Comparative Efficacy and Systemic Activity of Iprodione and the Experimental Anilide E-0858 for Control of Brown Rot on Peach Fruit

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ABSTRACT

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Iprodione, benomyl, or the experimental anilide E-0858 (SC-0858), when used as a pre- or postharvest spray, limited the size of brown rot lesions on fruit of the peach cultivar Elegant Lady inoculated with conidia of *Monilinia fructicola*. Fungicides applied 21, 14, 7, or 1 day before harvest reduced lesion diameters on inoculated fruit in postharvest storage compared with nontreated fruit. Brown rot lesions on fruit treated with E-0858 were significantly smaller than those on fruit treated with the other two fungicides. Postharvest hydrocooling using chlorinated water after fungicide treatment reduced the efficacy of iprodione or benomyl but not the efficacy of E-0858. Penetration of fungicides into fruit was demonstrated by injecting conidia of the fungus to a depth of 1 cm in fruit that were surface-treated 1–21 days before harvest or 1 day after harvest with E-0858, iprodione, or benomyl. In all treatments, fruit treated with E-0858 had significantly less decayed tissue than nontreated fruit or fruit treated with iprodione or benomyl. When sprays were applied 1 day or 7 days preharvest or after harvest, fruit treated with iprodione had significantly more nondecayed internal tissue than fruit treated with benomyl or not treated. In fruit treated with radioactively labeled fungicide, 51% of measurable radioactivity of ^{14}C-E-0858 and 2.8% measurable radioactivity of ^{14}C-iprodione was detected in mesocarp tissue. The remainder of the measurable radioactivity of each fungicide was recovered within 1 mm below the fruit surface. The efficacy of iprodione and E-0858 is attributed to their high activity against *M. fructicola* and their penetration into mesocarp tissue of peach fruit.

Additional keywords: radioactive fungicide

Brown rot, caused by *Monilinia fructicola* (G. Wint.) Honey or *M. laxa* (Aderhold & Ruhland) Honey, is one of the most destructive diseases of stone fruit crops in North America (2). In California, both species occur on peach (*Prunus persica* (L.) Batsch), but *M. fructicola* is more common (22). Control methods for brown rot of peach fruit include pre- and postharvest fungicide treatments that protect fruit from fungal decay and prolong their storage and shelf life. In the 1950s and 1960s, several fungicides, including sulfur, copper, captan, sodium orthophenylphenate, and dichloran formulations, were applied as pre- and postharvest treatments to control brown rot (8,10). Most fungicides recommended before 1970 were multisite inhibitors of relatively low activity with low biochemical specificity and were limited to protection of plant surfaces (18,23). These chemicals decreased the postharvest incidence of brown rot, but significant losses still occurred. In the 1970s, the introduction of benomyl and other benzimidazole fungicides virtually eliminated losses due to brown rot. The systemic activity of benomyl both protects against and suppresses infections (10). The exclusive use of benomyl in orchards, however, led to the development of benomyl-resistant populations of *M. fructicola* and *M. laxa*, rendering benzimidazole fungicides ineffective for brown rot control in many locations (6,8,9,20,24).

New fungicidal compounds have been developed since 1970 that are more active, generally biochemically specific with a narrow fungal spectrum of activity (18), and highly effective against benomyl-sensitive and benomyl-resistant populations of fungi. These alternative fungicides include the dicarboxamides (e.g., iprodione, vinclozolin) and the demethylation inhibitors (e.g., triforine, myclobutanil). Some of these have been registered for pre- or postharvest use and are currently the most effective fungicides available for the control of brown rot of stone fruits. The effectiveness of iprodione for control of diseases on turfgrasses, vegetable crops, and fruit crops has been attributed to its high toxicity against target fungi (5,19) and localized systemicity (3,4). Additionally, new fungicides, including a new chemical class, the anilides, are being developed that inhibit mycelial growth of both benomyl-sensitive and benomyl-resistant populations of *M. fructicola* and *M. laxa* (11) and are highly effective for the control of brown rot (24).

