Efficacy of Two New Systemic Fungicides and Ethazole for Control of Phytophthora Root Rot of Rhododendron, and Spread of *Phytophthora cinnamomi* in Propagation Benches

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ABSTRACT

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Three fungicides, SN 66752, CGA 48988, and ethazole were tested for efficacy against Phytophthora root rot of rhododendron, which frequently causes high levels of mortality on container-grown plants. Monthly drenches of CGA 48988 (75 μg/ml), initially applied prior to inoculation of container-grown rhododendrons, moderately controlled root rot, and were more efficacious than drenches with ethazole (300 μg/ml) or SN 66752 (1000 μg/ml). However, level of control decreased with increasing susceptibility of the cultivars. When applied after inoculation, ethazole exhibited very limited therapeutic activity, and SN 66752 and CGA 48988 were ineffective. These fungicides would have no practical value when used on container plants that had been previously infected. *Phytophthora cinnamomi* spreads among rooted cuttings in propagation benches, but many of the plants infected in this way may remain symptomless until late in the following season. Phytotoxicity of fungicides was studied to determine the practicality of applying such materials to young plants during propagation. Monthly applications of CGA 48988 or SN 66752 did not inhibit rooting or subsequent root development, whereas ethazole was phytotoxic.

Phytophthora root rot of rhododendron, caused by *Phytophthora cinnamomi* Rands, has long been considered one of the most devastating diseases of rhododendrons in commercial nurseries (6,7,18). With the recent trend towards producing rhododendrons in containers, losses from root rot have increased greatly (13).

In Rhode Island, mortality from root rot generally is severe only when plants are grown outdoors in containers. Consequently, chemical control measures are usually directed at this stage. Ethazole (Truban), a fungicide presently recommended for control of Phytophthora root rot, frequently does not provide adequate control (8). Two experimental systemic fungicides, SN 66752 and CGA 48988, were developed recently, with toxicity to certain Phycomycetes (1,4,9,10,11,15–17). For instance, Benson (1) found that CGA 48988 provided good control of Phytophthora root rot on azaleas in North Carolina. We tested efficacy of CGA 48988 on inoculated container-grown rhododendrons in Rhode Island with less satisfactory results. In this study pre- and post-inoculation efficacy of the two experimental fungicides were compared with that of ethazole for root rot control on 2-yr-old, container-grown, hybrid rhododendrons. Because post-inoculation efficacy was inadequate, and fungicides with preventative efficacy depend upon application to healthy plants, we investigated the extent to which rhododendrons may become infected during propagation in the greenhouse prior to planting in containers.

MATERIALS AND METHODS

Fungicides. The fungicides used in this study were: *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester (CGA 48988, proposed trade name, Subdue), supplied as the 50% WP by Ciba-Geigy Corp., Greensboro, NC 27409; 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (ethazole; trade name, Truban), supplied as the 30% WP by Mallinckrodt Chemical Works, St. Louis, MO 63147; and Propyl (3-(dimethylamino) propyl) carbamate HCl (SN 66752) supplied as the 70% EC by NOR-AM Agricultural Products, Inc., Chicago, IL 60606. Fungicides were applied as root drenches, 250 ml per 4-L container, or 10.8 L/m^2 of bench area. An equivalent amount of water was applied to controls. Concentrations expressed as active ingredient, were CGA 48988, 75 μg/ml; ethazole, 300 μg/ml; and SN 66752, 1,000 μg/ml. These concentrations equalled or exceeded the highest rates recommended by the manufacturers. Additional concentrations of CGA 48988 and ethazole were included in the phytotoxicity study in the experimental greenhouse rooting bench. Fungicide drenches were followed by irrigating with 3–5 cm water.

Plant culture. Cuttings were rooted in late summer under intermittent mist in raised greenhouse benches containing a mixture of peat and perlite (1:1, v/v). After approximately 3–4 mo in the rooting bench, cuttings were transferred to transplant benches also containing a peat/perlite medium, and watered as needed. For the study on spread of *P. cinnamomi* in greenhouse propagation areas, the bottoms of benches were amply perforated to insure good drainage and minimize lateral movement of water. The benches were filled with peat/perlite medium to a depth of 15 cm and, in this study only, covered with tarpaulins and steam pasteurized. In the spring, plants were replanted in a medium with equal volumes of peat, perlite, and sand in 4-L plastic containers, and grown outdoors in full sun. Container plants were fertilized weekly with liquid 20-20-20 (N-P-K) at 60 g/100 L. The container medium for the study on spread of *P. cinnamomi* in propagation benches was fumigated with methyl bromide (60 kg/M^3) prior to planting. The pH of propagation and container media was approximately 5.4.

