# Penetration of Iprodione into Mesocarp Fruit Tissue and Suppression of Gray Mold and Brown Rot of Sweet Cherries

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**ABSTRACT**

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The efficacy of iprodione in controlling postharvest decay of sweet cherries (*Prunus avium*) caused by *Botrytis cinerea* and *Monilinia fructicola* was related to penetration of the fungicide into the mesocarp tissue of the fruit. In laboratory studies, fruit sprayed with an aqueous suspension of iprodione at 600 or 1,200 μg a.i./ml (Rovral 50WP) 1 hr before inoculation or 24 hr after inoculation (20 μl of 25,000 conidia per milliliter), prevented decay or completely suppressed development of decay caused by *B. cinerea* or *M. fructicola*. Additionally, penetration of iprodione was shown when fruit surfaces were sprayed with iprodione at 1,200 μg a.i./ml and inoculated at the pit using a syringe containing a conidial suspension of *B. cinerea* or *M. fructicola*. Fruit treated with iprodione had significantly less internal decay at the pit caused by either fungus than did fruit sprayed with water. Furthermore, decay was inhibited near the epicarp in treated fruit but not in nontreated fruit. Diameter of internal lesions at the pit caused by *B. cinerea* decreased linearly with increasing concentration of iprodione. Residues of iprodione in mesocarp tissue of fruit surface-sprayed with iprodione at 1,200 μg a.i./ml ranged from 0.18 to 0.21 μg/g of tissue.

Additional keywords: systemic activity, dicarboximide fungicides

Sweet cherries (*Prunus avium* (L.) L.) are primarily susceptible to the postharvest decays gray mold, brown rot, Alternaria rot, and Rhizopus rot caused by *Botrytis cinerea* Pers.:Fr., *Monilinia fructicola* (G. Wint.) Honey, *Alternaria alternata* (Fr.:Fr.) Keissl., and *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill., respectively (10). Postharvest fungicide treatments of fruit applied before shipping and marketing reduce the incidence of decay. One of the most effective fungicides used for postharvest control of brown rot and gray mold of stone fruits was benomyl. This benzimidazole fungicide has localized systemic activity in harvested fruit (13) and has been used for many years in combination with dicloran (DCNA or Botran) as a postharvest treatment to reduce decays of sweet cherries and other stone fruit crops (10). Benomyl and other benzimidazole fungicides, however, became ineffective with the development of benzimidazole-resistant populations of *B. cinerea* and *Monilinia* spp. in orchards throughout North America (11).

During 1974–1976, several dicarboximide fungicides (e.g., iprodione, vinclozolin, and procymidone) were introduced for control of pre- and postharvest fungal diseases of fruit and vegetable crops (9). In postharvest decay control studies of stone fruit crops, iprodione was effective against *Monilinia* spp. and *B. cinerea,* and moderately effective against *R. stolonifer* on sweet cherries (2,6,17) and on peaches (1,8). In 1989, it was registered for use in the United States for control of postharvest decays of stone fruits including brown rot and gray mold.

In general, the dicarboximide fungicides inhibit spore germination and mycelial growth. A specific mechanism of action of the dicarboximides has not been identified, but proposed mechanisms include binding to microfilaments and spindle fibers that affect DNA synthesis, and formation of secondary toxic compounds, either by bioactivation with cytochrome P-450 or by reductase enzyme inhibition that affects lipid metabolism and membrane stability (18). Additionally, iprodione is known to be translocated in some plants (3,4,15,20), suggesting that this fungicide, like benomyl, may be systemic in fruit tissue. In preliminary studies, Shotwell and Ogawa (17) indicated that iprodione penetrates into cherry fruit. Thus, the effectiveness of dicarboximides has been attributed to their fungitoxicity and localized translocation in plant tissue. The objectives of this study were to determine the efficacy of iprodione in suppressing postharvest decay caused by *B. cinerea* and *M. fructicola,* and to determine the systemic activity of the fungicide in cherry fruit.

