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Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies

Elizabeth Dann[1], Brian Diers[2], Joe Byrum[2] and Ray Hammerschmidt[1,*]
[1] *Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, USA (Fax: +1-517-353 1926);* [2] *Dept. Crop and Soil Science, Michigan State University, East Lansing, MI 48824, USA;* * *Author for correspondence*

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Abstract

Field or greenhouse grown soybeans were treated with 2,6-dichloroisonicotinic acid or benzothiadiazole and subsequently assessed for severity of white mold disease caused by *Sclerotinia sclerotiorum*. Three or four applications of 2,6-dichloroisonicotinic acid to field plots in 1993–1995 reduced severity of white mold after natural infection by 20–70% compared with water-treated controls in soybean cultivars Elgin 87 and Williams 82, which are considered to be highly susceptible to the disease. The effect was not as large in the cultivars Corsoy 79 and NKS19-90 which are more resistant to white mold. Two or four applications of benzothiadiazole to field plots in 1995 and 1996 reduced white mold severity by 20–60%, with the greatest reductions again observed in the more susceptible cultivars. Corresponding yields were increased compared with controls, particularly for the susceptible cultivars under conditions of high disease pressure. In greenhouse trials multiple applications of either compound resulted in significantly smaller lesion diameters following subsequent leaf inoculations with the fungus. The compounds did not result in observable phytotoxicity or inhibit growth of *Sclerotinia* sp. *in vitro*. We hypothesize that the decrease in disease severity following treatment with INA or BTH is a result of resistance induction.

Abbreviations: INA – 2,6-dichloroisonicotinic acid; BTH – benzothiadiazole; a.i. – active ingredient; SIR – systemic induced resistance; DSI – disease severity index.

Introduction

The phenomenon of systemic induced resistance (SIR), in which resistance to disease is enhanced in tissues distant from the site of the prior inducing treatment, has been extensively reported for a number of plant/pathogen systems and has been the subject of recent reviews (Deverall, 1995; Hammerschmidt and Kuc, 1995; Hammerschmidt and Dann, 1997). The majority of these studies have been conducted under controlled environment conditions. However, for SIR to be incorporated into applied disease management programs, the resistance must withstand disease and environmental pressures encountered under commercial production conditions.

Systemic induced resistance has been demonstrated under field conditions for a limited number of plant/pathogen interactions. Tobacco (*Nicotiana* sp.), cucurbits (Cucurbitaceae) and green bean (*Phaseolus vulgaris*) are among those crops protected in the field against diseases by a prior treatment with the biotic inducers *Peronospora tabacina, Colletotrichum lagenarium, Fusarium oxysporum* f. sp. *niveum* and *C. lindemuthianum*, respectively (e.g. Tuzun et al., 1986; Caruso and Kuc, 1977; Martyn et al., 1991; Sutton, 1982). The abiotic inducer 2,6-dichloroisonicotinic acid (INA) has been shown to induce resistance under field conditions to various fungal and bacterial diseases of pear (*Pyrus* sp.), pepper (*Capsicum* sp.), tobacco and

rice (*Oryza sativa*) (Métraux et al., 1991) and to the rust disease of green brean (Dann and Deverall, 1996).

Extensive greenhouse studies with *Arabidopsis* and tobacco demonstrated that treatment with INA or benzo (1,2,3)-thiadiazole-7-carbothioic S-methyl ester (BTH) induced resistance to fungal, bacterial and viral pathogens with associated increases in the accumulation of mRNAs for pathogenesis-related (PR) proteins (Ward et al., 1991; Uknes et al., 1992; Lawton et al., 1996; Friedrich et al., 1996). In addition, BTH treatment protected wheat against diseases caused by *Erysiphe graminis* f. sp. *tritici*, *Puccinia recondita* and *Septoria* sp. (Görlach et al., 1996).

Despite an increasing number of reports concerning induced resistance in legumes, in particular green bean, there have been few reports concerning the phenomenon in soybean. Localized resistance in *Phytophthora megasperma* was induced in hypocotyls by *P. cactorum* or an avirulent strain of *P. megasperma* (Paxton and Chamberlain, 1967) and was associated with enhanced phytoalexin concentrations (Svoboda and Paxton, 1972). Wrather and Elrod (1990) reported apparent SIR in soybean seedling epicotyls where lesions arising from inoculation with *Colletotrichum truncatum* were significantly smaller than on controls if cotyledons had been injected 24–96 h earlier with conidial suspensions of *C. truncatum, C. lagenarium* or heat-killed *C. lagenarium*.

