Effects of Diphenamid on Rhizoctonia solani, Pythium aphanidermatum, and Damping-off of Tomato

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ABSTRACT

Diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide) reduced the growth of *Rhizoctonia solani* and *Pythium aphanidermatum* on artificial media, delayed the emergence of tomato seedlings from sterile soil without significantly reducing the final stand, and decreased the incidence of pre-emergence damping-off in soil artificially infested with *R. solani* and/or *P. aphanidermatum*. Although postemergence damping-off increased in infested soil with the high rate of diphenamid, final stands were significantly higher than in infested soil not treated with diphenamid.

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The effects of herbicides on final stands of crop plants have been studied with reference to plant injury as well as their possible interactions with disease-causing organisms. Phytotoxic effects of herbicides on crop plants are well documented, but reports of the in-soil effects of herbicides on causal organisms of soil-borne diseases are conflicting. Bollen (2) suggested that at normal rates of use under field conditions, the herbicides developed up until 1961 would have little effect on soil microbial populations. However, Smith et al., (9) reported that soil microorganism response to herbicides varied with some being stimulated in growth while others were inhibited. In the laboratory, Chappell and Miller (4) tested 18 herbicides at usual field rates and determined that two were inhibitory to several pathogenic fungi. Kaufman (7) noted a decrease in the number of *Fusarium* spp. in atrazine-treated soil when compared to simazine-treated soil, and suggested a possible stimulation of Fusarium-antagonistic microorganisms by atrazine. Eshel and Katan (5), using several closely related herbicides, determined that the response of *Fusarium oxysporum* Schl. and *Rhizoctonia solani* Kuehn was dependent upon the individual herbicide. Chandler and Santleman (3) found an interaction between trifluralin, nitralin, and *R. solani* which resulted in increased cotton injury and increased seedling disease. However, they indicated the same interaction was not present with other herbicides tested. Sugar beet stands were also reported to be reduced by an interaction of herbicide and *R. solani* (1).

Diphenamid (*N,N*-dimethyl-2, 2-diphenylacetamide) is a herbicide commonly used in direct-seeded tomatoes. The environmental influence on crop response to diphenamid was studied by Lynch and Sweet (8). They concluded that there would probably be no injury to tomato at normal rates of application. Eshel and Katan (6), using excessive rates of diphenamid on pepper, measured reduced stands in *R. solani*-infested soils. They attributed the increased damping-off to a decrease in soil microorganisms antagonistic to *R. solani.*

The objectives of this study were to determine the effect of diphenamid on growth of *R. solani, P. aphanidermatum,* and its effect on the incidence of pre- and postemergence damping-off of seedlings grown in soil infested with these organisms.

MATERIALS AND METHODS.—The organisms most commonly isolated from field-grown tomato seedlings showing symptoms of damping-off were *R. solani* and *Pythium aphanidermatum* (Edson) Fitzp. Cultures of these organisms were maintained on Difco potato-dextrose agar (PDA).

To determine the effect of diphenamid on the growth of *R. solani* and *P. aphanidermatum,* 20 ml of PDA was dispensed into 15 × 100 mm petri plates. Diphenamid was applied to the solidified agar by passing the plates under a spray tip calibrated to deliver 3.36 and 6.72 kg/hectare

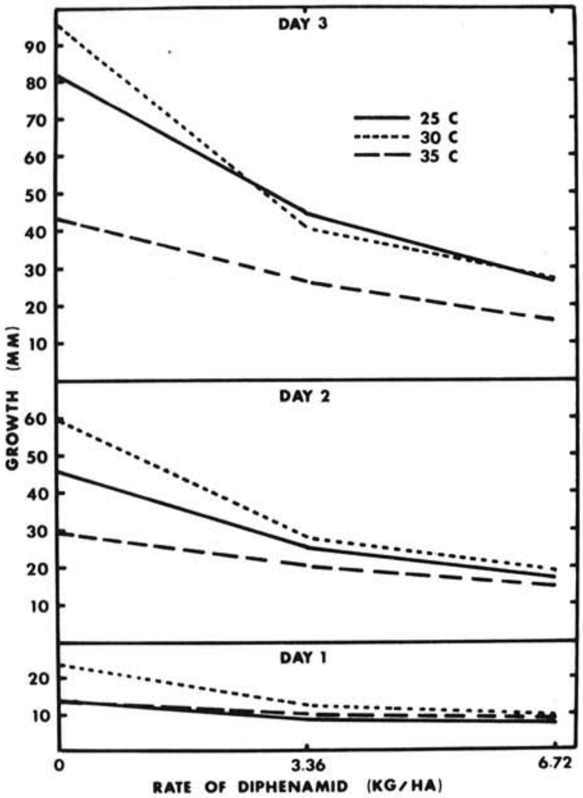

Fig. 1. Growth by day of *Rhizoctonia solani* on diphenamid-amended potato-dextrose agar at three temperatures.

