Interactions Between and Among Grain Sorghum, Sorghum Downy Mildew, and the Seed Herbicide Antidotes Concep II, Concep, and Screen

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ABSTRACT

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The herbicide antidote Concep II significantly increased the incidence of sorghum downy mildew in susceptible grain sorghum hybrids inoculated with oospores of *Peronosclerospora sorghi*, pathotypes 1 and 3. Other antidotes, Concep and Screen, had no effect, or Screen significantly decreased disease incidence. Concep and Concep II retarded growth of young sorghum seedlings when used at >0.5 g a.i./kg seed. Higher rates further inhibited plant growth and increased disease incidence. The length of time that Concep II inhibited seedling growth increased with dosage. Scanning electron microscopy revealed that Concep II affected sorghum root growth and development. A major morphological effect of Concep II appears to be that it delays natural maturation of root tissue, which may prolong occurrence of pathogen-susceptible juvenile tissue. Concep II had no bactericidal effect in vitro but had a fungicidal effect on microorganisms naturally associated with oospores of *P. sorghi*. There was no evidence that Concep II enhanced oospore germination. Seed treatment with metalaxyl at 0.5 g a.i./kg seed completely controlled the iatrogenic effect of Concep II. The fungicidal effectiveness of metalaxyl in controlling sorghum downy mildew was reduced when rates of Concep II higher than 1.25 g a.i./kg seed were used.

Additional keywords: *Sorghum bicolor*.

One of the most important diseases of sorghum (*Sorghum bicolor* (L.) Moench.) and corn (*Zea mays* L.) is sorghum downy mildew caused by *Peronosclerospora sorghi* (Weston et Uppal) C. G. Shaw. In the United States, the greatest production threat due to this disease has been in areas along the Texas Gulf Coast (8). Systemic infection of susceptible sorghum hybrids is initiated by germ tubes from oospores (2,21) or conidia (11) and results in partial or complete sterility of heads. Conidial infection remains restricted to the leaf tissue (local lesion) if the pathogen does not invade the apical meristem of the plant. Conidia are very short-lived but play an important role in the secondary spread of the pathogen (8). Oospores are formed between leaf veins of infected plants, and, before harvest, fall to the soil due to natural shredding of leaf tissues. The disease has been controlled with resistant sorghum hybrids (19), but new pathotypes of *P. sorghi* virulent on resistant hybrids recently have been reported (3,4). Use of systemic fungicides is effective in control of the disease (15,16).

Crop protection measures used in agricultural systems may affect nontarget organisms or physiological processes and thereby increase disease incidence and/or severity, constituting an iatrogenic plant disease (10). Such a situation occurred in 1984 in Texas when the herbicide antidote applied to protect grain sorghum from herbicide damage affected sorghum downy mildew incidence (5,6). Seed of the grain sorghum cultivar Dinero (Taylor Evans Seed Co., Tulia, TX) treated with Concep II herbicide antidote (∝-[(1,3-dioxolan-2-yl-methoxy) imino] benzene-acetonitrile; Ciba-Geigy Corporation, Basle, Switzerland)

produced plants with nearly four times greater incidence of downy mildew than plants grown from seed of Dinero treated with the herbicide antidote Screen (benzyl-2-chloro-4-[trifluoromethyl]-5-thiazole carboxylate; Monsanto Chemical Co., St. Louis, MO). Greenhouse research demonstrated that Concep II increased disease incidence (5). The objective of our study was to describe the nature of the iatrogenic disease caused by Concep II and to determine the effectiveness of chemical control of this disease.

MATERIALS AND METHODS

Effect of herbicide antidotes on seedling growth and development. A completely randomized design with eight replicates was used to test the effect of cyometrinil (Concep; α-[(cyanomethoxy)imino]benzeneacetonitrile), Ciba-Geigy Corporation), Concep II, and Screen on seed germination and initial seedling growth of sorghum cultivar R920 (Paymaster Seed, Plainview, TX). Treatments were Concep, Concep II, and Screen applied at 1.25 g a.i./kg seed (manufacturer's recommended rate), Concep II at 3.75 g a.i./kg seed, and an untreated control. Each replicate consisted of 100 seeds germinated on moist paper in a 15-cm-diameter plastic petri dish. A water-slurry method was used to treat seed with the herbicide antidotes. Treated seeds were incubated 3 days at 25 C in darkness; then germinability and seedling growth classes were assessed. Five classes of seedling growth, determined by average root and leaf lengths in millimeters, were as follows: 1 = ≤ 1 mm average root length and ≤ 1 mm average leaf length; 2 = 3 and 3 mm; 3 = 7 and 4 mm; 4 = 10 and 5 mm; 5 = 18 and 7 mm, respectively. The test was performed three times.

The effect of the herbicide antidotes on further development of seedlings of cultivar R920 was tested in a greenhouse at 24–32 C. The experimental design and treatments were as described above, with 29 replicates of each treatment. Each replicate comprised eight seedlings grown in peat pots 36 $cm^2 \times 6$ cm high. Pots were filled with sterile Baccto Potting Soil (Michigan Peat, Houston, TX). Emerged seedlings were counted and classified for stage of development 7 days after planting. Seedling development classes were as follows: 1 = 100% of seedlings had one leaf; 2 = 100% of seedlings had two leaves and aboveground growth was < 2.6 cm; 3 = 100% of seedlings had two leaves and aboveground growth was 3.1–2.6 cm; 4 = more than 80% of seedlings had two leaves and aboveground growth was > 3.1 cm; 5 = more than 80% of seedlings had three leaves.

A completely randomized design with 15 replicates was used to test the effect of the herbicide antidotes on root and leaf lengths of 8-day-old cultivar R920 seedlings in the paper hanger in vitro system (20). Treatments were as described in the previous experiment. Each replicate paper hanger contained 10 seedlings. Seedlings were incubated at 25 C under 12 hr of fluorescent light (approximately 15,000 $ergs \cdot cm^{-2} \cdot sec^{-1}$) and 12 hr of darkness. The test was performed three times.

