

Systemic Activity and Fungitoxicity of Iprodione and E-0858 (*N*-[5-(2-methoxypyridinyl)]-Cyclopropane-Carboxamide) in Almond Blossoms to *Monilinia laxa*

J. M. Osorio, J. E. Adaskaveg, J. M. Ogawa, R. M. Bostock, and A. D. Jones

First four authors: Department of Plant Pathology, and fifth author: Facility for Advanced Instrumentation, University of California, Davis 95616.

Current address of J. M. Osorio: Facultad de Agronomía, University of Panama, David, Chiriquí.

Supported by the Almond Board of California, Zeneca Ag Products, and Rhône Poulenc Ag Company.

Accepted for publication 21 March 1994.

ABSTRACT

Osorio, J. M., Adaskaveg, J. E., Ogawa, J. M., Bostock, R. M., and Jones, A. D. 1994. Systemic activity and fungitoxicity of iprodione and E-0858 (*N*-[5-(2-methoxypyridinyl)]-cyclopropane-carboxamide) in almond blossoms to *Monilinia laxa*. *Phytopathology* 84:656-660.

The structure of the experimental fungicide E-0858 was identified and affirmed as *N*-[5-(2-methoxypyridinyl)]-cyclopropane-carboxamide (molecular mass = 192 Da). The compound is a heterocyclic, anilide derivative with an empirical formula of C₁₀H₁₂N₂O₂ and represents a new class of fungicides. Translocation of iprodione and E-0858 into almond floral tissues was demonstrated by autoradiography, thin-layer chromatography (TLC), and combustion to radioactive carbon dioxide of blossoms treated with ¹⁴C-E-0858 and ¹⁴C-iprodione. These fungicides were applied either to the petals or the sepals of closed blossoms on detached shoots from 5-yr-old Drake and 10-yr-old Thompson almond trees, as well as 3-yr-old potted plants of Ne Plus Ultra almond. Translocation of ¹⁴C-E-0858 into stamens and pistils of blossoms on detached

shoots ranged from 4 to 8% of the recovered radioactivity, whereas the recovered radioactivity of ¹⁴C-iprodione in the same tissues ranged from 1 to 3%. On potted plants, translocation of fungicide into internal floral parts ranged from 1 to 2% for E-0858 and was 1% for iprodione. Higher radioactive counts were detected in stamens and pistils when fungicides were applied to sepals rather than to petals. Separation of constituents in extracts of ¹⁴C-E-0858-treated blossoms by TLC yielded three radioactive spots, but only the parent compound was fungitoxic to mycelium of *Monilinia laxa*. Some of the ¹⁴C-iprodione was converted into three radioactive products in almond blossoms, with the parent compound and one metabolite showing fungitoxic activity against mycelial growth of *M. laxa*. In bioassays, E-0858 and iprodione applied to closed blossoms provided stamen protection similar to that of the systemic fungicide benomyl.

Additional keywords: brown rot blossom blight, dicarboximides, fungicide translocation.

Translocation of fungitoxic materials into susceptible floral parts represents an ideal strategy in chemical control of blossom diseases of plants (7). Application time and number of applications of fungicide needed for control of brown rot blossom and twig blight of stone fruit, caused by *Monilinia laxa* (Aderhold & Ruhland) Honey or *M. fructicola* (G. Wint.) Honey, are influenced by systemic action and residual activity of the fungicide used in the spray program. Developmental studies on translocation of fungicides into blossoms of stone fruit, however, are limited. Ramsdell and Ogawa (12) showed that after ¹⁴C-benomyl was applied to closed blossoms, methyl 2-benzimidazolecarbamate (¹⁴C-MBC), a breakdown product, was translocated into pistils and stamens. The amount of ¹⁴C-MBC residue in the pistils and

stamens was sufficient to prevent infection by *M. laxa*. Furthermore, Ogawa et al (6) demonstrated that a single spray application of benomyl was equivalent to two spray applications of protectant fungicides.

Preliminary tests under laboratory conditions revealed that iprodione and the experimental fungicide E-0858 suppressed disease development after infection by *M. laxa* had occurred. Additionally, under field conditions, these fungicides reduced disease incidence when applied to unopened blossoms (8). From this evidence, we postulated that iprodione and E-0858 or their breakdown products penetrate the petals and sepals of almond blossoms and are translocated into the pistils and stamens. The objectives of this study were to characterize the chemical structure of E-0858, to determine if iprodione and E-0858 are systemic in almond blossom tissues, and to determine the fungitoxicity of these fungicides in blossom tissues to *M. laxa*.

