blending the suspension for 1 min, then filtering through eight layers of cheesecloth. The concentration of *M. fructicola* was measured with a hemacytometer and diluted to 10^6 , 10^5 , 10^4 , 10^3 , and 10^2 spores per ml.

Fruits were separated into two batches. In the first batch, one wound (2 mm deep and 2 mm wide) was made on the equator (top) of each fruit with a sterile nail. Twenty microliters of a suspension of *M. fructicola* was applied to the wound of each wounded fruit and at the same position on unwounded fruits. The test was done twice with three replicates per treatment and five to eight fruit per replicate. After inoculation, fruits were kept in the same chamber for 24 h to ensure about 12 h wetness on the inoculation sites of unwounded fruit, and then moved to a reach-in chamber also kept at 20°C but 50% RH for 5 to 7 days.

Incubated fruits were examined daily for 6 to 8 days after inoculation for brown rot development by measuring lesion diameter for nectarines and peaches and by rating severity for infected plums. The severity rating scale of infected plums was: 0, no symptoms; 1, the area exhibiting sporulation of *M. fructicola* restricted to <2 cm in diameter around the wound; 2, the area of sporulation extending beyond 2 cm in diameter but still covering a small portion of fruit surface (top half); and 3, sporulation covering the majority of the fruit surface. Daily brown rot severity was calculated for each replicate in all tests and subjected to analysis of variance using Proc. GLM of the SAS package (Version 6.12, SAS Institute, Cary, NC). The brown rot severity read 6 days after inoculation was further regressed against the logarithmic transformed inoculum density. The incidence of brown rot was summarized by fruit variety

at the inoculum density of two spores per wound or fruit to give indications of infection efficiency of *M. fructicola* on wounded and unwounded fruits. Lesions that did not originate from the inoculation points were considered natural infections. These were recorded separately and excluded from the calculations and statistical analysis of deliberate inoculations.

Efficacy of biological control agents against brown rot of peach and plum fruits. Four biological control agents at two concentrations plus one positive control (using 20 µl of sterile deionized water [SDW], then 20 µl of a suspension of *M. fructicola*) were included in the test. The test was repeated at least once on individ-

ual varieties of two peaches and one plum, with three to six replicate boxes and five to eight fruit per replicate in each test. Isolates Ta291 and New of *Trichoderma atroviride* Karsten were isolated originally from an apothecium of *M. fructicola* and from green grapes, respectively, and isolate 23-E-6 of *Trichoderma viride* Pers. ex S. F. Gray aggr. was isolated from kiwifruit. The yeast (*Rhodotorula* sp.) isolate BI-54 was a gift from J. L. Smilanick, USDA-ARS Horticultural Crops Research Laboratory, Fresno. The biological control agent isolates were cultured on acidified potato dextrose agar plates for 9 days, and suspensions of 10^7 and 10^8 spores per ml were prepared. In addition, a 10^3

Fig. 1. Brown rot development on wounded (W) and unwounded (U) nectarine (cv. Fantasia), peach (cv. Elegant Lady), and plum (cv. Casselman) fruits inoculated with 20 µl of a suspension containing 10^2 (2), 10^3 (3), 10^4 (4), or 10^5 (5) spores of *Monilinia fructicola* per ml. Fruit were incubated at 20°C and 95% relative humidity (RH) for 24 h, then at 20°C and 50% RH for the remainder of the incubation period.

Table 1. Incidence of postharvest brown rot of nectarine, peach, and plum inoculated^y with *Monilinia fructicola* into a wound at the fruit equator or at the same position on unwounded fruit

Fruit	Cultivar	Infected fruit (%)		Putative infection efficiency ^z	
		Wounded	Unwounded	Wounded	Unwounded
Nectarine	Fantasia	100	25	0.50	0.13
Peach	Elegant Lady	100	30	0.50	0.15
Plum	Casselman	54	0	0.27	0

^y Two spores were applied per wound in each fruit. Fruit were incubated at 20°C and 95% relative humidity (RH) for 24 h, then at 20°C and 50% RH for an additional 5 days.

^z Infection efficiency was calculated by dividing the proportion of infected fruit by 2 (number of spores applied in each drop to each fruit).