The effect of rate of application of Concep II on growth of cultivar R920 seedlings was tested in the greenhouse. The experimental design was completely randomized with 29 replicates. Treatments were rates of Concep II at 0.00, 0.50, 1.00, 1.25, 2.00, and 3.75 g a.i./kg seed. Each replicate comprised 10 seedlings germinated in 36 $cm^2 \times 6$ cm high peat pots. The pots were planted in 10-cm-diameter peat pots at 7 days after planting. Aboveground growth of plants was measured at 7-day intervals for 5 wk.

Effect of herbicide and antidotes on downy mildew incidence. Effect of the herbicide antidotes on downy mildew incidence was tested in the greenhouse in three separate experiments. Treatments were arranged in a completely randomized design. In the first experiment, treatments were Concep, Concep II, and Screen at 1.25 g a.i./kg seed, Concep II at 3.75 g a.i./kg seed, and an untreated control; in the second experiment, treatment was Concep II at 1.25 g a.i./kg seed, and an untreated control; and in the third experiment, treatments were Concep II at 0.50, 1.00, 1.25, 2.00, 3.75 g a.i./kg seed, and an untreated control. Each treatment had 29 replicates, and each replicate comprised 10 seedlings per pot. Seed of cultivars DK42Y (DeKalb Seed Co., Lubbock, TX), Dinero, TEY44R, TEY101R (Taylor Evans Seed Co., Tulia, TX), G522DR (Funk Seed International, Lubbock, TX), Pioneer 8222, Pioneer 8300 (Pioneer Hi-Bred International, Inc., Plainview, TX), and Tophand II (Conlee Seed Co., Waco, TX) were used in these trials. Peat pots, 36 $cm^2 \times 6$ cm high, were filled to two-thirds volume with oospore-infested soil collected from fields of the Windsaur Farms near Skidmore, TX, (pathotype 1 of *P. sorghi*) or with soil from La Ward, TX, (pathotype 3). Fifteen seeds were placed on the soil surface in the pots and covered with 20 cc of oospore-infested soil. The pots were kept in a growth chamber at 22 C. Seven days after planting, seedlings were thinned to 10 per pot, and the peat pots were removed from the growth chamber and planted in 10-cm-diameter peat pots in the greenhouse. Daily observations for downy mildew symptoms were made for 30 days, and plants with symptoms were removed from pots.

Combined effect of Concep II and metalaxyl on downy mildew incidence. The effect of combined application of Concep II and metalaxyl fungicide on downy mildew incidence in sorghum cultivar R920 seedlings was tested in the greenhouse. The experiment was a 6 × 6 factorial with 10 replicates arranged in a completely randomized design. Treatments were as follows: Concep II applied at 0.00, 0.50, 1.00, 1.25, 2.00, and 3.75 g a.i./kg seed in combination with metalaxyl at 0.00, 0.02, 0.05, 0.10, 0.50, and 1.00 g a.i./kg seed. Each replicate comprised 10 seedlings grown in one 10-cm-diameter pot. Fifty-gram seed samples were treated first with metalaxyl, followed 24 hr later with Concep II in a water slurry. Field soil infested with oospores of *P. sorghi* pathotype 1 was used as a source of inoculum. Planting, potting, and scoring of downy mildew incidence were as in the experiment described above.

Oospore inoculation of seedlings at different ages. The effects of Concep II and age of cultivar R920 seedlings at time of exposure to oospore inoculum on downy mildew incidence were tested in the greenhouse. The experimental design was an 8 × 2 factorial with 10 replicates of each treatment completely randomized. Treatments were ages of seedlings grown from seed untreated and treated with Concep II at 1.25 g a.i./kg seed. The ages were days that passed between initial planting in sand to the time when the seedlings were transplanted to infested soil, which were 0, 3, 5, 7, 9, 11, 13, and 15 days. Each replicate consisted of six seedlings per pot. Seed treated with Concep II and untreated seed were planted separately in sterile sand and watered. At 2-day intervals, 60 seedlings from Concep II-treated seed and 60 seedlings from untreated seed were transplanted from sand to 10-cm-diameter pots filled with soil infested with oospores of *P. sorghi* pathotype 1. Downy mildew incidence was evaluated through 28 days after the initial planting. The trial was performed twice.

Microflora associated with oospores and infested leaf residue. The effect of rates of in vitro applications of Concep and Concep II on number of bacteria and fungi associated with oospores and leaf tissue residue was tested. The experimental design was completely randomized with 20 replicates. Treatments were rates of Concep (12.6, 50.4, and 119.7 μg a.i./L 1% water agar) added to the agar at 45 C, Concep II (7, 14, 28, 70, and 140 μg a.i./L 1% water agar), and an untreated control. Dry leaves from mature sorghum plants systemically infected with *P. sorghi* were collected in a field at Windsaur Farms near Skidmore, TX, and shredded in a blender. The residue was sieved through a 53-μm-mesh screen. Sieved residue, containing oospores, was stirred in sterile distilled water for 5 min, the oospores were allowed to settle, and floating leaf residue was decanted from oospores. The procedure was repeated until the oospores were suspended in clear water. Oospore density was adjusted to 1×10^4 oospores/ml. Two milliliters of oospore suspension was spread over the surface of 1% water agar in a 15-cm-diameter plastic petri dish. Petri dishes were incubated in the dark for 10 days at 25 C, and the numbers of bacterial colonies and fungal colonies were counted daily. The test was performed three times.

Scanning electron microscopy. Seed of cultivars G522DR and TEY44R treated with Concep II (0.00, 1.25, and 6.00 g a.i./kg seed) were planted (one seed per pot) in 49 cm^2 × 10-cm-high plastic pots

filled with rinsed, sterile river sand. After 4 days incubation at 23 C, seedlings were removed from the sand and rinsed in distilled water, and 10-mm-long root segments (measured from the root cap) were cut off, prepared for examination, mounted on aluminum stubs with silver conductive paint, and coated with gold-palladium (18). Samples were examined in a scanning electron microscope (JEOL, Boston, JSM-25SII at 15 kV and a 10-mm working distance).