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## MATERIALS AND METHODS

**Evaluation of the efficacy of iprodione and dicloran in disease suppression.** Mature, ripe Bing cherries were harvested from an orchard at the University of California, Davis (Armstrong Research Station), where no fungicides were applied preharvest. Fruit were placed on wire screens in plastic containers (31 × 24 × 10 cm). Distilled water was added to the bottom of the container but was not in contact with the fruit. To determine the efficacy of iprodione (Rovral 50WP) or dicloran (Botran 75WDG) in suppressing disease, two inoculation–fungicide application procedures were evaluated. In the first procedure, fruit were sprayed with autoclaved distilled water or aqueous suspensions of iprodione at 600 or 1,200 μg a.i./ml or dicloran at 1,500 μg a.i./ml using an atomizer and compressed air as the propellant, and air-dried for 1 hr (25 C). Fruit were then inoculated by wounding (1 × 0.5 × 1 mm) one side with a glass rod and placing 20 μl of a conidial suspension ($2.5 \times 10^4$ conidia per milliliter) of either *B. cinerea* (isolate JMO-83-1) or *M. fructicola* (isolate JEA-90CH) on the wound, and incubated at 20 C and >95% RH. In the second inoculation–fungicide application procedure, fruit were wounded and inoculated, incubated for 24 hr, sprayed with water or fungicides, and then incubated as described previously. For each procedure, treatments were replicated three times with eight fruit per replication. The experiment was done twice. Data were analyzed for each decay fungus using analysis of variance and least significant difference (LSD) mean separation procedures (16). Experiments were compared using Bartlett's test for homogeneity of variances (16).

**Evaluation of iprodione efficacy in mesocarp tissue.** Mature, ripe Bing cherries from the plant pathology field station in Davis, and Rainier and Royal Ann cherries from a commercial orchard in Stockton, California, none of which had been treated with preharvest fungicides, were harvested and stored for 2–5 days at 1 C. Fruit were surface disinfested in NaOCl (400 μg/ml) for 2 min and placed on wire screens in plastic containers containing water as described previously. Cherry fruit were sprayed with autoclaved distilled water or iprodione at 1,200 μg a.i./ml and air-dried as described previously. After storage for 48 hr at 1 C, a 5-μl suspension containing $8 \times 10^4$ conidia per milliliter of *B. cinerea* (isolate JMO 83-1) or $3 \times 10^4$ conidia per milliliter of *M.*

*fructicola* (isolate JEA-90CH) was injected into each fruit (by 22-gauge Hamilton syringe) on the side of the pit opposite the suture and incubated for 5 days at 20 C and >95% RH. Fruit were then cut in half, and lesion diameter at the pit was measured. Treatments were replicated at least three times with a minimum of six fruit per replication. The experiment was done twice on Bing and Rainier fruit using *M. fructicola* and once on Rainier fruit using *B. cinerea.* Additionally, a duplicate set of Royal Ann fruit was treated, inoculated with a 5-$\mu$l suspension of $1 \times 10^5$ conidia per milliliter of *B. cinerea* as described above, incubated 7 days, cut in half, and photographed. Data were analyzed using analysis of variance, LSD mean separation procedures, and Bartlett's test for homogeneity of variances (16).

**Evaluation of concentration of iprodione and inhibition of gray mold.** To determine the concentration of iprodione effective to minimize lesion diameter in the mesocarp tissue at the pit, surface-disinfested Royal Ann fruit were sprayed with autoclaved distilled water or an aqueous suspension of iprodione at 300, 600, 900, or 1,200 $\mu$g a.i./ml and stored for 3 days at 1 C and >95% RH. Fruit were inoculated by injecting 5 $\mu$l of a spore suspension containing $6.5 \times 10^4$ conidia per milliliter of *B. cinerea* (isolate JMO 83-1) on opposite sides of the pit and incubated for 7 days at 20 C and >95% RH. Lesion diameter at the pit was measured for each half fruit, and values were averaged for each cherry. Treatments consisted of five replications of five fruit per replication, and the experiment was repeated using Rainier fruit. Data for each treatment level were averaged and were analyzed using linear regression and analysis of variance procedures (16).

