Systemic Fungistatic Activity of 1,1,1-Tricholoro-3-nitro-2-propanol Against Smut Fungi in Grasses

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Cooperative investigations of the Crops Research Division and the Oregon Agricultural Experiment Station. Published with approval of the Director as Technical Paper No. 2956, Oregon Agricultural Experiment Station.

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Acknowledgment is made to Nor-Am Agricultural Products, Inc., Woodstock, Illinois, for supplies of the experimental chemical and technical advice. We thank Nor-Am Agricultural Products, Inc., the Penncross Bentgrass Growers Association, and the Merion Bluegrass Association for financial support given to Oregon State University.

Accepted for publication 5 March 1971.

ABSTRACT

Systemic fungistatic activity against several smut fungi was noted for 1,1,1-trichloro-3-nitro-2-propanol (TCNP) after soil application and root uptake in several grasses. TCNP applied at 5-30 mg/600 ml soil (about 5-30 lb./acre) in the root zone of infected plants temporarily eliminated disease symptoms and prevented sporulation of *Ustilago striiformis* (stripe smut) in *Poa pratensis, Dactylis glomerata,* and *Agrostis palustris,* and of *Urocystis agropyri* (flag smut) in *P. pratensis* for 8-19 weeks. During suppression of the pathogens, plants regained normal color and leaf form, growth rate was restored, and root growth was increased. The pathogens were not killed, however, and sporulation and disease symptoms reappeared 8-19 weeks after TCNP was applied. Loose smut (*Ustilago nuda*) was controlled in Larker barley by TCNP applied at 15-30 mg/600 ml soil 10 days after seeds were planted. Lower dosages (5-10 mg) resulted in incomplete control, but TCNP prevented usual destruction of spikes in which sporulation of *U. nuda* was restricted. *U. bullata* in *Bromus marginatus* and *Tilletia caries* in *Triticum aestivum* were not controlled by treating soil around infected plants. TCNP was ineffective against *Puccinia striiformis* and *Helminthosporium vagans* in *P. pratensis*. Phytopathology 61:936-939.

Additional key words: systemic fungicides, chemotherapy, stripe rust.

We obtained 1,1,1-trichloro-3-nitro-2-propanol (TCNP) from Nor-Am Agricultural Products, Inc., under the Code EP-346 for trial against major grass diseases, although no promising fungicidal activity was known. In agar dilution plates, TCNP was only weakly active against six fungi (1). It was inactive against four plant-pathogenic fungi and four bacterial plant pathogens (3), and showed no promise as an insecticide (3) or herbicide (6). However, hexachloro-2-propanol, a related compound, was active as an antisporulant against six fungi in nutrient agar tests (2), and was effective against powdery mildews in foliar sprays (5).

We obtained promising systemic activity with TCNP against a grass smut disease in preliminary tests in October 1966; therefore, the objective of the present study was to evaluate TCNP for control of grass diseases with emphasis on smut fungi.

MATERIALS AND METHODS.—Systemic activity of TCNP was tested in vivo against *Ustilago striiformis* (Westend.) Niessl (stripe smut) in *Poa pratensis* L. 'Merion', *Dactylis glomerata* L., and *Agrostis palustris* Huds. 'Pennlu'; *Urocystis agropyri* (Preuss) Schröt. (flag smut) in *P. pratensis* 'Merion'; *Ustilago nuda* (Jens.) Rostr. (loose smut) in *Hordeum vulgare* L. 'Larker'; *Ustilago bullata* Berk. (head smut) in *Bromus marginatus* Nees; *Tilletia caries* (DC.) Tul. (common bunt) in *Triticum aestivum* L. 'Elgin'; *Puccinia striiformis* West. (stripe rust) in *P. pratensis* 'Newport'; and *Helminthosporium vagans* Drechs. (leaf spot) in *P. pratensis* 'WSU-402'.

Plants for the tests were grown in 600 ml of a sandy loam soil in 10-cm sq plastic pots with four bottom drainage holes. Three pots of each grass were treated with each chemical dosage, except for *B. marginatus,* in which four replications were treated. Plants infected with *U. striiformis* and *U. agropyri* that had been propagated in the greenhouse were divided into segments and transplanted to pots. The soil was treated after good root development. Seeds of *B. marginatus* inoculated with *U. bullata* were planted in soil in plant bands on 18 November, and germinated in a cold frame. Two of the resulting small plants were transplanted to 600-ml soil in pots on 21 February. TCNP was added to the soil on 10 March. The plants were grown to maturity in the same pots in the greenhouse. Larker barley seeds infected with *U. nuda* were planted in soil in the pots, and five seedlings were grown in each pot. TCNP was added to soil 10 days after planting, and the barley plants were grown to maturity in the same pots. Seeds of Elgin wheat were coated with *T. caries* teliospores and planted in soil individually in 4.5-cm plant bands. The wheat seedlings were kept outdoors from 18 November to 23 February, when two small plants were transplanted to each pot. The wheat plants each had three-five visible shoots on 10 March when the chemical was applied. Originally separated from field plants, small plants of *Poa pratensis* 'Newport' with seven-eleven shoots and *P. pratensis* 'WSU-402' with four-eight shoots were treated in pots for tests by inoculation with *P. striiformis* and *H. vagans,* respectively.