Statistical analyses. A combined analysis of data from repeated experiments was performed because mean square errors were homogeneous. (Relationships between different rates of Concep II and incidence of downy mildew, and between different rates of Concep or Concep II and relative to the control number of bacterial or fungal colony-forming units were determined by regression analyses (14).) The Statistical Analyses Systems version 21 statistical package (Statistical Analysis Systems, SAS Institute, Inc., Cary, NC) was used to analyze the effect of treatment (Concep II and control) and age of seedlings at time of exposure to inoculum on the incidence of downy mildew. The same program was used to analyze data of effect of seed treatment with herbicide antidotes on incidence of sorghum downy mildew in cultivars TEY44R and TEY101R. Because percentage of infected plants (PIP) was the response variable, the arcsin $\sqrt{PIP}$ transformation was used to stabilize variance. If interactions were present, multiple comparison (least significant difference) was applied to the means of one factor at a specific level of the other factor. Data from most other experiments were analyzed by ANOVA, and treatment means were separated with Duncan's multiple range test or *t*-test.

RESULTS

Effect of herbicide antidotes on seedling growth and development. Concep and Screen at 1.25 g a.i./kg seed and Concep II at 3.75 g a.i./kg seed decreased viability of sorghum seed compared with the control (Table 1). A Concep II rate of 3.75 g caused a significantly greater decrease in germination than Concep II at 1.25 g. Concep and Screen were the most phytotoxic of the herbicide antidotes as measured by seed germination. The herbicide antidotes' effect on growth of 3-day-old seedlings (root and leaf lengths) varied, with the lowest growth recorded for Concep. Retardation of seedling growth also occurred in the in vitro system for treatments with both Concep II rates, but not with Screen (Table 1). In greenhouse experiments, the number of seedlings that emerged 7 days after planting was significantly lower in both Concep II treatments as compared with other treatments (Table 1). Seedlings produced by seed treated with both rates of Concep II developed poorly. Seedlings grown from seed treated with Concep developed almost equally with the control plants after 7 days. Screen did not affect seedling emergence and only slightly decreased seedling development compared with the control (Table 1). Seedling growth retardation caused by Concep II at 0.50–2.00 g lasted for 4 wk, and after 5 wk, stunting was seen only on plants from seed treated with Concep II at the 3.75 g rate (Fig. 1). Stunting of seedlings caused by Concep II increased with increasing rates of this herbicide antidote (Fig. 1). The inhibition dissipated with age of the seedlings.

Effect of herbicide antidotes on downy mildew incidence. A significant interaction was found between the main treatment effects and sorghum cultivar for downy mildew incidence incited by oospores of *P. sorghi* pathotype 3, but not by pathotype 1 (Table 2). Concep II increased incidence of downy mildew incited by oospores of pathotypes 1 and 3 (Table 3). There was no significant effect of Concep on disease incidence. Screen decreased disease incidence or had no significant effect (Table 3). Concep II did not alter the resistance of sorghum hybrids in cases of infection by oospores of pathotype 1 or 3 (Table 4). The higher the rate of Concep II, the higher the level of downy mildew incidence (Figs. 2 and 3, the curve for metalaxyl at 0.00 g). Doses lower than commonly recommended (i.e., < 1.25 g a.i./kg seed) decreased the incidence of downy mildew.

Combined effect of Concep II and metalaxyl on downy mildew. Higher rates of metalaxyl caused low incidence of downy mildew (Fig. 3). Metalaxyl at 0.50 g a.i./kg seed fully controlled the disease

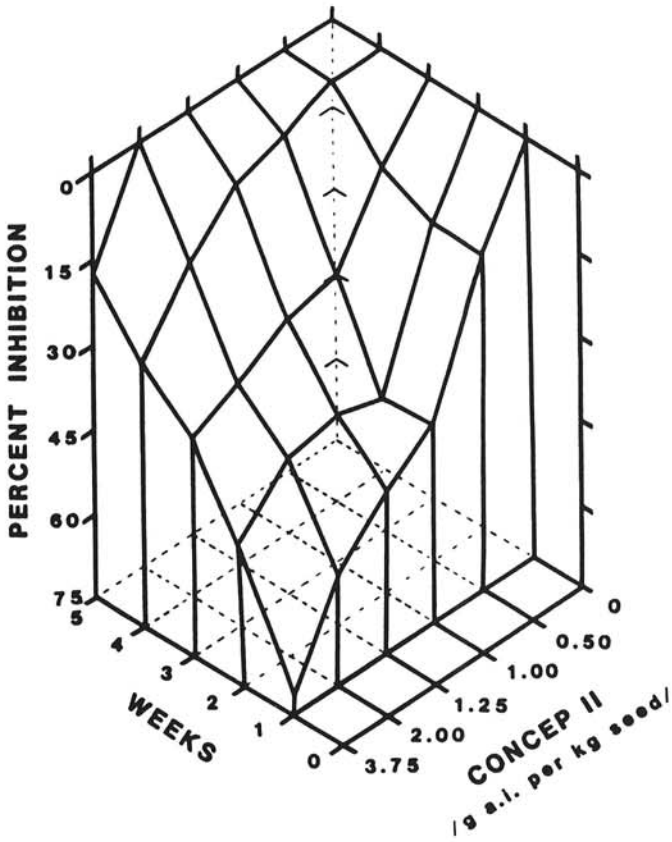

Fig. 1. Influence of different Concep II seed treatment on aboveground growth inhibition of cultivar R920 sorghum seedlings.

TABLE 1. Effect of seed treatment with herbicide antidotes on sorghum cultivar R920 seed germination, seedling growth, emergence, and development

		In vitro				Greenhouse	
Herbicide antidote	Rate (g a.i./kg)	Seed germination after 3 days (%)	Growth classes of 3-day-old seedlings[x]	Root length of 8-day-old seedlings (mm)	Leaf length of 8-day-old seedlings (mm)	Seedling emergence 7 days after planting (%)	Development classes of 7-day-old seedlings[y]
None	...	83.1 c[z]	5	70 d[z]	62 c[z]	59.5 b[z]	5
Concep	1.25	46.0 b	2	57 c	38 b	57.4 b	4
Concep II	1.25	75.2 c	3	45 b	35 b	38.7 a	3
Concep II	3.75	32.3 a	3	37 a	27 a	32.0 a	2
Screen	1.25	53.9 b	4	68 d	65 c	55.6 b	4

[x] Growth classes were determined by average root and leaf lengths and scaled 1–5 (1 = minimum growth, 5 = maximum growth).

[y] Development classes were determined by aboveground growth and number of leaves and were scaled 1–5 (1 = minimum development, 5 = maximum development).