Fig. 2. Comparison of brown rot development between wounded (W) and unwounded (U) nectarine (cv. Fantasia), peach (cv. Elegant Lady), and plum (cv. Casselman) fruits inoculated with 20 μ l of a suspension containing 10^2 , 10^3 , 10^4 , 10^5 , or 10^6 spores of *Monilinia fructicola* per ml and incubated at 20°C and 95% relative humidity (RH) for 24 h, then at 20°C and 50% RH for an additional 5 days.

spores per ml suspension of *M. fructicola* was prepared.

Peach (cvs. Elegant Lady and Fairtime) and plum (cv. Casselman) fruits were picked, surface-disinfested, and preconditioned as previously described. One wound was made on the equator of each plum with the sterile nail, but peaches were not wounded prior to the application of biological control agents. Twenty microliters of a suspension of *M. fructicola* was applied at each wound site for plums and at the same position on peaches immediately following the application of 20 μ l of biological control agent. Fruits were incubated for 6 to 10 days and examined daily for brown rot development as described previously. The incidence and severity of brown rot were calculated and analyzed as described above.

Timing of application of biological control agents to suppress brown rot of plums. Two or three timings of four isolates at two concentrations plus one positive control and one negative control (using SDW) were included in the test. The test was repeated twice, with three replicates per treatment and eight plums per replicate in each test. Plums (cv. Casselman) were harvested and surface disinfested, and inocula of *M. fructicola* and biological control agents were prepared as previously described. In the first two tests, three control agent timings were used relative to the time of application of 20 μ l of *M. fructicola*. An aliquot of 20 μ l of biological control agent was applied 12 h prior to pathogen application, immediately following the pathogen, or 12 h after the pathogen. For the first test, the concentration of suspension was 10^3 spores of *M. fructicola* per

ml; fruit were kept in a walk-in chamber (20°C and 95% RH) for 24 h after the challenge inoculation was made, then moved to a reach-in chamber (20°C and 50% RH) and incubated for an additional 6 days. For the second test, the concentration of *M. fructicola* was 4×10^4 spores per ml; fruit were incubated at 20°C and 95% RH until the end of the test (7 days). In the third test, only two timings were employed; fruits were inoculated with *M. fructicola* at 10^3 spores per ml, and the biological control agents were applied either 12 h earlier or 12 h later than the inoculation. Fruit were rated, and the incidence and severity of brown rot were calculated and analyzed as described previously.

Effect of concentrations of biological control agents on postharvest brown rot of plums. Plums (cv. Casselman) were harvested and surface disinfested as previously described. Three *Trichoderma* isolates (Ta291, 23-E-6, and New) at concentrations of 4×10^4 , 4×10^5 , 4×10^6 , 4×10^7 , and 4×10^8 spores per ml were included in the test. All inocula were prepared from 9-day-old cultures. Fruit wounds were inoculated with *M. fructicola* using 8×10^4 spores per ml immediately following the application of individual biological control agents. The test included three controls: positive applied with SDW/5-E-3, negative with SDW/nil, and the third with nil/nil on wounds. There were three replicates per treatment and eight plums per replicate in each of the two tests. Fruits were incubated for 7 days at 20°C and 95% RH, then rated. The incidence and severity of brown rot were calculated and analyzed as previously described.

Fig. 3. Brown rot development on unwounded peaches (cvs. Elegant Lady and Fairtime) inoculated with 20 μ l of a suspension containing 10^3 spores of *Monilinia fructicola* per ml immediately following the application of 20 μ l of biological control agent, incubated at 20°C and 95% relative humidity (RH) for 24 h, then at 20°C and 50% RH for the remainder of the incubation period. Number in parentheses following the isolate name represents the concentration applied, e.g., BI-54(7) denotes that BI-54 was applied at 10^7 spores per ml.

RESULTS

Effects of wounding and inoculum concentration of *M. fructicola* on infection of nectarine, peach, and plum fruits. Brown rot was first observed on nectarine and peach fruits 2 days and on plums 4 days after inoculation (Fig. 1). The disease severity (or lesion diameter) increased as the incubation progressed up to 6 days postinoculation, depending on the wounding and inoculum density (Fig. 1). Approximately 2% of fruits were naturally infected and developed brown rot symptoms during incubation. Symptoms resulting from natural infections were easily recognized by their distance from the inoculation points and by the time of their appearance. They usually developed more slowly than those resulting from artificial inoculation with high conidial concentrations. Brown rot was observed frequently on wounded nectarine, peach, and plum fruits inoculated with *M. fructicola* at a concentration of two spores per wound (20 μ l of 10^2 conidia per ml) and occasionally on unwounded nectarine and peach fruits at the same spore load (Table 1, Figs. 1 and 2).