Inoculum production and inoculation. *P. cinnamomi* was cultured on V8 sterol agar in 6-cm-diameter plastic petri dishes under near-UV light (200 μW cm^{-2}) for 2 wk, by which time abundant chlamydospores were produced (3). Sporangia were induced by flooding the colonies four times at 1 hr intervals with the salt solution described by Chen and Zentmeyer (2). Cultures were then flooded for 24–48 hr with a nonsterile rhizosphere effluent (14) freshly collected from beneath a *Rhododendron* 'Nova Zembla' growing in a 4-L container.

In the first season's tests of preinoculation fungicide efficacy, container-grown rhododendrons were inoculated by transferring the contents of one petri dish to the surface of the medium at the periphery of each container. Plants in pre- and post-inoculation trials during the second season were inoculated with a suspension of comminuted colonies (contents of 100 dishes blended and suspended in 13 L of H_2O) that had been incubated and treated as described above. Twenty-five milliliters of the inoculum suspension

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were poured on the surface of the medium in each container. In all cases, inoculation was followed with application of 5 cm of water by using overhead sprinklers.

In the study on spread of *P. cinnamomi* in greenhouse benches, rooted cuttings were gently washed and agitated to remove loose peat and perlite, then root balls were submerged for 24 hr in a zoospore suspension. Colonies with sporangia were obtained as described above, and zoospore release was induced by chilling colonies to approximately 5 C for 20 min, then returning them to room temperature. Distilled water was used in place of a zoospore suspension on control plants. Roots of inoculated plants were thoroughly rinsed three times in running tap water, and one inoculated plant was planted in the center of each block of uninoculated assay plants.

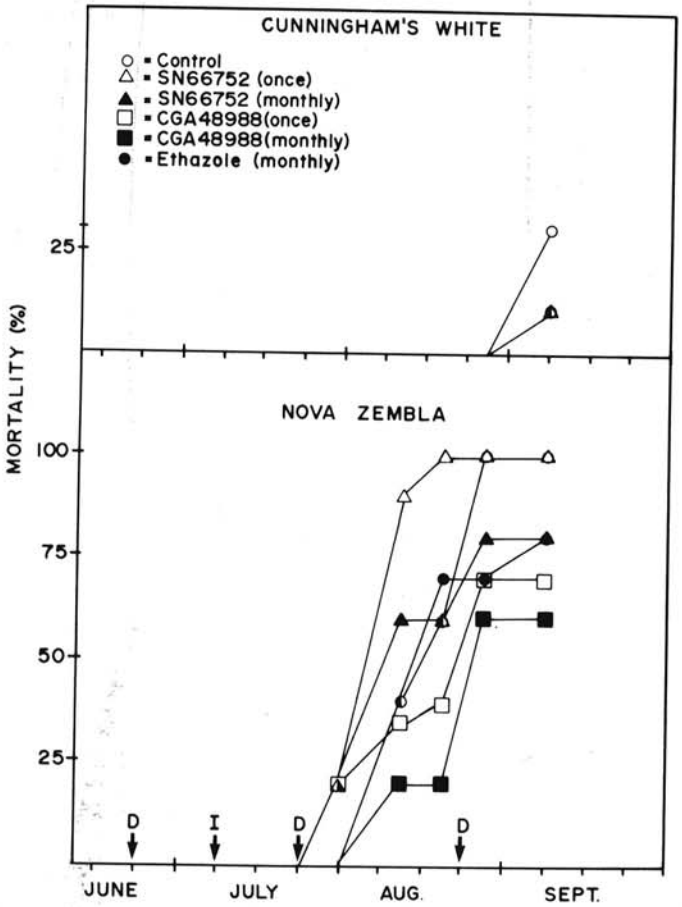

Fig. 1. Mortality over time of 2-yr-old container-grown *Rhododendron* 'Cunningham's White' and 'Nova Zembla' inoculated with *Phytophthora cinnamomi* 2 wk after initial root drenches with SN 66752 (1,000 μg/ml), CGA 48988 (75 μg/ml), or ethazole (300 μg/ml). Dates of drenches and inoculation are indicated with 'D' and 'I', respectively. There was no mortality during the season on Cunningham's White treated with SN 66752 monthly, or CGA 48988 either once or monthly.

