Laboratory and Glasshouse Studies of the Activity of Carboxamide Derivatives Against *Rhizoctonia solani* in Cotton

J. L. HUPPATZ, J. N. PHILLIPS, and B. WITRZENS, CSIRO, Division of Plant Industry, PO Box 1600, Canberra City, Australia

ABSTRACT

Huppatz, J. L., Phillips, J. N., and Witrzens, B. 1983. Laboratory and glasshouse studies of the activity of carboxamide derivatives against *Rhizoctonia solani* in cotton. Plant Disease 67:45-47.

Carboxin and several of its analogues, including pyracarbolid, fenfuram, methfuroxam, and furmetamid, as well as two experimental pyrazole carboxanilides, have been studied as fungitoxic agents against the soil pathogen *Rhizoctonia solani*. The activities of these compounds were compared both in vitro on mycelial growth and in vivo against damping-off disease in cotton seedlings grown under glasshouse conditions. Carboxin, methfuroxam, and furmetamid were the more active inhibitors in vitro, whereas furmetamid, the pyrazole derivatives, and carboxin were the more effective compounds in vivo.

The oxathiins carboxanilide, carboxin, and related carboxamide systemic fungicides (Fig. 1) have been shown to be selectively active against basidiomycetes (8,13,14). Several members of a new series of carboxin analogues, the pyrazole carboxanilides, have been reported as being comparable to carboxin in inhibiting cereal rusts and smuts (3). This has prompted a study of their activity against yet another basidiomycete, the soil pathogen *Rhizoctonia solani* Kühn.

Accordingly, the effects of two representative pyrazole derivatives (Fig. 1; see IIa and IIb) on the mycelial growth of *R. solani* in vitro and on damping-off caused by *R. solani* in glasshouse-grown cotton seedlings have been examined. The lack of data in the literature comparing the effectiveness of various carboxamide fungicides toward *R. solani* in vitro and in vivo has led to the inclusion in this study of carboxin and a number of its analogues—pyracarbolid, fenfuram, methfuroxam, and furmetamid (Fig. 1).

MATERIALS AND METHODS

Compounds. The compounds 1,3,5-trimethylpyrazole-4-carboxanilide (IIa) and 1,3,5-trimethylpyrazole-4-carbox-3′-toluidide (IIb) were synthesized as described by Carter et al (3). Formulations of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide (I) (carboxin, 75% a.i.); 3,4-dihydro-6-methylpyran-5-carboxanilide (III) (pyracarbolid, 50% a.i.); 2-methyl-3-furanilide (IVa) (fenfuram, 75% a.i.); and 2,4,5-trimethyl-3-furanilide (IVb) (methfuroxam, 97% a.i.) were supplied as wettable powders by R. Warner of ICI-Australia, Melbourne. The formulation *N*-cyclohexyl-*N*-methoxy-2,5-dimethyl-3-furancarboxamide (V) (furmetamid) was supplied as a 50% liquid (BAS 389F) by R. Saur of Badische Anilin and Soda-Fabrik A-G., Limburgerhof, Germany.

Laboratory studies. Compounds were incorporated in the growth medium by addition of 0.2 ml of an acetone solution of an appropriate concentration to 20 ml of liquid, sterilized potato-dextrose agar contained in an 8.5-cm petri dish. The medium was cooled and inoculated with agar disks (0.7 cm^2) taken from the periphery of plate cultures of *R. solani*, isolated from infected cotton seedlings, and incubated at 25 C. Treatments as well as untreated controls were replicated fivefold. The growth of the organism was measured in terms of the mean diameter of the colony after 7 days, by which time the untreated controls had just covered the plate. The ratio of treated to untreated colony diameter was plotted against the molar concentration of the test compound, and the ED_{50} value was determined.

