

Control of Rhizoctonia Stem Rot of Poinsettia During Propagation with Fungicides that Prevent Colonization of Rooting Cubes by *Rhizoctonia solani*

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ABSTRACT

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The effectiveness of several fungicides applied either as foliar sprays or as rooting-cube soaks was compared for control of Rhizoctonia stem rot of poinsettia during propagation. *Rhizoctonia solani* was introduced to the surface of rigid foam rooting cubes at the time cuttings were stuck. Benomyl at 0.3 g a.i./L, chlorothalonil at 1.8 ml a.i./L, flutolanil at 0.3 g a.i./L, iprodione at 0.6 g a.i./L, and metalaxyl + benomyl at 0.5 g a.i./L all prevented stem rot. Rooting-cube soaks of flutolanil, iprodione, and metalaxyl + benomyl were as effective as foliar sprays in disease control. Foliar sprays of quinterozone at 0.45 g a.i./L and ethazole + thiophanate methyl at 0.24 g a.i./L were not as effective in control of stem rot as the other fungicides tested. Root development on cuttings treated with flutolanil and metalaxyl + benomyl was not different from the untreated, uninoculated control. Root development was intermediate with benomyl, chlorothalonil, and iprodione and poorest with quinterozone and ethazole + thiophanate methyl. Untreated rooting cubes (47 cm³) were colonized by *R. solani* from inoculum placed on the cube surface in 2-5 days. Rooting cubes of poinsettia treated with metalaxyl + benomyl, flutolanil, and iprodione at 0.25 g a.i./L were not colonized as extensively as untreated cubes. Fungicide effectiveness in control of stem rot was related directly to the extent of colonization of rooting cubes by *R. solani*.

Additional keyword: *Euphorbia pulcherrima*

Rhizoctonia stem rot of poinsettia, caused by *Rhizoctonia solani* Kühn, is a widespread and destructive disease (2,6). Although most growers have devel-

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and be a source of inoculum for development of stem rot during propagation.

The purpose of this research was to evaluate the effectiveness of several fungicides, applied as foliar sprays or rooting-cube soaks during propagation, in prevention of Rhizoctonia stem rot of poinsettia and to determine if fungicides applied at the start of propagation inhibit root development. In addition, the effect of the fungicides on colonization of rooting cubes by *R. solani* was determined.

MATERIALS AND METHODS

Poinsettias. Stock plants of poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch 'Gutbier V-14 Glory') were maintained in 6-L polyvinyl containers on a shaded greenhouse bench. Plants were fertilized weekly with 200 mg/L of N (N-P-K, 21:7:7). Cuttings were taken just before being stuck in rooting cubes as described below.

Inoculum preparation. An isolate of *R. solani*, RS3, from a poinsettia cutting with stem rot was used in all experiments. Petioles 5-10 cm long were removed from extra stock plants and dipped in 95% EtOH for 60 sec. Five to six petioles were placed on moistened filter paper in a sterile petri plate. A 7-mm-diameter potato-dextrose agar (PDA) disk with mycelium from a 9-day-old culture of *R. solani* was placed between each petiole in the petri plate. Within 5-6 days, mycelium of *R. solani* had grown throughout the plate colonizing the petioles. Colonized petioles were cut aseptically into 5-mm-long segments for use as inoculum. In colonization experiments, *R. solani* was cultured on sterilized rice grains for 7 days.

oped management strategies, including sanitation and preventive fungicide programs, to avoid epidemics, the pathogen continues to cause losses in poinsettia. Recently, Powell (3) described the effectiveness of benomyl and quinterozone as soil drenches for control of Rhizoctonia stem rot in poinsettia cuttings. In each experiment, benomyl or combinations of benomyl + ethazole gave better control of stem rot than quinterozone (3).

