Rapid Assay for Systemic Fungicides Against Phytophthora Rot of Soybeans

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ABSTRACT

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A procedure for screening systemic fungicides for activity against *Phytophthora megasperma* var. *sojae* on soybean (*Glycine max*) is described. Roots of etiolated seedlings were soaked for 24 hr in solutions or suspensions of test compounds. Seedlings were then placed horizontally in trays and inoculated by placing droplets of a zoospore suspension from a compatible race of the fungus on the upper part of the hypocotyl. The trays were closed to maintain humidity, and disease development was recorded after 24 hr. Control ranged from complete (restriction of the fungus to the area beneath the droplet with light flecking of surface cells), through partial (varying degrees of browning and spreading), to nil (tissue was water-soaked and spread of the fungus unrestricted as in untreated hypocotyls). Ridomil (CGA 48988) and Chevron RE 26745 were the most effective of the compounds tested and Aliette the least. The procedure is rapid, simple, requires relatively small amounts of test compound, and gives uniform results.

Koch (2) observed that biological screens for detecting systemic fungicides tend to be slow, labor intensive, and expensive and require relatively large amounts of chemicals compared with in vitro assays. Although some rapid small-scale procedures have been described (1,4), usually test compounds are applied to roots or the surrounding medium and disease development is evaluated after inoculation of leaves or stems with an appropriate fungus. Probably because few compounds have been suitable until recently, reports of methods for diseases caused by Phycomycetes have been limited (3). In this article we describe a simple, rapid assay system using *Phytophthora megasperma* Drechs. var. *sojae* Hildeb. (*Pms*) on soybean (*Glycine max* L., Merr.).

MATERIALS AND METHODS

The chemicals used were Ridomil [DL-methyl *N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl) alaninate, Ciba Geigy 48988, 50% wettable powder (WP)]; Fongarid [DL-methyl *N*-(2,6-dimethylphenyl)-*N*-(2-furoyl) alaninate, Ciba Geigy 38140, 25% WP]; Chevron experimental fungicide RE 26745 [2-methoxy-*N*-(2,6-dimethylphenyl)-*N*-(tetrahydro-2-oxo-3-furanyl) acetamide, 50% WP]; Chevron experimental fungicide RE 20615 [2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(tetrahydro-2-oxo-3-furanyl) acetamide, 50% WP]; Pyroxychlor [2-chloro-6-methoxy-4-(trichloromethyl) pyridine, Dowco 269, (Lorvek) 6% EC]; Dupont experimental fungicide, DPX 3217 [2-cyano-*N*-([ethylamino] carbonyl)-2-(methoxyimino) acetamide, 80% WP]; Prothiocarb [S-ethyl-*N*-(3-dimethylaminopropyl)-thiolcarbamate hydrochloride, Nor-Am, SN 41703, 100%]; Aliette (aluminum ethyl phosphite, May and Baker LS 74-783, 80% WP). They were dissolved or suspended in water in the concentrations indicated in Tables 1 and 2; all concentrations refer to active ingredient.

P. megasperma var *sojae* race 6 and seeds of soybean cultivar Altona were obtained from R. I. Buzzell (Research Station, Agriculture Canada, Harrow, Ont.). Altona is susceptible to *Pms* race 6, but another compatible cultivar-race combination would serve equally well. Cultures of *Pms* race 6 were grown on V-8 juice agar in the dark at 25 C. For production of zoospores, cultures were grown in petri dishes containing 12 ml of V-8 juice agar for 4–6 days at 25 C. Commencing 24 hr before spores were required, the cultures were leached by filling the dishes with sterile distilled water and by changing the water every 30 min for 8 hr. Then 15 ml of sterile distilled water was added, and by the following day sporangia had formed and germinated. Zoospore numbers were determined with a hemacytometer and the suspension was adjusted to approximately 1×10^5 spores per milliliter with sterile distilled water.