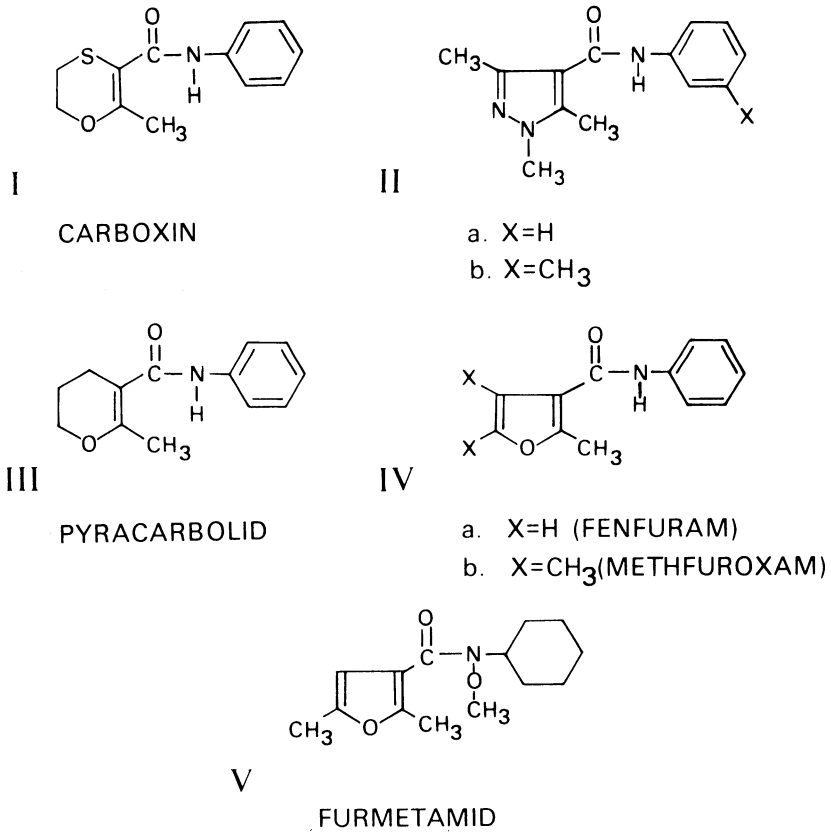

Fig. 1. Chemical structure of carboxin and several of its analogues: **(I)** Carboxin; 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide. **(IIa)** 1,3,5-Trimethylpyrazole-4-carboxanilide. **(IIb)** 1,3,5-Trimethylpyrazole-4-carbox-3′-toluidide. **(III)** Pyracarbolid; 3-4-dihydro-6-methylpyran-5-carboxanilide. **(IVa)** Fenfuram; 2-methyl-3-furanilide. **(IVb)** Methfuroxam; 2,4,5-trimethyl-3-furanilide. **(V)** Furmetamid; *N*-cyclohexyl-*N*-methoxy-2,5-dimethyl-3-furancarboxamide.

Accepted for publication 17 May 1982.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/83/01004503/$03.00/0

Glasshouse studies. Two procedures were used to assay compounds for the control of damping-off caused by *R. solani* in a susceptible cotton cultivar (Deltapine 16) growing in a steam-sterilized sandy loam at 24 ± 2 C. The compound was either incorporated in the growth medium prior to the cotton seeds being planted and the seedlings inoculated 10–12 days later (postemergence inoculation) or it was applied to the seeds, which were planted into *Rhizoctonia*-infested soil (preemergence inoculation).

Postemergence inoculation assay. Each compound was dissolved in acetone and mixed with 40 ml of sand; the acetone was allowed to evaporate, and the sand was placed as a layer over soil in 10-cm pots to give an equivalent surface application rate of compound of 8 kg/ha. Cotton seeds were sown in the sand layer and covered with 2 cm of soil. Each treatment, as well as untreated inoculated and untreated uninoculated controls, was replicated in 25 pots (four plants per pot).

The inoculum was prepared by autoclaving 60 g of wheat seed for 30–40 min with 120 ml of distilled water. Three 1-cm^2 pieces of agar covered with mycelium were added, and the wheat seed was incubated for 7 days at 25 C. A 3-g portion of this incubated mixture was blended with 1,200 ml of distilled water, and 50 ml of the resultant suspension was poured over the soil surface of a pot containing four 10- to 12-day-old cotton seedlings. The mycelial inoculum was then covered with a thin layer of soil. Such inoculation led to infection at the base of the stem, and seedlings began to wilt and collapse within 5 days. The level of disease control was assessed 14 days after inoculation in terms of the number of upright plants and the number of plants showing no evidence of infection (ie, with no macroscopic stem or root discoloration). This assay procedure also served as an indicator of the phytotoxicity of the compound.

Preemergence inoculation assay. In this assay, each compound was applied as a seed treatment at a rate of 5 g a.i./kg of seed. The compound was usually ground with an equal weight of talcum powder, and the mixture was applied to the cotton seeds by tumbling on a ball mill. Furmetamid, being a liquid formulation (BAS 389F), was added directly to the seeds prior to tumbling.

The inoculum was prepared from a 10-day-old culture of *R. solani* on potato-sucrose broth by blending the mycelial mat with 200 ml of distilled water (12). Flats (60 × 30 cm) containing moist soil were inoculated by distributing the fungal suspension evenly over the surface and covering with a thin layer of soil. The flats were kept moist, and after 2 wk the treated cotton seeds were planted (100 seeds per flat). There were two replicates of all treatments as well as of untreated inoculated and untreated uninoculated controls.