A large segment of the poinsettia crop is produced from cuttings taken from stock plants and stuck in rigid soilless rooting cubes. Cubes with cuttings are placed on greenhouse benches under intermittent mist for 2-4 wk until cuttings are rooted. Organic debris containing inoculum of *R. solani* may contaminate the rooting cube or cutting

Rooting cubes and petiole inoculum.

Dry strips of five foam rooting cubes (Oasis Rootcubes, Smithers-Oasis U.S.A., Kent, OH) were saturated with water or soaked in a fungicide solution as described below. A dry five-cube strip absorbed 200 ml of water. Each strip then was secured by rubber bands in a polystyrene sleeve provided by the manufacturer. The sleeve covered the sides and bottom of the strip. Individual rooting cubes were 25 mm wide × 51 mm long × 37 mm high (47 cm³). Before sticking poinsettia cuttings, the long axis of a 5-mm-long segment of petiole inoculum was placed on the top surface of the strip along the junction line separating cubes in the strip about 2 cm away from preformed holes in which the cutting was placed. With inoculum also placed at each end of the five-cube strip, there were a total of six inoculum pieces per strip.

Propagation. Cuttings were stuck with care in the rooting cubes as soon as the inoculum segments were in place to avoid contacting the cutting with the colonized petioles. A mist system was used to wet the cutting and cube for 2 min twice a day on a shaded greenhouse bench. Initially, cuttings wilted during the warmest portion of the day, but after a few days, foliage on healthy cuttings remained turgid.

Fungicides. Foliar sprays and rooting-cube soaks were used to apply fungicides. Foliar sprays were applied to run off in the afternoon following sticking after foliage had dried from the first misting. Each five-cube strip was used as a replication and sprayed or soaked individually. Fungicide contacted both foliage and the top surface of the cubes during spraying. Foliar sprays consisted of 60 ml of fungicide solution applied to each five-cube strip. For rooting-cube soaks, an application rate of 947 ml/929 cm² (2 pt/ft²) was chosen in which 24 ml of fungicide solution mixed with 176 ml of water was absorbed completely by the dry five-cube strip. The strips were soaked before sticking poinsettia cuttings.

The fungicides and rate of active ingredient used as foliar sprays included: benomyl, 0.3 g/L (Benlate 50W, DuPont Chemical Co., Wilmington, DE); ethazole + thiophanate methyl, 0.24 g/L (Banrot 40W, Grace Sierra Co., Milpitas, CA); chlorothalonil, 0.73 ml/L (Daconil 2787 40.4F, Fermenta Plant Protection Co., Cleveland, OH); flutolanil 50W, 0.30 g/L (Nor-Am Chemical Co. Wilmington, DE); iprodione, 0.60 g/L (Chipco 26019 50W, Rhone-Poulenc Agricultural Division, Research Triangle, NC); metalaxyl + benomyl, 0.50 g/L (Subdue 2W + Benlate 40W, Varsity 42W, Ciba-Geigy Agricultural Division, Greensboro, NC); and PCNB, 0.45 g/L (quintozene, Terraclor 75W, Uniroyal Chemical Co., Middlebury, CT). There were three replications per fungicide, each with five cuttings arranged in a randomized complete

block design. The experiment was repeated once.

In another experiment, the following fungicides with active ingredient were applied as foliar spray or rooting-cube soak treatments: flutolanil, 0.30 g/L; iprodione, 0.25 g/L; and metalaxyl + benomyl, 0.50 g/L. There were three replications per treatment and the experiment was repeated once.

Root development and disease assessments. Root development was assessed on a scale where 1 = no roots formed on cutting, 2 = roots initiated but not growing through to outside surface of cube, 3 = roots growing through one outside surface of cube, 4 = roots growing through two outside surfaces of cube, and 5 = roots growing through all three outside surfaces of the cube.

Poinsettias were rated for extent of stem rot after 29 days. A stem rot scale where 1 = no stem lesion, 2 = lesions covering less than 25% of stem surface, 3 = lesions covering 26–50% of stem, 4 = lesions covering 51–75% of stem, and 5 = stem completely girdled, cutting collapsed, was used.