TABLE 1. Control of *Ustilago striiformis* in *Poa pratensis* 'Merion' by 1,1,1-trichloro-3-nitro-2-propanol (TCNP) applied to soil in the root zone

TCNP/600 ml soil (mg)	Smutted shoots/total shoots					
	Weeks after chemical applied					
	0	4	9	12	14	18
15	31/31	0/40	0/213	294/366	365/458	508/508
20	44/44	0/33	0/202	10/318	356/503	645/645
25	38/38	0/24	0/101	0/315	79/474	696/696
30	38/38	0/25	0/134	1/283	99/369	457/457
35	36/36	0/34	0/161	0/372	18/479	634/638
40	45/45	0/44	0/168	0/312	1/570	710/725
None	42/42	56/56	172/172	228/228	264/264	266/266

All tests were conducted in a greenhouse. Fungicide activity resulted from root uptake after soil application of TCNP. Technical grade TCNP was dispersed in water. Desired quantities of the suspended chemical were placed in holes 2 cm deep in the soil at four locations surrounding each plant. Before addition of TCNP, the drainage holes were sealed with plastic adhesive tape to prevent loss of chemical.

Fungicide activity against stripe smut and flag smut was noted by the appearance of smut-free leaf tissue originating at the basal meristems. Healthy and smutted shoots were counted periodically. Control of head smut in *B. marginatus* and of common bunt in wheat was measured by percentage of smutted panicles or of bunted spikes, respectively. Control of loose smut in barley was noted by the number and time of appearance of smutted spikes and degree of sporulation in partially smutted spikes. Stripe rust control was evaluated at 14 and 18 days after inoculation by noting the presence or absence and type of rust infections. Control of *H. vagans* was measured by appearance of typical leaf spot lesions after inoculation.

RESULTS.—*Ustilago striiformis in P. pratensis 'Merion'*.—TCNP quickly inhibited *U. striiformis* in all new leaf growth of existing and new shoots. The disease was suppressed for 8 to 18 weeks, in proportion to the dosage applied to soil in relation to amount of plant tissue present to absorb the chemical. The chemical was strongly fungistatic for 3 months, but the fungus was not killed (Table 1). During the period of fungus suppression, stripe smut symptoms were prevented, leaves assumed normal color and form, and leaf growth apparently was stimulated. A striking effect was the much increased root growth of plants in treated soil. Root development is usually weak in infected plants. Numerous trials were made at severely phytotoxic dosages of TCNP, but eradication of the pathogen was never obtained.

Ustilago striiformis in Dactylis glomerata.—Strong inhibition of sporulation of *U. striiformis* was obtained by root uptake in *Dactylis glomerata* after application of TCNP at 2-30 mg/600 ml soil (Table 2). Suppression of stripe smut was lost first at the lower dosages. Orchard grass plants remained smut-free through the 14 to 16 weeks after TCNP was applied at 10 and 15 mg/600 ml soil. The pathogen was not eradicated at any dosage, and stripe smut eventually reappeared in all shoots of treated plants in several different tests.

Ustilago striiformis in Agrostis palustris.—Root uptake of TCNP in creeping bentgrass prevented stripe smut symptoms for 12 to 20 weeks, depending on the dosage applied. Sporulation and other disease symptoms were not apparent for 16 weeks in the small plants treated with 10 to 25 mg TCNP/600 ml soil. Plants treated with 30 mg/600 ml soil remained smut-free through 20 weeks, and only 3% of the stolons showed stripe smut after 24 weeks.

Activity against Urocystis agropyri.—TCNP showed fungistatic activity against *U. agropyri* in *P. pratensis* 'Merion'. In tests with plants, each with 10-13 shoots in 10-cm pots, TCNP at 5 mg/600 ml soil was fungistatic in only part of the shoots, and this partial control was lost after 10 weeks. TCNP at 10, 15, and 20 mg prevented sporulation and other flag smut symptoms for 8 weeks, but disease control was declining after 10 weeks (Table 3). Suppression of flag smut required heavier dosages of TCNP, and control was of shorter duration than for *U. striiformis*.

TABLE 2. Control of *Ustilago striiformis* in *Dactylis glomerata* by 1,1,1-trichloro-3-nitro-2-propanol (TCNP) applied to soil in the root zone

TCNP/600 ml soil (mg)	Smutted shoots/total shoots							
	Weeks after soil treatment							
	0	6	8	10	12	14	16	18
2	16/16	0/12	0/12	26/30	48/48	62/62	65/65	59/59
5	17/17	0/11	0/11	1/36	45/50	63/63	74/74	78/78
10	19/19	0/16	0/17	0/22	0/45	27/73	70/82	90/90
15	21/21	0/16	0/14	0/51	0/70	35/87	86/86	89/89
30	23/23	0/15	0/15	0/26	0/54	0/72	31/83	85/85
None	15/15	14/14	15/15	26/26	39/39	63/63	80/80	90/90