The effectiveness of a treatment in controlling the disease was assessed after 14 days in terms of seedling emergence, the number of upright plants, and the number of plants showing no evidence of infection.

RESULTS

The ED_{50} values of various carboxamide derivatives are indicated in Table 1. Carboxin (ED_{50} 0.4 μM), methfuroxam (0.4 μM), and furmetamid (0.6 μM) were an order of magnitude more active than fenfuram (4 μM), pyracarbolid (4 μM), and the pyrazoles IIa (10 μM) and IIb (4 μM).

The effectiveness of these same compounds in protecting cotton seedlings from damping-off disease caused by *R. solani* is also given in Table 1. In the postemergence tests, no phytotoxic symptoms were observed with any of the treatments up to the time the cotton seedlings were inoculated, ie, 10–12 days after planting. Moreover, a high percentage of plants was still standing at the time of assessment 14 days after inoculation, although untreated inoculated plants had collapsed by that time. The more effective compounds were furmetamid (94% upright plants), the pyrazoles IIb (93%) and IIa (85%), and carboxin (82%), whereas less effective were fenfuram (68%), pyracarbolid (64%), and methfuroxam (53%). When the effectiveness of compounds was evaluated in terms of the number of plants showing no evidence of disease symptoms, a similar order was observed with furmetamid and the pyrazole IIb giving 50% protection as against 16–23% for methfuroxam and fenfuram.

In the preemergence tests (Table 1), all treatments except the one involving fenfuram resulted in greater than 90% seedling emergence, and more than 90% of the plants were still standing after 14 days. This compares with 64% emergence and 36% upright plants in the case of untreated inoculated seedlings. In terms of seedlings showing no disease symptoms, carboxin (58%) and the pyrazole IIa (56%) were the most effective and fenfuram (28%) and methfuroxam (29%) the least effective.

DISCUSSION

The relative order of activity of the carboxamide derivatives in protecting cotton seedlings from damping-off was largely independent of the method of application, the inoculation technique, and the disease assessment procedure. Thus, furmetamid and the pyrazoles IIa and IIb were consistently better protectants than methfuroxam and fenfuram, regardless of whether the compounds were soil incorporated and the plants inoculated after emergence or whether the compounds were applied as a seed dressing and the seeds sown in infested soil. Carboxin, on the other hand, was more effective under the latter set of conditions, where it was able to make immediate contact with the organism in the soil. Its poorer performance in postemergence inoculation trials may have resulted from its oxidation to the less active sulfoxide derivative (4) during the 10- to 12-day interval between its application to the soil and the introduction of the organism.

Table 1. Effect of carboxamide fungicides on mycelial growth of *Rhizoctonia solani* and on Rhizoctonia damping-off in glasshouse-grown cotton

Compound	ED_{50}[v] (μM)	Postemergence[w] soil incorporation			Preemergence[x] seed treatment	
		% Plants standing	% Plants nondiseased	% Seedling emergence	% Plants standing	% Plants nondiseased
Carboxin (I)	0.4[y]	82 ac	26 bc	97	94	58
Pyracarbolid (III)	4.0	64 cd	28 bc	...[z]	...	...
Fenfuram (IVa)	3.2	68 cd	23 c	79	74	28
Methfuroxam (IVb)	0.4	53 d	16 c	94	90	29
Furmetamid (V)	0.6	94 a	50 a	100	97	44
Pyrazole (IIa)	8.0	85 ab	41 ab	93	90	56
Pyrazole (IIb)	4.0	93 a	50 a	93	91	48
Uninoculated control		100	100	100	100	100
Inoculated control		0	0	64	36	0

[v] The molar concentration of compound giving 50% inhibition of the mycelial growth of *R. solani* on potato-dextrose agar over 5 days.
[w] Compounds applied at 8 kg/ha in the soil prior to sowing the cotton. Seedlings (10–12 days old) inoculated with *R. solani* and assessed after 14 days. Values followed by the same letter are not significantly different ($P = 0.01$).
[x] Compounds applied as a 0.5% (w/w) seed dressing to cotton seeds sown in *R. solani*-infested soil. Seedlings assessed after 14 days.
[y] Literature ED_{50} values: 0.4 μM (13); 1.0 μM (15).
[z] Not tested.