Growth of rooted cuttings after transplanting was evaluated in longer experiments. At the time of transplanting, isolations were made from untreated and fungicide-exposed cubes to assess colonization of cubes by *R. solani*. One of the five cubes in the strip was chosen randomly for culture. The outer 5-mm layer of the cube was removed aseptically, then five blocks about 5 × 15 mm were cut at random from the inner portions of the cube and transferred to PDA. Culture plates were examined in 24 and 48 hr for typical colonies of *R. solani*. The pathogen was not isolated from rooting cubes of apparently healthy poinsettias treated with fungicides except for one of three cubes of poinsettia treated with either ethazole + thiophanate methyl or quintozene.

After selection of the cube for isolation, the remaining apparently healthy poinsettias in each strip of five per treatment were transplanted to Metro-Mix 260 (W. R. Grace Co., Cambridge, MA) in 10-cm-diameter pots and placed under drip irrigation (5 min twice a day). Plants with visual symptoms of stem rot were not transplanted. Plants were fertilized the same as stock plants. Sixty days after transplanting, plant height and stem rot symptoms were assessed.

Colonization of rooting cubes by *R. solani*. Colonization of the rooting cube by *R. solani* in the presence of the fungicides was assessed in another series of experiments. Fungicide sprays of metalaxyl + benomyl (0.5 g a.i./L), flutolanil (0.30 g a.i./L), or iprodione (0.25 g a.i./L) were applied to poinsettias in rooting cubes. The effect of spray application on colonization was compared with rooting-cube soaks of the same fungicide as described earlier. A 7-day-old rice grain colonized by *R. solani* was placed on the top of

the cube surface along the junction line between cubes as described for colonized petiole inoculum before spraying or after the cube soak. Treated rooting cubes of poinsettias were misted in the greenhouse as described earlier. There were two replications per treatment with enough strips set up for sampling at day 2, day 5, and day 13. A total of three interior cubes were removed at random from the two replications at sampling. At 14 days, the remaining unsampled poinsettias were rated for stem rot as described earlier.

The outer 5 mm of each sampled cube was removed aseptically and discarded. The inner core of the cube was divided into four columns about 10 mm wide. Each column was then divided into four rows about 6 mm high. Thus, each section was about 6 × 10 × 14 mm. Sections were placed on PDA as described earlier for detection of *R. solani*. Position of the individual sections relative to the original cube was recorded so that colonization of *R. solani* through the cube could be followed. In addition, an analysis of the number of sections colonized by *R. solani*, regardless of cube location, was done for each treatment. The experiment was repeated once.

RESULTS

Comparison of fungicides as foliar sprays. Untreated cuttings exposed to petiole inoculum of *R. solani* developed extensive stem rot and collapsed within 7–10 days (Fig. 1A). Poinsettias treated with foliar sprays of chlorothalonil, flutolanil, iprodione, or metalaxyl + benomyl had stem rot ratings less than 1.3 and were not different ($P = 0.05$) from the untreated, uninoculated control after 29 days (Fig. 1A). Quintozene and ethazole + thiophanate methyl gave the least control of Rhizoctonia stem rot of any fungicides tested (Fig. 1A). Stem rot was intermediate for cuttings treated with a benomyl spray.

Rooting ratings of cuttings treated with flutolanil and metalaxyl + benomyl were equivalent to ratings for the untreated, uninoculated control (Fig. 1B). Rooting ratings of poinsettias treated with chlorothalonil, iprodione, benomyl, ethazole + thiophanate methyl, and quintozene were less than the untreated, uninoculated control (Fig. 1B).

Comparison of foliar sprays to soaking rooting cubes in fungicide. Rooting cubes soaked in fungicide solutions were as effective as foliar sprays in preventing stem rot. For example, stem rot ratings for the metalaxyl + benomyl combination was 1 for either mode of application (Fig. 2A). Stem rot was less ($P = 0.05$) than the untreated, inoculated cuttings for all fungicides tested. However, only cuttings treated with flutolanil and metalaxyl + benomyl had stem rot ratings as low as the untreated, uninoculated control (Fig. 2A).

