Dowco® 269: A New Systemic Fungicide for Control of Phytophthora parasitica of Tobacco

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ABSTRACT

Dowco 269® [2-chloro-6-methoxy-4-(trichloromethyl)pyridine] exhibits unusual in vivo activity against *Phytophthora parasitica* of tobacco. Long-term control is obtained when the chemical is applied in transplant water. The following evidence indicates Dowco 269 systemically controls *P. parasitica* of tobacco: (i) in vivo effectiveness markedly exceeds in vitro activity; (ii) therapeutic activity can be demonstrated; and (iii) foliar applications are effective in controlling this root disease, supporting basipetal translocation. The versatility of a material effective as an at-plant or post-plant treatment offers new opportunities for control of this serious disease.

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Black shank of tobacco, caused by the fungus *Phytophthora parasitica* (Dastur) var. *nicotianae* (Breda de Haan) Tucker, is a major soil-borne disease of burley and flue-cured tobacco. Certain cultural practices are employed which predispose the plants to increased disease potential. These include liming to a pH of ~ 6.0 (17, 18, 19, 20, 34), and the use of high levels of nitrogen (1). Interactions with nematodes have also been shown to increase the incidence of black shank (14, 15, 21, 22, 24, 25, 26, 27, 28, 30). The use of moderately resistant varieties has been of major benefit in minimizing the destructive potential of this disease (4, 11, 30). Rotation practices (6, 8, 9), and chemical soil treatments (23), also have been shown to be of significant value.

Breakdown in resistance to black shank has been reported on certain cultivars (2, 3, 12, 29, 33). Depending upon relative infestation levels, there is need for a control program based upon a variety of cultural practices (23, 25, 32). North Carolina's "R-6-P" program (7) emphasizes the need for using resistant varieties in combination with crop rotation and chemical soil treatments.

Chemical treatments for black shank control have depended principally upon the use of pre-plant soil fumigants (23). Todd and Nusbaum (31) report the use of captan plus nematicide in transplant water as a combination treatment for black shank and nematode control. While showing reduced incidence of midseason disease expression, yields did not differ significantly from untreated checks.

Dowco 269 [2-chloro-6-methoxy-4-(trichloromethyl)pyridine] (The Dow Chemical Company, Midland, Michigan) prepared by Johnston and Tomita (16), exhibited excellent activity for control of *P. parasitica* of tobacco when applied in transplant water or as a foliar spray. The objective of this study was to characterize the unusual activity of this new experimental fungicide in laboratory and greenhouse investigations.

MATERIALS AND METHODS.—*Host plants.*—Tobacco plants, cultivar 402 (Coker Seed Company, Hartsville, S.C.), were used for all studies. Stock plants were cultured under greenhouse conditions in vermiculite plus Hoagland's basal medium (13). Four- to 5-week-old plants in the 3- to 4-leaf stage were used for the drench treatment studies summarized in Tables 1-3. Seven- to 8-week-old plants in the 4- to 5-leaf stage were used for the foliar spray (Table 4) or long-term control studies employing soil drench or combination soil drench plus foliar spray schedules (Fig. 1,2).

Inoculum.—*P. parasitica* virulent strain #1156 (10) was used for all experiments. Stock cultures were maintained on potato-dextrose agar (PDA) slants and inoculum for soil tests was grown in 0.23-liter bottles of wheat (per bottle: 24 g wheat, 40 cc distilled water, autoclaved 30 minutes). After 2 weeks of growth at 24 C, the contents from three bottles were transferred to a blender and homogenized for 20 seconds with 200 cc of distilled water. This suspension was incorporated into 16 kg of fumigated soil. For uniformity of infection, the artificially inoculated soil was spread to a depth of 10.2 cm in greenhouse flats and planted with 4- to 5-week-old tobacco seedlings. After two or more plantings, the

TABLE 1. LD_{100} of Dowco 269 and captan against *Phytophthora parasitica* var. *nicotianae* in in vitro and in vivo tests

	LD_{100}	μg/ml active
Treatment	In vitro[a]	In vivo[b]
Dowco 269	<16 > 4	< 1.6 > 0.4
Captan	< 4	<25.0 > 6.3

[a]Basic activity of chemicals in agar against colony growth of test organism measured after 1 week of incubation.

[b]Four- to 5-week-old tobacco plants were transplanted into infested soil, drenched with solution of test chemical and incubated 2 weeks before being graded.

infested soil was blended, then used for experiments or stored at 21 C until needed.