Sclerotinia sclerotiorum causes devastating soft rot and white mold diseases of a large number of vegetable and non-graminaceous field crops. Its wide host range and abilty to survive many years as sclerotia makes control of this disease particularly difficult (Agrios, 1997). There is one report of apparent localized induced resistance to *S. sclerotiorum* in tobacco (Bonnet et al., 1996). Aqueous droplets of cryptogein or capsicein, elicitins excreted by *Phytophthora* spp., were applied to the fresh wound site of decapitated plants. Two days later the plants were challenge inoculated by placing a mycelial plug of *S. sclerotiorum* at the site of the treated decapitated stem. Resulting stem invasion was approximately 70–90% lower for elicitin-treated plants compared with controls. Elicitin treatment had no effect on the rate of stem colonization by this fungus in rape (*Brassica napus*). While more detailed investigation of this system is necessary the report provided evidence for induced resistance to *Sclerotinia* sp.

In soybean, white mold has caused only localized problems until recently. However, disease outbreaks in Michigan, Ohio, Wisconsin, Minnesota and Ontario, Canada during the past few years have resulted in economic losses (Diers, 1993). The objective of this study was to determine if available abiotic resistance-inducing chemicals were effective in controlling *S. sclerotiorum* on soybean in both the field and greenhouse.

Materials and methods

Field studies

The studies were conducted in the growing seasons of 1993–1996 at the Botany and Plant Pathology research farm, Michigan State University, East Lansing, MI and at a producer's field near Zilwaukee, MI. The agronomic practices were essentially the same for each year. The cultivars Northrup King S19-90, Corsoy 79, Elgin 87 and Williams 82 were planted each year at 444 600 seeds/ha in 7-row plots, 6.1 m long with 17.8 cm between rows. The plots were trimmed to a length of 4.25 m at approximately the R5 growth stage (Fehr et al., 1971), when seeds were beginning to develop in pods at one of the four uppermost nodes with a completely unrolled leaf. Post-emergent herbicides 0.78 kg a.i. ha^{-1} bentazon (Basagran®, BASF Corporation, Research Triangle Park, North Carolina) with 0.21 kg a.i. ha^{-1} acifluorfen (Blazer®, BASF Corporation) plus crop oil concentrate at 2.9 l ha^{-1} were applied in 1993 and 1994 only, and the plots were hand weeded as necessary each year.

In 1990 and 1993 the East Lansing site was inoculated with *S. sclerotiorum* sclerotia collected from cull piles at a dry bean processing plant. The sclerotia were lightly incorporated into the top 3 cm prior to planting. The East Lansing site was overhead irrigated (max. 4 cm/week) to maintain wet canopy conditions during flowering. The Zilwaukee site was naturally infested with the pathogen and was not irrigated. However, it is in an area in which the environment is favorable for infection; low-lying with a high water table and frequent fog.

Chemical treatments

2,6-dichloro-isonicotinic acid (INA, CGA 41396) formulated as 25% active ingredient (a.i.) in a wettable powder and benzo[1, 2, 3] thiadiazole-7-carbothioic acid S-methyl ester, benzothiadiazole (BTH, CGA 245704) formulated as 50% a.i. in wettable granu-

lar form, were obtained from Novartis Crop Protection (formerly Ciba-Geigy Crop Protection), Greensboro, North Carolina, USA. The compounds were prepared at various concentrations in distilled water. Distilled water was applied alone as a control. The treatments were applied with a hand-held CO_2-pressurized sprayer with 1.45 m wide boom, equipped with flat fan type nozzles, delivering a volume of 186.7 l ha^{-1}. Crop injury was assessed after application. In a preliminary study, neither INA nor BTH inhibited growth of *S. sclerotiorum in vitro* at a range of concentrations up to 65 and 70 mg a.i. l^{-1}, respectively.