Rooting ratings between fungicide-soaked cubes and foliar sprays were not

different (Fig. 2B). Rooting ratings were not different ($P = 0.05$) among the untreated-uninoculated control and poinsettias treated with flutolanil and poinsettias treated with flutolanil and metalaxyl + benomyl (Fig. 2B). However, less rooting ($P = 0.05$) occurred on cuttings exposed to iprodione (Fig. 2B).

Plant growth and disease development after transplanting. Height of plants treated with flutolanil, iprodione, and metalaxyl + benomyl was the same ($P = 0.05$) as the untreated, uninoculated control 60 days after transplanting (Table 1). Plants treated with benomyl, chlorothalonil, ethazole + thiophanate methyl, and quinterozone were shorter ($P = 0.05$) than the untreated controls. No untreated, inoculated poinsettias were transplanted because stem rot killed all of them in the propagation phase of the experiment.

At least 40% or more of the rooted cuttings treated with one application of chlorothalonil, quinterozone, ethazole + thiophanate methyl, and benomyl at propagation developed symptoms of *Rhizoctonia* stem rot within 60 days after transplanting (Table 1). No stem rot symptoms were observed on the control

plants or those treated with metalaxyl + benomyl, whereas 8% of the plants treated with flutolanil had symptoms (Table 1).

Colonization of rooting cubes by *R. solani*. Within 2 days of placing inoculum on untreated rooting cubes, the pathogen was isolated from the top half of the cube

(Figs. 3 and 4). After 5 days, the pathogen was isolated from most cube sections at the bottom of the cube, 29 mm from the surface. (Figs. 3 and 4). Within 13 days, all cube sections were colonized by *R. solani*.

In contrast, colonization of rooting cubes of poinsettias treated with fungicide sprays or rooting-cube soaks was markedly less (Figs. 3 and 4). Whether applied as a fungicide spray or rooting-cube soak, poinsettias treated with metalaxyl + benomyl and flutolanil had very few of the cube sections colonized by *R. solani* even after 13 days. Colonization was limited to the upper half of the cubes with both fungicides (Figs. 3 and 4). In contrast, poinsettias treated with iprodione at 0.25 g a.i./L as a rooting-cube soak had almost no cube sections colonized even after 13 days, compared with several sections colonized for those treated with the iprodione spray at the same concentration (Figs. 3 and 4).

The number of sections colonized by *R. solani* within individual cubes for poinsettias treated with fungicides was less ($P = 0.05$) than for untreated poinsettias at all sampling dates (Fig. 5). At day 13, only the metalaxyl + benomyl spray and soak treatments had fewer sections colonized (zero sections) per cube ($P = 0.05$) than cubes of poinsettias treated with iprodione sprays (4.3 sections per cube) (Fig. 5).

Stem rot assessed at 14 days after sticking poinsettias in the rooting cubes not sampled for section colonization gave an estimate of disease development for poinsettias in the various treatments. Poinsettias in the untreated control had stem rot ratings averaging 5 (dead), compared with lower ($P = 0.05$) ratings for poinsettias in all fungicide treatments. Only poinsettias in iprodione treatments had stem rot ratings greater than 1 ($P = 0.05$), with the iprodione spray (2.2 rating) greater than ($P = 0.05$) the iprodione cube soak (1.6 rating). Cuttings had not rooted in any treatment