Soil.—Experiments were conducted with a pasteurized sandy loam soil with the following analysis: sand, 94.3%; silt, 1.3%; clay, 4.4%; organic matter, 0.08%; pH, 6.9. Drums (113.6 liters) were filled two-thirds full of soil, sealed, and treated with a 0.454-kg (1.0-pound) can of Dowfume® MC-2 (98% methyl bromide, 2% chloropicrin) (The Dow Chemical Company, Midland, Michigan) per drum. After 3 days at 22 C, containers were opened and aerated for 5 days before use.

Compounds tested.—In vitro or soil drench experiments were conducted with the following emulsifiable formulations of Dowco 269 (values represent amount of active ingredient on a weight basis): S-1805–95% (Tables 1-3); M-3852–6.7% (Fig. 1); and M-3858–7.2% (Fig. 2). Foliar spray treatments (Table 4 and Fig. 2) employed M-3860–12.4%. Standards included wettable powders of captan–*cis*-N-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide (50%) and Terrazole® 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole (30%) (Olin, Little Rock, Arkansas).

Experimental procedure.—In the in vitro test (Table 1), stock concentrates of Dowco 269 and captan were prepared in sterile distilled water. Appropriate aliquots were added to warm melted PDA to arrive at desired concentrations of test chemical in the agar. Fifteen-ml portions were added to each petri dish and allowed to cool. Colony growth from stock slants of *P. parasitica* was transferred onto the agar surface. Because Dowco 269 is moderately volatile (Hamaker, *unpublished*), the dishes were sealed with heavy rubber bands to minimize loss by diffusion. Four replicates were used for each concentration. The test was graded after 1 week of incubation at 21 C. The criterion for control was absence of fungal growth onto the agar surface from sites of inoculation. Results are expressed as concentrations of test chemical which provided complete growth inhibition (LD_{100}, μg/ml).

All in vivo tests were conducted in the greenhouse at 21-23 C. Chemical solutions were prepared in tap water. Plants were transplanted into 5.1-cm diameter plastic pots filled to 1.27 cm from the top with infested soil and drenched with 40 cc of test solution (Tables 1-3). Controls were transplanted in inoculated and uninoculated soil and drenched with 40 cc of tap water only. Treatments were replicated four times; the therapeutic test (Table 3) was replicated 10 times. All experiments were repeated twice; data presented are averages of all experiments.

TABLE 2. Residual effectiveness of Dowco 269, captan and Terrazole for control of *Phytophthora parasitica* of tobacco[a]

Treatment	% control (μg/ml active)					
	100	25	6.3	1.6	0.4	0
4 days[b]						
Dowco 269	100	100	100	100	50	...
Captan	100	100	100	50	0	...
Terrazole	100	100	100	50	0	...
Control, inoculated	...	...	...	...	...	0
Control, uninoculated	...	...	...	...	...	100
21 days[b]						
Dowco 269	100	100	100	0	0	...
Captan	100	0	0	0	0	...
Terrazole	50	0	0	0	0	...
Control, inoculated	...	...	...	...	...	0
Control, uninoculated	...	...	...	...	...	100

[a]Four- to 5-week-old tobacco transplants were placed in infested soil and drenched to field capacity (40 cc) with chemical solutions at the indicated concentrations.

[b]Exposure period from test initiation until treatments were graded.

For the foliar spray test, plants were transplanted into 10.2-cm diameter plastic pots filled 2.54 cm from the top with infested soil. After watering, the pots were filled to the top with dry vermiculite to prevent foliar spray treatments from contacting the soil surface or channeling down the stem to the roots. Solutions containing 2,400, 1,200, 600, and 300 μg/ml active of Dowco 269 or captan were prepared and sprayed onto the foliage to run-off. After leaves were dry, the vermiculite was removed. Subsequent waterings were made without wetting the foliage. Each treatment was replicated five times and the test was graded after 2 weeks. Results summarized in Table 4 are the average of two experiments.