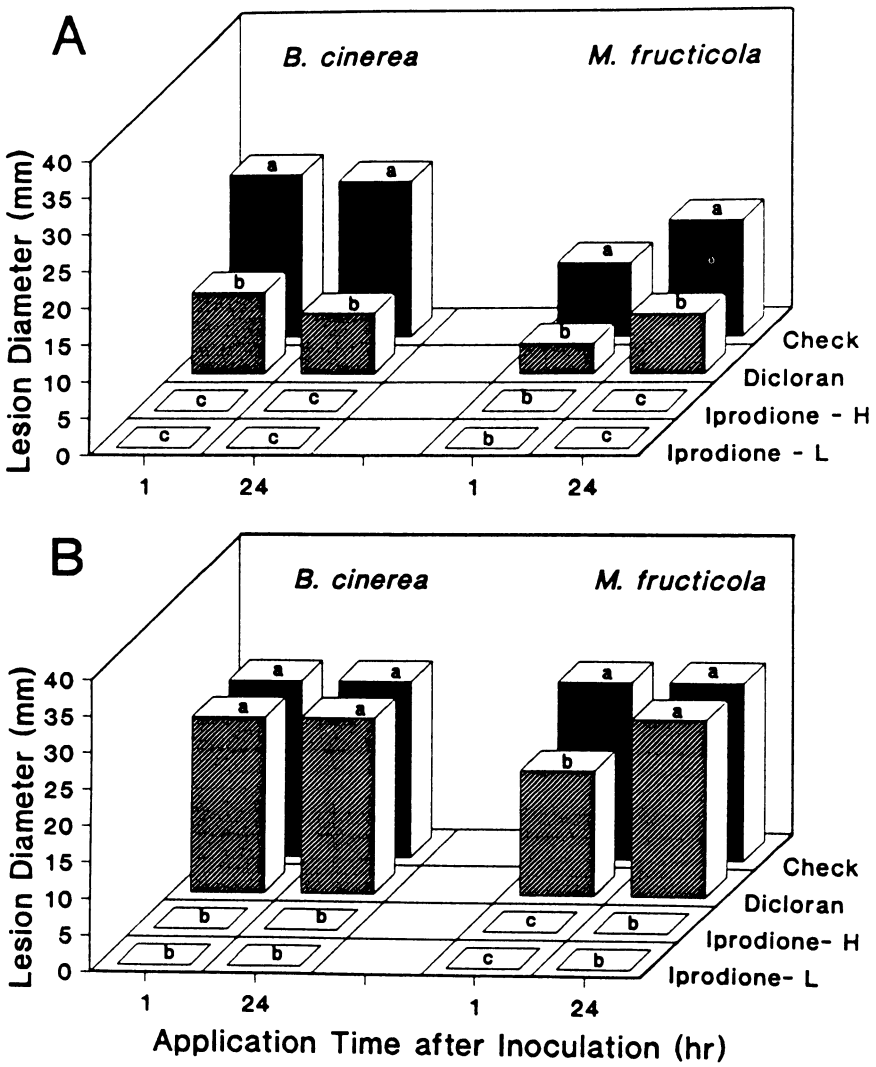


**Fig. 1.** Efficacy of iprodione and dicloran in protection and suppression of decay caused by *Monilinia fructicola* or *Botrytis cinerea* (20 $\mu$l of $2.5 \times 10^4$ conidia per milliliter) on Bing cherry fruit. Fruit were sprayed with iprodione-L or -H (600 or 1,200 $\mu$g a.i./ml, respectively), dicloran (1,500 $\mu$g a.i./ml), or distilled water (check) 1 or 24 hr after inoculation with either organism and evaluated **(A)** after 5 days or **(B)** after 9 days. The variances of two experiments were homogeneous, and the results are from one experiment. For each fungus and fungicide application time, treatments (bars) with the same letter are not significantly different ($P > 0.05$) according to least significant difference (LSD) mean separation.

**Residue analysis.** For iprodione residue analysis of the mesocarp tissue, three 500-g samples of healthy, noninjured Rainier cherries were sprayed with iprodione at 1,200 $\mu$g a.i./ml and incubated for 2, 3, or 5 days at 1 C and >95% RH. Additionally, a nontreated fruit sample (500 g) was incubated for 3 days. Following incubation, fruit were individually rinsed under running tap water for 15 sec, air-dried (25 C), and cut in half, and mesocarp tissue was removed with a small, curved metal spatula without contacting the fruit surface. Samples were frozen and sent to an analytical laboratory (Morse Laboratories, Sacramento, CA) for residue analysis of iprodione (RP26019) and its metabolites (RP32490 and RP30228) using gas-liquid chromatography (5).

## RESULTS

**Efficacy of iprodione and dicloran in disease suppression.** A significant difference ($P < 0.10$) was observed in lesion diameter caused by *M. fructicola* for application time, fungicide treatment, and the interaction of application time and fungicide treatment after 5 days (Fig. 1A) and 9 days (Fig. 1B) of incubation. For decay caused by *B. cinerea,* no significant differences ($P > 0.10$) in lesion diameter were observed between application time and the interaction of fungicide treatment and application time, whereas a significant difference ($P < 0.01$) was observed between treatments after 5 and 9 days of incubation (Fig. 1A and B). For fruit treated with iprodione, decay caused by *B. cinerea* or *M. fructicola* was completely inhibited for the 1-hr and completely suppressed for the 24-hr application time after inoculation for both incubation periods. Dicloran significantly reduced decay from both organisms from that of the nontreated fruit after 5 days (Fig. 1A). Lesion diameter of infections caused by *B. cinerea,* however, was not significantly different from the nontreated fruit for either application time after 9 days (Fig. 1B). Dicloran significantly reduced the lesion diameter of infections caused by *M. fructicola* in the 1-hr application time after inoculation, but failed to suppress decay in the 24-hr application time after inoculation and incubation for 9 days (Fig. 1B). Variances of the two experiments for either decay fungus were homogeneous.