Wounds greatly facilitated the infection of all fruits by *M. fructicola* at all concentrations tested (Figs. 1 and 2). Brown rot was observed only on wounded but not on unwounded plums, even at the highest inoculum concentration of 10^6 spores per ml (Fig. 2). A substantial increase in lesion diameter and daily growth rate was also observed on wounded nectarines inoculated with the suspensions of $\leq 10^3$ spores per ml and peaches with suspensions of $\leq 10^4$ spores per ml, compared with unwounded fruit (Figs. 1 and 2). In general, the severity of brown rot increased in a sigmoidal pattern on all wounded fruits and unwounded nectarine and peach fruits as inoculum density increased (Fig. 2).

Efficacy of biological control agents against brown rot of peach and plum fruits. Brown rot symptoms were observed 3 days after the inoculation of control Elegant Lady peaches and 4 days on Fairtime peaches. The diameter of brown rot lesions was significantly ($P = 0.003$) reduced on Elegant Lady peaches protected with BI-54 or Ta291 at either concentration of 10^7 and 10^8 spores per ml 4 days after inoculation (Fig. 3). Significant reduction of brown rot severity was achieved with Ta291 but not with BI-54 6 days postinoculation (Fig. 3). Reduction of brown rot severity was achieved on Fairtime peaches with four biological control agents only at the concentration of 10^8 spores per ml 5 days after inoculation (Fig. 3).

Brown rot was observed 5 days after inoculation with *M. fructicola* on control plums, and 1, 2, and 6 days later on plums protected with BI-54 and 23-E-6, Ta291, and New, respectively (Fig. 4). Brown rot was suppressed greatly ($P < 0.001$) on plums protected with all biological control

Fig. 4. Brown rot development on wounded plums (cv. Casselman) inoculated with 20 μ l of a suspension containing 10^3 spores of *Monilinia fructicola* per ml immediately following the application of 20 μ l of biological control agent, incubated at 20°C and 95% relative humidity (RH) for 24 h, then at 20°C and 50% RH for the remainder of the incubation period. Number in parentheses following the isolate name represents the concentration applied, e.g., BI-54(7) denotes that BI-54 was applied at 10^7 spores per ml.

Fig. 5. Effects of biological agent and inoculum concentration on the severity and incidence of brown rot of unwounded peach (cv. Fairtime) and wounded plum (cv. Casselman) fruits inoculated with 20 μ l of a suspension containing 10^3 spores of *Monilinia fructicola* per ml immediately following the application of 20 μ l of each biological control agent, incubated at 20°C and 95% relative humidity (RH) for 24 h, then at 20°C and 50% RH for 3 and 6 days, respectively. Number in parentheses following the isolate name represents the concentration, e.g., BI-54(7) denotes that BI-54 was applied at 10^7 spores per ml. Mean bars topped with different letters differed significantly according to Fisher's least significant difference ($P = 0.01$).

agents at both concentrations throughout the tests (11 days) except for BI-54 at 10^7 spores per ml (only 7 days) (Fig. 4).

Trichoderma isolates reduced the severity of peach brown rot by 63 to 98%; BI-54 reduced decay by 100% at a concentration of 10^8 spores per ml and 87% at 10^7 spores per ml (Fig. 5). Isolates Ta291 and BI-54 gave discernibly better control of brown rot on peaches than did the other isolates at 10^8 spores per ml (Fig. 5). However, the three *Trichoderma* isolates gave better control of brown rot on plums than did the yeast isolate BI-54 at comparable concentrations (Fig. 5). The isolate New gave discernibly better control of brown rot than did the other two *Trichoderma* isolates on plums. BI-54 reduced brown rot of plums only at 10^8 spores per ml but not at 10^7 spores per ml (Fig. 5).