The antifungal behavior of carboxin and its analogues has been attributed to their ability to inhibit the succinic dehydrogenase enzyme complex in the organism (15,16). They have also been shown to move systemically in plants (14). In the present experiments, infection of cotton seedlings inoculated after emergence occurred at the base of the stem. Thus, a compound applied in the soil would need to be taken up by the seed or developing root, moved to the site of infection in the stem, and survive in the plant for up to 2 wk if it were to be directly effective in combating the disease. Accordingly, the overall activity of a compound in vivo could be regarded as a function of its intrinsic activity against the organism in vitro and of its systemic behavior and metabolic stability within the plant. There appeared to be a broad correlation between effectiveness in vivo and inhibition of mycelial growth of *R. solani* in vitro for carboxin, furmetamid, fenfuram, and pyracarbolid. However, the pyrazoles were among the less effective compounds in vitro (ED_{50} = 4–10 μM) but the more effective in vivo, whereas the converse applied for methfuroxam, which was highly active in vitro (ED_{50} = 0.4 μM) but consistently inferior to the pyrazoles in vivo. Such discrepancies between in vitro activity and in vivo performance might be attributable to differences in either the metabolic stability or systemic behavior of these compounds.

There are several reports in the literature of carboxin and other carboxamide derivatives giving moderate to good control of damping-off caused by *R. solani* in both glasshouse and field-grown cotton (1,2,5–7,11). The recently introduced fungicide furmetamid appears to have shown particularly promising results (9,10) consistent with the data in Table 1, which indicate it to be one of the more effective compounds in vitro and in vivo. The pyrazoles IIa and IIb, although inferior to furmetamid in vitro, are comparably active in vivo. Further studies of their antifungal behavior would appear to be justified.

LITERATURE CITED

1. Al-Beldawi, A. S., and Pinckard, J. A. 1970. Control of *Rhizoctonia solani* on cotton seedlings by means of a derivative of 1,4-oxathiin. Plant Dis. Rep. 54:524-528.
2. Borum, D. E., and Sinclair, J. B. 1968. Evidence for systemic protection against *Rhizoctonia solani* with Vitavax in cotton seedlings. Phytopathology 58:976-980.
3. Carter, G. A., Huppatz, J. L., and Wain, R. L. 1976. The fungitoxicity and systemic antifungal activity of certain pyrazole analogues of carboxin. Ann. Appl. Biol. 84:333-342.
4. Chin, W. T., Stone, G. M., and Smith, A. E. 1970. Degradation of carboxin in water and soil. J. Agric. Food Chem. 18:731-732.
5. Cole, D. L., and Cavill, M. E. 1977. Use of selected fungicides as seed dressings for the control of *Rhizoctonia solani* in cotton. Rhod. J. Agric. Res. 15:45-50.
6. Darrag, I. E. A., and Sinclair, J. B. 1968. Techniques to evaluate chemotherapeutic activity of certain fungicides against *Rhizoctonia solani* in cotton seedlings. Plant Dis. Rep. 52:399-403.
7. Datta, T. P., and Sharma, B. D. 1976. A preliminary study on the control of soreshin disease of cotton in West Bengal. Pesticides 10:28-29.
8. Marsh, R. W., ed. 1972. Systemic Fungicides. Longmans, Green, New York. pp. 54-64.
9. Papavizas, G. C., Lewis, J. A., and O'Neill, N. R. 1979. BAS389, a new fungicide for control of *Rhizoctonia solani* in cotton. Plant Dis. Rep. 63:569-573.
10. Pommer, E. H., and Zeeh, B. 1977. Substituted 3-furamides with specific activity against basidiomycetes. Pestic. Sci. 8:320-322.
11. Sharma, K. B., and Sharma, N. D. 1976. Chemical control of root rot of cotton. Indian J. Mycol. Plant Pathol. 6:190-191.
12. Sinclair, J. B. 1957. Laboratory and greenhouse screening of various fungicides for control of Rhizoctonia damping off of cotton seedlings. Plant Dis. Rep. 41:1045-1050.
13. Snel, M., von Schmeling, B., and Edgington, L. V. 1970. Fungitoxicity and structure-activity relationships of some oxathiin and thiazole derivatives. Phytopathology 60:1164-1169.
14. von Schmeling, B., and Kulka, M. 1966. Systemic fungicidal activity of 1,4-oxathiin derivatives. Science 152:659-660.
15. White, G. A., and Thorn, G. D. 1975. Structure-activity relationships of carboxamide fungicides and the succinic dehydrogenase complex of *Cryptococcus laurentii* and *Ustilago maydis*. Pestic. Biochem. Physiol. 5:380-395.
16. White, G. A., and Thorn, G. D. 1980. Thiophene carboxamide fungicides: Structure-activity relationships with the succinic dehydrogenase complex from wild-type and carboxin resistant mutant strains of *Ustilago maydis*. Pestic. Biochem. Physiol. 14:26-40.