[z] Means within columns followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

unless seed was treated with Concep II at rates higher than recommended by the manufacturer. Concep II counteracted control of downy mildew by metalaxyl, and this effect of Concep II increased with its dosage.

Oospore inoculation of seedlings at different ages. A significant interaction was found between the main treatment effects (Concep II and an untreated control) and age of seedlings at inoculation for downy mildew incidence (Table 5). Also, the main effects were highly significant. Effect of Concep II on downy mildew incidence depended on age of seedlings (Table 6). Concep II decreased the rate at which seedlings escaped the disease because of aging.

Microflora associated with oospores and infested leaf residue. Concep II had no bactericidal activity in vitro even at the highest rate, but had fungicidal activity against fungi naturally associated with mature oospores of *P. sorghi* and infected leaf tissue (Fig. 4). Concep decreased the number of colonies formed by fungi (Fig. 4), but it was ineffective as a bactericide, except at the highest rate (Fig. 5).

TABLE 2. Analysis of variance of effect of seed treatment with herbicide antidotes on incidence of sorghum downy mildew incited by oospores of *Peronosclerospora sorghi* pathotype 1 and 3 in two sorghum cultivars

	Pathotype 1			Pathotype 3		
Source of variation	df	SS	F[x]	df	SS	F[x]
Model	7	20.928	251.05***	7	6.514	30.01***
Treatments (T)[y]	3	20.283	567.74***	3	5.303	57.00***
Cultivars (C)[z]	1	0.525	44.16***	1	0.504	16.26***
T × C	3	0.118	3.33 NS	3	0.706	7.60***
Error	224	2.667		224	6.946	
Total	231	23.595		231	13.460	

[x] *F*-test significance indicated as *** = $P < 0.001$. NS = not significant at $P < 0.01$.
[y] Treatments were Screen, Concep, and Concep II, applied at 1.25 g a.i./kg seed, and an untreated control.
[z] Sorghum cultivars TEY44R and TEY101R.

TABLE 3. Effect of seed treatment with herbicide antidotes on incidence of sorghum downy mildew incited by oospores of *Peronosclerospora sorghi* pathotype 1 and 3 in sorghum cultivars TEY44R, TEY101R, and G522DR

	Plants with downy mildew symptoms (%)				
	Pathotype 1		Pathotype 3		
Herbicide antidote[y]	TEY44R	TEY101R	TEY44R	TEY101R	G522DR
None	37.1 b[z]	40.4 b[z]	41.2 b[z]	34.0 b[z]	33.1 b[z]
Concep II	68.3 a	82.0 a	83.3 a	56.3 a	87.7 a
Concep	35.6 b	44.3 b	51.6 b	40.8 b	47.1 b
Screen	4.2 c	9.7 c	34.5 b	31.9 b	28.3 b

[y] Herbicide antidotes were applied at 1.25 g a.i./kg seed.
[z] Means within columns followed by the same letter are not significantly different at $P = 0.05$ according to LSD-test.

TABLE 4. Effect of seed treatment with herbicide antidote Concep II on disease incidence in four sorghum hybrids inoculated with oospores of pathotype 1 and 3 of *Peronosclerospora sorghi*

	Plants with downy mildew symptoms (%)							
	DK42Y[a]		Dinero[a]		Pioneer 8222[b]		Pioneer 8300[b]	
Treatment	P1[c]	P3[d]	P1[c]	P3[d]	P1[c]	P3[d]	P1[c]	P3[d]
Control	3.1	36.4	7.3	49.4	0.0	1.3	3.2	5.0
Concep II[e]	5.2	89.0[f]	5.2	97.0[f]	0.0	0.0	4.1	4.2

[a] Sorghums resistant to pathotype 1 and susceptible to pathotype 3.
[b] Sorghums resistant to pathotypes 1 and 3.
[c] P1 = pathotype 1 oospore-infested soil collected from Windsaur Farms near Skidmore, TX.
[d] P3 = pathotype 3 oospore-infested soil collected from La Ward, TX.
[e] Rate is 1.25 g a.i./kg seed.
[f] Significant difference ($P = 0.05$) between Concep II and the control according to *t*-test.

Scanning electron microscopy. Root tips of 4-day-old seedlings grown from seed treated with Concep II had less development of the root-cap region compared with the control (Fig. 6). Examination of root surfaces in the root-hair region revealed retarded development of the rhizodermis in both Concep II

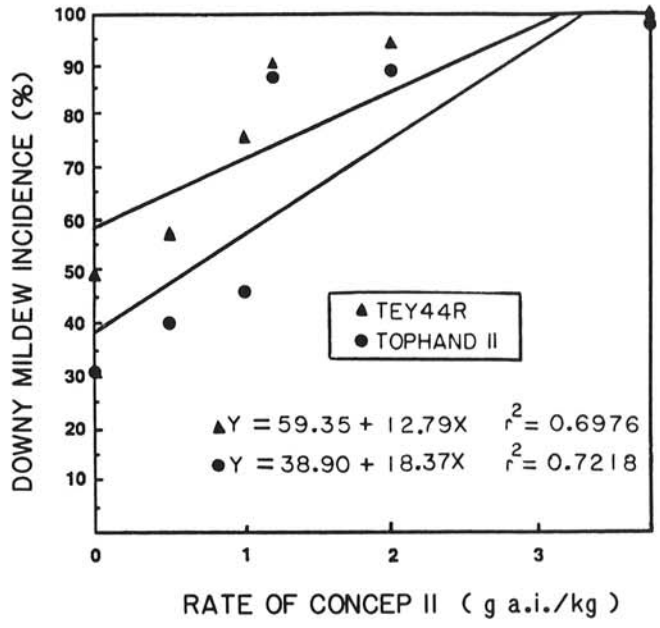

Fig. 2. Effect of seed treatment with different rates of herbicide antidote Concep II on the incidence of sorghum downy mildew incited by oospores of *Peronosclerospora sorghi* pathotype 1 in two sorghum cultivars.

