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CONTROL OF PHYTOPHTHORA WILT OF AZALEAS WITH CGA 48988

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Abstract. **Phytophthora wilt of azaleas, caused by** Phytophthora parasitica **Dastur., was successsfully controlled with the systemic fungicide, CGA 48988 (50 W.P.). In the greenhouse, control was better with a soil drench of 25 and 50 ppm a.i. than with a foliar spray at the same concn. In the field, a soil drench of 0, 12.5, 25, and 50 ppm a.i. applied 4 days after inoculation with a zoospore suspension (1 x 10^5/ml) resulted in 34, 15, 17, and 10 wilted plants (20.2, 8.9, 9.6, and 5.9%), respectively, 10 weeks after inoculation. In a second field trial, a soil drench of 0, 19, 38.5, 77, and 154 ppm a.i. (0, 1/2, 1, 2, and 4 oz/100 gals) was applied 3 days prior to inoculation with a zoospore suspension (7 x 10^4/ml) and was reinoculated every 2 weeks (1 x 10^4 zoospores/ml). This resulted in 164, 97, 15, 18, and 6 wilted plants (69.3, 53.9, 8.3, 10.0, and 3.3%), respectively, 8 weeks after the initial inoculation.** P. parasitica **was reisolated from basal stem sections of all wilted plants in this trial. Phytotoxicity was not observed at any of the above rates, nor up to 250 ppm a.i. in 2 separate tests.**

Phytophthora wilt of azaleas, caused by *Phytophthora parasitica* Dastur., is a major limiting factor in the commercial production of azaleas (*Rhododendron* sp.) in southwest Florida. The warm, wet, Florida summers greatly enhance disease development and control of the disease has been difficult. The ability of the fungus to survive in the soil and in irrigation water (10, 11) further increases the difficulty of control.

The disease is characterized by both a foliar and a crown and lower stem phase. Foliar infection results from inoculum splashing onto the leaves and is first seen as a dark brown to black, irregularly shaped necrotic lesion. In time the fungus invades the shoots causing dieback and the eventual death of the plant. Infection of the crown and lower stem is first observed as defoliation of the lower stem, or "basal leaf drop". This is followed by rapid wilting and death of the plant.

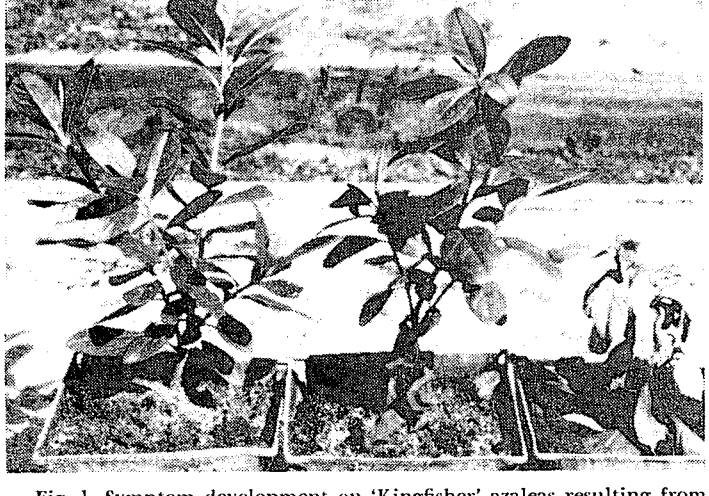

Fig. 1. Symptom development on 'Kingfisher' azaleas resulting from infection of the crown by zoospores of *P. parasitica;* healthy (left), basal leaf symptom (center), and wilt (right).

The fungicides, Manzate 200 and Daconil 2787, have provided control of the foliar phase of the disease. However, the crown and lower stem phase has proven more difficult to control. Available soil fungicides are limited and their effectiveness has been unsatisfactory.