For long-term tests (Fig. 1, 2), 15.24-cm diameter plastic pots were filled to within 2.54 cm of the top with infested soil. Test solutions containing Dowco 269, captan, or water were poured onto the roots of tobacco plants as they were transplanted into infested soil. Four replicates were used in each trial. Chemical concentrations and total liquid volume used in transplant water were calculated as follows: An average of 1,870

TABLE 3. Therapeutic effectiveness of Dowco 269 and captan for control of *Phytophthora parasitica* of tobacco[a]

Treatment	Concentration (μg/ml active)	% Control in plants incubated (days) before treatment				
		0	1	2	3	4
Dowco 269	40	100	100	100	80	20
Dowco 269	10	100	80	100	100	20
Captan	40	100	20	0	0	0
Control, Transplanted	...	100	100	20	0	0
Control, Inoculated	...	0	0	0	0	0
Control, Uninoculated		100	100	100	100	100

[a]Four- to 5-week-old tobacco transplants were incubated in infested soil at indicated days exposure prior to drenching solutions of test chemical. Transplanted controls were placed in infested soil then removed after the indicated number of days. Following washing in running tap water, they were transplanted into sterile soil. All treatments were graded 21 days after the initiation of the experiment.

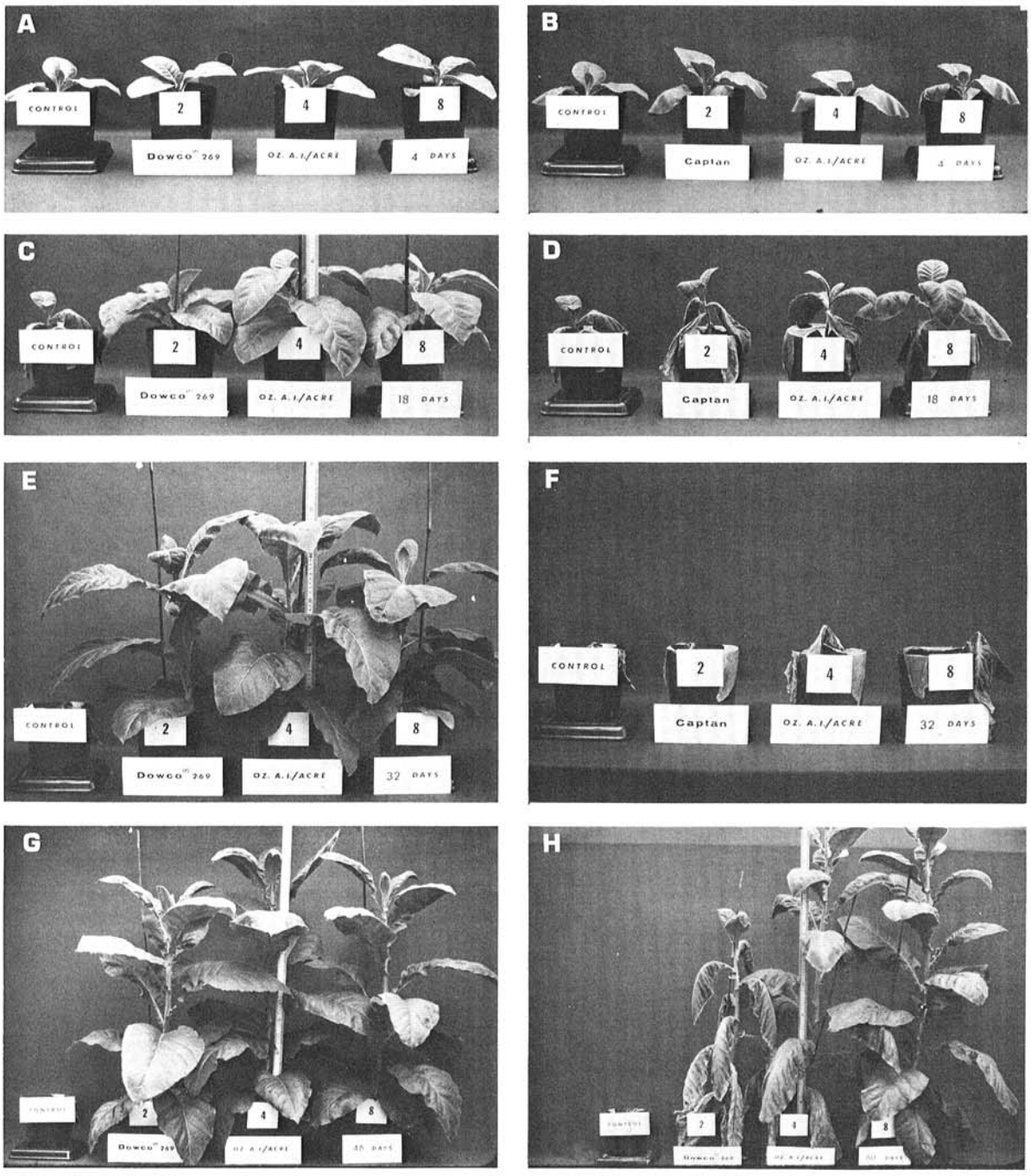
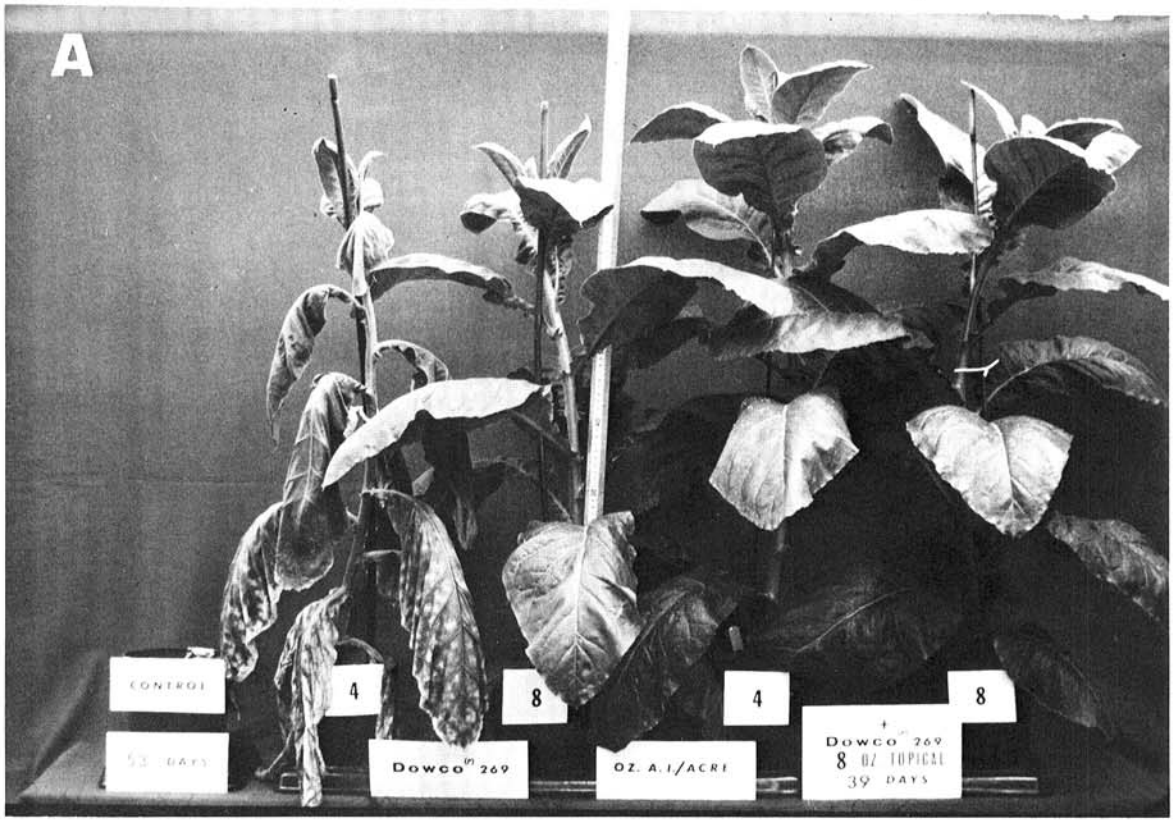


