Control of *Colletotrichum acutatum* in Strawberry Under Laboratory, Greenhouse, and Field Conditions

S. Freeman and **Y. Nizani,** Department of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250; and **S. Dotan, S. Even,** and **T. Sando,** Ministry of Agriculture, Extension Service, Ra'anana 43208, Israel

ABSTRACT

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Various fungicides and a heat treatment were assessed for their ability to control strawberry anthracnose caused by the fungus *Colletotrichum acutatum* under laboratory, greenhouse, and field conditions. The effective dose causing 50% inhibition of mycelial growth (ED_{50}) was 30.5, 12.2, 0.2, 0.15, 0.05, 0.07, and 0.05 µg/ml for the fungicides folpet, captan, propiconazole, difenoconazole, combined prochloraz-Zn/folpet, prochloraz-Zn, and prochloraz-Mn, respectively. In laboratory experiments, infection in segments of strawberry runners treated with prochloraz-Zn reached 60%, which was significantly reduced as compared to combined prochloraz-Zn/folpet (90%), captan, folpet, and water controls (100%). In the greenhouse, numbers of naturally infected transplants killed were significantly reduced by all fungicides and the heat treatment (5 min at 49°C) as compared to the non-treated control. Prochloraz-Zn was the most effective chemical control treatment but did not differ significantly from the heat treatment. In field experiments conducted during 1995 and 1996, numbers of naturally infected strawberry transplants killed were significantly reduced by all fungicide treatments relative to the non-treated control. Percent reduction of transplant mortality in the field was 93.3, 93.1, 66.7, 37.7, and 29.1 for prochloraz-Mn, prochloraz-Zn, combined prochloraz-Zn/folpet, propiconazole, and difenoconazole, respectively.

Species of the fungal plant pathogen *Colletotrichum* are responsible for strawberry (*Fragariae* × *ananassa* Duch.) anthracnose, one of the major diseases of this crop. Major species that cause strawberry anthracnose are *C. acutatum* J. H. Simmonds, *C. fragariae* Brooks, and *C. gloeosporioides* (Penz.) Penz. & Sacc. (7,10,11). Symptoms of the disease are manifested in a variety of phases. Infection of mother plants with anthracnose may result in collapse of the entire plant due to crown rot. In the nursery, lesions are formed on stolons that eventually girdle the runners, and unrooted daughter plants distal to the lesion wilt and die. Death of daughter plants in the nursery and in field transplants can also be caused by crown rot. Infected transplants are capable of spreading the disease from the nursery to the field. In strawberry fruit production fields, entire crops can be destroyed by anthracnose under conducive environmental and cultural conditions. Leaves, flowers, and green and ripe fruit may be extremely susceptible to anthracnose, which can cause loss of all fruit in some cases (7). In central Brazil, a recent epidemic of anthracnose caused high yield loss, ranging from 30 to 68% (6).

During the months of May to September in 1995 and 1996, severe outbreaks of strawberry anthracnose were observed in many strawberry-growing nurseries in Israel. Following transplantation, entire beds of strawberry plants collapsed in the field due to crown rot. The causal agent was shown to be *C. acutatum* (2,3). Recently, various molecular techniques have been used to accurately and reliably identify the species of *Colletotrichum* causing strawberry anthracnose (4,5). The polymerase chain reaction (PCR) method was consequently used to verify that more than 100 Israeli isolates are from the species *C. acutatum* (3).

Fungicide application for the control of strawberry anthracnose is limited due to restrictions imposed on pesticide usage and susceptibility of strawberry plants to *Colletotrichum* during all stages of development, including flowers and green and ripe fruit. Anthracnose infection in plant nurseries can be reduced by producing disease-free propagation material. This is a prerequisite for establishing disease-free nursery transplants. Control measures practiced in the United States rely on frequent applications of the protectant fungicides captan and thiram (13). However, benzimidazole fungicides are not effective due to the common occurrence of resistant *Colletotrichum* strains (13). Certain fungicides, including propiconazole, have been reported to successfully limit in vitro growth of *C. fragariae* (12), and fluazinam, a protectant fungicide, confers good control of strawberry anthracnose in post-harvest berries (15).