MATERIALS AND METHODS

Characterization of E-0858. The experimental fungicide E-0858 (formulated as a 50WP) was obtained from Zeneca Ag Products (Willmington, DE). The compound was analyzed by $^1\text{H-NMR}$ (nuclear magnetic resonance) and gas chromatography/mass spectroscopy (GC/MS). For $^1\text{H-NMR}$, the fungicide was dissolved in dimethyl sulfoxide ($\text{DMSO}-d_6$) (1 mg/0.5 ml), and a spectrum was acquired on a General Electric QE-300 spectrometer at 300 MHz.

For GC/MS, the E-0858 (100 $\mu\text{g}/\text{ml}$) was dissolved in ethyl acetate, and splitless injections of 1- μl samples were performed on a 15-m DB-5 column (0.25 mm i.d., 0.25 μm of film; J & W Scientific, Folsom, CA) with helium as a carrier at 35 cm/s and a temperature program from 150 to 275 C at 10 C/min. Mass spectra were acquired by electron ionization at 70 eV with a VG Trio-2 mass spectrometer (VG Masslab, Altrincham, England). A high-resolution mass spectrum also was obtained by fast atom bombardment ionization on a VG ZAB-2F-HS mass spectrometer (VG Analytical, Wythenshawe, England) with a xenon atom beam (8 keV, 1 mA) and polyethylene glycol-300 as a matrix and mass calibrant.

Radiolabeled fungicides used for systemic studies. ^{14}C -E-0858 (specific activity 1.16×10^5 dpm/ μg) was provided by Zeneca and ^{14}C -iprodione, 3-(3,5-dichlorophenyl)-*N*-(1-methylethyl)-2,4-dioxo-1-imidazolidine-carboxamide (specific activity 20 $\mu\text{Ci}/\text{mmol}$), was provided by Rhône-Poulenc, Inc. (Triangle Park, NC). Iprodione was uniformly labeled in the phenyl ring, whereas the position of ^{14}C in the structure of E-0858 was not disclosed by Zeneca. An aqueous suspension of E-0858 or iprodione (Rovral 50WP) was mixed with ^{14}C -E-0858 or ^{14}C -iprodione, respectively, to produce fungicide suspensions of 300 μg a.i./ml, consisting of 1.5 or 2.5 $\mu\text{Ci}/\text{ml}$. The solvent *N*-methyl-2-pyrrolidone (M-pyrol) was added to the iprodione suspension (1%, v/v) to increase the solubility of the ^{14}C -iprodione.

Determination of systemic activity of E-0858 and iprodione in almond blossoms. Unopened blossoms at the pink bud stage of bloom on detached shoots were taken from 5-yr-old Drake almond trees and from 3-yr-old potted Ne Plus Ultra plants and treated with 10 μl of 300 μg a.i./ml (1.5 $\mu\text{Ci}/\text{ml}$) E-0858 or iprodione. Unopened blossoms on detached shoots taken from 10-yr-old Thompson trees were treated with 10 μl of 300 μg a.i./ml (2.5 $\mu\text{Ci}/\text{ml}$) E-0858 or iprodione. The fungicides were applied to either the petals or sepals as a small droplet with a Hamilton 701-N microsyringe (Hamilton Co., Reno, NV). The treated blossoms were kept at 23 C and $65 \pm 5\%$ relative humidity (RH) for 3 days, then separated into petals, sepals, stamens, and pistils and frozen in liquid nitrogen. The samples were oxidized by combustion in the presence of oxygen (10,11). Released CO_2 was trapped in 30 ml of a scintillation cocktail consisting of toluene/methanol/phenylethylamine (4.3:3.0:2.7, v/v/v) with 0.5% 2,5-diphenyloxazole (PPO) and 0.05% 2,2'-*p*-phenylene bis-5-phenyloxazole (POPOP) (14). Radioactivity was determined by liquid scintillation counting with corrections made for efficiency, quenching, and background counts. Translocation was expressed as the percentage of radioactivity recovered per blossom. Each treatment was replicated five times in the detached-shoot experiments (one blossom per shoot) and seven times in the potted-plant experiments (one blossom per plant). Percent radioactivity of each fungicide recovered in each blossom part was compared by general linear model and *t* test procedures (SAS, version 6.04, SAS Institute Inc., Cary, NC).

Additionally, floral parts of treated blossoms on detached Drake shoots were separated 3 days after treatment and pressed onto Kodak XAR diagnostic film. Autoradiographs were developed after 4 wk exposure at 24 C with an X-ray film processor.