During ripening, peach fruit and other stone fruits become increasingly susceptible to infection by *M. fructicola* (1,7,24). Preharvest fungicide treatments protect fruit from infection in the field and reduce fruit decay in storage. Preharvest sprays with iprodione, benomyl, or the anilide fungicide E-0858 controlled brown rot after harvest, but postharvest treatments provide better control of postharvest decay. Currently, fungicide treatments used for control of postharvest decays of stone fruits include mixtures of dichloran for Rhizopus rot, caused by *R. stolonifer* (Ehrenb.:Fr.) Vuill.), and either iprodione or thiophanate-methyl for control of brown rot, caused by *Monilinia* spp., and gray mold, caused by *Botrytis cinerea* Pers.:Fr. An additional postharvest treatment used in California packinghouses includes hydrocooling of fruit in chlorinated water before treatment with fungicides. This treatment cleans fruit, reduces viable inoculum of microorganisms on fruit surfaces, and removes latent heat, thereby slowing the ripening process of fruit and the growth of decay organisms. Hydrocooling, however, may also remove field-applied fungicides from fruit surfaces and reduce disease control effectiveness. The objectives of this research were to compare the efficacy of the anilide E-0858 to that of iprodione or benomyl when applied as a pre- or postharvest treatment for control of brown rot of peach, to determine the effects of hydrocooling on preharvest fungicide treatments, and to determine the systemic activity of iprodione and E-0858 in peach fruit.

MATERIALS AND METHODS

Fungal isolate. A benomyl-sensitive isolate of *M. fructicola* (MUK-1) obtained from the University of California, Davis, was used. The fungus was grown on 2% Difco potato-dextrose agar for 5–10 days at 25 C under fluorescent light for conidial production.

Evaluation of pre- and postharvest fungicide treatments in reducing decay of surface-inoculated fruit. Field experiments were conducted on the peach cultivar Elegant Lady growing at Kearney Agricultural Center, Fresno County, California. Rainfall was recorded during

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field experiments. Treatments of E-0858 (formerly SC-0858, a heterocyclic anilide, M_r 192, formulated as 50WP, Zeneca Ag Products, Wilmington, DE), iprodione (Rovral 50WP), or benomyl (Benlate 50WP) at 1,200 μg a.i./ml were applied to runoff at 21, 14, 7, or 1 day before harvest using a handgun sprayer at a pressure of 1,694 kPa. Fruits from nonsprayed trees were used as controls. Twenty-one fruit were randomly harvested from each tree, placed in plastic trays (21 fruit per tray), and packed in commercial cardboard fruit boxes. Fruit were inoculated by making a semicircular puncture (approximately 0.5 × 0.5 × 1 mm deep) in the epidermis with a glass rod and placing 25 μl of a conidial suspension of *M. fructicola* (2×10^4 conidia per milliliter) on the injured areas. Boxes were stored at 20 C and ≥95% relative humidity (RH), and disease severity was evaluated 6 days after inoculation by measuring the lesion diameter. Treatments had three single-tree replications and were arranged in a randomized complete block design. The experiment was performed twice (once per year). Data were analyzed by regression, analysis of variance, and general linear model procedures (16).

In postharvest studies, fruit harvested from nonsprayed trees were immediately sprayed to runoff with E-0858, iprodione, or benomyl at 300 μg a.i./ml using a pressurized hand sprayer (68.9 kPa). Fruit sprayed with water were used as controls. Following fungicide treatment, fruit were stored for 16 hr at 4 C, and then one-half of the fruit were hydrocooled in chlorinated water at 1 C for 30 min in a commercial packinghouse; the remaining fruit were not hydrocooled. Both hydrocooled and nonhydrocooled fruit were packed, inoculated (24 hr after fungicide treatment), incubated, and evaluated as described previously. Treatments had four replications (21 fruit per replication), and the experiment was done twice (once per year). Data from postharvest treatments were analyzed by analysis of variance, and treatment means were separated using least significant difference (LSD) mean separation procedures (16).

Evaluation of pre- and postharvest fungicide treatments in reducing internal decay. Peaches were sprayed to runoff with a handgun sprayer 21, 14, 7, or 1 day before harvest or immediately after harvest with formulations of iprodione, benomyl, or E-0858 at 300 μg a.i./ml as described previously. Nonsprayed fruit were used as controls for preharvest treatments, and fruit sprayed with water were used as controls for postharvest treatments. Twenty-four hours after harvest, 200 μl of a conidial suspension of *M. fructicola* (2×10^3 conidia per milliliter) was injected with a syringe into each of two sites (2 cm apart, 1 cm from the stem end, and 1 cm deep) on each fruit. Fruit were incubated for 7 days at 20 C and ≥95% RH and then cut perpendicularly to the injection sites. The sections were photographed, and disease was evaluated on the basis of the ratio of nondecayed to decayed area expressed as a percentage, using a Quantimat 900 video image analyzer (Cambridge Instruments Inc., Edison, NJ). Treatments had six replications (three fruit per replication), and the experiment was done twice (once per year). Data were analyzed by analysis of variance, and treatment means were separated using LSD mean separation procedures (16).