Timing of application of biological control agents against brown rot of plums. All biocontrol agent isolates tested gave the best control of brown rot on plums when they were applied to wounds 12 h earlier than the inoculation with *M. fructicola* (Fig. 6). The yeast isolate BI-54 suppressed brown rot only when it was applied 12 h earlier than the challenge inoculation and only in the first test but not in the second, a more critical test conducted under continuously high humidity. In contrast, at a concentration of 10^8 spores per ml, the isolate New significantly suppressed brown rot even when it was applied to the wounds 12 h later than the inoculation with *M. fructicola* in these tests (Table 2). Similarly, at a concentration of 10^8 spores per ml, the other two *Trichoderma* isolates (Ta291 and 23-E-6) also suppressed brown rot substantially, even when they were applied 12 h later than the inoculation with *M. fructicola* during the first test. Similar data were obtained from the third experiment (data not shown).

Effect of concentration of biological control agent on postharvest brown rot of plums. The incidence of brown rot decreased as the concentration of biological control agent (New or Ta291) increased (Fig. 7). On plums, satisfactory control was achieved with isolate New at 10^6 spores per ml, and with isolate Ta291 at 10^7 spores per ml, but not with isolate 23-E-6 at any concentrations tested.

DISCUSSION

Effects of wounding or inoculum concentration on decay of stone fruits by *M. fructicola* have been investigated previously (20,34,37,46). This study further provides quantitative information on the effects of interaction between wounding and inoculum concentration on postharvest brown rot of nectarine, peach, and plum. Brown rot was not observed on any unwounded plums inoculated with *M. fructicola* at concentrations from 10^2 to 10^6 spores per ml. This result is consistent with a previous observation (46). The signifi-

Table 2. Biocontrol of postharvest brown rot of plums (cv. Casselman), caused by *Monilinia fructicola*, at different timings of application of biological agents^y

Biological agent	Concentration (spores/ml)	Control (%)		
		12 h earlier	Simultaneous	12 h later
New	10^8	100 a ^z	100 a	91 a
	10^7	100 a	100 a	19 bc
Ta291	10^8	93 a	98 a	36 b
	10^7	100 a	76 a	8 bc
23-E-6	10^8	100 a	69 a	-1 bc
	10^7	100 a	12 b	-12 bc
BI-54	10^8	1 b	1 b	-10 bc
	10^7	-3 b	-12 b	-19 c

^y Treated fruit were incubated at 20°C and 95% relative humidity for 7 days, then incubated at 20°C and 50% RH for 6 days. The control of brown rot by biological agent was calculated as the difference in disease severity between untreated and treated fruit divided by the severity of untreated fruit.

^z Numbers followed by the same letter within a column did not differ significantly according to Duncan's multiple range test ($P = 0.01$).

Fig. 6. Effects of biological agent and concentration on brown rot between wounded plum (cv. Casselman) fruit inoculated with 20 μ l of a suspension containing 1×10^3 (top) and 4×10^4 spores (bottom) of *Monilinia fructicola* per ml 12 h earlier or 12 h later than the application of 20 μ l of the biological control agent or at the same time. Fruit were incubated at 20°C and 95% relative humidity (RH) for 24 h, then at 20°C and 50% RH for 6 days (top), and incubated at 20°C and 95% RH for 7 days (bottom). Number in parentheses following the isolate name represents the concentration, e.g., BI-54(7) denotes that BI-54 was applied at 10^7 spores per ml. Mean columns of the same isolate and concentration topped with different letters differed significantly according to Fisher's least significant difference ($P = 0.05$).

cance of wounds as entry points for *M. fructicola* to initiate infection increased as the spore load on the fruit surface decreased, particularly from ≤ 5 spores per mm^2 for nectarines and ≤ 50 spores per mm^2 for peaches. In orchards, spore load of *M. fructicola* on fruit surface builds up as the season progresses (15,16) and can be extremely high at the end of the season (20,36). For example, an average of 0.22 to 1.7×10^5 spores per fruit were detected by spreading washings from California fruit on plates of selective medium (36). A higher spore load of 0.3 to 2.33×10^6 infective units per peach fruit was monitored in Canberra, Australia (20). If we suppose that the surface area of a stone fruit is 200 cm^2 , the above numbers are equivalent to a spore load of 1 to 116 infective units per mm^2 of fruit surface. Therefore, any measures taken to reduce injury to fruits during picking and packing processes would greatly reduce postharvest brown rot of stone fruits, particularly in years when preharvest brown rot is not severe or spore load is low.