Experiments were based on a randomized complete block design, with 2 and 5 blocks respectively, at the East Lansing and Zilwaukee sites in 1993, and four blocks at each site in 1994–1996. The 1995 and 1996 experiments were designed as a split plot with cultivars as main plots and treatments as sub-plots. Separate treatments were applied to each plot within each block as outlined below. The plots were harvested for seed yield determination after the pods in all plots were approximately two weeks past the R8 stage. The seed yield is expressed at 13% moisture. Yields were not obtained for the Zilwaukee site in 1993.

Applications of the treatments varied in number and concentration across the four years as outlined below. However, the initial application was always made prior to the commencement of flowering, which is defined as the time when 50% of the plants in the plot were at the R1 growth stage i.e., when one flower was present at any node on a plant (Fehr et al., 1971). The initial application of 2,6-dichloroisonicotinic acid (INA) in 1993 was made on 5 July at either 15, 25 or 35 mg a.i. l^{-1}. Two further applications at the same rates were made 10 and 20 days later. INA was applied in 1994 at 35, 45 or 65 mg a.i. l^{-1} either once on 25 June, three times (additional applications 9 and 20 days later) or four times, which included a final application 33 days after the initial treatment date. INA was applied in 1995 at 50 mg i.a. l^{-1} once or four times. The initial treatment was on 30 June and the three repeat applications at ten day intervals thereafter. Benzothiadiazole (BTH) at 35 mg a.i. l^{-1} was also applied four times on the same dates as INA in 1995. BTH was applied in 1996 at 375 mg a.i. l^{-1} twice or four times. The initial treatment was on 11 July and the other applications were 11, 21 or 32 days later.

Disease assessment and analyses

The trials were assessed for white mold severity between mid to late September at approximately the R7 growth stage, when pods were yellowing and 50% of leaves were yellow (Fehr et al, 1971). Fifty plants in 1993 and thirty plants in 1994–1996 from the center three rows in each plot were rated. Each plant was rated for white mold on a 0–3 scale based on a previously reported method (Grau et al., 1982) where 0 = no symptoms, 1 = lesions on lateral branches only, 2 = lesions on the main stem but little or no effect on pod-fill and 3 = lesions on main stem resulting in plant death and poor pod-fill. A disease severity index (DSI) was calculated for each plot by summing the scores of the fifty or thirty plants, and expressing the value as a percentage using the formula below. A DSI of 0 was given to plots where no disease was present, and 100 to plots where all plants rated were assigned a score of 3. Analysis of variance was performed for each year on yield and DSI data with the program Statistical Analysis System (Cary, North Carolina).

$$\text{DSI} = \frac{\Sigma \text{ (ratings of each plant)}}{\text{number of plants rated} \times 3} \times 100$$

Greenhouse studies

Williams 82 and NKS19-90 seedlings were grown in 13 or 15 cm pots in Baccto® Professional planting mix (Michigan Peat Co., Houston, Texas) in the greenhouse (temperature 18–40 °C, 15 h/day, 80–100 $\mu Em^{-2}s^{-1}$ additional lighting provided by sodium vapor lights). Aqueous solutions of INA at 25 mg a.i. l^{-1} or BTH at 35 mg a.i. l^{-1} were each applied with plastic household sprayers, delivering a fine mist to the leaf surface. Distilled water alone was applied as a control treatment. The treated pots were placed randomly on the greenhouse bench.

The initial applications were made approximately 11–14 days after planting to the expanding unifoliate leaves prior to the emergence of the first trifoliate leaf. Repeat applications to unifoliate leaves were made at various times thereafter as indicated in Figure 1. Challenge inoculations were made by excising 8–10 unifoliate leaves, or terminal leaflets of the first trifoliate per treatment and placing them on moist germination paper in opaque plastic boxes. Mycelial plugs (2.5 mm diameter, 2–3 mm long) were taken from colony margins and placed mycelium-down on the adaxial surface of each leaf or leaflet between major lateral veins. Lids