Evaluation of radioactive and nonradioactive fungicide treatments in penetrating fruit. ^{14}C-E-0858 (specific activity 188 μCi/μg), uniformly labeled on the heterocyclic ring, was provided by Zeneca Ag Products, and ^{14}C-iprodione (specific activity 0.06 μCi/μg), uniformly labeled in the phenyl ring, was provided by Rhône-Poulenc Ag Company (Research Triangle Park, NC). Radioactive fungicide was added to an aqueous suspension of the nonlabeled formulation such that the final concentration of each fungicide was 300 μg a.i./ml and 1.5 μCi/ml. 2-Pyrrolidone was added to the iprodione suspension (1% v/v) to increase the solubility of the ^{14}C-iprodione.

Mature peach fruit were placed in plastic containers (30 × 20 × 10 cm), treated by placing 150 μl of radioactively labeled E-0858 or iprodione on the surface of each fruit, and stored at 0 C for 1 day. A cork borer was used to remove a 13-mm-diameter core extending to the pit from each fruit where the fungicide was applied. Cores were cut into a 1-mm section containing the epidermis and three subsequent 5-mm sections of mesocarp extending to the pit. A 6-mm-diameter core was taken from each of these four sections, and the tissue was frozen in liquid nitrogen. The cork borers and knives were rinsed in 95% ethanol after each cut to prevent cross-contamination. Samples were combusted in the presence of oxygen (12,13), and the resulting CO_2 was trapped in 30 ml of a scintillation cocktail consisting of toluene:methanol:phenylethylamine (4.3:3.0:2.7, v/v) with 0.5% PPO (2,5-diphenyloxazole) and 0.05% POPOP [1,4-bis(5-phenyloxazolyl)benzene] (21). Radioactivity was determined by liquid scintillation counting in a Beckman LS-3133T counter (Beckman Scientific Instruments, Irvine, CA); corrections were made for efficiency, quenching, and background counts. Values were expressed as the percentage of measurable radioactivity recovered per sample. Each treatment was replicated five times, and the experiment was done twice. Data were analyzed by analysis of variance and *t* test mean separation procedures (16).

Additional statistical procedures. Variances of treatments for repeated experiments were evaluated by Bartlett's test of homogeneity of variance. Data from experiments with homogeneous variances were combined and analyzed by procedures described previously.

RESULTS

Efficacy of pre- and postharvest fungicide treatments in reducing decay of surface-inoculated fruit. Lesion diameter increased linearly on fruit sprayed with E-0858 ($P < 0.01$, $R^2 = 0.89$), iprodione ($P < 0.05$, $R^2 = 0.89$), or benomyl ($P < 0.05$, $R^2 = 0.86$) with increasing preharvest time intervals of fungicide application (Fig. 1). Preharvest sprays of E-0858, iprodione, or benomyl at 21, 14, 7, or 1 day before harvest significantly ($P \leq 0.01$) reduced lesion size from that of nonsprayed fruit as indicated by midpoint, slope, and *Y*-intercept values of regression models. When regressions among fungicides were compared, the midpoint of the regression of E-0858 was significantly ($P < 0.01$) lower than that of iprodione and benomyl; midpoints for iprodione and benomyl were not significantly ($P > 0.10$) different. No rainfall was recorded following preharvest spray applications for experiments conducted in each year.

In postharvest studies comparing brown rot lesions of fruit that were sprayed before harvest and either hydrocooled with chlorinated water after harvest or not hydrocooled, all fungicide treatments significantly ($P \leq 0.05$) reduced decay from that of nontreated fruit, with or without hydrocooling (Fig.

