

In Vitro and Field Comparison of Six New Fungicides with Iprodione and Vinclozolin for Control of Leaf Drop of Lettuce Caused by *Sclerotinia sclerotiorum*

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ABSTRACT

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In vitro and field studies were initiated to identify compounds having fungicidal activity against *Sclerotinia sclerotiorum* and potential for improving control of lettuce leaf drop. At a concentration of 0.5 µg/ml, radial growth of the pathogen was significantly reduced by bitertanol, chlozolate, diniconazole, iprodione, terbuzazole, vinclozolin, and CGA-449. At the ED₅₀ concentration of tested compounds, sclerotia production was reduced in the presence of diniconazole or SC-0858 and was increased in the presence of chlozolate or iprodione. In field tests, levels of disease control equivalent to those provided by iprodione and vinclozolin were observed for at least 2 yr with diniconazole, CGA-449, and SC-0858. Potential new fungicides apparently exist for control of *Sclerotinia* leaf drop of lettuce, should iprodione and vinclozolin become ineffective in the future.

Sclerotinia leaf drop of lettuce (*Lactuca sativa* L.) is a destructive but sporadic disease in Arizona. *Sclerotinia sclerotiorum* (Lib.) de Bary is the primary causal agent of this disease in Arizona, although *S. minor* Jagger is occasionally recovered from diseased plants. The disease was first reported in Arizona (3) in 1925.

Approximately 85% of the 16,000 ha of lettuce produced in Arizona during the 1987-1988 season was located in Yuma and La Paz counties in western Arizona, where the growing season typically begins in mid-August and ends the following year about mid-April. The incidence of lettuce drop can be high during February, March, and April, when cool wet periods favor disease development (1).

The efficacy of the dicarboximide fungicides iprodione and vinclozolin against *Sclerotinia* species has been demonstrated (4,7-9,12-14). These materials currently are providing effective control of lettuce drop in Arizona. However, recent studies have reported the in vitro development of resistance to dicarboximide fungicides by *S. minor* (2,10,11) and *S. homoeocarpa* F. Bennett (5). These developments necessitate the need for continued testing of new compounds for efficacy in the control of *Sclerotinia* leaf drop of lettuce.

The objectives of this study were to determine the effects of six new fungi-

cides, in addition to iprodione and vinclozolin, on mycelial growth and sclerotium formation by *S. sclerotiorum* and to test the performance of these materials for control of *Sclerotinia* leaf drop of lettuce in the field.

MATERIALS AND METHODS

Mycelial growth and sclerotia production. The following fungicides were used in these tests: bitertanol (Baycor, 50W, Mobay Chemical Co., Kansas City, MO); chlozolate (SDS-65311, 50W, Fermenta Plant Protection, Painesville, OH); diniconazole (Spotless, 25W, Chevron Chemical Co., San Francisco, CA); iprodione (Rovral, 50W, Rhone Poulenc Ag. Co., Research Triangle Park, NC); terbuzazole (Folicur, 1.2 EC, Mobay Chemical Co.); vinclozolin (Ronilan, 50W, BASF Corp., Parsippany, NJ); CGA-449 (50W, Ciba-Geigy Corp., Greensboro, NC); and SC-0858 (50W, ICI Americas, Inc., Goldsboro, NC). Various concentrations of fungicide suspensions were prepared in sterile distilled water then added into autoclaved Difco potato-dextrose agar (PDA) cooled to 60 C. The medium was thoroughly mixed after addition of the fungicide to ensure uniform distribution within the agar. It was then dispensed at 20 ml per 85-mm-diameter plastic petri dish.