In this study, we evaluated the fungicides prochloraz-Zn, prochloraz-Mn (both protectant sterol-biosynthesis inhibitors), captan, folpet, a combined formulation of prochloraz-Zn and folpet, propiconazole, difenoconazole, and a heat treatment for the control of *C. acutatum* in naturally infected strawberry plants under laboratory, greenhouse, and field conditions. Fungicides were applied at the transplant stage to determine whether a single-dip treatment is sufficient for disease control.

MATERIALS AND METHODS

Fungal cultures and plant material. Three isolates of *C. acutatum* from naturally infected strawberry (cv. Oso Grande) plants (TUT-40A from stolons, TUT-112A from fruit, and TUT-127A from roots) were used in this study. Isolates were maintained on modified Mathur's medium (MS) at 25°C in the dark, as previously described (14). Cultivars Ofra, Malach (clone 156), and Hadas (clone 543), which are susceptible to strawberry anthracnose and were naturally infected with *C. acutatum*, were used in laboratory, greenhouse, and field experiments.

In vitro fungicide assay. Fungicide-amended petri plates (9-cm-diameter) were used for assaying in vitro activity of captan (Marpan, 50WP, Makhteshim Chemical Works Ltd., Beer-Sheva, Israel), folpet (Folpan, 50WP, Makhteshim), prochloraz-Zn (Mirage, 50WP, Makhteshim), prochloraz-Mn (Octave, 50WP, Agrevo Ltd., Cambridge, UK), combined prochloraz-Zn and folpet (Mirage-F, 15 and 60WP, respectively, Makhteshim), propiconazole (Tilt, 25% EC, Ciba-Geigy Corp., Basel, Switzerland) and difenoconazole (Score, 25% EC, Ciba-Geigy Corp.) towards *C. acutatum*. Mycelial disks (5-mm-diameter removed from the margins of 4- to 5-day-old cultures) were transferred to MS amended with the tested fungicides at various concentrations, from 0 to 500 µg/ml. Radial growth of the mycelium was meas-

Corresponding author: S. Freeman
E-mail: vpsfreem@volcani.agri.gov.il

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ured periodically to determine percent control (8). The effective dose causing 50% inhibition of mycelial growth (ED_{50}) was calculated by log/probit transformation of percent control. The experiment was conducted twice using each of the three *C. acutatum* isolates. Similar results were reproduced and thus data were pooled. Because no significant differences were observed between prochloraz-Mn and prochloraz-Zn, in further experiments, except for the field trials, only prochloraz-Zn was assessed.

Control of *C. acutatum* in naturally infected strawberry runners. Naturally infected strawberry runners (cv. Hadas) were segmented into 1.5-cm sections and surface-disinfested for 30 s in 3% sodium hypochlorite. The segments were then submerged for 1 min in sterile tap water (untreated control) or in an aqueous solution of the following fungicides: captan (2.5 g/liter), prochloraz-Zn (1 g/liter), folpet (4 g/liter), or combined prochloraz-Zn (1 g/liter) and folpet (4 g/liter). Similar concentrations of prochloraz have been used in the past for control of almond anthracnose caused by *C. gloeosporioides*. Runner segments were dried on sterile Whatman filter paper and then plated on MS supplemented with 2.5 µg (a.i.) iprodione (Rovral 50WP, Rhone Poulenc Inc., Lyons, France) per ml to reduce contaminating fungi. Each treatment consisted of 25 segments, five segments per plate. Control efficacy was evaluated by growth of *C. acutatum* from the disinfested, fungicide-treated runners. The proportion of runner segments showing visible growth of *C. acutatum* was evaluated over 12 days. The experiment was conducted three times and data were pooled. Evaluations of captan and folpet were discontinued in greenhouse and field experiments due to the high concentrations required and their limited success in mycelial inhibition and pathogen control under laboratory conditions.