Assessment of mortality and phytotoxicity. In fungicide efficacy tests, mortality was indicated by the general wilting of the plant and browning of the cambium in the basal stem region. *P. cinnamomi* was isolated consistently from the discolored cambium of wilted plants.

Quantitative evaluations of fungicide phytotoxicity to root development of uninoculated plants were made by carefully lifting plants to expose the root ball, and calculating the average of two perpendicular root-ball diameters measured on the horizontal axis of each plant (5). Qualitative evaluations were made with the assistance of an experienced nurseryman, on plants treated at a commercial nursery.

Plant material and experimental design. In tests where the initial application of fungicide preceded inoculation, there were six fungicide treatments (including controls without fungicide), three cultivars, and ten plants per treatment per cultivar, with a total of 180 plants in each of two growing seasons of tests. *Rhododendron* 'Cunningham's White' (*caucasicum* × *ponticum* var. *album*), 'Mrs. P. den Ouden' (*atrosanguineum* × Doncaster), and 'Mrs. C. S. Sargent' (*catawbiense* hybrid) were used the first season, with cultivar Nova Zembla (Parsons' *grandiflorum* × hardy red hybrid) in place of 'Mrs. C. S. Sargent' the second year. *Rhododendron* 'Laetevirens' (also referred to as 'Wilsoni'; *carolineanum* × *ferrugineum*) was used in tests where fungicides were applied after inoculation. There were ten plants per fungicide per postinoculation interval, a total of 120 plants. In the study on spread, 49 rooted Nova Zembla cuttings were used in each of three replicate plots. Phytotoxicity tests included 12 treatments (fungicides × concentrations × application schedules), two cultivars (Nova Zembla and Cunningham's White), and three replicate groups with ten plants each, for a total of 720 plants. For phytotoxicity tests in a commercial nursery, plots with approximately 125 Nova Zembla cuttings were delineated on a bench, with five replicate plots per fungicide treatment. Control plots contained approximately 1,000 plants per replication.

RESULTS

Fungicide applications to container-grown rhododendrons prior to inoculation. The efficacy of the two experimental systemic fungicides, CGA 48988 (CG) and SN 66752 (SN), and ethazole, were evaluated during 1977 and 1978 for reducing mortality of container-grown rhododendrons inoculated with *P. cinnamomi* 2 wk after the initial application of fungicides (Table 1). CG applied once or monthly, and SN applied monthly, consistently prevented mortality of Cunningham's White, a cultivar considered relatively resistant to Phytophthora root rot (13). On the relatively susceptible cultivars (Mrs. P. den Ouden and Mrs. C. S. Sargent), mortality reduction by preinoculation application of either of the systemic fungicides or ethazole was less impressive. Generally, three applications at monthly intervals were more effective than a single drench. However, losses were high in all fungicide treatments

TABLE 1. Efficacy of fungicides applied once or at monthly intervals beginning prior to inoculation of several *Rhododendron* cultivars with *Phytophthora cinnamomi*

Treatment	Concentration and frequency of application[b]	Percent mortality of cultivars[a]					
		Cunningham's White[c]		Mrs. P. den Ouden		Mrs. C. S. Sargent	Nova Zembla
		1977	1978	1977	1978	1977	1978
Control	...	30	30	60	60	50	100
Ethazole	300 μg/ml monthly	0	10	30	60	50	80
SN 66752	1,000 μg/ml once	10	10	60	40	50	100
SN 66752	1,000 μg/ml monthly	0	0	30	30	20	80
CGA 48988	75 μg/ml once	0	0	10	50	50	70
CGA 48988	75 μg/ml monthly	0	0	10	30	0	60

[a] Total mortality observed during the season.
[b] Initial root drenches (250 ml/4-L container) with fungicides were applied 2 wk before inoculation. Subsequent drenches were repeated at monthly intervals where noted, for a total of three drenches during the season. Concentrations are active ingredients.
[c] Two-year-old plants growing in a mixture of peat, perlite, and sand (1:1:1, v/v) in 4-L containers.

on Nova Zembla, a highly susceptible cultivar.