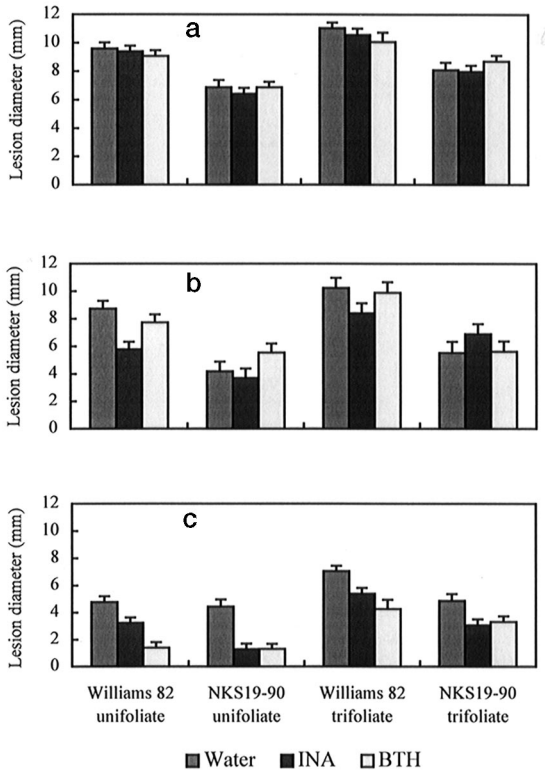

Figure 1. Effect of treating Williams 82 or NKS19-90 seedlings once, twice or five times with 2,6-dichloroisonicotinic acid (INA) or benzothiadiazole (BTH) on lesion diameter in unifoliates and first trifoliate leaflets after challenge with *S. sclerotiorum*. (a) Treatments applied day 0, leaves excised and challenged day 5; (b) Treatments applied days 0 and 2, leaves excised and challenged day 5; (c) Treatments applied days 0, 1, 3, 5 and 7, leaves excised and challenged day 10. INA and BTH were applied at 25 and 35 mg a.i. l^{-1}, respectively; lesion diameters were measured 2 days after challenge inoculation; each figure is based on means of two replicate experiments; bars represent positive standard errors ($P < 0.05$).

were placed on the boxes and boxes were kept on the laboratory bench at 22 °C. Two days later three diameters per resulting lesion were measured with digital calipers. Data were analyzed using the Minitab statistical software (Minitab Inc., State College, Pennsylvania).

Results

Field studies

There were often large differences in the severity of white mold disease and seed yields between each field site and over the four years studied. Disease severity and yield data for water-treated control plots averaged across cultivars are presented for each site and year in Table 1. The disease severity was greater at the irrigated East Lansing site than the Zilwaukee site for each year except 1995, where it is likely that unusually hot, drying winds attributed to the lack of disease. The yields were similar between sites in 1994 and 1995, however yields were greatly suppressed at East Lansing in 1996.

Table 1. Effect of location and growing season (year) on severity of white mold and yields of soybean in water treated controls

Year[3]	DSI[1]		Yield (t ha^{-1})[2]	
	East Lansing	Zilwaukee	East Lansing	Zilwaukee
1993	16.2 ± 0.9	12.6 ± 0.9	3.42 ± 0.1	no data
1994	41.0 ± 2.5	36.7 ± 2.5	2.84 ± 0.1	2.78 ± 0.1
1995	3.1 ± 1.0	15.8 ± 1.9	3.52 ± 0.1	3.53 ± 0.1
1996	48.5 ± 3.5	7.2 ± 2.1	2.85 ± 0.1	3.59 ± 0.1

[1] DSI per plot ± standard errors from water treated control plots averaged across test cultivars.
[2] Yield per plot ± standard errors from water treated plots averaged across test cultivars; expressed at 13% moisture.
[3] Data obtained for 2 or 5 blocks per cultivar at East Lansing or Zilwaukee, respectively in 1993 and 4 blocks per cultivar at each site in 1994–1996; data were not analyzed statistically across years.

There were large differences in the disease severity and yields among the four cultivars for plots which had received no treatment (Tables 2–6). In each year the ranking of the cultivars in increasing severity was NKS19-90, Corsoy 79, Elgin 87 and Williams 82. Conversely, the greatest yields were harvested from NKS19-90, then Corsoy 79 and Elgin 87, which had similar yields, and the lowest yields were from Williams 82. This corresponds with the ranking of the cultivars in previous testing (Diers, 1993). This current study and previous observations suggest that NKS19-90 and Corsoy 79 have partial resistance to the disease.