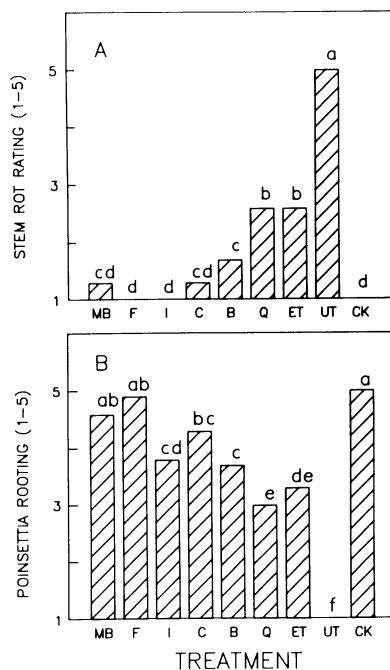


Fig. 1. Efficacy of foliar fungicide sprays on control of *Rhizoctonia* stem rot of poinsettia caused by *Rhizoctonia solani* and on rooting of poinsettia cuttings in rooting cubes. (A) Stem rot rating where 1 = healthy and 5 = stem girdled, cutting collapsed, and (B) poinsettia rooting where 1 = no roots formed on cutting and 5 = roots formed on all three sides of rooting cube, at 29 days, for metalaxyl + benomyl (MB), flutolanil (F), iprodione (I), chlorothalonil (C), benomyl (B), quinterozone (Q), ethazole + thiophanate methyl (ET), untreated inoculated (UT), and untreated uninoculated control (CK). Bars capped with the same letter are not significantly different as tested by an F protected Waller-Duncan k ratio; $k = 100$, $P = 0.05$.

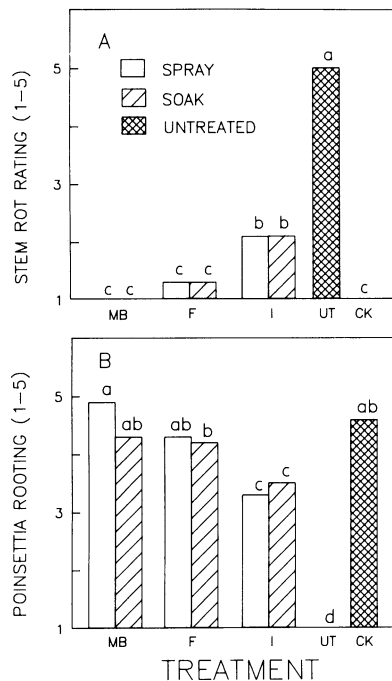


Fig. 2. Efficacy of several fungicides as foliar sprays compared to rooting-cube soaks for control of *Rhizoctonia* stem rot of poinsettia caused by *Rhizoctonia solani* and for rooting of poinsettia cuttings in rooting cubes. (A) Stem rot rating where 1 = healthy and 5 = stem girdled, cutting collapsed, and (B) poinsettia rooting where 1 = no roots formed on cutting and 5 = roots formed on all three sides of rooting cube, at 33 days, for metalaxyl + benomyl (MB), flutolanil (F), iprodione (I), untreated inoculated (UT), and untreated uninoculated control (CK). Bars capped with the same letter are not significantly different as tested by an F protected Waller-Duncan k ratio; $k = 100$, $P = 0.05$.

Table 1. Plant height and *Rhizoctonia* stem rot caused by *Rhizoctonia solani* for poinsettias transplanted as apparently healthy rooted cuttings treated in rooting cubes with foliar fungicide sprays 89 days earlier

Fungicide	Rate (g a.i./L)	Rooted cuttings (no.) ^w	Plant ht (× cm) ^x	Stem rot (%) ^y
Chlorothalonil 40.4F	0.73 ^z	11	9.3 cd	55
Quinterozone 75W	0.45	6	8.5 d	50
Ethazol + thiophanate methyl 40W	0.24	7	10.9 bcd	43
Benomyl 50W	0.30	10	10.9 bcd	40
Iprodione 50W	0.60	11	16.4 ab	17
Flutolanil 50W	0.30	12	14.8 abc	8
Metalaxyl + benomyl 42W	0.50	12	17.9 a	0
Control	...	12	18.3 a	0

^w Rooted cuttings transplanted 29 days after fungicide treatment.