Sclerotia of *S. sclerotiorum* from two different locations in Yuma County were collected from naturally infected lettuce plants. Sclerotia were surface-sterilized by agitation in a 5.25% solution of NaClO (undiluted household bleach) for 10 min, rinsed in sterile distilled water, then plated onto PDA plates. One actively growing colony arising from a sclerotium from each of two locations

was subcultured on PDA and designated SS-1 and SS-2. These two isolates were used in all subsequent in vitro and field tests. To assess the effect of each fungicide on mycelial growth of *S. sclerotiorum*, a 6-mm-diameter agar disk from the periphery of an actively growing colony of isolates SS-1 and SS-2 was placed at the edge of petri plates containing PDA amended with fungicide. Plates were incubated at 24 C in darkness for 48 hr, and radial growth was then measured. Each treatment contained eight replicates, four from isolate SS-1 and four from SS-2. The test was performed three times. Percent inhibition was determined by comparing the rate of growth on fungicide-amended and unamended PDA. Levels of inhibition were plotted as a function of fungicide concentration on log-probit graphs (6). Linear regression was used to determine dosage levels for 50% inhibition of growth (ED₅₀).

The effect of each fungicide on sclerotia production was determined by counting the number of sclerotia produced on 25-day-old cultures on PDA amended with fungicides.

Field experiments. Three field trials in artificially infested soils (15) were conducted from 1986 to 1988. The medium for producing sclerotia for soil infestation was prepared by boiling 5.5 kg of barley grain in 15 L of tap water for 90 min. After boiling, the liquid was discarded and 1,000 cm³ of moist grain was put into 2-L widemouth flasks. After 24 hr, the flasks were autoclaved for 60 min, cooled for 24 hr, and autoclaved again. When flasks were cool, the grain was seeded with a single sclerotium taken from a colony of *S. sclerotiorum* growing on PDA. Cultures of the pathogen were started by placing surface-sterilized sclerotia from naturally infected lettuce onto PDA medium. Inoculated flasks were incubated at room temperature (24-26 C) for 3 mo under ambient light. Following the incubation period, the contents of each flask were removed, spread on a clean surface, and air-dried. Dried inoculum contained an average of 2,000 sclerotia per kilogram of material.

Lettuce (cultivar Vanguard 75) was seeded from mid to late November at the Yuma Agricultural Center in double rows, 30 cm apart, on beds 102 cm wide. After thinning at the three leaf to four

leaf stage to a 20- to 25-cm spacing within each row, 400 cm³ of the dried mixture of sclerotia and infested barley grain was distributed evenly on each lettuce bed in a band 51 cm wide and 15.2 m long. Fungicide treatments were applied to the entire surface of treated beds and lettuce plants immediately after inoculum distribution and again 3 wk later with a tractor-mounted boom sprayer that delivered 935 L/ha at a pressure of 689 kPa to nozzles spaced 30 cm apart. Treatments were replicated four times in a randomized complete block design, with each replicate consisting of 15.2 m of double-row bed. Treated beds of lettuce were separated by single nontreated but inoculated beds. Final disease incidence was evaluated by recording the number of collapsed lettuce plants at crop maturity, which occurred approximately 130 days after seeding. Values for

potential increase in yield resulting from usage of a fungicide were derived from a comparison of the number of diseased plants in plots treated with a fungicide with the number of diseased plants in nontreated plots.

RESULTS

Mycelial growth and sclerotium production. At a concentration of 0.5 µg/ml, vinclozolin was highly inhibitory to mycelial growth of *S. sclerotiorum* (Table 1). A comparison of this registered fungicide with other tested compounds at the same concentration revealed large differences in fungicide activity. Only CGA-449 was as inhibitory as vinclozolin to mycelial growth of the pathogen. Iprodione, the other compound currently registered for use on lettuce, reduced mycelial growth, but to a lesser extent than vinclozolin. With the exception of

SC-0858, all tested materials significantly reduced mycelial growth on PDA at a concentration of 0.5 µg/ml (Table 1).

The dosage response curves for the tested fungicides reveal a broad range in activity among the different compounds (Fig. 1). The ED₅₀ values, calculated from these regression lines, range from 0.026 µg/ml for CGA-449 to 158 µg/ml for SC-0858 (Table 1). When compared with terbutrazole, diniconazole, and bitertanol, the steeper slope of the regression line for CGA-449, vinclozolin, iprodione, chlozolinate, and SC-0858 suggests a more pronounced response to an incremental increase in dose (Fig. 1).