Fig. 1-(A to H). Comparative performance of Dowco 269 and captan for control of *Phytophthora parasitica* of tobacco. Compounds were applied in the transplant water to tobacco plants placed in infested soil. Each illustration shows inoculated control and chemical treatments at 0.14, 0.28, and 0.56 kg/ha of active ingredient (2, 4, and 8 oz per acre). Results with time (days) from treating are as follows: **A)** Dowco 269 and **B)** captan after 4 days; **C)** Dowco 269 and **D)** captan after 18 days; **E)** Dowco 269 and **F)** captan after 32 days; **G)** Dowco 269 after 46 days; and **H)** Dowco 269 after 60 days.

Fig. 2-(A, B). Comparative performance of Dowco 269 transplant and transplant-plus-topical-spray treatments for control of *Phytophthora parasitica* of tobacco. Dowco 269 was applied in transplant water to tobacco plants at 0.28 and 0.56 kg/ha of active ingredient (4 and 8 oz per acre). One set also received a topical (foliar) application of 600 μg/ml of active ingredient (8 oz per 100 gallons) sprayed to runoff, 39 days after the transplant treatment. Results with time (days) are as follows: **A)** inoculated control, Dowco 269 transplant and transplant-plus-topical treatments after 53 days (14 days after applying the topical spray); and **B)** inoculated control, Dowco 269 transplant and transplant-plus-topical treatments after 60 days (21 days after applying the topical spray).