INA was applied to plots during the 1993–1995 growing seasons. INA at three concentrations or water alone were applied three times in 1993. There was no significant location by treatment interaction so these values are presented across locations. The INA treatments resulted in a significant ($P < 0.05$) reduction in DSI for Elgin 87 and Williams 82 at each rate, but not for Corsoy 79 and NKS19-90 (Table 2). At the East Lansing site yields were greater for Corsoy 79 at each rate of INA and for Williams 82 when data across rates was combined, compared with water controls (Table

Table 2. Effect of 2,6-dichloroisonicotinic acid (INA) treatment on the severity of white mold disease for soybean averaged over both locations in 1993

Treatment[2]	DSI[1]				
	NKS19-90	Corsoy 79	Elgin 87	Williams 82	Mean
Water control	0.3 a	1.8 a	14.5 a	37.9 a	13.6 a
INA 3× 15 mg a.i. l^{-1}	0.3 a	0.9 a	8.7 b	20.9 b	7.7 b
INA 3× 25 mg a.i. l^{-1}	0.3 a	0.6 a	3.6 c	16.6 b	5.3 b
INA 3× 35 mg a.i. l^{-1}	0.0 a	1.1 a	2.7 c	17.7 b	5.5 b
INA mean	0.2 a	0.9 a	5.0 bc	18.4 b	6.2 b

[1] DSI per plot from 7 blocks per treatment per cultivar; within each column, means followed by the same letter are not significantly different ($P < 0.05$).
[2] Initial treatment applied 5 July, with two additional treatments at 10 day intervals thereafter.

Table 3. Effect of 2,6-dichloroisonicotinic acid (INA) treatments on soybean yields at East Lansing in 1993

Treatment[2]	Yield (t ha^{-1})[1]				
	NKS19-90	Corsoy 79	Elgin 87	Williams 82	Mean
Water control	4.04 a	3.29 a	3.55 a	2.78 a	3.42 a
INA 3× 15 mg a.i. l^{-1}	4.44 a	3.96 b	3.84 a	3.24 ab	3.87 b
INA 3× 25 mg a.i. l^{-1}	4.39 a	4.10 b	4.06 a	3.25 ab	3.95 b
INA 3× 35 mg a.i. l^{-1}	4.43 a	4.04 b	3.52 a	3.12 ab	3.78 b
INA mean	4.42 a	4.03 b	3.81 a	3.20 b	3.86 b

[1] Yield per plot from 2 blocks per treatment per cultivar, expressed at 13% moisture; within each column means followed by the same letter are not significantly different ($P < 0.05$).
[2] Initial treatment applied 5 July, with two additional treatments at 10 day intervals thereafter.

3). Yields were not obtained for the Zilwaukee site in 1993.

The number of INA applications and concentrations were varied in 1994. There was no visible phytotoxicity in the 1993 trial thus higher INA rates were tested in 1994. There was no location by treatment effect so these values are presented across locations. Also, there were no differences in DSI or yield among the different INA concentrations tested (35, 45 and 65 mg a.i. l^{-1}) so the data were averaged across concentrations. A significant decrease in severity after treatment was observed only for Williams 82. The plots treated once, three or four times with INA had lower DSIs than water-treated control plots (Table 4) and plots treated three or four times had greater yields than the controls or once-treated plots (Table 5). Surprisingly, disease severity was greater for once-treated Corsoy 79 plots compared with water-treated controls (Table 4).

INA was applied once or four times at a fixed concentration in 1995. There was a significant difference in the severity of disease and yields between locations, so the data were not averaged across locations. The disease severity at East Lansing was very low and there were no differences in disease severity or yields between INA-treated or water controls for any cultivar (results not shown). However, there were treatment effects on disease severity but not yields at the Zilwaukee site. The plots treated four times with INA had less severe disease than once-treated or water control plots and this effect was significant ($P < 0.05$) for Elgin 87 and Williams 82 cultivars (Table 6).

Treatments of BTH were applied in the 1995 and 1996 growing seasons. It was applied four times at a fixed concentration in 1995. As mentioned previously, there was very low disease severity at the East Lansing site in 1995 and there were no differences in DSI or yields among treatments. At the Zilwaukee site in 1995 four applications of BTH resulted in less severe white mold than water-treated control plots for each cultivar, but the effect was significant only for Elgin 87 and Williams 82 (Table 6). BTH treatment had no effect on yields in 1995 (results not shown).