Detection of metabolic products of E-0858 and iprodione in almond blossoms. Floral parts of Drake blossoms, treated and frozen as described in the preceding section, were macerated in acetone (5 ml per blossom per sample), the mixture was filtered through quartz fibers, and the filtrate was concentrated to 0.5

ml by rotary evaporation under a stream of N_2 .

E-0858, iprodione, and their metabolites were separated by thin-layer chromatography (TLC) on 250- μm silica-gel HLF plates (Analtech, Inc., Newark, DE). Aliquots of blossom extracts and of the proprietary fungicide suspensions amended with the ^{14}C -labeled compounds were applied to the TLC plates (5×10^5 dpm per spot). Chromatograms were developed with a mixture containing toluene/pyridine/methanol (70:15:15, v/v/v) as the solvent system for E-0858 and toluene/ethyl acetate/acetic acid (75:20:5, v/v/v) as the solvent system for iprodione. The plates were dried overnight, exposed to Kodak XAR diagnostic film for 4 wk at 24 C, and developed in an X-ray film processor. Radioactive spots in the plates were scraped, and radioactivity was quantified by liquid scintillation counting. Each chromatographic run was replicated six times, and the experiment was performed twice. Variances in treatments for experiments were evaluated by Bartlett's test for homogeneity of variances (SAS, version 6.04). Data with homogeneous variances were combined.

Fungitoxicity assays. Fully opened blossoms from 5-yr-old Drake trees not sprayed (control) or sprayed at the pink bud (unopened) stage with E-0858 or iprodione at 1.12 kg a.i./ha were frozen, ground in liquid nitrogen, extracted in acetone (5 ml per blossom per sample), and concentrated to 0.5 ml by rotary evaporation as described previously. Twenty-five microliters of extract for each treatment and 10 μl of E-0858 or iprodione aqueous suspension at 150 μg a.i./ml were separately applied to individual lanes on silica-gel HLF plates. Chromatograms were developed as described above, and plates were dried overnight. Dry plates were inoculated by spraying with a conidial suspension of *M. laxa* (1×10^5 conidia per milliliter) in a nutrient medium (1), using a chromist spray unit. The plates were placed in sterile plastic containers (10 \times 20 \times 30 cm) that contained 20 ml of sterile distilled water in opened petri dishes (HLF plates were not in contact with water), and the containers were sealed with tape. The samples were kept at 23 C in the dark for 3 days. Fungitoxicity of the compounds was based on the zone of mycelial inhibition formed around each spot and rated as positive (inhibition) or negative (no inhibition). Each treatment was replicated four times, and the experiment was performed twice.

Bioassays. Five-year-old Drake almond trees were sprayed at the pink bud (unopened) stage with E-0858, iprodione, or benomyl (Benlate 50WP) at 1.12 kg a.i./ha, using a handgun sprayer at 1,694 kPa. Trees that were not sprayed were used as controls. One day after the trees were sprayed, shoots with blossoms were excised from the trees, placed with stem ends in water, and incubated at 24 C until the blossoms were fully opened. Opened blossoms were detached from the shoots, placed with peduncles inserted in sterile moist sand in a plastic container (10 \times 20 \times 30 cm), and inoculated by spraying with a conidial suspension (1.2×10^4 conidia per milliliter) of a benomyl-sensitive isolate of *M. laxa*. Blossoms were incubated for 3 days at 23 C and $>95\%$ RH. The number of healthy stamens was determined for each blossom and was expressed as a percent for each treatment. The treatments were replicated four times (10 blossoms per replication), and the experiment was performed twice. Variances in treatments for the two experiments were evaluated by Bartlett's test for homogeneity of variances. Data with homogeneous variances were combined and analyzed by analysis of variance and least significant difference procedures (SAS, version 6.04).

RESULTS

Structure of E-0858. $^1\text{H-NMR}$ spectra of E-0858 indicated chemical shifts (expressed in parts per million) as follows: 10.18 (s, 1H); 8.32 (s, 1H); 7.87 (d, 1H, $J = 8$ Hz); 6.77 (d, 1H, $J = 8$ Hz); 3.79 (s, 3H); 1.727 (m, 2H); and 1.081 (t, 2H). Mass spectra of ethyl acetate preparations of E-0858 indicated: m/z 192 (M^+ , 36% of base peak); 124 (97%); 69 (100%); 41 (63%); and 137 (28%). High-resolution mass spectra indicated that m/z measured 193.0950 calculated for $\text{C}_{10}\text{H}_{13}\text{N}_2\text{O}_2[\text{M}+\text{H}]^+$ = 193.0977. The structure of E-0858 was described as *N*-[5-(2-methoxy-pyridinyl)]-cyclopropane-carboxamide, a heterocyclic anilide