A
CONTROL
53 DAYS
4
8
DOWCO 269
4
OZ. A.I./ACRE
8
+
DOWCO 269
8 OZ TOPICAL
39 DAYS

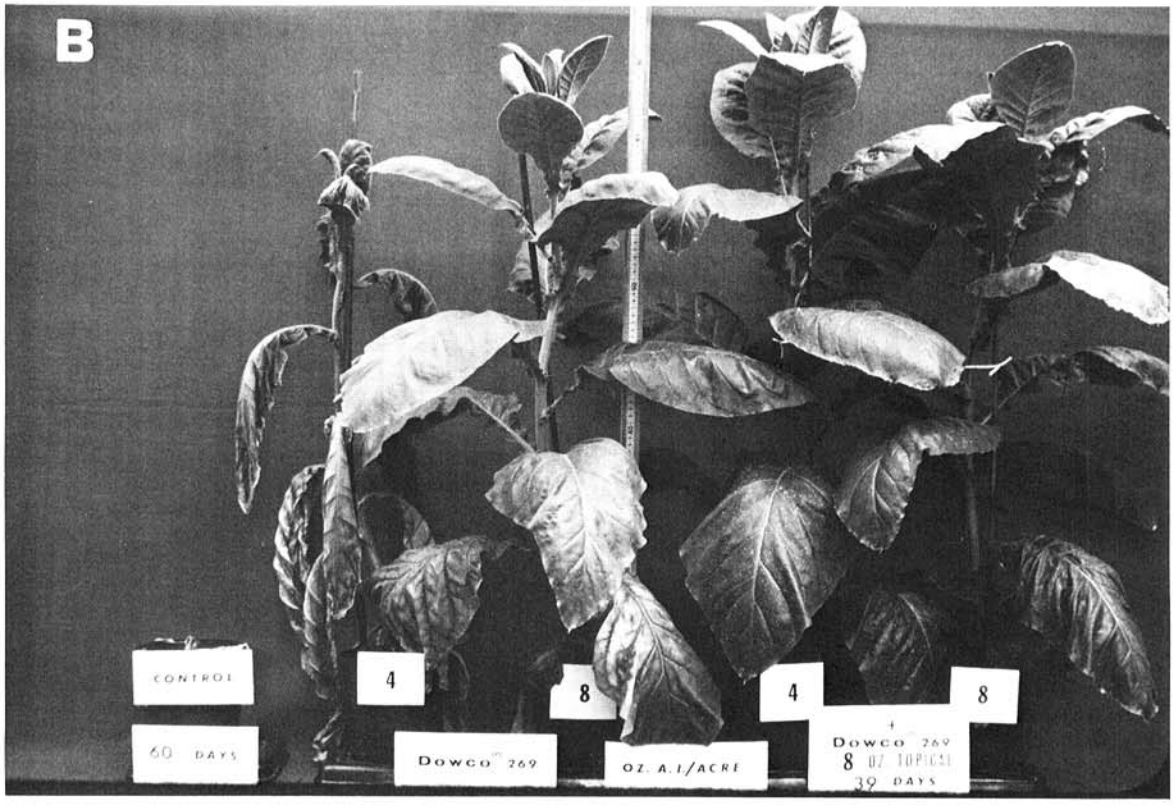

B
CONTROL
60 DAYS
4
8
DOWCO 269
4
OZ. A.I./ACRE
8
+
DOWCO 269
8 OZ TOPICAL
39 DAYS

TABLE 4. Foliar systemic activity of Dowco 269 and captan for control of *Phytophthora parasitica* of tobacco

Treatment	% Control at indicated concentration of active (μg/ml)[a]				
	2,400	1,200	600	300	0
Dowco 269	100	100	90	50	...
Captan	0	0	0	0	...
Control	...	...	...	...	0

[a]Tobacco plants in the 4- to 5-leaf stage were transplanted in infested soil. The soil surface was shielded to prevent availability of chemical to roots from the foliar application. Plants were sprayed to runoff with the above indicated concentrations of active and graded 2 weeks later for control.

liters/ha of transplant water is used in planting flue-cured tobacco at a planting rate of 17,297 plants per hectare, averaging 108 cc of solution per plant. Concentrations were calculated to deliver 0.14, 0.28, and 0.56 kg/ha (2, 4, and 8 ounces per acre) of active ingredient in 1,870 liters/ha of water by using 108 cc of solution per plant containing 75, 150, and 300 μg/ml, respectively.