The experimental fungicide CGA 48988 (also referred to as GA-1-82), introduced by Ciba-Geigy Corp., has been shown to have potential in controlling diseases caused by pythiaceous fungi (i.e. Pythium and Phytophthora). Kelley observed that GA-1-82 at 1 μg/ml inhibited growth of *P. parasitica* in culture by 68.8% after 5 days (5). Disease control with the fungicide has been observed for late blight of potatoes (*Phytophthora infestans* (Mont.) de Bary) (2, 3, 4, 6, 8), Pythium blight of creeping and colonial bentgrass (*Pythium aphanidermatum* (Edson) Fitz.) (1, 9), and downy mildew of cabbage (*Peronospora parasitica* (Pers. ex. Fr.) Fr.) (12). In addition, the systemic nature of the fungicide greatly increases its potential for disease control.

The purpose of this investigation was to determine whether CGA 48988 controlled Phytophthora wilt of azaleas, and to determine the rates and frequency of application.

Materials and Methods

Inoculum preparation. An isolate of *P. parasitica* was obtained from a naturally infected azalea by plating stem sections on Potato Dextrose Agar (PDA). The isolate was maintained on PDA by transferring to fresh plates every 7-10 days. Inoculum was produced on V-8[1] Juice Agar (7) and consisted of a zoospore suspension adjusted to the desired concentration using a hemacytometer. V-8 Juice Agar plates were inoculated with mycelial plugs of the fungus grown on PDA and were incubated in the dark at room temperature (ca. 26 C) for 3 days. Ten ml of sterile deionized water was then added to each plate and the plates were reincubated for an additional 4-14 days. Each plate was then flooded with 20 ml of cold (4 C) sterile deionized water to release the zoospores. After reaching room temperature, the water was collected from the plates and the concentration of the resulting zoospore suspension was adjusted to the desired level.

Inoculation. Inoculations consisted of pouring 20 ml of the zoospore suspension onto the soil around the base of each plant. All field trials were watered for approximately 30 minutes following inoculation by means of overhead Chapin spray stakes.

Disease readings. All plants were observed weekly for symptom development; the number of plants exhibiting basal leaf drop or wilt were noted. Wilted plants were rogued and basal stem sections were cultured on PDA to recover the pathogen.

Plant maintenance. In the greenhouse, rooted 'Kingfisher' cuttings were planted in Peace River peat in four-inch-square plastic pots. The plants were maintained in a clear polyethylene-covered growth chamber on a greenhouse bench for 4 weeks prior to treatment. The chamber was heated by means of a network of heating coils which permitted the temperature to be held within a range of *20-35 C.*

[1]A canned mixture of various vegetable juices. Ed.

A high relative humidity was maintained by periodic misting of the chamber with a fogger nozzle. Plants were watered 3 times a week, and fertilized once a week. Care was taken to avoid splashing when watering.

In the field, rooted 'Kingfisher' cuttings were planted in Peace River peat in four-inch-square wooden berry baskets. The baskets were sunk into the beds so that at least three-quarters of the basket was below the soil surface. Plants were watered as needed and fertilized 3 times a week. Water and fertilizer were applied with 360° overhead Chapin spray stakes. Plants were maintained for 4 weeks prior to treatment.

Greenhouse experiments

Two preliminary trials were conducted in the greenhouse. In each case, inoculations were done with a suspension of 1 x 10^5 zoospores/ml. Each trial contained 2 control groups, inoculated and uninoculated.

Experiment 1. Eighty plants were divided into 8 treatments of 10 plants per treatment. Treatments consisted of soil drenches of 50 ml per pot 3 days prior to inoculation. Fungicides and rates tested were: Difolitan 6F (cis-N [(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide), 1 pt/100 gals; Thiram (Thylate 75 WP) (bis(dimethylthiocarbamoyl) disulfide), 2 lbs/100 gals; Truban 30 WP (5-ethyoxy-3-(trichloromethyl)-1,2,4-(thiadiazole), 12 oz/100 gals; Captan 50 WP (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide), 2 lbs/100 gals; Dexon 35 WP (p- dimethylamino) benzenediazo sodium sulfonate), 12 oz/100 gals; and CGA 48988 50 WP (N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-*D L*-alanine methyl ester), 1.3 oz/100 gals (50 ppm a.i.).