TABLE 1. Percent recoverable radioactivity detected in floral parts of detached branches of Drake, Ne Plus Ultra, and Thompson almond 3 days after petals or sepals were treated with a mixture of labeled (^{14}C -E-0858 or ^{14}C -iprodione) and unlabeled fungicide

Cultivar ^a	Tissue treated	Treatment	Percent radioactivity (dpm) ^{y,z}			
			Petals	Sepals	Stamens	Pistils
Drake	Petal	E-0858	95.6 a	3.7 a	0.6 a	0.1 a
		Iprodione	98.7 b	1.2 b	0.1 b	0.0 a
	Sepal	E-0858	2.5 a	95.6 a	1.7 a	0.2 a
		Iprodione	1.6 b	97.9 b	0.4 b	0.1 a
Thompson	Petal	E-0858	95.3 a	3.6 a	0.8 a	0.3 a
		Iprodione	98.5 b	1.0 b	0.4 b	0.1 b
	Sepal	E-0858	4.5 a	91.5 a	3.2 a	0.8 a
		Iprodione	1.7 b	97.2 b	0.7 b	0.4 b
Ne Plus Ultra	Petal	E-0858	99.1 a	0.8 a	0.1 a	0.0 a
		Iprodione	99.2 a	0.7 a	0.1 a	0.0 a
	Sepal	E-0858	1.9 a	97.7 a	0.3 a	0.1 a
		Iprodione	0.9 b	99.0 b	0.1 b	0.0 a

^aDrake and Ne Plus Ultra blossoms were treated with 10 μl of E-0858 or iprodione at 1.5 $\mu\text{Ci}/\text{ml}$ applied to either the petals or the sepals. Thompson blossoms were treated with 10 μl of E-0858 or iprodione at 2.5 $\mu\text{Ci}/\text{ml}$.

^bSamples were oxidized by combustion and radioactivity assayed by liquid scintillation counting.

^cValues followed by the same letter for each paired fungicide treatment are not significantly different ($P > 0.05$) based on t test comparisons of radioactivity in different floral tissues. Values are the average of five replications for Thompson and Drake almond and seven replications for Ne Plus Ultra almond.

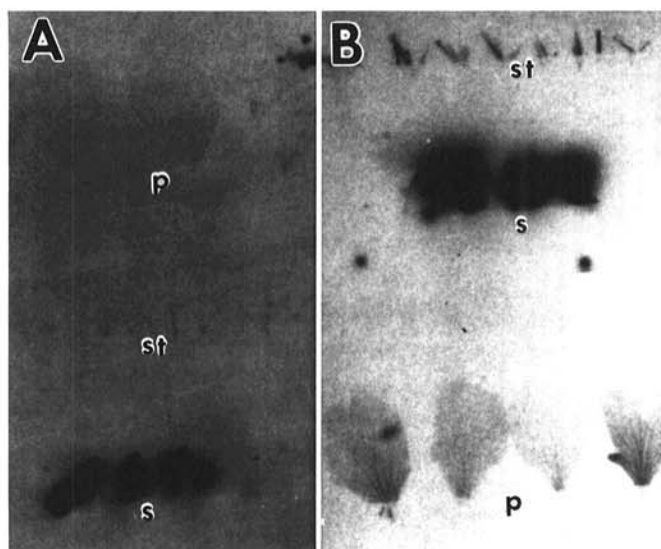


Fig. 1. Autoradiographs of blossom petals (p) and stamens (st) after sepals (s) of Thompson almond were treated with 10 μl of A, iprodione or B, E-0858 at 2.5 $\mu\text{Ci}/\text{ml}$.

with an empirical formula of $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_2$ (molecular mass = 192 Da).

Translocation of E-0858 and iprodione into almond blossoms.

^{14}C -E-0858 and ^{14}C -iprodione and their metabolites were recovered from stamens and pistils of blossoms treated at the pink bud (unopened blossoms) stage. On detached shoots (Drake and Thompson), the total percentage of recoverable radioactivity from floral tissues not in contact with the fungicide at the time of treatment ranged from 4 to 8% for E-0858 and from 1 to 3% for iprodione (Table 1). In pairwise comparisons, the amount of radioactivity detected in floral parts not directly in contact with the fungicides was generally higher ($P < 0.05$) in blossoms treated with E-0858 than with iprodione (Table 1). On potted plants (Ne Plus Ultra), translocation of E-0858 from the petals and sepals to other blossom parts ranged from 1 to 2%, whereas translocation of iprodione was 1% (Table 1). The amount of fungicide translocated was significantly lower ($P < 0.05$) than was observed on detached shoots but followed the same distribution pattern.