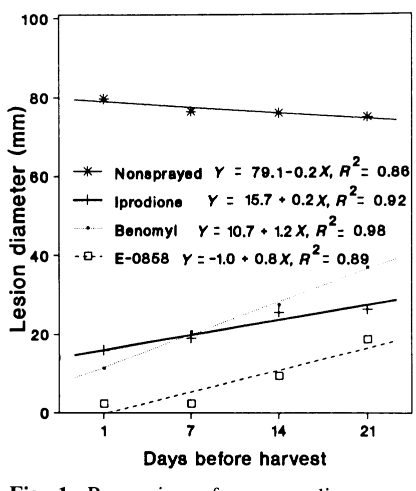

Fig. 1. Regression of average diameter of brown rot lesions of surface-inoculated peach cv. Elegant Lady fruit on time of fungicide application before harvest. Fruit were sprayed with 1,200 μg a.i./ml of manufacturer formulations of iprodione, benomyl, or the experimental anilide E-0858 at 21, 14, 7, or 1 day before harvest and postharvest, surface wound-inoculated with 25 μl of a conidial suspension of *Monilinia fructicola* (2×10^4 conidia per milliliter). Lesion diameters were measured 7 days after incubation at 20 C and ≥95% RH. Values are the combined means from two experiments (four replications of 21 fruit per experiment).

2). Fruit treated with E-0858 had smaller diameters ($P \leq 0.05$) of lesions caused by *M. fructicola* than fruit treated with iprodione or benomyl. Lesion diameters on fruit treated with iprodione or benomyl were similar and significantly ($P \leq 0.05$) smaller than those on nonsprayed fruit. Fruit treated before harvest with iprodione or benomyl and not hydrocooled had significantly ($P \leq 0.05$) smaller lesions than fruit treated with either of these fungicides and hydrocooled after harvest. Lesion diameters on fruit treated with E-0858, however, were not significantly affected by the additional hydrocooling treatment (Fig. 2).

Efficacy of pre- and postharvest fungicide treatments in reducing internal decay. Pre- or postharvest treatments of E-0858 had significantly ($P \leq 0.05$) higher percent nondecayed area of mesocarp tissue than fruit sprayed with either iprodione or benomyl, whereas fruit treated with iprodione had significantly more percent nondecayed area than fruit treated with benomyl for the 7- or 1-day pre- and postharvest treatments (Table 1). No difference ($P > 0.05$) in percent internal decay was observed between benomyl, iprodione, and the nontreated control for the 21- and 14-day preharvest spray applications.

Systemic movement of iprodione and E-0858 in peach fruit. Measurable radioactivity of both ^{14}C-E-0858 and ^{14}C-iprodione was recovered in mesocarp tissue of peach. In fruit treated with ^{14}C-E-0858, approximately 50% of the radioactivity recovered was detected in the epidermal tissue (< 1 mm) and 50% in the mesocarp tissue. Most (49.1%) of the radioactivity in the mesocarp tissue was found within the first 5 mm, whereas 1.9% was found 6–10 mm deep (Fig. 3). Over 97% of ^{14}C-iprodione was detected in the epidermal tissue (< 1 mm), whereas 2.7% was detected 1–5 mm deep in the mesocarp tissue. Only trace amounts of radioactivity of either fungicide were detected at the 11- to 15-mm depths. Significantly ($P \leq 0.01$) more iprodione than E-0858 was found in epidermal tissue, whereas significantly more E-0858 was found at 1–5 mm ($P \leq 0.01$) and 6–10 mm ($P \leq 0.05$) (Fig. 3).

DISCUSSION

In both pre- and postharvest fungicide treatments, lesion diameters of surface-wounded fruit treated with any of the three fungicides were smaller than lesions in nontreated fruit. Lesions on fruit treated with E-0858 were significantly smaller than those in fruit treated with either iprodione or benomyl. Furthermore, the residual activity of E-0858 under field conditions was longer than that of either of the other two fungicides (Figs. 1 and 2). Hydrocooling did not affect the efficacy of E-0858 but did significantly reduce the efficacy of iprodione and benomyl (Fig. 2). Regardless of the hydrocooling treatment, all fungicides were effective in reducing decay when compared with the controls. The ability of E-0858 to penetrate peach fruit possibly contributed to differences observed in the efficacy of the three fungicides in controlling brown rot when fruit were given a chlorinated-hydrocooling treatment.