The number of sclerotia produced by *S. sclerotiorum* in the presence of the ED₅₀ concentration of each fungicide is presented in Table 1. Diniconazole and SC-0858 caused a significant reduction in the number of sclerotia produced on PDA. Conversely, iprodione and chlozolinate caused a significant increase in the quantity of sclerotia produced by this pathogen. The ED₅₀ concentrations of bitertanol, terbutrazole, and CGA-449 had no apparent effect on sclerotium production.

No perceptible differences were detected in the size of sclerotia formed at the ED₅₀ concentration of each fungicide. Sclerotia of *S. sclerotiorum* are quite variable in size and shape, which makes the detection of changes in size difficult.

Field experiments. The results of three field trials are summarized in Table 2. Iprodione and vinclozolin, the two compounds registered for use on lettuce against *Sclerotinia* leaf drop, were consistently among the most effective fungicides for disease control. Levels of disease control equivalent to those provided by iprodione or vinclozolin were observed with CGA-449 in 1986; CGA-449, SC-0858, and diniconazole in 1987; and CGA-449, SC-0858, chlozolinate, terbutrazole, and diniconazole in 1988. Bitertanol and terbutrazole did not provide consistent disease control in these tests. Values for potential percent increase of yield resulting from usage of test fungicides are presented in Table 2. For 1987 and 1988, application of diniconazole, iprodione, vinclozolin, CGA-449, and SC-0858 at a rate of 1,121 g a.i./ha resulted in potential yield increases of 54, 48, 62, 50, and 42%, respectively, over values recorded from nontreated lettuce.

Disease incidence was rated at the onset of leaf drop and periodically thereafter until crop maturity. Development of disease during each field trial from 1986 to 1988 was linear for all treatments. Highly significant ($P < 0.01$) linear correlation coefficients were observed, ranging from $r = 0.975$ to 0.996 in 1987 (Fig. 2). The steeper slope of the regression line for nontreated plants (1.37), as compared with those for lettuce

Table 1. Effect of eight different fungicides on in vitro mycelial growth and sclerotium production by *Sclerotinia sclerotiorum*

Treatment	Radial growth (mm) in presence of fungicide ^x	ED ₅₀ ^y (µg a.i./ml)	No. of sclerotia at ED ₅₀ concentration of fungicide ^z
Bitertanol	20 b	1.09	46 bc
Chlozolinate	22 b	0.63	61 a
Diniconazole	14 c	0.34	31 d
Iprodione	12 c	0.42	62 a
Terbutrazole	8 d	0.15	50 b
Vinclozolin	2 e	0.18	43 c
CGA-449	0 e	0.026	50 b
SC-0858	33 a	158.0	15 e
Check	33 a	...	46 bc

^xFungicide was applied at 0.5 µg a.i./ml. Radial growth was measured after incubation on amended PDA medium for 48 hr at 24 C. Each value is an average of 16 replicate measurements from two experiments. Values within each column with a different letter are significantly different ($P = 0.01$) according to Duncan's multiple range test.

^yDetermined from a linear regression of the percent of mycelial inhibition plotted against the log of the concentration of fungicide.

^zEach value is an average of 16 replicate, 26-day-old cultures from two experiments. Values within each column with a different letter are significantly different ($P = 0.01$) according to Duncan's multiple range test.