Experiment 2. Sixty plants were divided into 6 treatments of 10 plants per treatment. Treatments consisted of soil drenches (100 ml/pot) or foliar sprays (applied to run-off) with CGA 48988 50 WP at 25 and 50 ppm a.i., and 2 control groups. All treatments were applied 4 days prior to inoculation.

Field experiments

Expt 1. Ninety-six plants of each of the 6 azalea varieties: 'Kingfisher', 'Prize', 'Roadrunner', 'Solitaire', 'Gloria', and 'Skyliner'; 72 plants of the variety 'Whitewater'; and 24 plants of the variety 'Kiwi' were divided into 4 treatments each replicated 4 times. Treatments consisted of soil drenches (250/ml basket) of CGA 48988 50 WP at 0, 12.5, 25, and 50 ppm a.i. applied 4 days after inoculation with a suspension of 1.5 x 10^5 zoospores/ml.

Expt 2. One thousand six hundred and eighty plants were divided into 4 treatments, each replicated twice. Treatments consisted of soil drenches (100 ml/basket) with CGA 48988 50 WP at 1/2, 1, 2, and 4 oz product per 100 gals. Each treatment was divided into 7 subtreatments (30 plants/subtreatment). Subtreatments consisted of different frequencies of reapplication: either every 4, 6, 8, 10, or 12 weeks. Two plots remained undrenched. Three days after the initial drench, all treated and one untreated plot were inoculated with a suspension of 7 x 10^4 zoospores/ml. These plots were reinoculated with a suspension of 1 x 10^4 zoospores/ml every 2 weeks for 16 weeks.

Phytotoxicity evaluations

Two trials were conducted in the field to evaluate phytotoxicity. In the first trial, 120 three-month old plants of each of the varieties 'Kingfisher', 'Prize', 'Gloria', and 'Roadrunner' were divided into 5 treatments, each replicated twice. Treatments consisted of a single drench with CGA 48988 50 WP at 0, 100, 250, and 1,000 and 5,000 ppm a.i. at 100 ml/basket. In the second trial, 144 plants of each of the varieties 'Kingfisher', 'Gloria', and 'Prize' were divided into 12 treatments. Treatments consisted of applications of CGA 48988 50 WP at 0, 50, 100, 250, 1,000 and 5,000 ppm a.i. Six plots received a soil drench of 100 ml/basket, 6 plots received a foliar spray to run-off, and 2 plots remained untreated. Two months after the initial treatment, all treatments were reapplied with the exception of 5,000 ppm a.i.

Results and Discussion

Greenhouse experiments

In experiment 1, the best control 10 weeks after inoculation was obtained with Difolatan and CGA 48988 with no plants showing wilt symptoms (Table 1). One of 10 plants had wilted in each of the Thiram, Captan and Dexon treatments. Truban was least effective, with 4 of 10 plants wilted. However, 9 of 10 plants had wilted in the inoculated control group. No symptoms developed in the uninoculated control group. Although no phytotoxicity was observed in any of the treatments, further studies showed that Difolatan, Thiram and Captan caused chlorosis and stunting when applied as soil drenches of 100 ml/basket in the field. The fact that CGA 48988 successfully controlled wilt at 1.3 oz/100 gals (50 ppm a.i.) indicated the potential of this fungicide in controlling Phytophthora wilt of azaleas.

Table 1. Effect of soil drenches on infection of greenhouse grown 'Kingfisher' azaleas inoculated with zoospores of *Phytophthora parasitica.*

Treatment and Rates[x] (per 100 gals.)	Number of wilted plants[y] Number of weeks after inoculation[z] 2	4	7	10
CGA 48988; 1.3 oz.	0	0	0	0
Difolatan; 1 pt.	0	0	0	0
Thiram; 2 lbs.	0	0	0	1
Captan; 2 lbs.	1	1	1	1
Dexon; 12 oz.	1	1	1	1
Truban; 12 oz.	1	3	4	4
Control; inoculated	7	7	9	9
Control; uninoculated	0	0	0	0

[x]Soil drenches were applied at 50 ml/pot 3 days prior to inoculation.
[y]Number of wilted plants per 10 plants per treatment.
[z]Inoculation consisted of 20 ml of a zoospore suspension (1 x 10^5 zoospores/ml) poured around the base of each plant.