Translocation of iprodione and E-0858 occurred primarily to the sepals when the fungicides were applied to the petals (Table 1). In sepal-treated blossoms, however, recovered radioactivity of

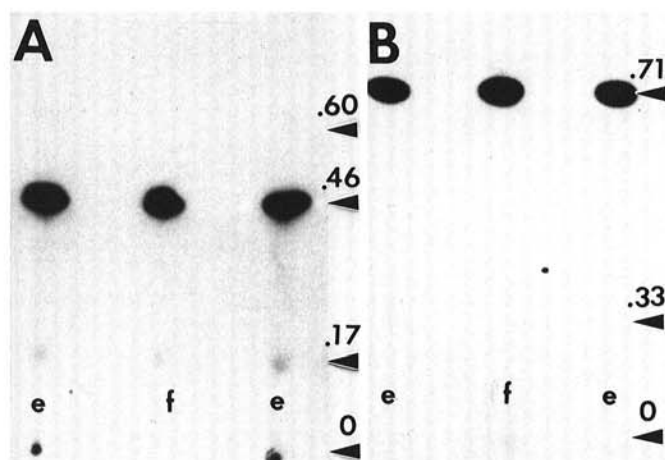


Fig. 2. Autoradiochromatograms of ^{14}C -labeled iprodione or E-0858 (f) or extracts of blossoms treated with ^{14}C -iprodione or ^{14}C -E-0858 (e). A, Iprodione. B, E-0858. Arrowheads indicate R_f values (distance of solute/distance of solvent) for parental and breakdown products of each fungicide.

either fungicide in stamens was significantly greater ($P < 0.05$) than that recovered from stamens of petal-treated blossoms (Table 1). No significant differences ($P > 0.10$) in amount of iprodione or E-0858 translocated in almond blossoms were observed between the 1.5 and 2.5 $\mu\text{Ci}/\text{ml}$ levels when these concentrations were applied to petals. Similarly, no differences in translocation of iprodione were observed when either level (1.5 and 2.5 $\mu\text{Ci}/\text{ml}$) of the radioactive fungicide was applied to sepals. However, when E-0858 at 2.5 $\mu\text{Ci}/\text{ml}$ was applied to the sepals, a significant increase ($P < 0.05$) or nearly two times as much translocation (8.5%) was detected, with 3.2 and 0.8% of the recovered radioactivity found in the stamens and pistils, respectively (Table 1).

Autoradiographs of floral parts of blossoms treated with ^{14}C -E-0858 or ^{14}C -iprodione at the pink bud stage also revealed translocation of both fungicides into internal floral tissues (Fig. 1). In blossoms treated with ^{14}C -E-0858, however, stamens and petals not directly in contact with fungicide at the time of treatment emitted more radiation (darker image) on X-ray films than did stamens and petals of iprodione-treated blossoms (Fig. 1).

Metabolism of E-0858 and iprodione in almond blossoms. The fungicide ^{14}C -E-0858 was degraded into additional metabolites in blossom tissues (Fig. 2), with R_f values of 0.00, 0.33, and 0.71 for two metabolites and the parent compound, respectively. E-0858 accounted for 97.6% of the radioactivity recovered from the plate,

whereas the other two compounds each accounted for 1.2% of the radioactivity recovered (Table 2). In aqueous solution, some breakdown of E-0858 occurred within 6 h of solution preparation, as evidenced by the presence of radioactivity at the origin of the chromatograms (Fig. 2).

Chromatograms of extracts of blossoms treated with ^{14}C -iprodione revealed radioactive regions at $R_f = 0.00, 0.17, 0.46,$ and 0.60 (Fig. 2). Most of the radioactivity recovered from the plates, 97.2%, was in the unreacted parent compound ($R_f = 0.46$), with 1.0, 0.9, and 0.9% of the radioactivity in the spots with R_f values of 0.00, 0.17 and 0.60, respectively (Table 2). Variances

TABLE 2. Fungitoxicity (Fungitox.) to *Monilinia laxa* and percent radioactivity (PR) of aqueous suspensions of iprodione and E-0858 or metabolized products of labeled ^{14}C -fungicides extracted from treated blossoms of Drake almond and separated by thin-layer chromatography (TLC)

Treatment	Preparation ^a	R_f	PR ^b (dpm)	Fungitox. ^c
E-0858	Blossom extract	0.00	1.2	—
		0.33	1.2	—
		0.71	97.6	+
	Aqueous suspension	0.00	1.4	—
		0.70	98.6	+
Iprodione	Blossom extract	0.00	1.0	—
		0.17	0.9	+
		0.46	97.2	+
	Aqueous suspension	0.60	0.9	—
		0.16	0.8	—
		0.48	99.2	+

^aExtracts of blossoms treated with ^{14}C -E-0858 or ^{14}C -iprodione were applied to TLC plates. A total of 5×10^3 dpm of each extract or aqueous solution of fungicide was applied to the plates.

