# A New Systemic Fungicide Against the Downy Mildew Disease of Cucumbers

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## ABSTRACT

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Propyl-(3-[dimethylamino]-propyl)carbamate-monohydrochloride (SN66752, propamocarb, a new compound by Schering AG) and prothiocarb (SN41703) exhibited systemic antifungal activity against *Pseudoperonospora cubensis*, the cause of downy mildew disease of cucumbers. Aqueous solutions of the chemicals applied as soil drenches to potted cucumbers protected them from the mildew for a period of 25 days. The fungicides were highly toxic to sporangia of the pathogen, induced an alteration in symptoms of the disease, and inhibited sporulation of the fungus on developed lesions.

*Additional key words: Cucumis sativus.*

Downy mildew of cucumbers caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rost. is a destructive disease of world-wide distribution (5). Present control measures include the use of resistant cultivars (1) and of fungicides, although both methods are only partially successful. Recently fungicides with systemic antifungal activity against plant-pathogenic species of the *Peronosporales* have been developed; prothiocarb was found to control downy mildew of lettuce (3,4) and the Pythium blight of beans (6). In the present study, results on the systemic antifungal activity of two fungicides, prothiocarb and propamocarb (SN66752, a new derivative of prothiocarb), against downy mildew of cucumbers are reported.

## MATERIALS AND METHODS

**Plants, inoculation method, and assessment of disease development.** All experiments were done with the susceptible cucumber (*Cucumis sativus* L. 'Bet-Alfa'). Plants from cotyledonary stage to the seven-leaf stage were used in different experiments. Plants were grown in 250-ml (10 cm diameter) plastic pots (10 plants/pot) to their cotyledonary stage; plants were grown in similar pots but with two plants per pot to the two- to three-leaf stage; and to the seven- to twelve-leaf stage in 10-L (25 cm diamter) containers, two plants in each. A mixture of garden soil: vermiculite: compost (1:1:1, v/v) was used in all experiments. A 250-ml and a 10-L pot contained ~ 210 g and ~ 8.5 kg air-dried soil mixture, respectively. Plants were grown in the greenhouse at 20–34 C.

Plants were inoculated at the cotyledonary stage by placing one 6-mm diameter Whatman No. 1 filter paper disk, saturated with about 0.1 ml sporangial suspension of *P. cubensis* (about $10^5$ sporangia/ml), on the lower leaf surface. Plants with true leaves were inoculated with the aid of a Schein inoculator (7) on a circular 4.5-$cm^2$ target on the lower leaf surface (one target/leaf) with 100-300 sporangia/$cm^2$. Inoculated plants were kept in a dew chamber at 20 C in the dark for 20 hr, and then transferred to a 20 C cabinet (50–60% RH) illuminated for 12 hr/day with cool-white fluorescent light of about 150 $\mu$Eins/$m^2$/sec for 7 days for symptom production. Disease index and infection type were assessed according to the method described recently (2). Two features were recorded for each leaf: (i) the area of target infected (the largest observed was about 700 $mm^2$; it served as 100% of target area infected) and (ii) the color of the infected tissue. The color index used was as follows: A, greenish (very weak chlorosis)=1; B, yellow (intense chlorosis)=3; and C, brown (necrosis)=5.

Infection type was characterized, therefore, by the color of the infected tissue (A, B, or C) and the area of target infected. By

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multiplying the percentage of the target infected area by the color index a visual disease index of 0–5 was obtained.

Frequently necrotic tissue was observed in a chlorotic infected area. In such cases the total infected area and the necrotic area included were rated separately and the two values were combined for final disease index determination. Thus, an infection type of B6C4 meant about 700 $mm^2$ of chlorotically infected area of which about 300 $mm^2$ became necrotic. This type of infection was rated as 3.3+(2.1−1.3)=4.1.

To measure sporangial yield of the pathogen on lesions, plants at 8 days after inoculation were kept in a saturated atmosphere in the dark for 24 hr, and sporangia from individual leaves were brushed into a fixative solution and counted with a cytometer (four counts/leaf). In some experiments, sporulation intensity was assessed visually by estimating the area of the lower leaf surface covered with sporangia.