In our study, penetration of E-0858 and iprodione into fruit was demonstrated in bioassays evaluating internal decay (Table 1) and in studies using ^{14}C-labeled fungicide (Fig. 3). Sufficient amounts of both iprodione and E-0858 penetrated mesocarp tissue to reduce the internal decay caused by *M. fructicola* when conidia of the fungus were injected 1 cm deep into the mesocarp. E-0858 resulted in greater suppression than either of the other two fungicides. As indicated in our radioactive-labeled fungicide studies, more E-0858 than iprodione penetrated the mesocarp tissue of peach fruit. A sharply decreasing gradient was observed for iprodione from the

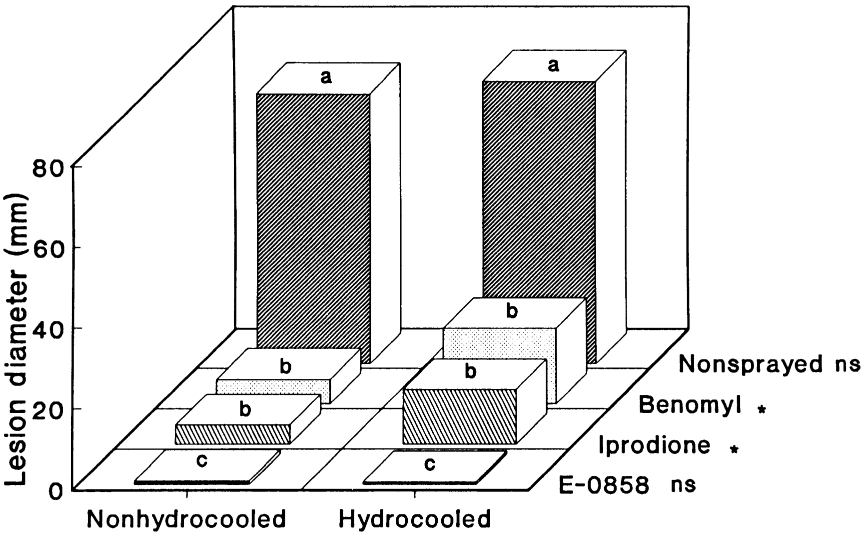

Fig. 2. Lesion diameter of peach fruit sprayed after harvest with iprodione, benomyl, or the experimental anilide E-0858 at 300 μg a.i./ml, not hydrocooled or hydrocooled in chlorinated water at 1 C for 30 min (16 hr after fungicide treatment), and postharvest surface wound-inoculated with 25 μl of a conidial suspension of *Monilinia fructicola* (2×10^4 conidia per milliliter). Fruit were incubated at 20 C and ≥95% RH, and lesion size was measured 6 days after inoculation. Statistical analysis was made on arcsine-transformed data. Fungicide treatment means for either hydrocooled or nonhydrocooled treatments marked with the same letter are not significantly different according to analysis of variance and least significant difference multiple comparison test ($P > 0.05$). Means of hydrocooled and nonhydrocooled treatments for each fungicide were separated by Student's *t* test ($P \leq 0.05$); ns = no significant difference, * = significantly different. Values of treatments are the means of eight replications (21 fruit per replication) from two experiments (four replications per experiment).

Table 1. Percent nondecayed mesocarp tissue of peach cv. Elegant Lady fruit surface-sprayed with 300 μg/ml of iprodione, benomyl, or the experimental anilide E-0858 at 21, 14, 7, or 1 day preharvest or immediately postharvest and internally inoculated with conidial suspensions of *Monilinia fructicola*[x]

	Percent nondecayed area				
	Preharvest spray (days)				Postharvest
Treatment	21	14	7	1	spray
Nontreated[y]	0.9 a[z]	0.9 a	0.8 a	0.7 a	1.1 a
Benomyl	1.7 a	2.4 a	2.3 a	3.4 a	4.3 a
Iprodione	2.6 a	3.7 a	13.0 b	17.6 b	20.9 b
E-0858	10.9 b	34.4 b	51.2 c	62.0 c	74.6 c

[x] Mesocarp tissue of fruit was inoculated 24 hr after harvest. Two sites (2 cm apart, 1 cm from the stem end, and 1 cm deep) on each fruit were injected with 200 μl of a conidial suspension of *M. fructicola* (2.5×10^3 conidia per milliliter). Seven days after incubation at 20 C and ≥95% RH, fruit were cut perpendicularly to the sites of inoculation and the percentage of nondecayed area was determined using a Quantimat 900 video image analyzer. Values are the average of 12 single-fruit replications from two experiments (six replications per experiment).

[y] Postharvest nontreated fruit were sprayed with water, and preharvest nontreated fruit were not sprayed.

[z] Statistical analysis was on arcsine-transformed data. Means within a column followed by the same letter are not significantly different according to analysis of variance and least significant difference multiple comparison ($P > 0.05$).