Control of *C. acutatum* in naturally infected strawberry transplants in the greenhouse. Various fungicides and a heat treatment, routinely used in California, United States (13), were evaluated for their control of *C. acutatum* in naturally infected transplants (cvs. Ofra, Malach, and Hadas). Transplants were submerged for 10 min in tap water (non-treated control) or in aqueous solutions of prochloraz-Zn (1 g/liter), combined prochloraz-Zn (1 g/liter) and folpet (4 g/liter), propiconazole (2.5 g/liter), or difenoconazole (2.5 g/liter). The experiment also included a heat treatment in which transplants were submerged for 5 min in a heated water bath at 49°C. Plants were then potted (in 11-cm-diameter pots) in a vermiculite/peat soilless mix (1:1, vol/vol). Plants were maintained under supplementary incandescent light for a 12-h photoperiod in the greenhouse at 25°C, and watered daily as necessary. Each treatment consisted of 25 plants set in a randomized design. Disease incidence was expressed as percent transplant mortality after 6 weeks. The experiment was conducted four times and data were pooled.

Control of *C. acutatum* in naturally infected field transplants. Field control of *C. acutatum* in naturally infected transplants was evaluated in the Sharon region in 1995 and in the Lev Hasharon region in 1996. Transplants were submerged for 10 min in tap water, prochloraz-Mn (1 g/liter), prochloraz-Zn (1 g/liter), combined prochloraz-Zn (1 g/liter) and folpet (4 g/liter), propiconazole (2.5 g/liter), or difenoconazole (2.5 g/liter). In the 1995 experiment, treated transplants (cv. Malach) were then planted in a randomized design in the field on 2 October, with four replicates of 32 plants each per treatment. The percentage of plants killed was assessed 34 days later, on 5 November. In the 1996 experiment, treated transplants (cv. Ofra) were planted in a randomized design in the field on 18 September, with five replicates of 50 plants each per treatment. Percent plant mortality was assessed 20 days later, on 7 October. Both field experiments were terminated before complete mortality of the plants in the control plots to allow the farmers adequate time to fumigate the soil and replant healthy plants. Mean separation of the data was analyzed according to Fisher's protected least significant difference (LSD) test, at $P < 0.05$.

RESULTS

In vitro inhibition of *C. acutatum*. The effective dose causing 50% inhibition (ED_{50}) was calculated for the fungicides folpet, captan, difenoconazole, propiconazole, combined prochloraz-Zn/folpet, prochloraz-Zn, and prochloraz-Mn (Table 1). Prochloraz-Zn and prochloraz-Mn, whether alone or in combination with folpet, were the most effective fungicides in inhibiting *C. acutatum* in vitro. A twofold to three-

Table 1. The effective dose of various fungicides causing 50% inhibition (ED_{50}) of *Colletotrichum acutatum* in vitro

Fungicide	ED_{50}(µg/ml)
Folpet	30.455 ± 5.6
Captan	12.225 ± 2.3
Propiconazole	0.199 ± 0.05
Difenoconazole	0.156 ± 0.05
Prochloraz-Zn/Folpet	0.053 ± 0.012
Prochloraz-Zn	0.077 ± 0.023
Prochloraz-Mn	0.054 ± 0.017