**Fungicides and fungicide application.** Two chemicals were investigated in this study: (i) S-ethyl-N-(3-dimethyl aminopropyl)-thio carbamate hydrochloride as a 70% aqueous solution (prothiocarb, SN41703, Previcur® manufactured by Schering-AG, Mueller Strasse 170-178, 1 Berlin 65, West Germany); and (ii) propyl-[3-dimethylamino-propyl]carbamate-monohydrochloride as a 70% aqueous solution (propamocarb, SN66752, also by Schering-AG). In most experiments, fungicides at a concentration

TABLE 1. The effect of prothiocarb and propamocarb on the development of downy mildew disease of pot-grown cucumber plants

| Volume of fungicide solution[a] (ml/pot) | Prothiocarb | | | Propamocarb | | |
|---|---|---|---|---|---|---|
| | Disease index[b] | Infection type[c] | Sporulation index[d] | Disease index[b] | Infection type[c] | Sporulation index[d] |
| 0 | 4.1 | B6C4 | ++++ | 4.1 | B6C4 | ++++ |
| 5 | 1.3 | B3→B4 | + | 1.8 | B4→B5 | ++ |
| 10 | 0.7 | A4→B4 | − | 0.8 | B3 | − |
| 15 | 0.8 | B3 | − | 0.4 | A3→B3 | − |
| 20 | 0.5 | A3→B3 | − | 0.4 | A4 | − |

[a] Various volumes of the fungicides, both at a concentration of 0.6%, were applied to potted plants (two two-leaf plants in a 250-ml pot) 2 days before inoculation. Disease symptoms were recorded 7 days after inoculation.

[b] A visual 0–5 index: 0=no symptoms, 5=inoculated target fully necrotized. (For details, see Materials and Methods section and Y. Cohen [1977] Can. J. Bot. 55:1478-1487).

[c] Infection type coding: A=greenish, B=yellow, C=brown lesions; the numbers 1 to 6 indicate area index of target infected (1=50$mm^2$, and 6=about 700 $mm^2$ area of target infected). Figures on left side of arrow show the lowest infection type, and those on the right side the highest infection type. B6C4 = mixed infection type.

[d] The symbols − = no sporangia were observed microscopically; + → ++++ = small amount (1–5 × $10^4$ sporangia per target) to abundant amount (3–5 × $10^5$ sporangia per target) of sporangia covering the lower leaf surface.

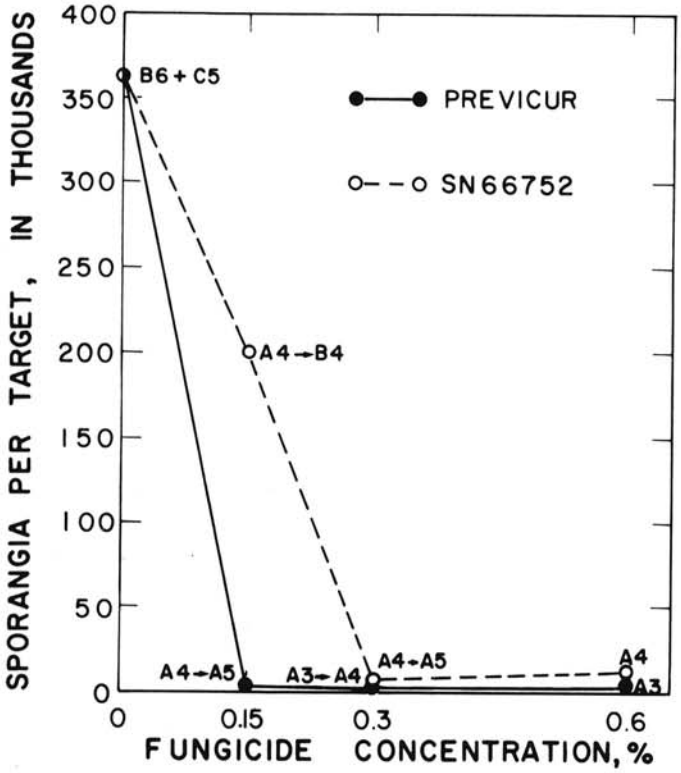


**Fig. 1.** The effect of prothiocarb and propamocarb on symptom production of downy mildew and on the sporulation potential of *Pseudoperonospora cubensis* on cucumber leaves. Two-leaf plants (eight per treatment, two in a 250-ml pot) were watered with 10 ml of fungicide solution per pot two days prior to inoculation and with another 10 ml/pot at time of inoculation. Disease symptoms were recorded at 7 days after inoculation. Sporulation took place in a dew chamber at 20 C in the dark (24 hr). Figures on curves indicate the range of infection types observed on lesions (see Material and Methods section and Y. Cohen (1977) Can. J. Bot. 55:1478-1487.