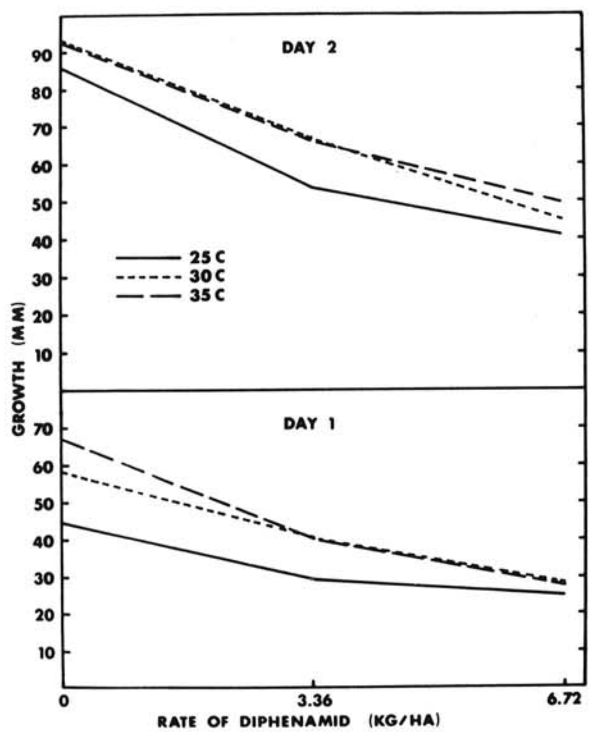

Fig. 2. Growth of *Pythium aphanidermatum* on diphenamid-amended potato-dextrose agar at three temperatures.

(ha). Unsprayed plates served as controls. Plates were covered and autoclaved 15 minutes at 1.0 atm. After resolidification 5-mm diameter agar plugs, cut from 7-day-old cultures of *R. solani* and *P. aphanidermatum*, were placed, growth surface down, in the center of each plate. Plates were incubated at 25, 30, or 35 C. The diameter of each colony was calculated from two measurements, taken at right angles to each other, and averaged. Measurements of colony diameter were taken at 24-hour intervals until the checks had overgrown the plate. Each rate of diphenamid was replicated five times for each organism at each temperature.

The effect of diphenamid on the incidence of damping-off was studied using a mixture of sand and Marietta silty clay loam (1:1, v/v) which had been steamed before being infested with *R. solani* and/or *P. aphanidermatum*. Inoculum was grown on a medium consisting of 500 cm^3 perlite, 50 cm^3 commercial corn meal, and 100 ml of Czapek's solution. Three hundred ml of this medium were placed in 75 × 100 mm storage dishes, autoclaved for 45 minutes at 1.0 atmosphere gauge pressure, and seeded with *R. solani* or *P. aphanidermatum*. Cultures were allowed to grow for 21 and 7 days, respectively. Cottage cheese cups (453-ml = 16-oz.) were filled to a uniform depth of 6 cm with steamed soil, watered, and infested with inoculum. The perlite medium was broken into individual particles and 0.5 g of a given inoculum was spread uniformly over the surface of the soil in five cups. A third series of five cups were infested with a mixture of 0.5 g of each inoculum. The inoculum was covered with 1 cm of steamed soil upon which 25 untreated TAMU Chico III tomato seed were placed. The tomato seed were covered with an additional 1 cm of steamed soil, and the cups were watered from overhead. Approximately 12 hours were allowed for soil moisture to stabilize before diphenamid was applied at rates of 0, 3.36, and 6.72 kg/ha. The 6.72 kg/ha rate is the maximum rate recommended for use in tomato production. Unsprayed, noninfested cups served as controls. Each treatment was replicated five times and the experiment was conducted twice. Emergence and postemergence damping-off of tomato seedlings were recorded daily for 15 days after planting. Final stand was recorded at 15 days and pre-emergence damping-off calculated as the difference between seed planted and total emergence in sterile soil.

RESULTS.—The growth response of *R. solani* to diphenamid at three temperatures is presented in Fig. 1. In general, the growth of *R. solani* was greater at 30 C than at the other temperatures tested. Diphenamid reduced the growth of *R. solani* at all temperatures, with the maximum reduction occurring at 30 C. After the first day, the amount of reduction, at each temperature, was proportional to the rate of diphenamid application with the 3.36 and 6.72 rates reducing the growth by approximately 50% and 75%, respectively. *Pythium aphanidermatum* initially grew best at 35 C but after 1 day was growing equally well at 30 C (Fig. 2). Although diphenamid was inhibitory to *P. aphanidermatum*, its effect on growth was less evident than that observed for *R. solani*. With *P. aphanidermatum* the 6.72 kg/ha of diphenamid was required to achieve a 50% growth reduction.

The addition of diphenamid to steamed soil delayed tomato emergence by 1-2 days depending upon the rate applied. A significantly greater percentage of tomato seedlings had emerged 7 days after planting in untreated soil, than in soil to which diphenamid had been added (Fig. 3). At 8 days, emergence at the 6.72 rate was significantly lower than in untreated soil, but after 9 days no significant differences existed in percent emergence in treated and untreated soils. Although not significant; total emergence at day 15 (in this case, the final stand) was lower in steamed soil to which diphenamid had been added.