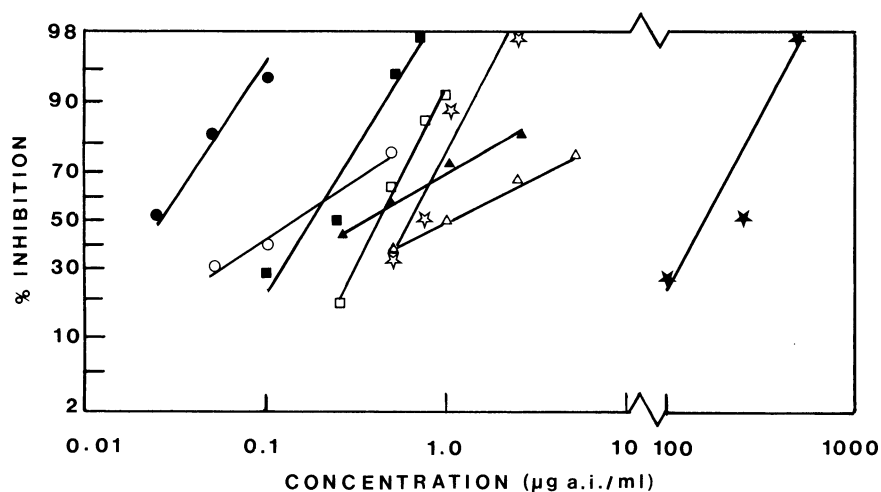


Fig. 1. Dosage response curves for bitertanol (open triangle), chlozolinate (open star), diniconazole (solid triangle), iprodione (open square), terbutrazole (open circle), vinclozolin (solid square), CGA-449 (solid circle), and SC-0858 (solid star) on log-probit axes.

treated with diniconazole (0.78), iprodione (0.74), vinclozolin (0.69), CGA-449 (0.95), or SC-0858 (0.81), suggests a higher rate of disease development in the absence of these fungicides.

DISCUSSION

All eight fungicides provided significant levels of disease control in at least one field trial, whereas chlozolinate, diniconazole, iprodione, vinclozolin, CGA-449, and SC-0858 significantly reduced levels of *Sclerotinia* leaf drop of lettuce whenever they were included in field tests. The potential new fungicides for control of *Sclerotinia* leaf drop of lettuce were often equal to but never more efficacious than iprodione and vinclozolin, which are currently registered for disease control.

No consistent relationship was found between ED₅₀ values for inhibition of mycelial growth and performance of fungicides in the field. For example, in field tests, SC-0858 provided levels of disease control comparable to iprodione or vinclozolin, whereas the ED₅₀ value for SC-0858 (158 µg/ml) is much larger than the value for iprodione (0.42 µg/ml) and vinclozolin (0.18 µg/ml). One possible explanation for this discrepancy is that in field tests all fungicides were applied at concentrations far in excess of ED₅₀ values. At the rate of 1,121 g/ha in 935 L of water, the concentration of fungicide applied in the field was 1,190 µg/ml. At this concentration, mycelial growth would be completely inhibited by all tested fungicides. Also, field performance of fungicides cannot be predicted by only evaluating the effect of the chemical on mycelial growth. The effects of fungicides on other stages of the life cycle of the fungus, such as sclerotium germination and apothecium development, would give a more complete picture of the potential utility of a compound.

Increased sclerotium production in the presence of ED₅₀ concentrations of chlozolinate and iprodione may be of importance in disease development and fungal population dynamics in the field. Brenneman et al (2) also found that the ED₅₀ concentration of iprodione caused increased sclerotium production by *S. minor*. The concentration of fungicides in our field tests far exceed the ED₅₀ values derived from in vitro tests. Therefore, predicting increased sclerotium production in the field from in vitro test data would be highly speculative.

These field tests evaluated the efficacy of fungicides in preventing infection initiated by soilborne inoculum of *S. sclerotiorum*, but did not evaluate control of disease arising from aerial infections caused by ascospores of the fungus. The linear development of disease over time suggests that there was

no multiplication of inoculum. This is not unusual for development of leaf drop of lettuce caused by *S. sclerotiorum* in Arizona, as the levels of moisture, humidity, and temperature required for development of apothecia and ascospores do not occur regularly. Infection of lettuce plants by soilborne inoculum led to rapid plant death, with no observation of partially infected plants. No aerial infections were observed in these field experiments.

Development of resistance to tested fungicides by *S. sclerotiorum* was not examined in this study. The original

isolates of the fungus collected from diseased lettuce plants were used in all subsequent in vitro and field tests. Additional work, similar to that of Brenneman et al (2) and Porter and Phipps (10,11), could provide a forewarning of possible development of resistance to these compounds in the field by *S. sclerotiorum*.