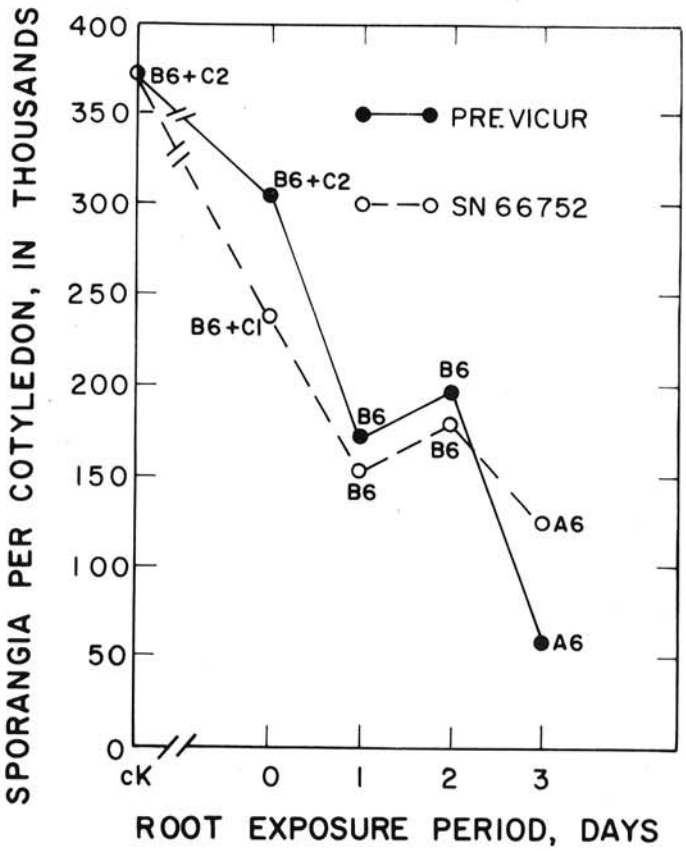


**Fig. 2.** The relationships between the exposure period to prothiocarb and propamocarb of cucumber roots and the control of *Pseudoperonspora cubensis*. Plants were watered at the cotyledonary stage with 0.3% solution of a fungicide (10 ml per pot, 10 plants per 250-ml pot) and inoculated at various intervals afterwards. Disease symptoms and sporulating potential were recorded as in Fig. 1.

of 0.075–0.6% (0.525–4.2 g active ingredient per liter) were applied to potted plants: 5–20 ml per 250-ml pot or 500 ml per 10-L container. Usually 2–3 days elapsed between fungicide application and inoculation.

All experiments were repeated at least twice with 8–40 plants per treatment.

## RESULTS

Both prothiocarb and propamocarb gave good control of the disease when used as soil drenches. Prothiocarb, at a concentration of 0.15%, and propamocarb at a concentration of 0.3%, each applied to two-leaf plants in two 10-ml doses, reduced symptom production and suppressed formation of sporangia (Fig. 1). Infection type of the lesions on treated plants did not exceed the B4 chlorotic stage; these lesions did not become necrotic. With higher fungicide concentrations, lesions reached only the A5 infection type. These lesions consisted of many small greenish spots which resembled the reaction of resistant cultivars (1).

Disease control was greatly dependent upon the length of the exposure period before inoculation. In cotyledonary plants, extending the exposure period from 0 to 3 days (at 20 C) resulted in a marked supression of disease development and in a sharp decrease in the sporangial yield of the fungus on lesions (Fig. 2). Similar results were obtained with two-leaf plants. However, both fungicides had no effect on sporangial formation of *P. cubensis* on normally developed chlorotic B6-lesions, if applied at the beginning of the sporulation period (on the 8th day after inoculation).

Soil drench at 5 ml per pot of a 0.6% solution of either fungicide resulted in a marked decrease in disease index (as a result of the change in infection type), whereas 10 ml/pot were needed to completely nullify spore formation (Table 1). Further increase of the fungicide solution volume to 15–20 ml/pot resulted in the production of greenish lesions of the A type.

A similar experiment was conducted with propamocarb at four different concentrations. Complete protection of the plants from the disease was achieved with the fungicide at low volume and high concentration or vice versa (Table 2).

The antifungal activity of a single soil drench with prothiocarb or propamocarb lasted more than 16 days. When applied to germinating cucumber seeds, both fungicides protected the developing plants up to their three-leaf stage (Table 3). A short exposure period of 2 days was much less effective compared with a 9- or 16-day exposure period.

With cucumbers grown in 10-L containers it was possible to

TABLE 2. Infection type of downy mildew symptoms on cucumber plants treated with various amounts of propamocarb

| Volume of fungicide solution[a] (ml/pot) | Infection type[b] obtained with fungicide concentration of: | | | |
|---|---|---|---|---|
| | 0.15% | 0.3% | 0.6% | 1.2% |
| 5 | B3→B4 | B3→B4 | B2→B3 | A1→B1 |
| 10 | B3→B4 | B3→B4 | 0 | 0[c] |
| 15 | 0→B1 | 0→B1 | 0 | 0[c] |
| 20 | 0→B1 | 0 | 0 | 0[c] |

[a] Plants (two per 250-ml pot) were watered with fungicide solutions at time of germination, and inoculated 18 days later at the three-leaf stage. Untreated plants developed lesions of a B4 infection type (data not shown).
[b] Disease index: See Table 1, footnote b, for details.
[c] Plants showed phytotoxic symptoms: periphery of lower leaf lamina became necrotic.