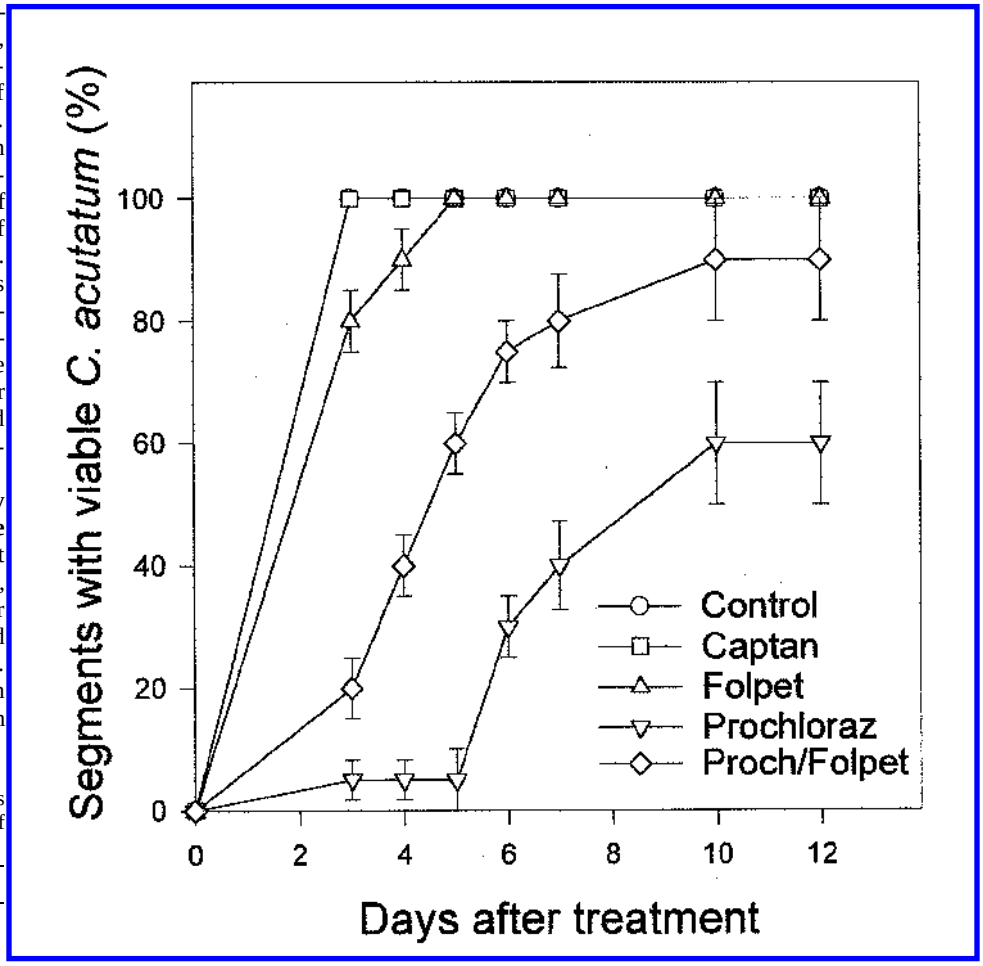

Fig. 1. Effect of various fungicides on control of *Colletotrichum acutatum* in naturally infected strawberry runners. Infected 1.5-cm-long segments were treated with the fungicides captan (2.5 g/liter), prochloraz-Zn (1 g/liter), folpet (4 g/liter), combined prochloraz-Zn (1 g/liter) and folpet (4 g/liter), and water (control), then plated to determine percent viability of *C. acutatum* in segments. Vertical lines in the graph denote SE (standard error) for each sampling date.

fold concentration of the -azole fungicides and an approximately 200- to 500-fold concentration of captan and folpet were required for 50% fungal-growth inhibition relative to that required of the prochloraz fungicides (Table 1).

Control of *C. acutatum* in infected strawberry runners. Results obtained in naturally infected runners resembled those from the in vitro experiments (Fig. 1). Captan and folpet had little or no effect on control of *C. acutatum.* However, survival of the fungus in runners was significantly reduced by prochloraz-Zn and the combined prochloraz-Zn/folpet fungicides as compared to the other treatments and the non-treated control during the course of the entire experiment (Fig. 1). After 12 days, recovery of *C. acutatum* from runners treated with prochloraz-Zn reached 60%, which was significantly lower than that observed with the combined prochloraz-Zn/folpet fungicide and other treatments, including the water control (90 to 100% infection).