Examination of mortality data over time during the 1978 tests showed that the onset of foliar wilt symptoms occurred much later in the season with the more resistant cultivar Cunningham's White, than with Mrs. P. den Ouden or Nova Zembla (Fig. 1). Also, foliar symptoms on plants of Mrs. P. den Ouden and Nova Zembla that received SN fungicide treatments appeared earlier than in most other treatments and the controls.

Fungicide applications to container-grown rhododendrons after inoculation. The ability of fungicides to eradicate *P. cinnamomi* from infected roots and container medium was evaluated by applying fungicide drenches either 2, 7, or 18 days after inoculation of the highly susceptible cultivar, Laetevirens, with *P. cinnamomi*. The number of dead plants, and time of initial foliar symptom expression was similar on plants treated with any of the fungicides either 7 or 18 days after inoculation. Plants treated with SN or CG 2 days after inoculation exhibited high mortality rates, similar to control plants (Fig. 2). Ethazole showed some postinoculation efficacy when applied 2 days after inoculation, delaying foliar symptom onset nearly 1 mo and reducing mortality to less than 50% that of control plants by the end of the season.

Spread of *P. cinnamomi* among rooted cuttings in the greenhouse. Rooted Nova Zembla cuttings were planted 11 cm apart in pasteurized peat and perlite in replicate plots each with a square grid arrangement, seven plants on each side, with a total of 49 plants per plot. Plants were labeled by location with reference to an inoculated (or uninoculated control) cutting centrally located in each plot. After 5 mo, plants were transplanted to 4-L containers, and were placed outdoors in a random arrangement. Periodic observations were made from the time of inoculation through the end of the season 9 mo later, and mortality was recorded according to the original location of the plant with respect to the intentionally inoculated cuttings in the greenhouse plots.

None of the plants from the uninoculated control plots died, while 14.6, 20.8, and 18.8% of the assay plants, respectively, in the three inoculated plots died. Initial proximity of dead plants to intentionally inoculated plants is graphically represented in Fig. 3.

During the 5-mo period in which the cuttings were in the transplant bench adjacent to the intentionally inoculated cuttings only about one-tenth of the eventual mortality was observed; the remainder occurring during the 4 mo following transplant of symptomless cuttings to containers.

Fungicide phytotoxicity to rooting and newly rooted cuttings. We have not observed phytotoxicity by the fungicides CG, SN, or ethazole (at the rates used in this study) on 2-yr-old plants in containers. However, cuttings in the process of rooting, and young plants, may be more sensitive. The effect of CG, SN, and ethazole on rooting and root system development was determined in tests in an experimental greenhouse and under commercial conditions in a nursery.

In the experimental greenhouse study, fungicide drenches were applied at various concentrations immediately upon placement of the cuttings of Nova Zembla and Cunningham's White in the medium, and certain treatments were continued on a monthly schedule. Root-ball diameters were measured 82 days after the initial fungicide treatment (Table 2). Root system size of Nova Zembla and Cunningham's White plants drenched with CG or SN at all concentrations and application schedules tested were similar to the appropriate controls. Root balls of Nova Zembla cuttings treated with ethazole at either 30 or 300 μg/ml were significantly

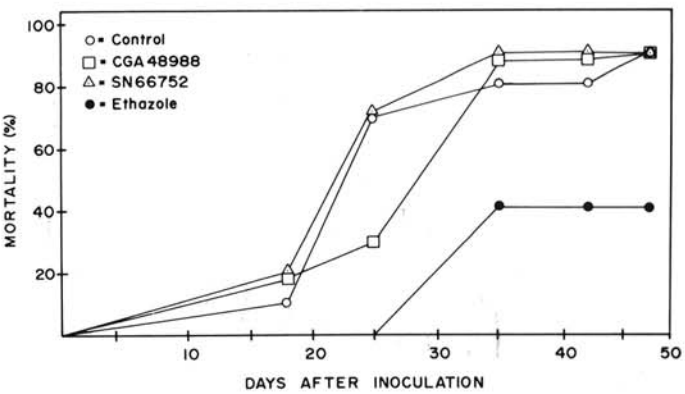

Fig. 2. Mortality over time of 2-yr-old container-grown *Rhododendron* 'Laetevirens' inoculated with *Phytophthora cinnamomi* 2 days before the application of root drenches of SN 66752 (1,000 μg/ml), CGA 48988 (75 μg/ml), or ethazole (300 μg/ml).