This study has identified some potential fungicides for control of leaf drop of lettuce caused by *S. sclerotiorum*. Further tests should be conducted in regions where *S. minor* causes extensive leaf drop of lettuce.

Table 2. Control of lettuce drop caused by *Sclerotinia sclerotiorum* in yearly field trials

Treatment ^w	Rate (g a.i./ha)	Disease incidence ^x			Potential percent yield increase ^y		
		1986	1987	1988	1986	1987	1988
Bitertanol	561	40 bc ^z	22
	1,121	...	38 ab	52 a	...	14	0
Chlozolinate	1,121	22 bc	54
	224	38 c	25
Diniconazole	1,121	...	26 c	16 bc	...	41	67
	1,121	18 ef	25 c	22 bc	65	43	54
Iprodione	249	...	37 ab	16	...
	1,121	26 bc	46
Vinclozolin	1,121	15 f	21 c	14 c	70	52	71
	1,121	26 de	31 bc	15 bc	49	30	69
CGA-449	1,121	...	26 c	28 b	...	41	42
SC-0858	...	51 a	44 a	48 a
No fungicide

^wFungicides applied after thinning (three leaf to four leaf stage) and again 3 wk later, completely covering lettuce plants and plant bed.

^xPercentage of lettuce plants collapsed from disease at maturity. Each value is a mean of four replicates representing a total of 61 m of double-row lettuce.

^yEach value was derived from a comparison of the number of diseased plants in plots treated with fungicide with the number of diseased plants in nontreated (control) plots.

^zValues within each column with a different letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.

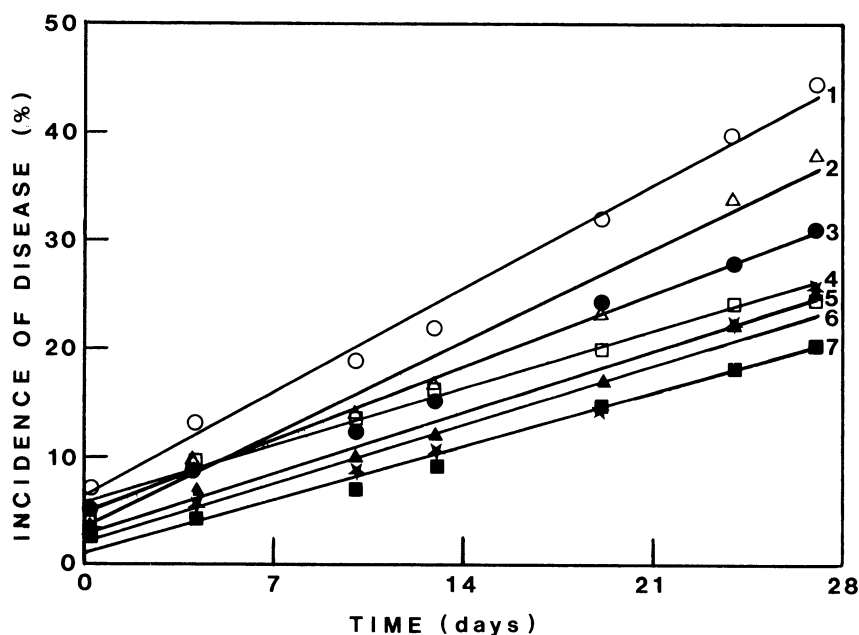


Fig. 2. Incidence of *Sclerotinia* leaf drop on lettuce during 1987 field trial. Treatments included no fungicide (1, open circle) and 1,121 g a.i./ha bitertanol (2, open triangle), diniconazole (6, solid star), iprodione (4, open square), vinclozolin (7, solid square), CGA-449 (3, solid circle), or SC-0858 (5, solid triangle). Linear correlation of disease development with time was highly significant ($P < 0.01$) for all treatments. Similar responses were observed during 1986 and 1988 tests.

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