BTH was applied two or four times in 1996 at a tenfold higher concentration than in 1995, based on rec-

Table 4. Effect of 2,6-dichloroisonicotinic acid (INA) treatments on the severity of white mold disease for soybean averaged over both locations in 1994

Treatment[2]	DSI[1]				
	NKS19-90	Corsoy 79	Elgin 87	Williams 82	Mean
Water control	13.8 a	26.5 a	50.0 a	65.0 a	38.8 a
INA 1×	14.5 a	33.8 b	54.3 a	58.5 b	40.3 a
INA 3×	9.1 a	30.5 ab	50.7 a	51.4 b	35.4 a
INA 4×	9.5 a	31.0 ab	45.3 a	49.9 b	33.9 a
INA mean	11.0 a	31.8 ab	50.1 a	53.3 b	36.5 a

[1] DSI per plot from 8 to 24 blocks per cultivar for control or INA treatments respectively; within each column means followed by the same letter are not significantly different ($P < 0.05$).
[2] Initial treatment applied 25 June, with the second, third and fourth applications at 9, 11 and 13 day intervals respectively, thereafter.

Table 5. Effect of 2,6-dichloroisonicotinic acid (INA) treatments on soybean yields averaged over both locations in 1994

Treatment[2]	Yield (t ha^{-1})[1]				
	NKS19-90	Corsoy 79	Elgin 87	Williams 82	Mean
Water control	3.66 a	2.89 a	2.76 a	1.92 a	2.81 a
INA 1×	3.65 a	2.88 a	2.90 a	2.06 a	2.87 a
INA 3×	3.57 a	2.88 a	2.96 a	2.32 b	2.94 a
INA 4×	3.49 a	2.75 a	3.06 a	2.49 b	2.95 a
INA mean	3.57 a	2.84 a	2.97 a	2.29 b	2.92 a

[1] Yield per plot from 8 to 24 blocks per cultivar for control or INA treatments respectively, expressed at 13% moisture; within each column means followed by the same letter are not significantly different ($P < 0.05$).
[2] Initial treatment applied 25 June, with the second, third and fourth applications at 9, 11 and 13 day intervals respectively, thereafter.

ommendations by Ciba Geigy. As in 1995 there were large differences in disease severity between locations, with the most severe disease occurring at the East Lansing site. The DSI for water controls for East Lansing and Zilwaukee were 48.5 and 7.2, respectively, and the corresponding yields were 2.85 an 3.59 t ha^{-1}, respectively. Two or four applications of BTH resulted in similar disease severity and yields compared with water controls for each cultivar (results not shown).

Greenhouse studies

Two days after challenge inoculation of excised leaves and leaflets with *S. sclerotiorum*, soft brown lesions had developed and each were uniform in diameter except where the fungus had ramified and rotted along a lateral vein. Lesion diameters on unifoliate and trifoliate leaves of Williams 82 were significantly larger ($P < 0.05$) than on corresponding leaves of NKS19-90 (Figure 1a, b), except for unifoliates treated five times, where there was no difference in lesion size between the two tested cultivars (Figure 1c). Also, lesions were significantly larger on the first trifoliate leaflets than on unifoliate leaves of the same cultivar. There were no significant differences in lesion diameters among treatments after one or two treatments (Figures 1a, b). Lesion diameters were significantly smaller ($P <0.05$) after five applications of INA or BTH, compared with water-treated controls for each cultivar and leaf type (Figure 1c).