CGA 48988 successfully controlled wilt when applied as a soil drench of 25 and 50 ppm a.i. (Table 2). No wilted plants were observed in either treatment 10 weeks after inoculation. A foliar spray of 50 ppm a.i. was less effective with 1 of 10 plants rogued for wilt. A foliar spray of 25 ppm a.i. gave better control than no treatment, but was the least effective of the 4 treatments with 5 of 10 plants rogued 10 weeks after inoculation. Nine of 10 plants wilted in the inoculated control group and no symptoms developed in the uninoculated control group. No phytotoxicity was observed in any of the treatments. The results of this trial confirmed the conclusion of the previous trial indicating the potential of this fungicide in controlling Phytophthora wilt. In addition, the systemic nature of the fungicide suggests useage as either a soil drench or a foliar spray. Studies currently in progress indicate that weekly foliar sprays provide excellent control with no evident phytotoxicity.

Table 2. Effect of different concentrations and methods of application of CGA 48988 on infection of greenhouse grown 'Kingfisher' azaleas inoculated with zoospores of *Phytophthora parasitica*.

Treatment[x]	Concentration (ppm a.i.)	Number of wilted plants[y] Number of weeks after inoculation[z] 3	7	10
Soil drench	25	0	0	0
Soil drench	50	0	0	0
Foliar spray	25	4	4	5
Foliar spray	50	0	0	1
Control; inoc.	—	5	7	9
Control; uninoc.	—	0	0	0

[x]Soil drenches were applied at 100 ml/pot. Foliar sprays were applied to run-off. Treatments were applied 4 days prior to inoculation.
[y]Number of wilted plants per 10 plants per treatment.
[z]Inoculation consisted of 20 ml of a zoospore suspension (1 x 10^5 zoospores/ml) poured around the base of each plant.

Field experiments

Expt 1. Control of Phytophthora wilt of field grown azaleas was obtained with CGA 48988 at 50 ppm a.i. when applied as a post-inoculation soil drench (Table 3). An average of 1.75 and 4.00 plants had wilted 10 and 15 weeks after inoculation, respectively, compared to 5.50 and 8.75 wilted plants in the inoculated control plots. Control was less effective with treatments of 12.5 and 25 ppm a.i. with no obvious difference between the 2 treatments. No phytotoxicity was observed in any treatments. The ability of the fungicide to control wilt when applied as a post-inoculation drench again indicates its potential. As well as being systemic, some eradicative properties were also indicated. Even though this trial was carried out during the winter months when the environmental factors affecting disease development were least favorable, the results were encouraging.

Expt 2. One aspect of this study was to evaluate CGA 48988 when disease pressure was high. The 4 rates, 1/2, 1, 2, and 4 oz product/100 gals, applied as a single soil drench, successfully controlled wilt for 6 weeks with 3 alternate weekly inoculations (Table 4). After 8 weeks and 4 inoculations, the 1/2 oz rate no longer effectively controlled the disease. While the higher rates ranged from 1 to 3 wilted plants/replication, 16.2 and 20.5 plants/replication were wilted in the 1/2 oz and non-treated plots, respectively.

A second aspect of this study was to determine the frequency of application necessary to obtain disease control with repeated inoculations. The best control 24 weeks after the initial inoculation followed by alternate weekly inoculations was obtained with the 4 oz rate (Table 5A) with

Table 3. Effect of different concentrations of CGA 48988 applied as a post-inoculation soil drench on disease development of *Phytophthora parasitica* on field grown azaleas.

Concentration[x] (ppm a.i.)	Average number of wilted plants[y] Number of weeks after inoculation[z] 5	10	15
0	2.25	5.50	8.75
12.5	1.00	3.75	6.75
25	1.75	4.25	6.00
50	0.00	1.75	4.00

[x]Soil drenches were applied at 250 ml/basket 4 days after inoculation.
[y]Average of 4 replications with 42 plants per replication.
[z]Inoculation consisted of 20 ml of a zoospore suspension (1.5 x 10^5 zoospores/ml) poured around the base of each plant on Dec. 3, 1976.