^bPercentage of total radioactivity recovered in each compound. Values are the averages of six replications from each of two experiments.

^cPlates were developed and inoculated with a conidial suspension of *M. laxa* (1×10^4 conidia per milliliter) in a nutrient medium and incubated in the dark for 3 days at 25 C. + = mycelial inhibition; — = no mycelial inhibition.

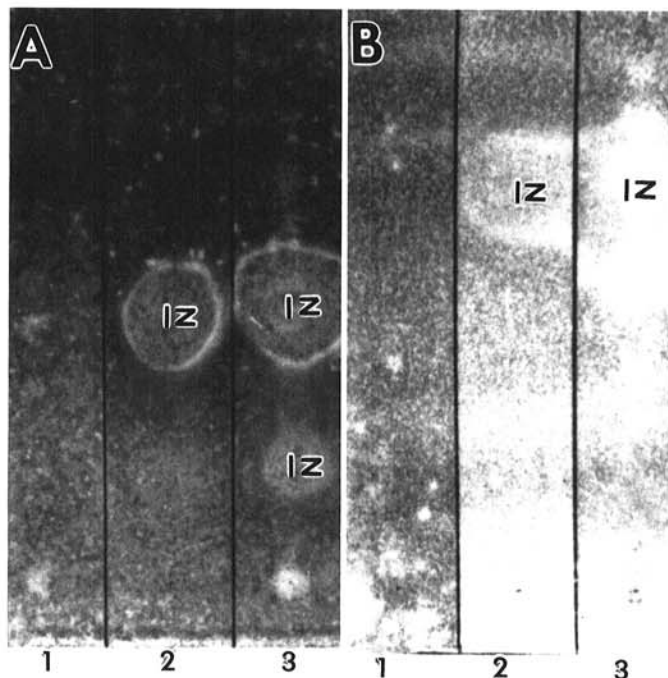


Fig. 3. Bioautographs of standard solutions of iprodione or E-0858 and extracts from nontreated and fungicide-treated blossoms. A, Iprodione and B, E-0858: lane 1, 15 μl of nonsprayed blossom extract; lane 2, 10 μl of iprodione or E-0858 at 150 μg a.i./ml in aqueous solution; and lane 3, 10 μl of extract from the fungicide-treated blossoms. Circular zones are areas of mycelial inhibition (iz).

of treatments from the two experiments were homogeneous, and data were combined.

Fungitoxicity of E-0858 and iprodione. Silica-gel HLF plates inoculated with a conidial suspension of *M. laxa* had one zone of mycelial growth inhibition on chromatograms of both aqueous suspensions of E-0858 and extracts of E-0858-treated blossoms (Fig. 3). The zone of inhibition corresponded to the area containing the parent compound, $R_f = 0.71$. Chromatograms of extracts of iprodione-treated blossoms showed two zones of mycelial growth inhibition. One inhibition zone was centered around $R_f = 0.17$, corresponding to one of the metabolites, and the other inhibition zone centered around the parent compound, $R_f = 0.46$ (Fig. 3). In aqueous solution, iprodione showed an inhibition zone at the parent compound, whereas the zone at $R_f = 0.17$ discolored mycelia but did not inhibit fungal growth.

Control of anther infection. Almond blossoms sprayed with benomyl, E-0858, or iprodione at the pink bud stage had fewer infected stamens than did the nonsprayed controls ($P < 0.05$). The percentage of healthy stamens was 22, 20, and 18 for benomyl, E-0858, and iprodione, respectively, compared to 4% for nonsprayed blossoms (Table 3). Variances of treatments from the two experiments were homogeneous, and data were combined.

DISCUSSION

The fungicide E-0858 is characterized as *N*-[5-(2-methoxy-pyridinyl)]-cyclopropane-carboxamide. The compound is a heterocyclic, anilide derivative with a molecular mass = 192 Da and an empirical formula of $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_2$. E-0858 was developed and originally characterized by Zeneca and represents a new class of pyridyl fungicides derived from intermediates in a herbicide-synthesis program (2). Our research affirmed the novel structure of this compound and provides $^1\text{H-NMR}$ and mass spectroscopy data.