Control of *C. acutatum* in infected strawberry transplants in the greenhouse. Mortality of transplants caused by *C. acutatum* was significantly reduced by all fungicides and by the heat treatment relative to the non-treated control (Table 2). Prochloraz-Zn was the most effective treatment (85.6% control), but did not differ significantly from the heat treatment (75.6% control). Prochloraz-Zn/folpet (50.6% control), propiconazole (33.8% control), and difenoconazole (28.8% control) were less effective than prochloraz-Zn and the heat treatment in reducing plant mortality (Table 2). It should be noted that the prochloraz fungicides, difenoconazole, and propiconazole caused slight phytotoxicity of the treated plants which was manifested by mild stunting and scorching of leaves.

Control of *C. acutatum* in field transplants. Mortality caused by *C. acutatum* in naturally infected strawberry transplants was significantly reduced by all fungicide treatments as compared to the non-treated control during both 1995 and 1996 field trials (Table 3). Prochloraz-Mn and prochloraz-Zn alone were the most effective in reducing transplant mortality among the tested fungicides. Reduction of transplant mortality in 1995 using prochloraz-Mn was 93.3%, whereas reduction in mortality in 1996 for prochloraz-Mn (86.8%) and prochloraz-Zn (93.1%) did not differ significantly. Prochloraz-Zn/folpet, propiconazole, and difenoconazole were less effective than prochloraz alone (Table 3). However in 1995, transplant mortality was reduced by 66.7 and 37.7% using prochloraz-Zn/folpet and propiconazole, respectively. Similarly, in 1996, transplant mortality was reduced by 52.9 and 29.1% using prochloraz-Zn/folpet and difenoconazole, respectively. As with the greenhouse experiments, the prochloraz fungicides, difenoconazole, and propiconazole were slightly phytotoxic.

DISCUSSION

High concentrations of the protectant fungicides captan and folpet were not effective in vitro and failed to control the disease in naturally infected runners. Their use in further greenhouse and field experiments was therefore not considered. The -azole fungicides, propiconazole and difenoconazole, were effective in reducing in vitro growth of *C. acutatum.* However, prochloraz in two forms, prochloraz-Zn and prochloraz-Mn, as well as a combined prochloraz-Zn/folpet formulation, were twofold to threefold more effective in inhibiting in vitro growth of the pathogen. This prompted us to proceed with in vivo studies using the -azole and prochloraz fungicides for control of *C. acutatum* in naturally infected plant material. In greenhouse and field studies, prochloraz-Mn and prochloraz-Zn alone were highly effective in controlling the pathogen in naturally infected plant material, as was the prochloraz-Zn/folpet formulation, albeit to a lesser extent. It should be noted that the combined treatment was also less effective than prochloraz alone in disease control under laboratory conditions, despite the fact that the prochloraz concentration in the combined formulation was equivalent to that of prochloraz alone. This may be attributed to an antagonistic effect of folpet in the formulation which interfered with the fungicidal activity of prochloraz. Although propiconazole has been reported to inhibit *C. fragariae* in vitro (11), it was not sufficiently effective in our experiments.

Prochloraz was effective in controlling strawberry anthracnose under all tested conditions, however, this fungicide under the current formulation had a residual taste effect on strawberries (S. Freeman and S. Dotan, *unpublished data*). Therefore, prochloraz should be thoroughly tested before widespread use in strawberry fruiting fields. Additional caution should be taken regarding resistance or tolerance of *C. acutatum* and other species of *Colletotrichum* to prochloraz in particular, and to other fungicides in general. Isolates of *C. kahawae*, which are usually highly sensitive to prochloraz, have been shown to acquire tolerance to this fungicide in culture (9). Similarly, isolates of *C. acutatum* from various hosts (including strawberry) were resistant to benzimidazole fungicides as compared to the sensitivity of *C. gloeosporioides* isolates (1).