**Efficacy of iprodione in mesocarp tissue.** Mean lesion diameter of decay in mesocarp tissue at the pit of Rainier and Bing fruit sprayed with iprodione on the surface and inoculated at the pit with conidia of *M. fructicola* was $1.5 \pm 1.9$ mm (standard deviation) and was significantly less ($P < 0.01$) than the $9.8 \pm 2.2$ mm mean lesion diameter of nontreated fruit. Variances of these experiments were homogeneous and data were com-

bined. For Rainier fruit inoculated with conidia of *B. cinerea,* mean lesion diameter at the pit of fruit treated with iprodione was 1.1 ± 0.3 mm and was significantly less ($P < 0.01$) than that of nontreated fruit, with a mean lesion diameter of 9.0 ± 0.03 mm. In nontreated fruit, decay formed a continuous region from the pit to the epicarp. In treated fruit, decay was observed in sharply delimited regions of the mesocarp tissue that did not extend to the epicarp (Fig. 2).

**Effect of iprodione concentration on inhibition of internal decay caused by *B. cinerea*.** A significant difference ($P < 0.01$) in lesion diameter of infections caused by *B. cinerea* was observed between iprodione- and nontreated Royal Ann or Rainier fruit. Lesion diameter decreased linearly on Royal Ann ($P < 0.01$) and on Rainier fruit ($P < 0.01$) with increasing concentration of iprodione (Fig. 3).

**Residual iprodione in mesocarp tissue.** Residual concentrations of iprodione (RP26019) were 0.19, 0.19, and 0.21 μg/g in cherries incubated for 2, 3, and 5 days after spraying, respectively. Residue concentrations of iprodione in fruit sprayed with water (check) were less than 0.05 μg/g. The limit of detectability of iprodione and its metabolites (RP32490 and RP30228) was 0.05 μg/g, whereas recovery from samples with a known amount of iprodione was 98%. Concentrations of iprodione metabolites were both below the level of detectability.

## DISCUSSION

The efficacy of iprodione against brown rot and gray mold of stone fruit crops is well documented (2,6,8,19). In our studies, iprodione protected cherry fruit against new infections, completely suppressed infections caused by either *B. cinerea* or *M. fructicola* established 24 hr before fungicide application, and provided complete control over a 9-day period. Dicloran protected and suppressed decay of both organisms after 5 days of incubation. After 9 days of incubation, however, dicloran only slightly reduced decay (lesion diameter) caused by *M. fructicola* and did not suppress infections of either decay organism as indicated by a significant interaction of postharvest fungicide application time and fungicide treatment. Suppression of brown rot and gray mold in fruit inoculated 24 hr before fungicides were applied suggests that iprodione penetrated into the mesocarp tissue of surface-treated fruit.

Internal decay was restricted to a region of mesocarp tissue adjacent to the pit when fruit were treated with iprodione and then inoculated at the pit with a syringe. A zone of healthy tissue near the epicarp suggests that a gradient of iprodione was established in the mesocarp of cherry fruit surface treated with iprodione. Ravetto (12) and Ravetto and Ogawa (13) demonstrated that dicloran and benomyl penetrate into peach fruit to control decay caused by *M. fructicola* and *R. stolonifer,* and that a decreasing concentration gradient of radioactively labeled dicloran or methyl-2-benzimidazole (MBC) occurred in the mesocarp toward the pit. As they suggested for dicloran and MBC (12,13), gradients may indicate that the fungicides moved into the fruit by simple diffusion. In our study, residue analysis confirmed that iprodione (0.20 μg/g) penetrated into the mesocarp tissue of fruit, and the amounts detected were well below the established tolerance level of 20 μg a.i./g of tissue. Penetration of iprodione in fruit tissue was expected to be similar to that of dicloran since dicarboximides are chemically similar to aromatic hydrocarbon fungicides (AHF) such as dicloran. Dicarboximides are often grouped with AHF because fungal isolates resistant to dicarboximides are also resistant to aromatic hydrocarbons (18). Furthermore, the efficacy of iprodione against several pathogens of potato, tomato, lettuce, and turfgrass also has been linked to the systemicity of the fungicide in plant tissue (3,4, 15,20). Thus, based on the initial research of Shotwell and Ogawa (17), this is the first report of systemic activity of iprodione in mesocarp tissue of a harvested stone fruit crop.