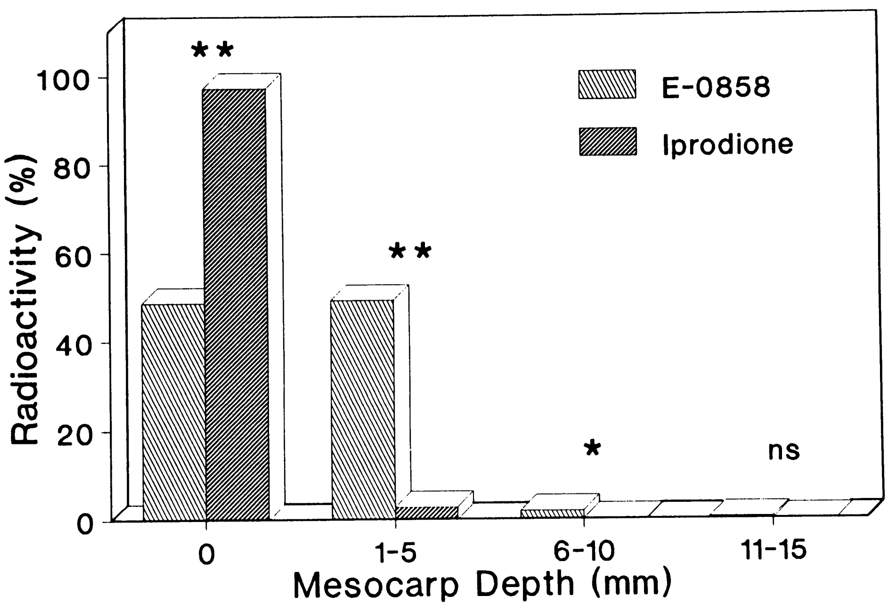

Fig. 3. Percent recoverable radioactivity distributed in peach cv. Elegant Lady fruit 24 hr after surface treatment with ^{14}C-iprodione or ^{14}C-E-0858 (an experimental anilide). Fruit were cut in half, sampled from epidermal ($\leq$ 1 mm = 0 on figure) and mesocarp tissues (depths of 1–5, 6–10, and 11–15 mm), and combusted, and radioactivity was measured with a liquid scintillation counter. Student's t test was used for comparisons of means of radioactivity between paired treatments of iprodione and E-0858 at each depth; ns = no significant difference, * = significantly different at $P \leq 0.05$, ** = significantly different at $P \leq 0.01$. Means are the average of 10 replications from two experiments (five replications per experiment).

surface to the mesocarp tissue, whereas the gradient for E-0858 was less pronounced. Ravetto (14) and Ravetto and Ogawa (15) showed a gradient for methyl-2-benzimidazole (MBC) in peach fruit similar to that for iprodione in our study; most of the MBC was on the surface (14). E-0858 has a solubility in water of 6,000 μg/ml, compared with 13 μg/ml for iprodione and 3.8 μg/ml for benomyl (17). Perhaps the greater solubility of E-0858 and iprodione in water than that of benomyl allows for absorption or penetration of these compounds through the fruit epidermis into mesocarp tissue of peach fruit. Thus, in our study, the differences in efficacy observed among the fungicides evaluated (E-0858, iprodione, followed by benomyl applied as postharvest treatment) in suppressing internal decay at the 1-cm depth correlate with the solubility of the fungicides in water and with the amount of fungicide that penetrates mesocarp tissue. Furthermore, in studies evaluating penetration of iprodione in cherry fruit, iprodione suppressed internal decay of mesocarp tissue at the pit caused by *B. cinerea* and *M. fructicola*. Residues of iprodione were detected in mesocarp tissue when the fungicide was applied to the surface of cherry fruit. Comparing the study with cherry with our study with peach, internal decay was suppressed more in cherry fruit than in peach fruit surface-treated with iprodione. Differences in suppression between cherry and peach fruit are probably due to the inherent differences in amount of mesocarp tissue between cherry and peach fruit and to penetration of iprodione into the upper 1–5 (-10) mm of mesocarp tissue.

The experimental anilide E-0858 as a pre- or postharvest treatment for control of brown rot is promising, but its future is uncertain because other properties of the compound, including mammalian toxicities, have not been disclosed by the manufacturer. Although iprodione did not inhibit lesion development as well as the experimental compound E-0858, it suppressed brown rot and was similar in its performance to benomyl. The efficacy of iprodione in controlling both benomyl-sensitive and benomyl-resistant populations of *M. fructicola* (11) and its low mammalian toxicity, as well as our observations of its penetration of fruit, make iprodione a valuable component in the brown rot disease control program in California.

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