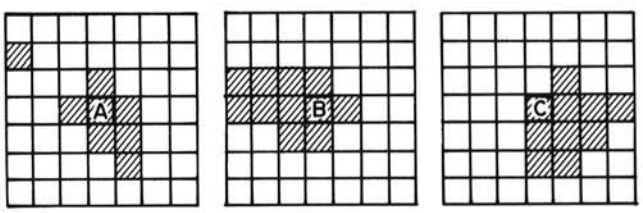

Fig. 3. Total mortality during 9 mo following inoculation of a centrally located plant (A, B, or C) in each plot in the greenhouse bench. Assay plants were transplanted to containers after 5 mo in the greenhouse. Shaded areas indicate original locations of dead plants in reference to the inoculated plant. Approximately 90% of the mortality occurred after transplanting.

TABLE 2. Effect of fungicide drenches on average root-ball diameters of *Rhododendron* cultivars rooted in greenhouse benches in a mixture of peat and perlite

Treatment[x]	Concentration[y] (μg/ml)	Application schedule	Average root-ball diameter (cm)[z] Nova Zembla	Cunningham's White
Control	0	...	4.13 ab	8.31 a
CGA 48988	0.75	once	3.82 ab	9.13 a
CGA 48988	0.75	monthly	4.27 ab	9.08 a
CGA 48988	7.5	once	5.38 a	7.94 a
CGA 48988	7.5	monthly	5.37 a	8.29 a
CGA 48988	35	once	5.13 a	8.89 a
CGA 48988	35	monthly	3.65 ab	8.78 a
CGA 48988	75	once	5.76 a	9.10 a
CGA 48988	75	monthly	3.50 ab	8.69 a
SN 66752	1,000	once	4.92 a	8.52 a
Ethazole	30	once	2.29 c	8.97 a
Ethazole	300	once	2.41 c	7.78 a

[x] Drenched at 10.8 L/m^2 either once when cuttings were taken or three times at monthly intervals.

[y] Expressed as active ingredient.

[z] Means of three replications of 10 plants each, measured 82 days after planting. Means in the same column with the same letter are not significantly different, according to the Duncan's multiple range test, $P = 0.05$.

smaller than those in other treatments.

In a commercial nursery, newly taken Nova Zembla cuttings were drenched immediately and monthly thereafter with CG, SN, or ethazole. After 118 days, quality of root systems were evaluated. Plants were classified into three categories: well-rooted cuttings, with an average root-ball diameter of ≥ 3 cm on a horizontal plane; replants, cuttings with relatively few roots or, in the absence of roots, with callus formation; and discards, cuttings with neither callus nor roots (Table 3). In the control plots, which received no fungicides, 63.6% of the cuttings were well-rooted, and 9.6% were classified as discards, while a significantly higher percentage were in the well-rooted category (87.3 and 85.5% when treated with CG or SN, respectively). However, only 47.2% of the cuttings treated with ethazole rooted. This was significantly fewer than in controls.

In accordance with standard practice at this nursery, plants in the well-rooted category were transferred to the transplant bench. After relocation from each treatment, plants continued to receive the same fungicide treatments as before. After 85 days in the transplant bench, a total of 48 plants were selected randomly from each treatment, and average root-ball diameters were determined. Monthly treatments with CG or SN did not significantly inhibit root system development (8.70 and 8.30 cm diameter, respectively) compared to that of the control (8.25 cm diameter). However, monthly applications of ethazole significantly inhibited root system development (6.62 cm diameter).

DISCUSSION

We believe that none of the fungicides tested provided practical control of Phytophthora root rot in container-grown rhododendrons. Even with relatively high concentrations of fungicides there were marked temporal limitations on therapeutic activity, and preventive treatments resulted in only moderate suppression of mortality on some of the commercially desirable but more susceptible cultivars.

The manufacturers claim that ethazole has fungicidal, protectant, and curative activity, and that CGA 48988 has systemic residual-protectant and curative activity, and SN 66752 has systemic, fungistatic activity. In our tests, neither CGA 48988 nor SN 66752 exhibited therapeutic activity when applied two days after inoculation of the highly susceptible cultivar, Laetevirens. Ethazole delayed and suppressed mortality when applied 2 days but not 7 days after inoculation. Because the precise time of natural inoculation of plants during the lengthy rhododendron production process cannot be predicted, it appears that neither ethazole, with its limited period of action, nor the systemic fungicides, have any practical value for controlling Phytophthora root rot of container-grown plants.