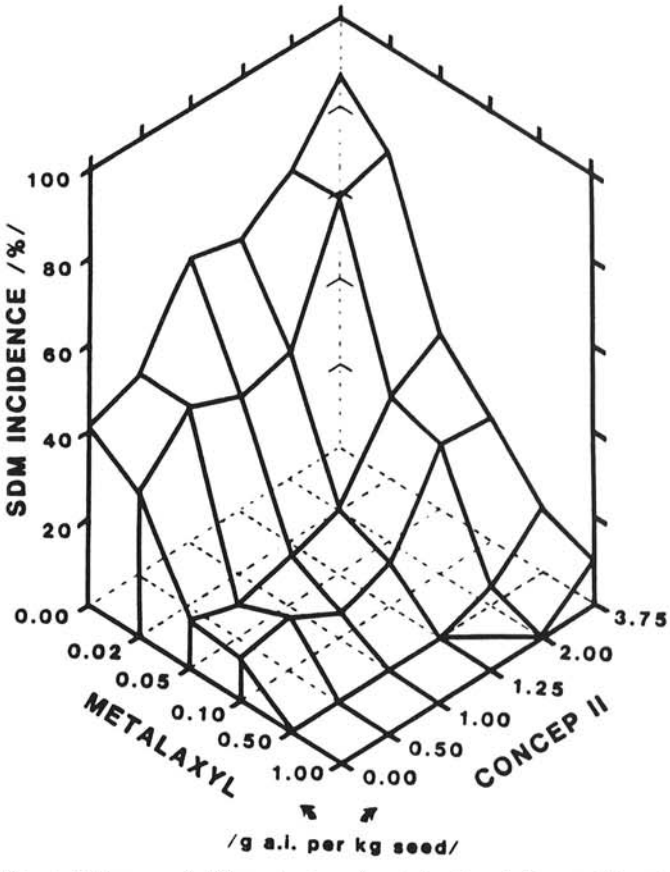

Fig. 3. Influence of different rates of metalaxyl and Concep II seed treatments on sorghum downy mildew incidence in cultivar R920 sorghum seedlings.

treatments (Fig. 7). The higher Concep II rate slowed the development of root hairs. In the control treatment, trichoblasts ceased elongating when root hairs emerged, producing a terminal location of root hairs within ends of trichoblasts (Fig. 8). Concep II not only inhibited root hair development, but also slowed trichoblast development compared with the control. Trichoblasts in both Concep II treatments continued to elongate at the time when trichoblasts in the control treatment had matured.

DISCUSSION

This research confirms previous reports (5,6) that Concep II caused an iatrogenic disease by increasing incidence of downy mildew in sorghum and that Screen had no effect or decreased the incidence. The mechanisms by which Screen can decrease disease incidence are still unknown. Craig et al (6) suggested that Screen can cause changes in the sorghum plant that are unfavorable to disease development. Concep did not significantly increase disease incidence in experiments reported herein, but its potential for iatrogenic effect is worth investigating further.

Our research strategy included bioassays to evaluate the effect of Concep II on the pathogen, host plant, host-parasite system, efficacy of metalaxyl, and microflora associated with inoculum. In the herbicide antidote-pathogen relationship, our assumption was that Concep II might stimulate the frequency and/or speed of oospore germination. Many of our experiments performed in vivo, following Pratt's technique (17), or in vitro, using French and Schmitt's methods (9), did not support that assumption. However, in such experiments, it is very difficult to note the difference between the control and a herbicide antidote treatment because few oospores germinate (usually less than 1%). In addition, direct light-microscopic examination of the nature and rate of colonization of young sorghum roots by oospore germ tubes did not support the hypothesis of increased germination or speed of germination (Szerszen et al, *unpublished*).

Oospores commonly are parasitized by naturally occurring bacteria or chytridiomycetous fungi (12,17). Lack of bactericidal activity of Concep II, its low fungicidal activity (Fig. 4), and lack of an effect of this chemical on oospore germination in vivo or in vitro suggest that Concep II increases downy mildew incidence in a way other than by improving oospore germination. However, fungicidal and bactericidal activity of these chemicals is worth further investigations. During seed treatment, each sorghum seed is covered with approximately 3.75×10^{-5} g a.i. of herbicide antidote, if the rate is 1.25 g a.i./kg seed. It is not known how much

TABLE 5. Analysis of variance of effect of seed treatment with herbicide antidote Concep II on the incidence of downy mildew incited by *Peronosclerospora sorghi* pathotype 1 oospore inoculation of cultivar R920 sorghum seedlings at different ages

Source of variation	df	SS	F[x]
Model	15	23.669	635.96***
Treatments (T)[y]	1	5.793	2334.96***
Ages (A)[z]	7	17.178	989.06***
T × A	7	0.697	40.16***
Error	144	0.357	
Total	159	24.025	
R^2	0.98		
C.V.	8.52		
Mean	35.69		

[x] *F*-test significance indicated as *** = $P < 0.001$.
[y] Treatments were Concep II at 1.25 g a.i./kg seed, and an untreated control.
[z] Ages of seedlings in days at which they were transplanted to oospore-infested soil.

TABLE 6. Effect of Concep II herbicide antidote seed treatment on sorghum downy mildew incidence incited by *Peronosclerospora sorghi* pathotype 1 oospore inoculation of cultivar R920 sorghum seedlings at different ages

Age of seedlings[x]	Sorghum downy mildew (%)	
	Concep II[y]	Control
0	83 ab[z]	56 a[z]
3	86 a	42 b
5	81 b	33 c
7	61 c	18 d
9	47 d	7 e
11	33 e	8 e
13	14 f	0 f
15	2 g	0 f

[x] Age of seedlings in days at which they were transplanted to oospore-infested soil.
[y] The herbicide antidote was used at 1.25 g a.i./kg seed.
[z] Means within columns followed by the same letter are not significantly different at $P = 0.05$ according to LSD-test.