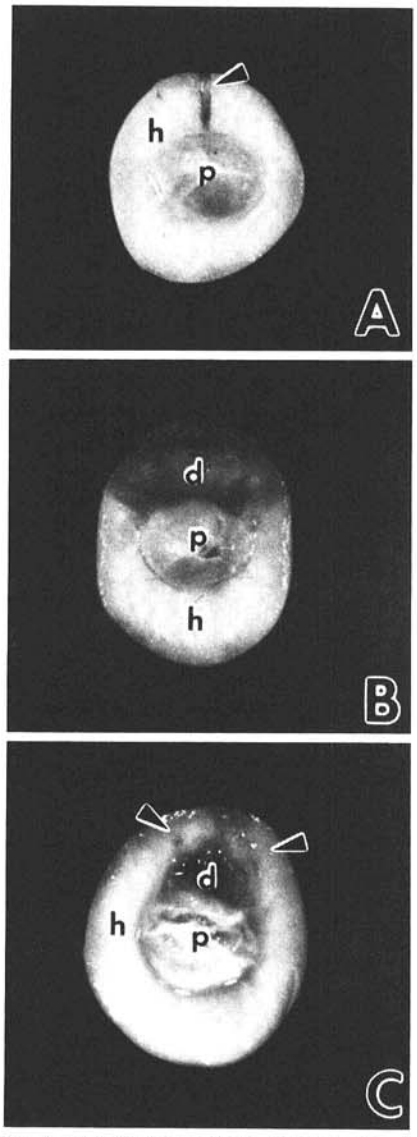


**Fig. 2.** Transverse sections through Royal Ann cherry fruit inoculated with *Botrytis cinerea* (5 μl of $1 \times 10^5$ conidia per milliliter) at the pit using a syringe treated with distilled water or iprodione (1,200 μg a.i./ml), and incubated 7 days at 20 C and >95% RH. Sections show healthy mesocarp tissue (h), cherry pit (p), and decayed mesocarp tissue (d). **(A)** Healthy fruit showing pit inoculation and syringe injury (arrowhead); **(B)** nonfungicide-treated fruit with extensive decay; and **(C)** iprodione-treated fruit showing limited decay near pit and a nondecayed zone of mesocarp tissue near epicarp (arrowheads).

The regression of lesion diameter of mesocarp tissue at the pit of cherry fruit caused by *B. cinerea* on iprodione concentration indicates that iprodione inhibited internal decay at concentrations as low as 300 μg/ml applied to fruit surfaces, and the degree of inhibition increased with increasing concentrations of iprodione. Possibly, the greater amount of iprodione applied on the surface resulted in a greater amount penetrating into the mesocarp tissue. Values less than 300 μg of iprodione per milliliter were less effective in reducing internal decay (J. M. Ogawa, *unpublished*). Residues of iprodione ranged from 0.18 to 0.21 μg/g of fruit tissue when the fungicide was applied at 1,200 μg of iprodione per milliliter. In Japan, minimum inhibitory concentrations of iprodione against nonresistant populations of *Botrytis* spp. ranged from 0.39 to 13.0 μg/ml (14).

The high activity of iprodione against species of *Botrytis, Monilinia,* and other fungal genera (7,9,19); the penetration of

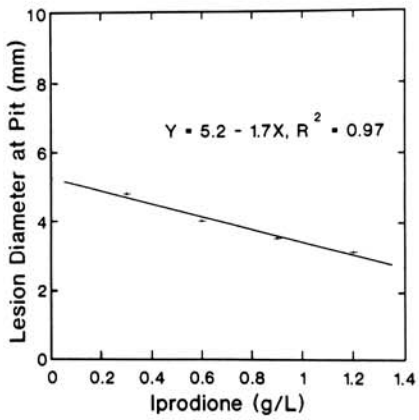


**Fig. 3.** Regression of average lesion diameter of gray mold at the pit of Royal Ann and Rainier cherry fruit on concentration of iprodione. Fruit were inoculated with *Botrytis cinerea* (5 μl of $6.5 \times 10^5$ conidia per milliliter) at the pit using a syringe treated with iprodione and incubated 7 days at 20 C and >95% RH.

iprodione observed in cherry fruit; and the suppression of established infections caused by *B. cinerea* and *M. fructicola* found in our study explain the high performance of iprodione as a commercial postharvest treatment of stone fruit crops. In California, iprodione and iprodione-dicloran mixtures have been used in commercial postharvest treatments of cherries since 1984 and thus confirm the benefits of these fungicides in preventing decays.


## ACKNOWLEDGMENTS

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