TABLE 3. Control of *Urocystis agropyri* in *Poa pratensis* 'Merion' by 1,1,1-trichloro-3-nitro-2-propanol (TCNP) applied to soil in the root zone

TCNP/600 ml soil (mg)	Smutted shoots/total shoots					
	Weeks after application of chemical					
	0	8	10	14	16	18
5	33/33	14/32	45/45	112/112	150/150	273/273
10	38/38	0/27	31/31	107/109	190/190	346/346
15	37/37	0/30	7/37	91/91	118/118	249/249
20	35/35	0/29	4/45	74/124	170/182	400/400
None	36/36	32/32	35/35	46/46	53/53	93/93

Activity against Ustilago nuda.—TCNP was applied to soil 10 days after planting Larker barley seed infected with *U. nuda*. Five seedlings were grown in 600 ml of soil in each 10-cm plastic pot. Vigorous root development was present throughout the soil at the time the chemical was added. An average of 14% of spikes in the 15 untreated plants were destroyed by *U. nuda*. At 2 mg TCNP/600 ml soil, no reduction in loose smut was noted. The 5- and 10-mg treatments reduced the incidence of loose smut, but more significantly, the spikes of infected plants remained fairly intact, and sporulation was restricted in the inflorescence. No loose smut was apparent at maturity in plants in soil treated at 15, 20, and 30 mg/600 ml soil.

Control of other smuts.—No significant control of *Ustilago bullata* in *Bromus marginatus* resulted from TCNP at 2-30 mg/600 ml soil applied around infected adult plants. Similarly, no apparent control of *Tilletia caries* in *Triticum aestivum* 'Elgin' plants resulted from TCNP at 2-30 mg/600 ml soil applied 16 weeks after seeding.

Control of other grass diseases.—No inhibition of *Puccinia striiformis* in *P. pratensis* 'Newport' resulted from TCNP at 1-30 mg/600 ml soil applied 1 month before inoculation or at 5-40 mg applied 2 weeks before inoculation of small plants that had been transplanted directly to test pots. No apparent control of *Helminthosporium vagans* in *P. pratensis* 'WSU-402' resulted from 2-30 mg TCNP/600 ml soil applied 1 month before conidial inoculation of field plants that had been transplanted directly to the 10-cm square pots for these tests.

Phytotoxicity.—With healthy Merion Kentucky bluegrass plants, each with six to eight shoots, no reduction in growth resulted from 1-10 mg TCNP/600 ml soil, and leaf growth apparently was increased by the lower dosages. Growth was temporarily reduced with moderate leaf tip burning from treatment with 15-20 mg/pot. Severe leaf injury and retardation of leaf growth resulted from 25- and 30-mg treatments. Similar injury was noted in other grasses studied.

Root uptake of TCNP quickly suppresses sporulation of *U. striiformis* and *U. agropyri* in grasses. Inhibition of the pathogens results in rapid recovery of plant vigor, improved leaf and root growth, and restoration of plant form. While the chemical was actively suppressing the pathogens, the treated plants were restored to normal growth and color and had the appearance of healthy plants. Root growth, particularly, was much improved in treated plants. Unfortunately, the control of several smut pathogens is temporary, because the pathogens are not killed. Sporulation and other disease manifestations reappear within 8-18 months as the chemical effects dissipate. Many attempts were made to effect eradication of these pathogens by TCNP by increasing the dosage to severely phytotoxic levels without success.

DISCUSSION.—Application of TCNP to prevent infection of healthy plants does not appear promising, because control of *U. agropyri* was not obtained by treatment of wheat seed with TCNP at 2 and 4 oz/bu (4). Because the chemical does not eradicate the pathogens, application of TCNP could complicate stripe smut and flag smut control by aiding the perpetuation of diseased plants that otherwise would succumb, owing to moisture stress. Conversely, by restricting systemic mycelial development and restoring plant vitality, TCNP might aid in stripe smut control by increasing the tolerance of infected plants to other chemicals. Reduction in pathogen development by pretreatment with TCNP may permit eradication by smaller dosages of smut-active systemic chemicals, thus reducing cost.

Horsfall & Rich (2), in studies of the antisporulant properties of hexachloro-2-propanol, recognized the desirability of disease control by eliminating inoculum at the source in preference to providing protection at the infection court. Control by antisporulant activity should function best where the infection period is brief. Possibilities for practical disease control by TCNP or a similar antisporulant chemical are indicated by results with *U. nuda*. Even though loose smut (sporulation) occurred, its appearance was delayed in treated plants. Thus, flower infection possibly could be avoided by elimination or reduction of inoculum during the brief infection period which is confined to anthesis.

Poor activity of TCNP in agar plate tests (1) contrasts with systemic activity following root uptake in the present study. Thus, failure of a chemical in in vitro tests does not predict its possible value as a chemotherapeutant. TCNP should be inexpensive (advice from Nor-Am Agricultural Products, Inc.). Since low cost fungicides are necessary in most grass seed crops, analogs of TCNP should be evaluated.

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