A standard heat treatment (5 min at 49°C) of bare-rooted transplants for nursery use is practiced in the United States for the control of foliar nematodes and anthracnose of strawberry (13). In this study the same heat treatment had no visible detrimental effect on transplants and was very effective in reducing percent mortality caused by *C. acutatum* (Table 2). However, heat treatment may be detrimental to plant vigor and should not be used if transplants are destined for production fields (13). Although heat treatment may be a welcome alternative to chemical fungicides for protective and curative measures of strawberry anthracnose, its long-term effect on plant development and yield needs to be addressed. On the other hand, nonchemical means of control may minimize the appearance of fungicide-tolerant or resistant fungal pathogenic strains.

The establishment of transplants in the field is one of the most critical stages of strawberry production in Israel. Plants that are killed by strawberry anthracnose during

Table 2. Effect of various fungicides and a heat treatment on percent mortality of strawberry plants caused by *Colletotrichum acutatum* in the greenhouse

Treatment[a]	Mortality[b] (%)
Control	80.0
Difenoconazole	57.2
Propiconazole	53.5
Prochloraz-Zn/Folpet	39.5
Heat	19.5
Prochloraz-Zn	11.5
LSD[c]	13.5

[a] Plants were immersed for 10 min in the following treatments: control (tap water), prochloraz-Zn (1 g/liter), combined prochloraz-Zn (1 g/liter) and folpet (4 g/liter), difenoconazole (2.5 g/liter), propiconazole (2.5 g/liter), and heated water (49°C for 5 min).

[b] Means are the average plant mortality of four experiments containing 25 plants each per treatment. Mortality was assessed 6 weeks after planting.

[c] Least significant difference (LSD), calculated according to Fisher's protected test, at $P < 0.05$.

Table 3. Percent mortality[a] of strawberry transplants caused by *Colletotrichum acutatum* in the field during the years 1995 and 1996

	Mortality (%)[c]	
Treatment[b]	1995	1996
Control	70.3	75.6
Prochloraz-Zn/Folpet	23.4	35.6
Prochloraz-Zn	…	5.2
Prochloraz-Mn	4.7	10.0
Propiconazole	43.8	…
Difenoconazole	…	53.6
LSD[d]	22.8	13.6

[a] Mortality was assessed 34 (1995) and 21 (1996) days after transplanting.

[b] Transplants were dipped for 10 min in the following treatments: control (tap water), prochloraz-Mn (1 g/liter), prochloraz-Zn (1 g/liter), combined prochloraz-Zn (1 g/liter) and folpet (4 g/liter), difenoconazole (2.5 g/liter), and propiconazole (2.5 g/liter).

[c] Means are the average transplant mortality of four replicates containing 32 plants each (1995), and five replicates containing 50 plants each (1996), per treatment.

[d] Least significant difference, calculated according to Fisher's protected test, at $P < 0.05$.

the first 3 to 4 weeks following transplantation are routinely replaced by new transplants. Fruit production is thereby delayed, resulting in extensive loss of revenue by fruit not accessing the European markets before January. As a result, single-dip prochloraz treatments, both at the nursery establishment and especially at the transplant stages, have become routine practice for all farmers in Israel, regardless of whether or not plants have visible anthracnose symptoms. Strawberry root necrosis caused by *C. acutatum* resulting in stunting, chlorosis, and plant mortality has become an increasing problem in Israel (3). The dip treatments are therefore likely to expose infected root tissues to prochloraz and contribute to reduction of disease incidence. In addition, single-dip treatments have minimal impact on the environment as entire fields are not sprayed after planting. Although mild prochloraz phytotoxicity was observed, crop yield does not appear to be affected.

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