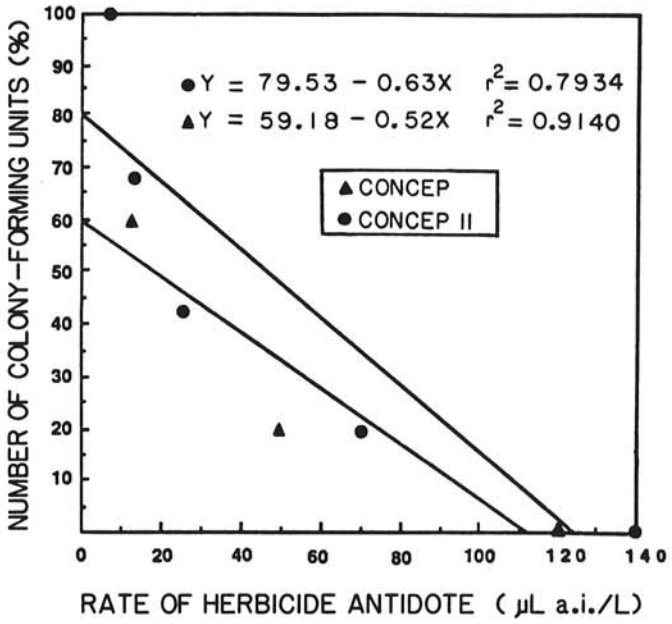

Fig. 4. Effect of Concep and Concep II herbicide antidotes on number of colony-forming units of fungi (relative to the untreated control) naturally associated with oospores of *Peronosclerospora sorghi*.

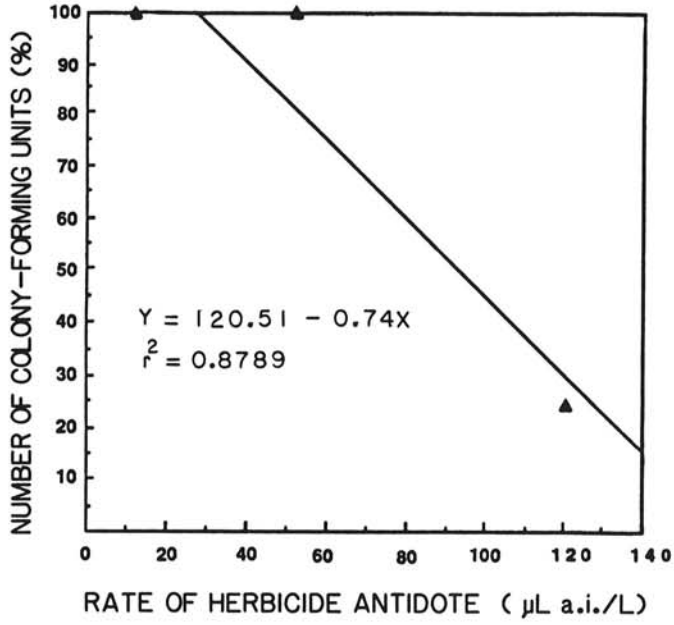

Fig. 5. Effect of Concep herbicide antidote on number of colony-forming units of bacteria (relative to the untreated control) naturally associated with oospores of *Peronosclerospora sorghi*.

of the chemicals is dissipated from treated seed into soil.

Iatrogenic diseases can be initiated by chemical agents that induce changes in plant structure. Chlormequat chloride causes dwarfing in wheat, and a side effect of this dwarfing is an increase in head infection by *Septoria nodorum* and *Fusarium culmorum* (1). We observed that growth of 3-day-old sorghum seedlings produced from seed treated with Concep or Concep II was retarded. The retardation of growth in plants from Concep-treated seed had a shorter duration compared with the plants from the Concep II treatment (Table 1). However, this observation was derived from two experiments carried out under different environmental conditions, and no developmental stages of 3-day-old seedlings were scored because of their very young age. Different environmental conditions may explain the higher percentage of seedling emergence in the greenhouse trial than germinability percentage in the in vitro trial.

The ability of Concep II to reduce growth and respiration rates of sorghum seedlings previously was reported by Ketchersid and Merkle (13). In experiments reported herein, Concep II also slowed the growth in plants at various ages (Table 1 and Fig. 1). Although higher rates of Concep II caused greater growth retardation and increased disease incidence (Fig. 3, the curve for metalaxyl at 0.00 g a.i./kg seed), there was no evidence that growth retardation was directly responsible for increased disease incidence. Scanning electron microscopy revealed that Concep II extended the time in which root trichoblasts elongated to their full size (Fig. 8). A root hair is initiated in the form of a small papilla at or near the apical end of a trichoblast. If longitudinal extension of rhizodermal cells is in progress after the appearance of the papilla, the root hair emerges at some distance from that end; otherwise, its position remains terminal (7). Ongoing growth of trichoblasts from untreated plants was not observed, and they had normally developed root hairs that occurred near apical ends of trichoblasts (Fig. 8). Seedlings from the Concep II treatment were the same age as the untreated seedlings, but their trichoblasts, with small root-hair papillae, were still elongating because the papillae were located away from the apical ends of the trichoblasts (Fig. 8). According to plant growth concept (7), there is a sequence of cell division, elongation, and maturation. If so, these observations suggest that Concep II-treated seedlings maintain juvenile tissue,