^x Plant height means followed by the same letters are not significantly different according to the Waller-Duncan k ratio; $k = 100$, $P = 0.05$.

^y Percentage of transplanted rooted cuttings with stem rot symptoms 60 days after transplanting.

^z Rate expressed as milliliter a.i./L.

at 14 days, although callus and root initials were present on healthy stems.

DISCUSSION

The excellent control of *Rhizoctonia* stem rot of poinsettia observed with foliar sprays of chlorothalonil, flutolanil, iprodione, and metalaxyl + benomyl and rooting-cube soaks of flutolanil and metalaxyl + benomyl was probably attributable to the lack of colonization

of rooting cubes by *R. solani*. On untreated rooting cubes, *R. solani* grew on and through the cube to infect cuttings. Foliar sprays of effective fungicides contacted the rooting cube surface as well as the cutting during application and, thus, prevented growth of *R. solani* on and in the rooting cube.

The effectiveness of the rooting-cube soak in control and in prevention of cube colonization provided evidence of the importance of protecting the rooting cube from colonization by *R. solani*. With rooting-cube soaks, the cutting was exposed to fungicide only at the point of contact where the cutting meets the rooting cube. It is unlikely that enough fungicide was transferred to the cutting to protect it. Instead, hyphae of *R. solani* must have been prevented from growing to the cutting from the petiole inoculum. In situations where cuttings became infested with debris containing *R. solani* before they are stuck in the cube, poor control of stem rot by rooting-cube soaks would be expected.

In the rooting cube colonization studies, fungicides applied as rooting-cube soaks were generally more effective than fungicides applied as foliar sprays. Not only was less fungicide active ingredient applied in rooting-cube soaks, but nontarget fungicide drift associated with fungicide sprays was avoided.

Flutolanil provided excellent control of stem rot either as a foliar spray or as a rooting cube soak. Apparently, this is the first report of the effectiveness of flutolanil in control of *R. solani* on ornamentals.

Iprodione as a drench effectively controlled *Rhizoctonia* stem rot on rooted cuttings of poinsettia in soilless peat

medium at rates of 0.2–0.8 g a.i./L, although control did not improve significantly with an increase in rate (4). In the present study, control of stem rot was not as good with foliar sprays of iprodione at 0.25 g a.i./L compared with 0.6 g a.i./L. However, at 0.25 g a.i./L, iprodione controlled stem rot and prevented cube colonization by *R. solani* better as a rooting-cube soak than as a foliar spray.

Powell (3) reported that benomyl at rates effective in control of *Rhizoctonia* spp. did not inhibit root development on poinsettia cuttings. In the present study, foliar sprays or rooting-cube soaks of the most effective fungicides, metalaxyl + benomyl and flutolanil, did not affect root development of cuttings. However, there were lower rooting values on cuttings treated with other less effective fungicides. This should not be attributed to phytotoxicity, because the root development rating is correlated negatively with the extent of stem rot. Therefore, cuttings with more stem rot had less root development. On the other hand, Powell (3) found that ethazole was phytotoxic to poinsettia cuttings, because the addition of ethazole with benomyl drenches resulted in better disease control than benomyl drenches alone, but root development was less. Because ethazole + thiophanate methyl did not adequately control stem rot in the present study, phytotoxicity attributable to ethazole could not be assessed. For any but the most effective fungicide tested here, additional experiments in the absence of *R. solani* would be needed to assess phytotoxicity.

Strider (5) demonstrated the importance of fungicide applications before the introduction of inoculum for control of *Rhizoctonia* root rot of rooted poinsettia cuttings. Timing was more important than the method of benomyl application (5). A delay of 1 day in drenching rooted cuttings with benomyl in a medium infested with *R. solani* resulted in a 35% increase in the number of dead plants 30 days after transplanting. Delaying the drench 4 days after transplanting resulted in 90% mortality (5). The rapid growth rate of hyphae of *R. solani* from a food base through soil (1) would account for the poor control observed with delayed applications of fungicides.