In soil artificially infested with *R. solani,* percent emergence of tomato seedlings increased as the rate of diphenamid application increased (Table 1). Postemergence damping-off decreased with the addition of 3.32 kg/ha but began to increase again at the 6.72 rate of diphenamid. Final stands of tomato increased as the rate of diphenamid increased with 3.36 kg/ha being sufficient to create a significant increase in stand.

Seedling emergence in soil infested with *P. aphanidermatum* was very low (Table 2). However, the addition of diphenamid to the soil did result in a significant increase in emergence. Unlike in the results with *R. solani,* however, the addition of diphenamid resulted in an increase in postemergence damping-off.

Emergence, postemergence damping-off, and final stand in soil infested with both organisms was similar to that obtained in soil infested with *P. aphanidermatum* alone.

DISCUSSION.—Damping-off greatly interferes with obtaining a stand of direct-seeded tomatoes. Any agent which delays emergence or causes injury to young seedlings can increase pre- and postemergence damping-off. Diphenamid applied at the rates recommended for tomato production delayed emergence and increased postemergence damping-off at the higher rates but, nevertheless, led to increased final stands in soils infested with *R. solani* and/or *P. aphanidermatum*. These results, while supporting in part the findings of Eshel and Katan (6) on pepper, add important information regarding the effects of diphenamid on pre-emergence damping-off, and offer a more direct explanation for the effects of diphenamid on seedling disease.

The increased final stands obtained were primarily due to a marked decrease in pre-emergence damping-off in

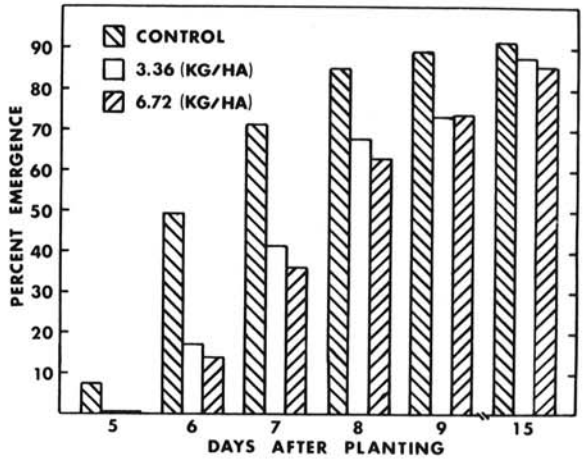

Fig. 3. Emergence of tomato seedlings in diphenamid-treated steamed soil.

TABLE 1. Effect of diphenamid on the incidence of damping-off of tomato in soil infested with *Rhizoctonia solani*

Diphenamid rates (kg/hectare)	Emergence[a] (%)	Pre-emergence damping-off[b] (%)	Postemergence damping-off[c] (%)	Final stand[d] (%)
0	49.3 C[e]	50.9 A	13.2 A	41.2 B
3.32	64.0 BC	36.1 AB	5.5 B	58.6 A
6.72	71.5 AB	28.6 B	6.6 B	65.1 A

[a]Expressed as a percentage of emergence in steamed soil.
[b]Emergence in steamed soil minus emergence in infested soil, expressed as a percentage of emergence in steamed soil.
[c]Seedlings that died expressed as a percentage of those that emerged.
[d]Total emergence minus those that died, expressed as a percentage of the final stand in steamed soil.
[e]Means within a column followed by a common letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 2. Effect of diphenamid on the incidence of damping-off of tomato in soil infested with *Pythium aphanidermatum*

Diphenamid rates (kg/hectare)	Emergence[a] (%)	Pre-emergence damping-off[b] (%)	Postemergence damping-off[c] (%)	Final stand[d] (%)
0	2.2 B[e]	97.8 A	7.2 B	1.3 B
3.36	9.0 A	91.1 A	38.0 A	4.9 A
6.72	11.2 A	90.7 A	31.8 A	5.4 A

[a]Expressed as a percentage of emergence in steamed soil.
[b]Emergence in steamed soil minus emergence in infested soil, expressed as a percentage of emergence in steamed soil.
[c]Seedlings that died expressed as a percentage of those that emerged.
[d]Total emergence minus those that died, expressed as a percentage of the final stand in steamed soil.
[e]Means within a column followed by a common capital letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

diphenamid-treated soils. This decrease apparently resulted from the direct inhibitory effect of diphenamid on *R. solani* and *P. aphanidermatum,* as indicated by growth studies. The increase in postemergence damping-off, attributed by Eshel and Katan (6) to an increase in *R. solani* resulting from the detrimental effect of diphenamid on soil microorganisms antagonistic to *R. solani,* is believed to be due to increased seedling injury. Although diphenamid may adversely affect antagonistic soil microorganisms, increased injury seems to be a more plausible explanation for increased postemergence damping-off, in light of the direct inhibitory effects of diphenamid on *R. solani* and *P. aphanidermatum.* Injured seedlings would be more susceptible to *R. solani* or *P. aphanidermatum* even though their population in the soil might be reduced. The fact that diphenamid is less effective in inhibiting the growth of *P. aphanidermatum,* and that postemergence damping-off was greater in *P. aphanidermatum*-infested soil, adds credence to this explanation of the effects of diphenamid on damping-off and two of its causal organisms.

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