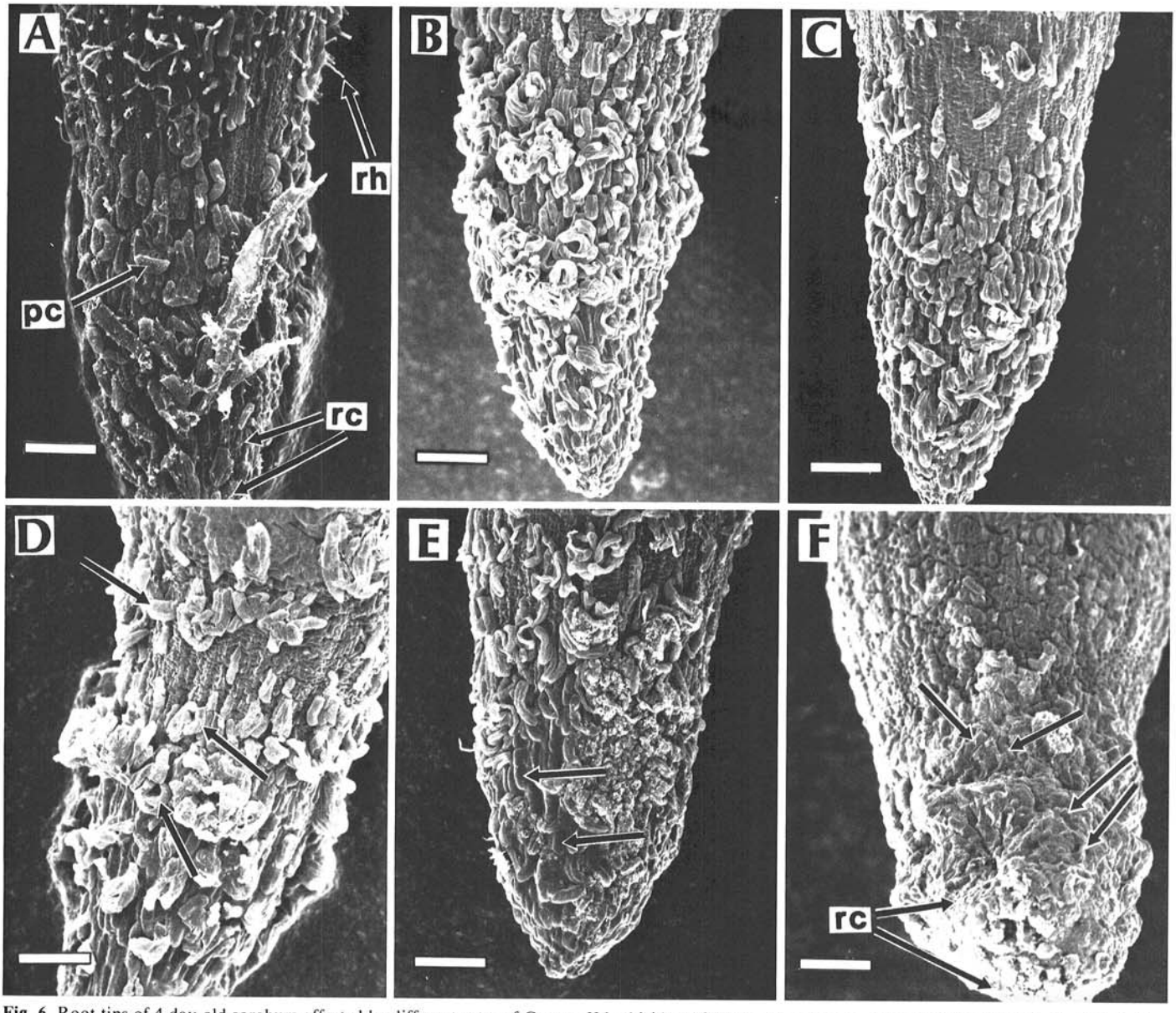

Fig. 6. Root tips of 4-day-old sorghum affected by different rates of Concep II herbicide antidote applied to seed. **A–C,** Hybrid TEY44R. **D–F,** Hybrid G522DR. **A, D,** Control. **B, E,** Concep II at 1.25 g a.i./kg seed. **C, F,** Concep II at 6.00 g a.i./kg seed. Note different development of root tips: **A,** Well-developed root hairs (rh), root-cap (rc), and parenchyma cells (pc). **B,** Root hairs are not developed. **C,** Lack of root hairs; parenchyma cells are less abundant. **D,** Well-developed parenchyma cells (arrows). **E,** Poorly developed parenchyma cells (arrows). **F,** Very poorly developed root-cap (rc) with small parenchyma cells (arrows). Bars = 100 μm.

at least in the root region, for a longer time than untreated seedlings. Juvenile plant tissue is usually more vulnerable to pathogen attack than mature tissue (10). Age of sorghum seedlings is a very important factor for infection by oospores (Tables 5 and 6) or conidia (22) of *P. sorghi* because susceptible sorghum seedlings gain resistance with aging. If Concep II causes longer than normal maintenance of sorghum root juvenile tissue, such tissue probably could be more easily infected by pathogens congenial with young root tissue, e.g., *Pythium* spp. However, Concep II did not influence infection of *P. arrhenomanes* in our paper-hanger in vitro experiments, or in soil-greenhouse experiments (Szerszen et al, *unpublished*). The specific root and root-hair developmental changes caused by Concep II probably could not be exploited by the pathogen in improvement of the infection-incubation process.