We found that CGA 48988 provided much less control of *P. cinnamomi* on rhododendrons than Benson (1) reports with azaleas. Possibly, this is due to differences between azaleas and rhododendrons, which are considered separate groups within the genus, *Rhododendron,* based on physiological and morphological differences (12). Benson speculated that rhododendrons generally may be more susceptible to *P. cinnamomi* than azaleas (1). We agree with this hypothesis, having observed nursery blocks of rhododendrons with high mortality due to root rot on numerous occasions, while adjacent blocks of various cultivars of azaleas were symptomless. Furthermore, azaleas and rhododendrons apparently differ in sensitivity to phytotoxicity by CGA 48988. Benson reports severe phytotoxicity to foliage and root development of azaleas drenched once every 2 mo with CGA 48988 (72 μg/ml) in greenhouse tests (1). In our studies, monthly applications of CGA 48988 (75 μg/ml) did not induce symptoms of phytotoxicity on the foliage of any of the cultivars of rhododendrons grown outdoors in containers. Greenhouse studies on cuttings and young plants revealed no phytotoxicity of CGA 48988 (75 μg/ml applied monthly) to root development (Tables 2 and 3).

Despite the demonstrated lack of efficacy of single postinoculation fungicide treatments (Fig. 2), continued monthly applications of CGA 48988 or SN 66752 generally decreased mortality over that obtained with a single preinoculation drench. This may be due to the accumulation of fungicides during the season in roots or soil, or the continuous suppression of inoculum production or infection in secondary cycles of *P. cinnamomi* in the rhizosphere of inoculated plants. In this regard, Hoitink and Schmitthenner (6) report that infected, root rot resistant cultivars of rhododendrons growing under favorable horticultural conditions may remain symptomless, with root regeneration compensating for root rot.

Fungicide efficacy most likely is influenced by cultural practices that alter disease pressure, and this may account for variation in results among studies. The increased severity of rhododendron root rot with elevated root-ball temperatures on container-grown plants in full sun (13,18), and a reduced level of chemical control on root rot of azaleas when in containers in an unshaded, outdoor site (1), indicate fungicide efficacy can be diminished by certain cultural conditions. More work is critically needed on the epidemiology of the rhododendron root rot disease in regard to the effects of the components of the physical environment to which container-grown plants are subjected.

The fungicides used here provided some protection when applied prior to inoculation, however, they did not express effective eradicant or therapeutic abilities when applied after inoculation. In fact, the poor chemical control of Phytophthora root rot observed on container-grown rhododendrons in commercial nurseries may be due to the inadvertent use of infected, albeit symptomless, plants. Natural infection may occur at any time during the plant production process. We have isolated *P. cinnamomi* on numerous occasions during the winter and spring from wilted plants in rooting and transplant benches in commercial nurseries. We have shown that *P. cinnamomi* may spread through rhododendron propagation benches, resulting in numerous symptomless, but infected plants which are thus unresponsive to therapy by fungicides having only protectant capability. Therefore, what is needed is: testing of routine applications of nonphytotoxic fungicides, beginning early in the propagation process (we found

TABLE 3. Effect of monthly fungicide drenches (10.8 L/m^2) on qualitative evaluations of root systems of *Rhododendron* cultivar Nova Zembla cuttings after development in the rooting bench in a commercial nursery for 118 days

Treatment	Concentration (μg/ml)	Root system evaluations: Well-rooted[a] No. of plants	Well-rooted[a] Total (%)	Replants[b] No. of plants	Replants[b] Total (%)	Discards[c] No. of plants	Discards[c] Total (%)
Control	0	3,145	63.6	1,325	26.8	475	9.6
CGA 48988	75	583	87.3[d]	75	11.2[d]	10	1.6[d]
SN 66752	1,000	591	85.5[d]	97	14.0[d]	3	0.4[d]
Ethazole	300	273	47.2[d]	272	47.0[d]	34	5.8[d]

[a] Root-ball diameter ≥3 cm.
[b] A few roots or some callus was present.
[c] Neither roots nor callus was present.
[d] Significantly different from the control $P = 0.05$.

that SN 66752 and CGA 48988 were not phytotoxic to rooting or root development); the development of a fungicide characterized by excellent persistence and eradicative properties; or the discovery and elimination of the sources of inoculum in the propagation process.

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