The fungicides ^{14}C -E-0858 and ^{14}C -iprodione were translocated from the sepals and petals into the inner blossom parts, especially into the stamens. E-0858 displayed greater systemic activity than did iprodione in all tests (Figs. 1 and 2). The amount of radioactivity recovered from internal floral tissues not in contact with the fungicides at the time of treatment was higher in blossoms of detached shoots of mature trees than that recovered from blossoms of potted plants. Furthermore, sepal-treated blossoms absorbed and translocated more radioactive materials into the stamens and pistils than did petal-treated blossoms. Possibly, greater translocation of ^{14}C -fungicide from sepals than from petals was due to greater metabolic activity of green sepals than achlorophyllous petals. Nakamura (5) has shown that sepals of persimmon fix CO_2 and export photosynthates. Thus, the fungicides may have been cotransported with photosynthate to adjacent floral tissues. Alternatively, sepal application placed the fungicide

TABLE 3. Efficacy of benomyl, E-0858, or iprodione, applied in the field to unopened blossoms, in preventing stamen infection in blossoms opened and inoculated with *Monilinia laxa* in the laboratory^a

Treatment	Rate (μg a.i./ml) ^b	Healthy stamens ^c (%)
Benomyl	300	21.6 a
E-0858	300	20.0 a
Iprodione	300	18.4 a
Control	—	4.3 b

^aBlossoms were detached from shoots, placed with peduncles inserted in moist sand in a plastic container, inoculated with a conidial suspension of *M. laxa* (1×10^4 conidia per milliliter), and incubated for 3 days at 23 C.

^bBlossoms were sprayed with E-0858, iprodione, or benomyl at a rate of 1.12 kg a.i./ha, using a handgun sprayer at 1,694 kPa. Nonsprayed trees were used as controls.

^cPercentage of healthy stamens is the average of four replications (10 blossoms per replication) from two experiments. Values followed by the same letter are not significantly different according to ANOVA and LSD mean separation procedures ($P \leq 0.05$).

closer to internal floral tissues than did petal application. Considering this, and that less than 8% of either fungicide was detected in the internal floral tissues, placement of fungicide on external floral parts in close vascular proximity to internal floral tissues and diffusion of fungicides also may explain the movement of the fungicides into the stamens and pistils.

Translocation of E-0858 in blossoms of detached shoots was similar to that reported for benomyl (12). Although the methods of fungicide application and extraction were different, Ramsdell and Ogawa (12) showed that ¹⁴C-MBC (a derivative of benomyl) was translocated into internal blossom tissue when the fungicide was applied at green (no petals exposed) and pink bud (petals exposed) stages of mature almond trees under field conditions (0.3 cm total rainfall, 5–27 C, and 25–100% RH during experiments). In our tests, treated blossoms were kept at 23 C and 60% RH, and fungicide droplets dried soon after application. Shephard (13) pointed out that after spray droplets evaporate, absorption by underlying tissues of surface residues ceases until rewetting occurs. Under field conditions, a longer absorption period could result in slower drying or rewetting depending on environmental conditions after fungicide application. Thus, greater translocation of E-0858 or iprodione may occur under prolonged wetness periods.

Of the ¹⁴C-E-0858 applied to almond blossoms, 98% was recovered in the form of the parent compound, with the remainder present in two metabolites. Studies on the fungitoxicity of breakdown products of E-0858 revealed that only the parent compound was inhibitory to mycelium of *M. laxa*. Similarly, ¹⁴C-iprodione was recovered primarily in the form of the parent compound (97%), with the remainder present as three metabolites. Studies on the fungitoxicity of breakdown products of iprodione showed two zones of mycelial growth inhibition from blossom extracts. Although one of these metabolites ($R_f = 0.17$) accounted for only 1% of the radioactivity recovered (Table 2), this metabolite had a distinct zone of inhibition of mycelial growth on the chromatogram inhibition assay (Fig. 3). Compared to the benzimidazole fungicides, thiophanate-methyl and benomyl, which are rapidly transformed into the fungitoxic compound MBC in aqueous solution and in plant tissues (3,4,9), the conversion rates of the parent compounds of E-0858 and iprodione was much lower, and fungitoxicity was detected for only one breakdown metabolite of iprodione.