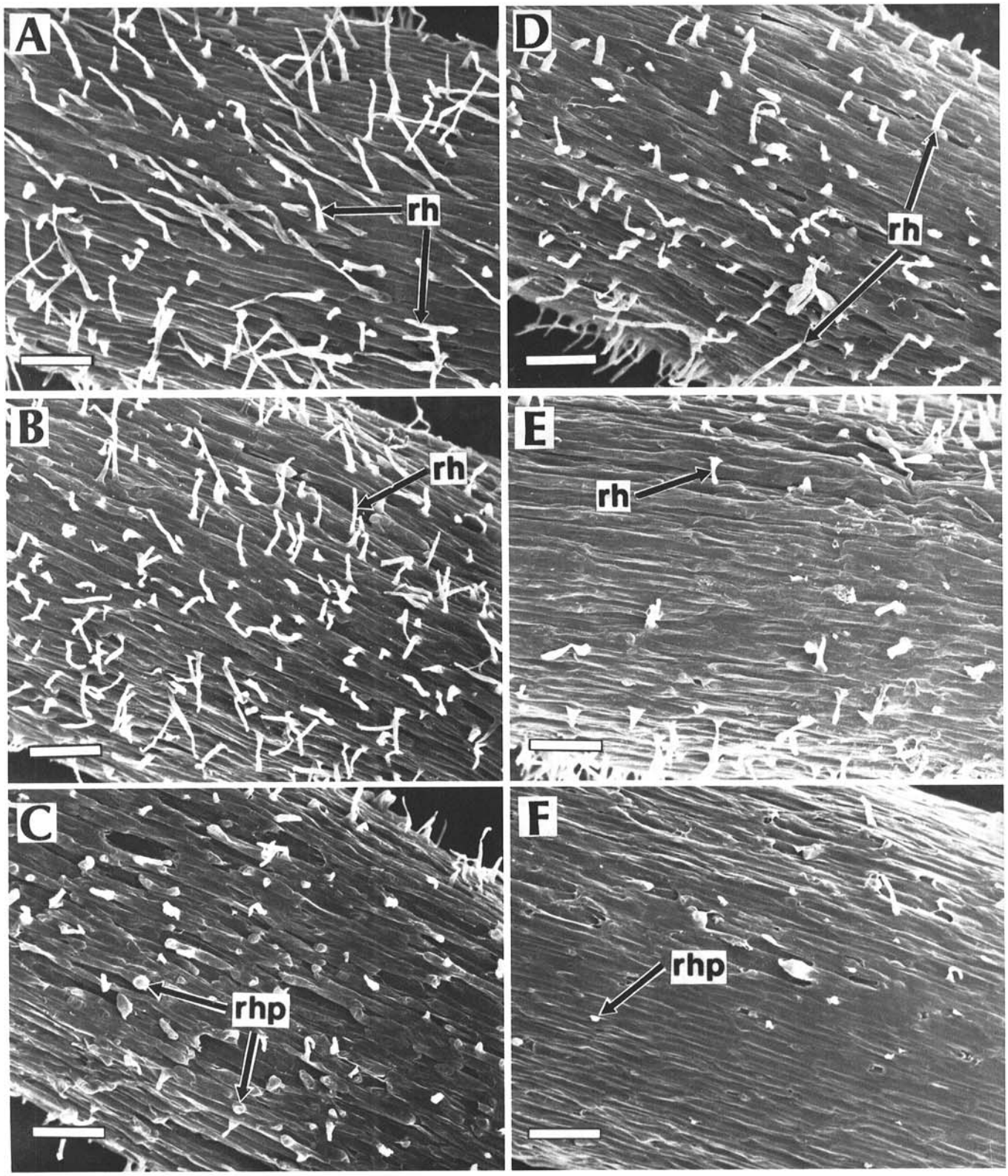

Fig. 7. Root surface regions of 4-day-old sorghum seedlings 1,000 to 1,700 μm from root tips. **A–C,** Hybrid TEY44R. **D–F,** Hybrid G522DR. **A, D,** Control. **B, E,** Concep II at 1.25 g a.i./kg seed. **C, F,** Concep II at 6.0 g a.i./kg seed. Note much shorter and less abundant root hairs (rh) with Concep II treatment at 1.25 g a.i./kg seed than in the control. Increase of Concep II rate to 6.00 g a.i./kg seed resulted in growth retardation of root hairs, which are seen as small root-hair papillae (rhp). Bars = 100 μm.

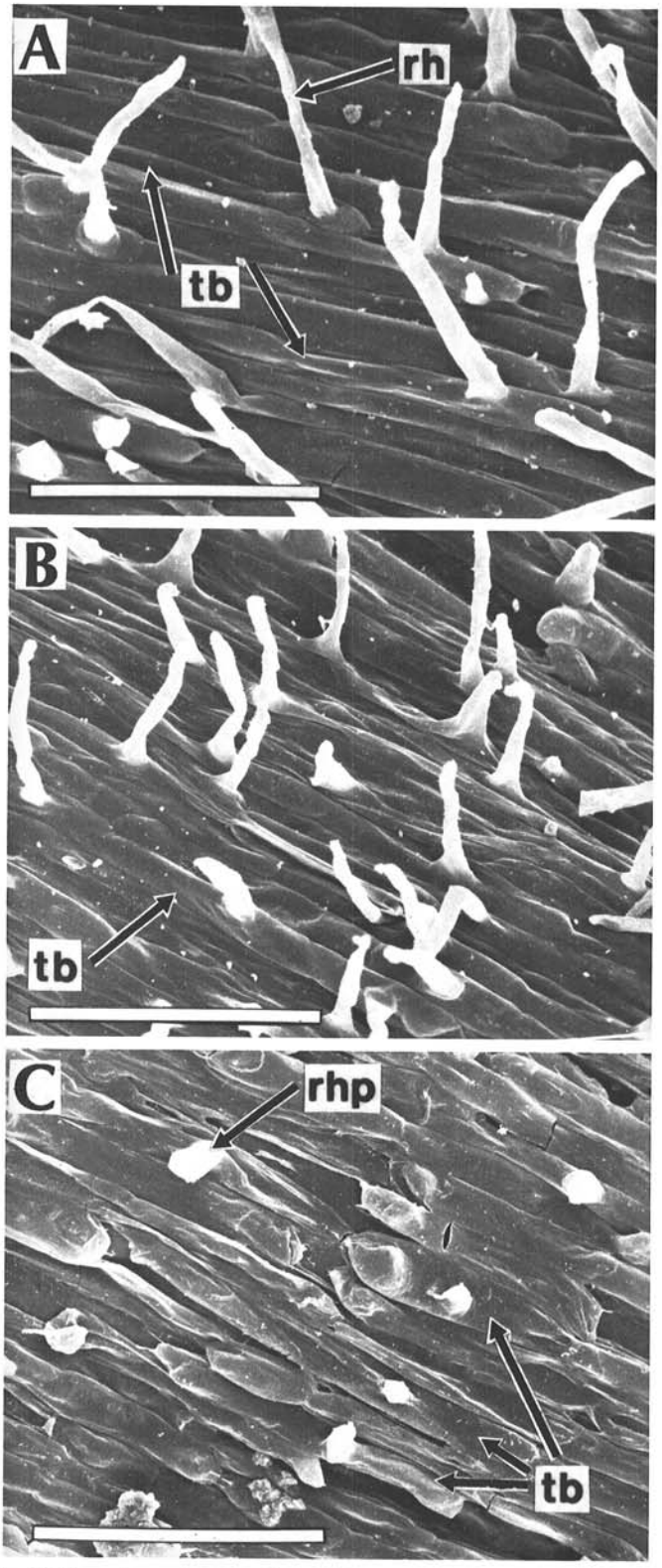

Fig. 8. Close views of root surfaces 1,300 to 1,550 μm from root tips of 4-day-old sorghum seedlings grown from seed treated with Concep II. **A,** Untreated control; root hairs (rh) are long and thick. **B,** Concep II at 1.25 g a.i./kg seed; root hairs are shorter than in the control. **C,** Concep II at 6.00 g a.i./kg seed; root hairs begin to emerge in the form of small root-hair papillae (rhp). Note that root hairs in the control treatment occur near apical ends of trichoblasts (tb). In both Concep II treatments, root hairs or root-hair papillae are located away from apical ends of trichoblasts. Bars = 100 μm.

Also, stability of host resistance in the presence of Concep II (Table 4) indicates that these changes did not influence sorghum's mechanism of resistance to certain pathotypes of *P. sorghi*.

Because downy mildew causes economically significant losses in sorghum production in the United States (8), further enhancement of the disease by seed-applied herbicide antidote is highly undesirable. Combined seed treatment with Concep II and metalaxyl can and should be used to counteract disease enhancement.

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