Seeds of soybean cv. Altona were planted in vermiculite in greenhouse flats, steeped overnight in 15-30-15 (N-P-K) fertilizer solution (2.25 g/L), and placed in a dark growth chamber (7 hr at 16 C, 5 hr increasing at 2.2 C/hr, 7 hr at 27 C, 5 hr decreasing temperature). This temperature regimen was programmed previously to represent a typical summer day and was found suitable for our purpose. The plants were fertilized again on the fourth day (4.5 g/L), removed from the vermiculite on the fifth day, rinsed in running tap water, and placed in beakers containing sufficient water or solutions of test compounds to cover the roots. Plants were incubated an additional 24 hr in the

Table 1. Control of Phytophthora rot by systemic fungicidal compounds on soybean seedlings (cv. Altona) hypocotyl-inoculated with zoospores of *Phytophthora megasperma* var. *sojae* race 6

Compound[a]	Concentration (μg/ml)	Disease control[b]
Aliette	2,000	0
	500	0
DPX 3217	150	+++
	50	++
RE 20615	50	++++
	25	++++
RE 26745	50	+++++
	25	+++++
Prothiocarb	150	+
	50	+
Ridomil	25	+++++
	10	+++++
Fongarid	25	+++++
	10	++++
Pyroxychlor	150	++++
	50	+++
H_2O control		0

[a] Roots of 5-day-old seedlings were immersed in solutions or suspensions of the test compounds for 24 hr, before inoculation of the hypocotyls with droplets of *Pms* zoospore suspensions.

[b] Rated 24 hr after inoculation: +++++ = complete control, light necrotic flecking of surface tissue covered by inoculum droplet; ++++ = increasing necrosis in area of droplet; +++ = spreading of browning beyond area of the droplet; ++ = extensive browning of tissue outside area of droplet; + = browning with water-soaking; 0 = no control.

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Table 2. Comparison of four fungicidal compounds for controlling Phytophthora rot on soybean (cv. Altona) seedlings hypocotyl-inoculated with zoospores of *Phytophthora megasperma* var. *sojae* race 6

Concentration (μg/ml)	Disease Control[a]			
	Ridomil[b]	Fongarid	RE 20615	RE 26745
1	++++	0	0	+++
2.5	++++	0	+	+++
5.0	+++++	+	+	+++++
10	+++++	+++	++	+++++
15	+++++	+++	+++	+++++
25	+++++	++++	++++	+++++
50	+++++	+++++	++++	+++++

[a] Rated 24 hr after inoculation: +++++ = complete control, light necrotic flecking of surface tissue covered by inoculum droplet; ++++ = increasing necrosis in area of droplet; +++ = spreading of browning beyond area of the droplet; ++ = extensive browning of tissue outside area of droplet; + = browning with water-soaking; 0 = no control.

[b] Roots of 5-day-old seedlings were immersed in solutions of Ridomil and the other compounds for 24 hr, before inoculation with droplets of *Pms* zoospore suspensions.

dark and then transferred to 35 × 21 cm glass trays where they were held in place by two slotted Plexiglas racks (1.2 cm wide), one just above the roots and the other immediately below the cotyledons. The roots were covered with a layer of moistened Cellucotton. The seedlings were inoculated by placing one or more 10-μl droplets of zoospore suspension on the upper one-third of the hypocotyl. The trays were sealed with plastic film and incubated for 24 hr at 25 C, after which the extent of infection was recorded.

RESULTS AND DISCUSSION

Untreated plants developed pale water-soaked lesions, which spread extensively beyond the area covered by the inoculum droplet (control rating = 0). Where