Bioassays showed that when manufactured formulations of E-0858 and iprodione were applied to closed blossoms, they provided protection of stamens similar to that of the systemic fungicide benomyl. These bioassays are consistent with the studies on absorption and translocation of ¹⁴C-E-0858 and ¹⁴C-iprodione. Field tests indicate that when these compounds are applied to closed blossoms they reduce disease incidence significantly (8). This study shows that iprodione and E-0858 are translocated to the inner blossom parts in sufficient concentration to protect the stamens of almond blossoms from infection by *M. laxa*. The parent compound serves as the fungitoxic material of E-0858, whereas both the parent compound and one breakdown product of iprodione in blossom tissues are fungitoxic to the mycelium of *M. laxa*. The systemic activity of these compounds may, in

part, explain their effectiveness in control of brown rot blossom blight of almond. Like benomyl, iprodione or E-0858 may be applied to unopened blossoms (e.g., pink bud stage) and provide protection of inner floral parts against *M. laxa* when blossoms open. Management strategies for fungicidal control of brown rot blossom blight are often limited to one or two spray applications during years of high rainfall and brown rot epidemics. Systemic fungicides provide greater flexibility in timing of fungicide application and reduction in the total number of sprays compared to contact fungicides that require multiple applications for protection of susceptible tissues (7). Compounds with characteristics for translocation in plants similar to or greater than iprodione, the experimental fungicide E-0858, and benomyl (12) should be developed to provide greater blossom blight control with minimal frequency of fungicide application.

LITERATURE CITED

- Allen, E. H., and Kuć, J. 1968. α -solanine and α -chaconine as fungitoxic compounds in extracts of Irish potato tubers. *Phytopathology* 58:776-781.
- Baker, D. R. 1992. A novel class of pyridyl fungicides. Pages 8-9 in: 8th Assem. Pestic. Design Res.: Basis Exploit. New Pestic. 21st Century. Pesticide Science Society of Japan, Kyoto.
- Clemons, G. P., and Sisler, H. D. 1969. Formation of a fungitoxic derivative from Benlate. *Phytopathology* 59:705-706.
- Clemons, G. P., and Sisler, H. D. 1971. Localization of the site of action of a fungitoxic benomyl derivative. *Pestic. Biochem. Physiol.* 1:32-43.
- Nakamura, M. 1967. Physiological and ecological studies on the calyx of the Japanese persimmon fruit. *Res. Bull. Fac. Agric. Gifu Univ. (Jpn.)* 23:1-62.
- Ogawa, J. M., Cañez, V. M., and Walls, K. M. 1987. Fungicides and bactericides used in management of plant diseases. Pages 25-45 in: *Fate of Pesticides in the Environment*. J. W. Biggar and J. N. Seiber, eds. Univ. Calif., Agric. Exp. Stn., Publ. 3320. 157 pp.
- Ogawa, J. M., Manji, B. T., and Sonoda, R. M. 1985. Management of the brown rot disease on stone fruits and almond in California. Pages 8-15 in: *Proc. Brown Rot Stone Fruit Workshop*. N.Y. State Agric. Exp. Stn. Bull. 55, Cornell Univ., Ithaca. 22 pp.
- Osorio, J. M., Bostock, R. M., and Ogawa, J. M. 1989. Systemicity in almond blossoms and efficacy of E-0858 and iprodione for control of brown rot blossom blight (Abstr.) *Phytopathology* 79:911.
- Peterson, C. A., and Edgington, L. V. 1969. Quantitative estimation of the fungicide benomyl using a bioautograph technique. *J. Agric. Food Chem.* 17:888-889.
- Peterson, J. I. 1969. A carbon dioxide collection accessory for the rapid combustion apparatus for preparation of biological samples for liquid scintillation analysis. *Anal. Biochem.* 31:204-210.
- Peterson, J. I., Wagner, F., Siegel, S., and Nixon, W. 1969. A system for convenient combustion preparation of tritiated biological samples for scintillation analysis. *Anal. Biochem.* 31:189-203.
- Ramsdell, D. C., and Ogawa, J. M. 1973. Systemic activity of methyl 2-benzimidazolecarbamate (MBC) in almond blossoms following pre-bloom sprays of benomyl + MBC. *Phytopathology* 63:959-964.
- Shephard, M. C. 1985. Fungicide behavior in the plant: Systemicity. *Br. Crop Prot. Council. Monogr.* 31:99-106.
- Takeda, F., Ryugo, K., and Crane, J. C. 1980. Translocation and distribution of ¹⁴C-photosynthates in bearing and nonbearing pistachio branches. *J. Am. Soc. Hortic. Sci.* 105:642-644.