Table 4. Effect of different rates of CGA 48988 applied as a soil drench on infection of field grown 'Kingfisher' azaleas inoculated with zoospores of *Phytophthora parasitica*.

Rate[x] (oz. per 100 gals.)	Concentration (ppm a.i.)	Average number of wilted plants[y] Number of weeks after initial inoculation[z] 4	6	8
0	0	11.4	15.9	20.5
1/2	19.25	1.3	4.5	16.2
1	38.5	0	0.3	2.5
2	77	0	0.3	3.0
4	154	0	0.3	1.0

[x]Soil drenches were applied at 100 ml/basket 3 days prior to the initial inoculation.
[y]Average number of wilted plants per replication with 30 plants per replication. Averages are for 6 replications per treatment for all treatments except 0 oz. which is the average of 8 replications.
[z]Initial inoculation consisted of 20 ml of a zoospore suspension (7 x 10^4 zoospores/ml) poured around the base of each plant on April 29, 1977.

soil drenches applied either every 4, 6, 8, 10, or 12 weeks. At these reapplication frequencies, only 1.5, 2.5, 3.5, 7.5, and 2.0 plants had wilted per replication, respectively, compared to 25.0 wilted plants per replication in the inoculated control plots. In all cases, *P. parasitica* was recovered from basal stem sections of the wilted plants. Similar control was obtained with the 2 oz rate applied either every 4 or 6 weeks with 2.5 and 3.0 wilted plants per replication, respectively, with the inoculated control plots showing 30 wilted plants/replication. Control with the 1 oz rate was best when applied every 4 weeks with 4.5 wilted plants per replication at the end of 24 weeks (Table 5B). This compared to 18 wilted plants/replication in the inoculated control plots. The 1/2 oz rate did not provide satisfactory control of Phytophthora wilt even for 10 weeks. No phytotoxicity was observed in any of the treatments.

Table 5A. Effect of different rates and frequencies of application of CGA 48988 on infection of field grown 'Kingfisher' azaleas inoculated with zoospores of *Phytophthora parasitica*.

Rates[x] (per 100 gals.)	Frequency of application	Average number of wilted plants[y] Number of weeks after initial inoculation[z] 5	10	15	24
4 oz.	No Drench; inoc.	10.5	20	24.5	25
	4 weeks	0	0.5	1.5	1.5
	6 weeks	0.5	2	2.5	2.5
	8 weeks	0	2.5	3	3.5
	10 weeks	0	1	5.5	7.5
	12 weeks	0	1	2	2
	No Drench; uninoc.	0	1	1	1
2 oz.	No Drench; inoc.	23	29	30	30
	4 weeks	0	0.5	2.5	2.5
	6 weeks	0	0	2	3
	8 weeks	0	5.5	9.5	10
	10 weeks	0	4	11.5	11.5
	12 weeks	0	4	10	10
	No Drench; uninoc.	0	0	0.5	0.5

[x]Soil drenches were applied at 100 ml/basket beginning 3 days before the initial inoculation.
[y]Disease incidence is expressed as the average number of plants rogued for wilt per 2 replications with 30 plants per replication. *P. parasitica* was recovered from basal stem sections of all wilted plants.
[z]Initial inoculation consisted of 20 ml of a zoospore suspension (7 x 10^4 zoospores/ml) poured around the base of each plant. Inoculation was repeated with a zoospore suspension (1 x 10^4/ml) every 2 weeks thereafter for 16 weeks.

Table 5B. Effect of different rates and frequencies of application of CGA 48988 on infection of field grown 'Kingfisher' azaleas inoculated with zoospores of *Phytophthora parasitica*.