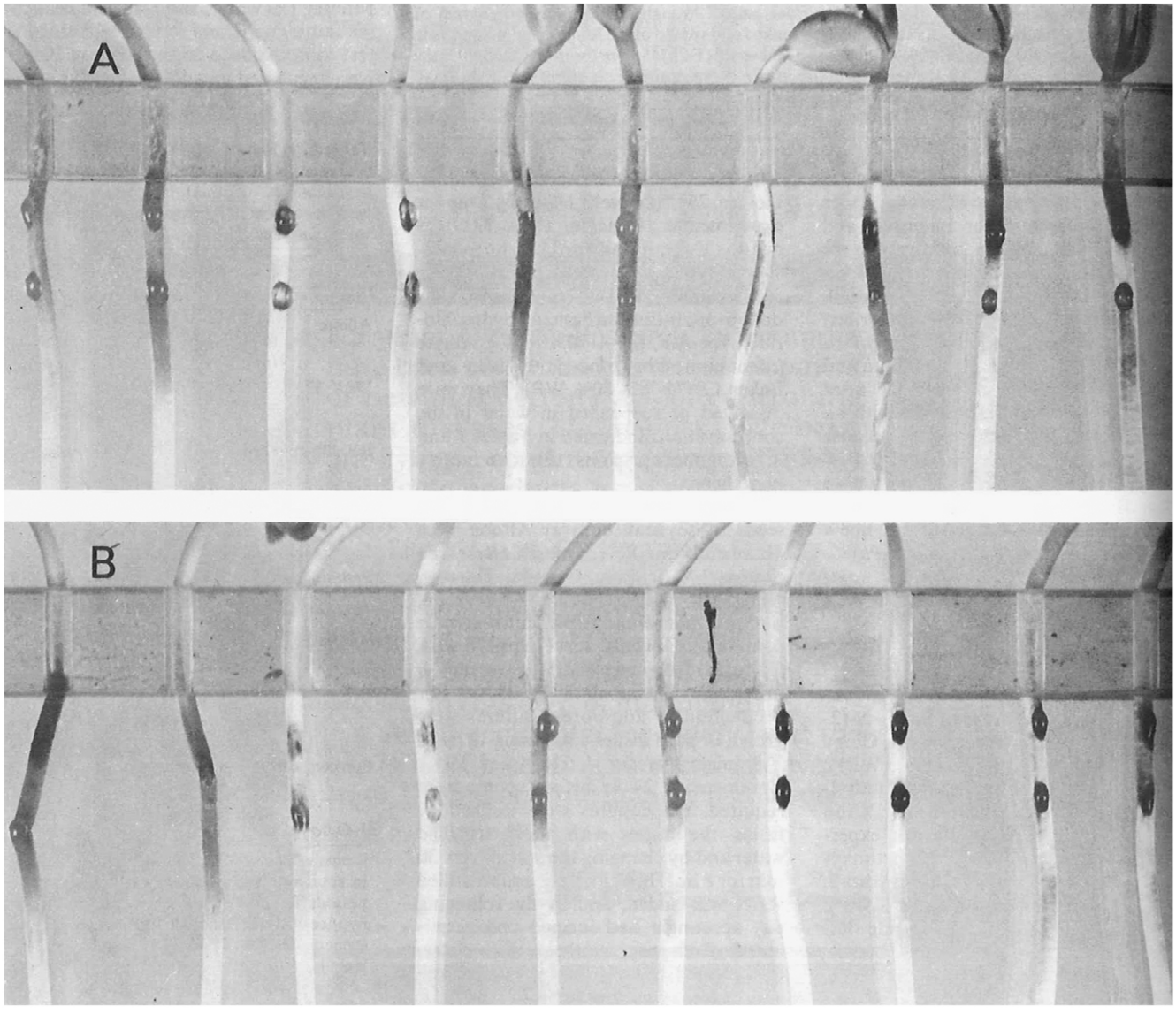

Fig. 1. Assay of systemic fungicides against Phytophthora rot on soybean seedlings. Fungicides were applied to roots, and hypocotyls were inoculated 24 hr later with droplets of zoospore suspensions of *Phytophthora megasperma* var *sojae*. **(A)** Two seedlings per treatment (L to R): control, Ridomil (CGA 48988) 5 μg/ml, Pyroxychlor 50 μg/ml, Prothiocarb 150 μg/ml, DPX 3217 50 μg/ml. **(B)** (L to R): control, Ridomil (CGA 48988) 5 μg/ml, Fongarid (CGA 38140) 5 μg/ml, RE 26745 5 μg/ml, RE 20615 5 μg/ml.

complete control was obtained, lesions with light necrotic flecking of the surface tissue were restricted to the area covered by the inoculum droplet (Fig. 1B). Degrees of partial control were typified by increasing necrosis within the area of the droplet, spreading of browning beyond the edge of the droplet, extensive browning of tissue outside the area of the droplet, and browning with watersoaking (Fig. 1A and B).

In a comparison of several compounds with reported activity against Phycomycete diseases (Table 1, Fig. 1), Ridomil, Fongarid, and Chevron RE 26745 gave complete control at one or both of the concentrations used, but Aliette was ineffective. Ridomil, Fongarid, RE 20615, and RE 26745 are structurally closely related and were compared over the concentration range given in Table 2. Ridomil and RE 26745 were highly effective even at 1 μg/ml, but the other two compounds failed to provide complete control at the lower concentrations.

Initially 10 plants were used per treatment in these experiments, but the response was so uniform that five plants were adequate. The lesions are particularly conspicuous on the white etiolated hypocotyls to which inoculum droplets readily adhere by surface tension. The method is simple, rapid, and reproducible and it may be applicable for screening compounds for systemic activity against Phycomycete diseases in general.

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