TABLE 3. Systemic antifungal activity of prothiocarb and propamocarb against *Pseudoperonospora cubensis* on leaves of cucumber plants[a]

| Plant growth stage at fungicide application | Interval between fungicide application and inoculation (days) | Fungicide concentration (%) | Prothiocarb | | Propamocarb | |
|---|---|---|---|---|---|---|
| | | | Infection type[b] | Sporangia per target (× 1,000) | Infection type[b] | Sporangia per target (× 1,000) |
| Germination | 16 | 0.3 | A4→A6 | 8 | A4→A6 | 0 |
| | | 0.6 | A4 | 1 | A4→A6 | 2 |
| First true leaf | 9 | 0.3 | A4→B4 | 4 | A4→B4 | 0 |
| | | 0.6 | A4→B4 | 1 | A6→B6 | 1 |
| Second true leaf | 2 | 0.3 | B4→B6 | 74 | B4→B5C2 | 57 |
| | | 0.6 | B6 | 14 | B4→C6 | 24 |

[a] Plants (16 per treatment, two per 250-ml pot) were treated through the soil with 10 ml of fungicide solutions per pot at either stage of germination, the first-leaf stage or at the two-leaf stage. All were inoculated at the three-leaf stage. Note: Control untreated plants exhibited C5 lesions with 122,000 sporangia per target.
[b] See Table 1 for details.

TABLE 4. The control of *Pseudoperonospora cubensis* on adult cucumber plants[a] by propamocarb

| Fungicide concentration[b] (%) | Infection type[d] | | Group III[a,f] | |
|---|---|---|---|---|
| | Group I[a,c] | Group II[a,d] | Disease index[e] | Sporangia per target × $10^{-3}$ |
| 0 | B6C5 | C6 | 2.8 | 346 |
| 0.075 | B4 | B3 | 0.7 | 55 |
| 0.15 | A3 | B2→B3 | 0.3 | 5 |
| 0.3 | 0 | 0→A1 | 0.1 | 0 |
| 0.6 | 0[g] | 0[g] | 0.0[g] | 0 |

[a] Plants in Group I were treated with the fungicide at the three-leaf stage and inoculated at the seven-leaf stage; those in Group II were treated at the seven-leaf stage and inoculated at the 12-leaf stage; those in Group III were treated at the first-leaf stage and inoculated at the eight-leaf stage.
[b] Fungicide solutions (500 ml) were applied (as a single soil drench) to two plants growing in a 10-L container.
[c] The interval period between fungicide application and inoculation was 9 days. Four plants/treatment.
[d] Interval period—8 days. Four plants per treatment.
[e] See Table 1 for details.
[f] Interval period—25 days. Eight plants per treatment.
[g] Phytoxicity symptoms on lower leaves.

TABLE 5. The effect of foliar spray with propamocarb on the development of downy mildew on cucumber cotyledons[a]

| Fungicide concentration (%) | Infection type[b] | Sporulation index |
|---|---|---|
| 0 | B6C2 | ++++ |
| 0.15 | A6→B6 | ++ |
| 0.3 | A6→B6 | ++ |
| 0.6 | A6 | ± |
| 1.2 | 0→A6 | traces |

[a]Plants were inoculated 2 days after receiving foliar treatment.
[b]See Table 1 for details.

demonstrate that propamocarb could protect the plants for a growth period of up to seven leaves (Table 4).

Propamocarb was only partially effective against *P. cubensis* when sprayed (0.15–0.6%) to run off onto plants at the cotyledonary stage (Table 5).

## DISCUSSION

Frequent applications of fungicides, sometimes as many as 10 during the life of a crop, usually are required to protect cucumbers from downy mildew. Prothiocarb and propamocarb had a very good systemic antifungal activity against *Pseudoperonospora cubensis* on cucumbers. These compounds were more effective in controlling the disease when applied as soil drenches than when applied directly to the leaves as foliar sprays. Their antifungal activity increased when the interval between application and inoculation was lengthened. Both chemicals, at low concentration, reduced sporulation of the pathogen but did not prevent symptom appearance. At higher concentrations, they reduced symptoms to, at most, small greenish spots or even inhibited lesion production. A single treatment with either of these compounds, in the appropriate combination of volume × concentration, could protect the growing leaves for a period of up to 25 days. Longer periods were not tested.

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