In this study, *R. solani* began to colonize the rooting cubes within 2 days. Complete colonization of untreated rooting cubes occurred within 5–13 days. Delaying the application of protective fungicides would result in rooting cube colonization and subsequent development of stem rot. In commercial greenhouses where rooting cubes are used, *R. solani* that contaminated rooting cubes from crop debris sources could grow throughout the rooting cube (given an appropriate food base) within a few days of moistening the cubes. Without the protection afforded by fungicides either as foliar sprays or rooting-cube soaks,

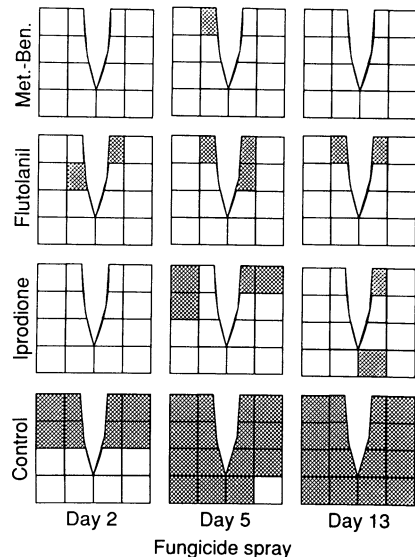


Fig. 3. Colonization of rooting cubes of poinsettias by *Rhizoctonia solani* treated with fungicide sprays of metalaxyl + benomyl, flutolanil, iprodione, or left untreated as a control. Shaded sections indicate recovery of *R. solani*. One representative replication presented of three sampled at day 2, 5, and 13 after applying fungicide.

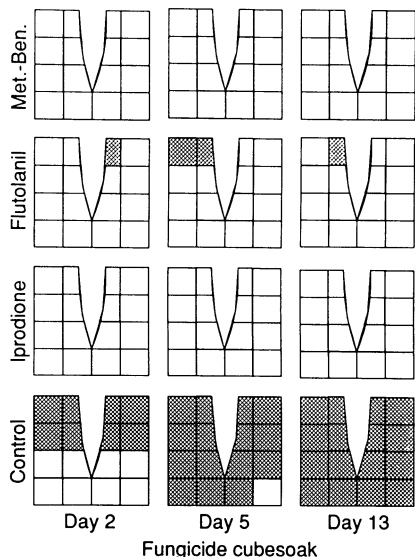


Fig. 4. Colonization of rooting cubes of poinsettias by *Rhizoctonia solani* treated with fungicide soaks of metalaxyl + benomyl, flutolanil, iprodione, or left untreated as a control. Shaded sections indicate recovery of *R. solani*. One representative replication presented of three sampled at day 2, 5, and 13 after applying fungicide.

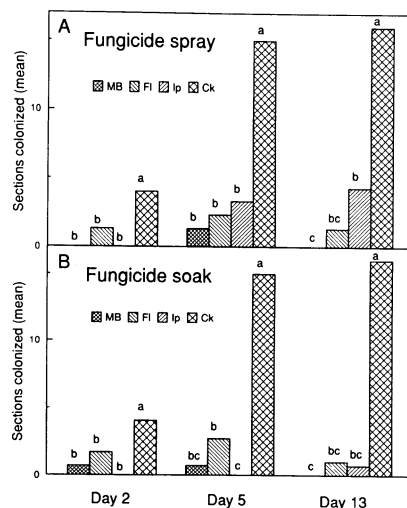


Fig. 5. Mean number of cube sections colonized by *Rhizoctonia solani* after treatment with (A) fungicide sprays or (B) rooting-cube soaks of metalaxyl + benomyl (MB), flutolanil (Fl), iprodione (Ip), or left untreated as a control (Ck). Bars within (A) or (B) capped by the same letters are not significantly different according to the Waller-Duncan *k* ratio; $k = 100$, $P = 0.05$.

Rhizoctonia stem rot will continue to cause disease losses.

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