The infection efficiency of *M. fructicola* (isolate 5-E-3) was 0.13 on nectarines and 0.15 on peaches in our tests (Table 1). Higher infection efficiencies were observed on wounded fruits of 0.5 for both nectarines and peaches and of 0.27 on plums (Table 1). These infection efficiencies are extremely high when compared with that (0.09) of *Uromyces appendiculatus* on *Phaseolus vulgaris*, a model system of one spore-one infection provided initially by Schein in 1964 (41) and represented by Zadoks and Schein in their pioneering work (49). The lowest spore load used in this study was two spores per wound or fruit; therefore the actual infec-

tion efficiency may be even higher than 0.5 on wounded nectarine and peach fruits under conditions similar to ours. This finding that *M. fructicola* has an extremely high infection efficiency, in combination with large spore loads on fruit surfaces (20,36), can help in explaining the explosive outbreaks of brown rot in orchards at the end of the season during wet years and severe decay (commonly $>50\%$) of fruits during storage and transport to markets (44).

Many biological agents have been investigated for their potential to control brown rot of stone fruits (19,38). *Aureobasidium pullulans*, *Epicoccum purpurascens*, and *Gliocladium roseum* were tested for controlling blossom blight and latent infections of sweet cherry by *M. fructicola* in Oregon (48). *Penicillium frequentans*, *P. purpurogenum*, and *Epicoccum nigrum* were investigated for control of twig blight of peach by *M. laxa* in Spain (4-6,22-24,28-31). *Bacillus subtilis* (B-3) was demonstrated to effectively control decay of peach in commercial packing lines (39,40), and *Pseudomonas corrugata* and *P. cepacia* were demonstrated to control the decay of nectarine and peach fruits at postharvest (44) and processing stages (3). For the first time, this study identified three isolates of *Trichoderma* spp. for their potential to control postharvest brown rot of stone fruits. Our tests indicate that *Trichoderma* spp. possess great potential for controlling postharvest brown rot of stone fruits. The *Trichoderma* isolates tested reduced brown rot by 63 to 98% on peaches and by 67 to 100% on plums. In general, control of brown rot increased as the concentration of *Trichoderma* conidial suspensions increased, which can be described by the models proposed by Johnson (21) and Montesinos and Bonaterra (33).

Interactions among fruit, *Trichoderma* isolate, and concentration of biological agent were observed with regard to the degree of decay control. At 10^8 spores per ml, isolate New completely suppressed decay on plums (100%) but only by 75% on peaches. In contrast, isolate Ta291 provided a better control of decay on peaches (98%) than on plums (89%). However, isolate 23-E-6 exhibited about the same degree of decay control on peaches (63 to 87%) as on plums (67 to 85%).

We observed that *Trichoderma* isolates not only reduced infections but also greatly suppressed the sporulation of *M. fructicola* on infected plum fruit. It was obvious that *M. fructicola* sporulated in smaller areas and in a much more sparse fashion on plums treated with *Trichoderma* than on control plums. Suppression of sporulation is an important positive aspect of controlling plant disease and is rarely accomplished by biological control agents (12). This implies that the *Trichoderma* isolates could also suppress secondary infections, and consequently they are important for the postharvest control of brown rot. The bio-

chemical mechanism by which brown rot was controlled by these isolates is currently under investigation.

At a concentration of 10^8 spores per ml, the yeast isolate BI-54 reduced decay of Fairtime peaches by 100% and decay of Casselman plums by 41%. Variations were observed with respect to decay control efficacy between tests and between different cultivars of peaches. Less control of decay was observed on Elegant Lady peaches, and variation among tests with this peach cultivar was noted (Fig. 3). This yeast isolate is being further tested.

Wounding, inoculum density, and biological control agents tested affected not only the infection of stone fruits by *M. fructicola*, but also the incubation period of brown rot. The time required for the development of brown rot symptoms was 1 to 2 days less on wounded than on unwounded fruits at all concentrations of the pathogen (Fig. 1). The incubation period decreased as the concentration of *M. fructicola* increased on both wounded and unwounded fruits (Fig. 1); similar data were obtained with brown rot of detached cherries (34) and dark leaf and pod spot of oilseed rape by *Alternaria brassicae* (14). The isolates of *Trichoderma* and a yeast tested all increased the incubation period by delaying the development of brown rot symptoms.

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