Rates[x] (per 100 gals.)	Frequency of application	Average number of wilted plants[y] Number of weeks after initial inoculation[z] 5	10	15	24
	No Drench; inoc.	7.5	16.5	17.5	18
	4 weeks	0	0.5	4.5	4.5
1 oz.	6 weeks	0	3	7	7
	8 weeks	0	2	7	7
	10 weeks	0.5	7	11.5	12
	12 weeks	0	9	14.5	14.5
	No Drench; uninoc.	0	0.5	1	1
	No Drench; inoc.	11.5	22.5	24	24.5
	4 weeks	3	14.5	17	17
1/2 oz.	6 weeks	1	14	17.5	17.5
	8 weeks	2	15.5	20	20
	10 weeks	3.5	21	24	24
	12 weeks	2.5	20	23	23
	No Drench; uninoc.	0	0	1	2.5

[x]Soil drenches were applied at 100 ml/basket beginning 3 days before the initial inoculation.
[y]Disease incidence is expressed as the average number of plants rogued for wilt per 2 replications with 30 plants per replication. *P. parasitica* was recovered from basal stem sections of all wilted plants.
[z]Initial inoculation consisted of 20 ml of a zoospore suspension (7 x 10^4 zoospores/ml) poured around the base of each plant. Inoculation was repeated with a zoospore suspension (1 x 10^4 zoospores/ml) every 2 weeks thereafter for 16 weeks.

Phytotoxicity evaluations

In 2 separate studies, no phytotoxicity was observed when CGA 48988 was applied as a soil drench or foliar spray at 250 ppm a.i. or less. When applied at 1,000 ppm a.i., slight inter-veinal chlorosis occurred after 4 weeks. Following a second application and a pinch, new growth was slower and stunted in comparison to the untreated plants. Plants treated at 5,000 ppm a.i. developed a slight inter-veinal chlorosis within 2 weeks. Severe chlorosis and marginal leaf burn were observed after 4 weeks. Heavy defoliation was observed following a pinch, and new growth was stunted and slower than the untreated plants. In all cases, the same reaction was observed whether the fungicide was applied as a soil drench or a foliar spray, but the reaction was less severe in the case of the foliar sprays. The reactions observed were similar for all varieties treated. However, whereas excellent control was obtained with 4 oz/100 gals (154 ppm a.i.) and no phytotoxicity was observed at 250 ppm a.i., the fungicide can be safely used with no plant damage.

Control of Phytophthora wilt of azaleas with the systemic fungicide, CGA 48988, has been demonstrated. Eradicative properties of the material are suggested by control with post-inoculation treatments. Both soil drenches and foliar sprays are effective. Its potential in controlling other diseases caused by pythiaceous fungi further contributes to its usefulness. It remains to be seen whether the fungi it controls will develop resistance as readily as in the case of the widely used systemic fungicide, Benlate. Regardless, the value of CGA 48988 has been demonstrated and its registration for commercial use is greatly anticipated.

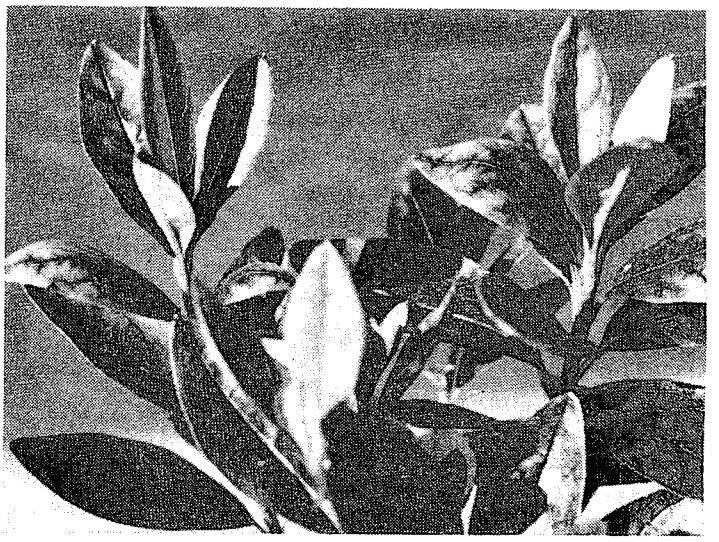

Fig. 2. 'Gloria' azalea exhibiting inter-veinal chlorosis resulting from a soil drench of CGA 48988 at 5,000 ppm a.i.

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