In another long-term experiment, a topical (foliar) application of Dowco 269 was sprayed onto plants 39 days after treatment with Dowco 269 at 0.28 and 0.56 kg/ha of active ingredient in the transplant water. The concentration of active in the topical spray solution was 600 μg/ml, sprayed to run-off.

RESULTS.—LD_{100} values for Dowco 269 and captan against *P. parasitica* were determined in in vitro agar and in vivo soil drench tests (Table 1). The in vivo activity of Dowco 269 exceeded anticipated performance as established by its basic activity and performance compared to captan.

The residual effectiveness of Dowco 269, captan and Terrazole against *P. parasitica* was compared in a drench experiment (Table 2). After 21 days. Dowco 269 was approximately 16 times more effective.

Therapeutic effectiveness of Dowco 269 is shown in Table 3. It was effective as a post-plant treatment through 3 days. At 4 days, plants were showing disease symptoms before chemical treatments were made. Captan was effective as an at-plant treatment but showed no therapeutic activity. As transplanted controls became irreversibly infected between 1 and 2 days, Dowco 269 appears to possess therapeutic effectiveness.

The systemic activity of Dowco 269 by foliar application is presented in Table 4. Dowco 269 was effective in controlling *P. parasitica* at 2,400, 1,200, and 600 μg/ml after 2 weeks, indicating an active principle moved basipetally into the roots.

Long-term control of *P. parasitica* by Dowco 269 and captan as transplant water treatments is compared in Fig. 1. Under severe disease conditions, plants treated with Dowco 269 (Fig. 1-A, C, E, G) retained excellent control through 46 days at all dosages tested (0.14, 0.28 and 0.56 kg/ha); higher rates (Fig. 1-H) provided some control as plants approached maturity (60 days). Plants treated with captan (Fig. 1-B, D, F) never grew vigorously and were dead at all treatment rates by 32 days.

Effectiveness of Dowco 269 for control of *P. parasitica* by a combination schedule was determined by applying chemical in the transplant water followed by a foliar spray 39 days later (Fig. 2). Results are summarized 53 and 60 days after transplanting (14 and 21 days after the topical application). Excellent control was achieved by the combination schedule.

DISCUSSION.—Dowco 269 is the first compound reported capable of controlling fungal infections of roots via foliar applications. Byrde (5) called attention to the complex interaction involved when systemic control depends upon the presence of host factors as well as the disease organism. As Dowco 269 appears capable of basipetal translocation as well as therapeutic effectiveness, the complexity of host membrane transport, translocation and bio-accumulation must be considered in relating ultimate fungicidal performance.

Dowco 269 is a moderately volatile compound, highly soluble in organic solvents with a low water solubility (11 μg/ml). Its vapor pressure is 6.5×10^{-3} mm Hg at 25 C (Hamaker, *unpublished*). It is unusual that a compound of moderate volatility would find application versatility as a foliar spray; possibly the highly lipophilic nature of this compound promotes rapid absorption into plant foliage.

Results reported suggest a possible mode of action. Dowco 269 appears to undergo rapid biodegradation in soil (Kurihara and Laskowski, *unpublished*) but is appreciably stable in aqueous solutions (Meikle, *unpublished*). Long-term disease control may be obtained by bioaccumulation in the root system where the parent compound or metabolites persist for extended time periods. This is consistent with known performance to date and serves to qualify the unexpected in vivo performance of a compound which exhibits only moderate in vitro activity.

The overall distribution of Dowco 269 in the plant is as yet unknown. *P. parasitica* can enter lower leaves which come in contact with infested soil. Root drenches of Dowco 269 arrest development of the disease organism before it can reach the petiole via leaf infection; thus, at least portions of the lower leaves of plants contain an active principle. On a practical basis, such a mechanism appears necessary; Flowers and Hendrix (11) showed that the numbers of propagules of *P. parasitica* in field soils are highest in the upper 7.62 cm of the soil profile. Captan did not provide practical control as a transplant water treatment in field trials (31), indicating its unsuitability as a contact soil fungicide.

As a tobacco transplant treatment, Dowco 269 appears capable of providing near season-long control of *P. parasitica*. In combination with a topical spray treatment, longer protection is realized. Field trials are in progress to determine disease control potential.

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