Discussion

Severity of white mold disease in field-grown soybeans was significantly reduced by sprays of INA (2,6-dichloroisonicotinic acid) in two of three years of testing. Applications of BTH (benzo[1,2,3] thiadiazole-7-

Table 6. Effect of 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) treatment on disease severity at Zilwaukee in 1995

Treatment[2]	DSI[1]				
	NKS19-90	Corsoy 79	Elgin 87	Williams 82	Mean
Water control	3.6 a	7.2	25.8 a	26.4 a	15.8 a
INA 1× 50 mg a.i. l^{-1}	5.6 a	8.6 a	25.8 a	20.8 ab	15.2 a
INA 4× 50 mg a.i. l^{-1}	0.8 a	3.6 a	11.4 b	13.6 b	7.4 b
BTH 4× 35 mg a.i. l^{-1}	0.8 a	0.8 a	14.2 b	19.5 ab	8.8 b

[1] DSI per plot from 4 blocks per cultivar per treatment; within each column means followed by the same letter are not significantly different ($P < 0.05$).
[2] Initial treatment applied 30 June, with the second, third and fourth application at 10 day intervals respectively thereafter.

carbothioic acid S-methyl ester) significantly reduced white mold disease in one of two years of testing. Applications of the compounds to greenhouse-grown plants also resulted in smaller lesions in treated and systemic leaves. The compounds have no direct antimicrobial activity against many fungal and bacterial pathogens, including *S. sclerotiorum*. Both compounds have been shown to induce disease resistance in a number of plants including another legume, green bean (Dann and Deverall, 1995; Ciba Geigy, 1995), against a broad range of pathogens. We suggest, therefore, that in soybean INA and BTH treatments may stimulate inherent defense mechanisms so that the plant can respond more quickly against the invading, colonizing fungus.

In many plants investigated so far, INA and BTH treatment is associated with increases in activities of many classes of pathogenesis-related (PR) proteins. It is thought the INA and BTH compounds most likely act as artificial endogenous signals in the plant, inducing such defense mechanisms as they are rapidly translocated from the site(s) of application. As so little is known of the soybean/*Sclerotinia* sp. interaction, it is difficult to speculate which aspects of the resistance mechanism are activated and important in the containment of this necrotrophic fungus.

Multiple INA or BTH applications were necessary in both field and greenhouse studies to observe the decrease in disease severity. This corroborates a report in glasshouse-grown sugar beets, where fewer than 3 applications of INA were ineffective in inducing resistance against *Cercospora beticola* (Nielsen et al., 1994). Similarly, repeated BTH applications to tobacco and other vegetable crops, including green bean, were necessary to induce resistance (Kessmann et al., 1995; Ruess et al., 1995). This differs from other studies, however, which demonstrate that one application is sufficient to induce significant disease resistance, for example, field-grown green bean could be protected throughout the growing season against rust disease caused by *Uromyces appendiculatus*, after only one INA spray at the seedling stage (Dann and Deverall, 1996). Similarly, Métraux and co-workers (Métraux et al., 1991) demonstrated that single foliar sprays of INA protected field-grown pear, pepper and tobacco against *Erwinia amylovora*, *Xanthomonas vesicatoria* and *Peronospora tabacina*, respectively. In addition, application of INA to field water also protected rice against *Pyricularia oryzae* and *Xanthomonas oryzae*. Single BTH applications to wheat and rice provided long-lasting protection against fungal diseases (Görlach et al., 1996). It is not known why multiple applications of BTH are necessary to induce and maintain disease resistance in the dicots tested. These observations highlight the need to examine and evaluate each activator/host/pathogen interaction separately.

The INA and BTH treatments were particularly effective in Williams 82 and Elgin 87 which were more susceptible to the disease than Corsoy 79 and NKS19-90. It is likely that resistance mechanisms were activated more strongly in these cultivars by the INA and BTH treatments which contributed to the enhanced resistance. It is conceivable that the avoidance and/or defense mechanisms of the more resistant varieties, NKS19-90 and Corsoy 79, are such that they already resist infection and colonization to a high degree, thus any further enhancement of physiological resistance by the chemicals is not as readily observed. This is an area requiring further detailed investigation.

Induced pest and disease resistance has great potential in crop protection strategies for the majority of horticultural and broad-acre crop species. BTH has recent-

ly been registered and released by Novartis Crop Protection in Germany, under the name 'Bion®' for use as a resistance activator, to help control powdery mildew disease on wheat. It is pertinent to note that chemical activators may have limitations, for example being most effective for specific plant/pathogen interactions, or causing phytotoxic symptoms on some plants. However, the formulation of chemical activators for use in combination with existing crop protection practices is conceivably the most logical and cost-effective means to introduce the induced resistance phenomenon into commercial production situations.

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