

## Effect of 2,3,5-Triiodobenzoic Acid on the Susceptibility of Soybeans to *Macrophomina phaseolina*

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### ABSTRACT

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The growth regulator 2,3,5-triiodobenzoic acid (TIBA) was tested for its effects on the susceptibility of the soybean cultivars Amsoy 71 and Wayne to disease incited by *Macrophomina phaseolina*. Foliarly applied TIBA, 70 g/ha, at flowering decreased microsclerotial development on Amsoy 71 and changed the pattern of vessel elements. Vessel elements were produced only during early and late season growth. TIBA was directly fungitoxic to *M. phaseolina* at 100 µg/ml Czapek Dox broth medium. When the organism was cultured on TIBA-treated soybean root and stem tissue agar media or on untreated tissue agar media amended with TIBA, no direct or induced fungitoxic response was noted.

The growth regulator 2,3,5-triiodobenzoic acid (TIBA) has been used in attempts to decrease lodging and increase yields in *Glycine max* (L.) Merrill (4). It also decreases the severity of charcoal rot of soybean (7), Fusarium wilt of tomato (1), oak wilt (3), and susceptibility of

strawberry to Phytophthora crown rot (6).

The objective of this investigation was to determine if TIBA-induced changes in the susceptibility of soybeans to *Macrophomina phaseolina* (Tassi) Goid. as noted by Oswald and Wyllie (7) were due to morphologic changes in the plant or to a fungitoxic effect of TIBA.

### MATERIALS AND METHODS

For the field study, a solution was prepared to provide the equivalent of 70 g

of TIBA per hectare by dissolving 0.582 g of TIBA (98% pure) in 20 ml of 95% ethanol, bringing the volume to 1.3 L with distilled water, adjusting the pH to 7.0 with 0.1N KOH, and adding 0.65 ml of Tween 20. A stock solution used in the in vitro studies was prepared similarly and provided a concentration of 1,000 µg of TIBA per milliliter of 1% ethanol (v/v).

The field plots (sandy clay loam soil) at the Plant Pathology Research Center (Blacksburg, VA) were arranged in a split plot design. *M. phaseolina* inoculated or uninoculated treatments were the main plots, and TIBA-treated or untreated plants were the subplots. Two soybean cultivars were randomized in each subplot. Three replicates, consisting of 0.75 m long rows (15 plants per row) with 0.9 m between rows, were used. Amsoy 71 and Wayne were seeded on 2 June 1978, one seed every 5 cm to a depth of about 4 cm.

The *M. phaseolina* culture was obtained from Dr. J. B. Sinclair (University of Illinois). Inoculum was grown in 250 µl

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of potato-dextrose broth at room temperature (19–22 C) for 14 days. The broth was decanted; the mycelial mat was ground in a blender for 1 min and then diluted to three times its original volume.

Around each row, a furrow was made within 4 cm of the base of the plants and approximately 3 cm deep. The soil was infested on 4 July and 20 July 1978 by dispensing 400 ml of the diluted inoculum into each furrow and working the soil with a hoe.

On 15 and 22 July 1978, the subplots were sprayed with the TIBA solution by using a backpack sprayer calibrated to deliver 230 L/ha. At that time, 5–10% of the Amsoy 71 flowers and 40–60% of the Wayne flowers had emerged.

Mature plants were harvested between 23 and 27 September 1978. Ten plants from each replicate were evaluated. The percent microsclerotial formation was calculated by dividing the length of *M. phaseolina* microsclerotial development

on the stem exterior by the total stem height.

Ten TIBA-treated and 10 untreated plants taken from the uninoculated plots were fixed in formalin acetic acid; a free hand cross section was taken 30–50 cm above the soil line and stained in safranin. The radius length of each stem cross section was divided into four equal measurements sequentially from the pith outward, and the average number of vessels per micrometer for each division was determined.

The growth of *M. phaseolina* on TIBA-amended medium was determined. Ten replicates of each concentration of 0.0 (containing ethanol) 0.1, 10.0, and 100 µg of TIBA per milliliter of Czapek Dox broth (CDB) were seeded with *M. phaseolina* mycelia. After 14-day growth in the dark as standing cultures at 28 C, the mycelium was harvested and dry weights were determined (5). This experiment was conducted twice.

The in vitro fungitoxicity of TIBA-treated soybean tissues was determined in Amsoy 71 plants. Plants were grown in a greenhouse and were sprayed either with 120 ml of a 1:10 dilution of the TIBA stock solution or with a 0.05% Tween 20-distilled water solution when the flowers began to emerge. One week later, the stems and roots were harvested, separated, frozen in a methanol-ice mixture, and weighed. Tissues were freeze-dried and ground in a Wiley mill to pass through a 60-mesh screen.

The ground tissues from the TIBA-treated and one-half of the Tween 20-treated plants were mixed with molten water agar to yield 1:1 (w/w) fresh tissue/water agar media. The remaining Tween 20-treated ground tissues were each mixed with an agar solution containing 1.2 mg of TIBA. The media were poured into petri plates, chemically sterilized with propylene oxide, and seeded with the fungus (5). Six replications of the stems and three replicates of the roots were incubated at 20 C, and radial growth measurements were recorded after 5 days. This experiment was conducted twice.

The data were analyzed for statistical significance at the  $P=0.05$  level using the two group t test, analysis of variance, or least significant range procedures.

## RESULTS

The extent of *M. phaseolina* microsclerotial formation was significantly reduced in TIBA-treated Amsoy 71 plants but not in TIBA-treated Wayne plants (Table 1). There was no *M. phaseolina* microsclerotial formation on the control plants.

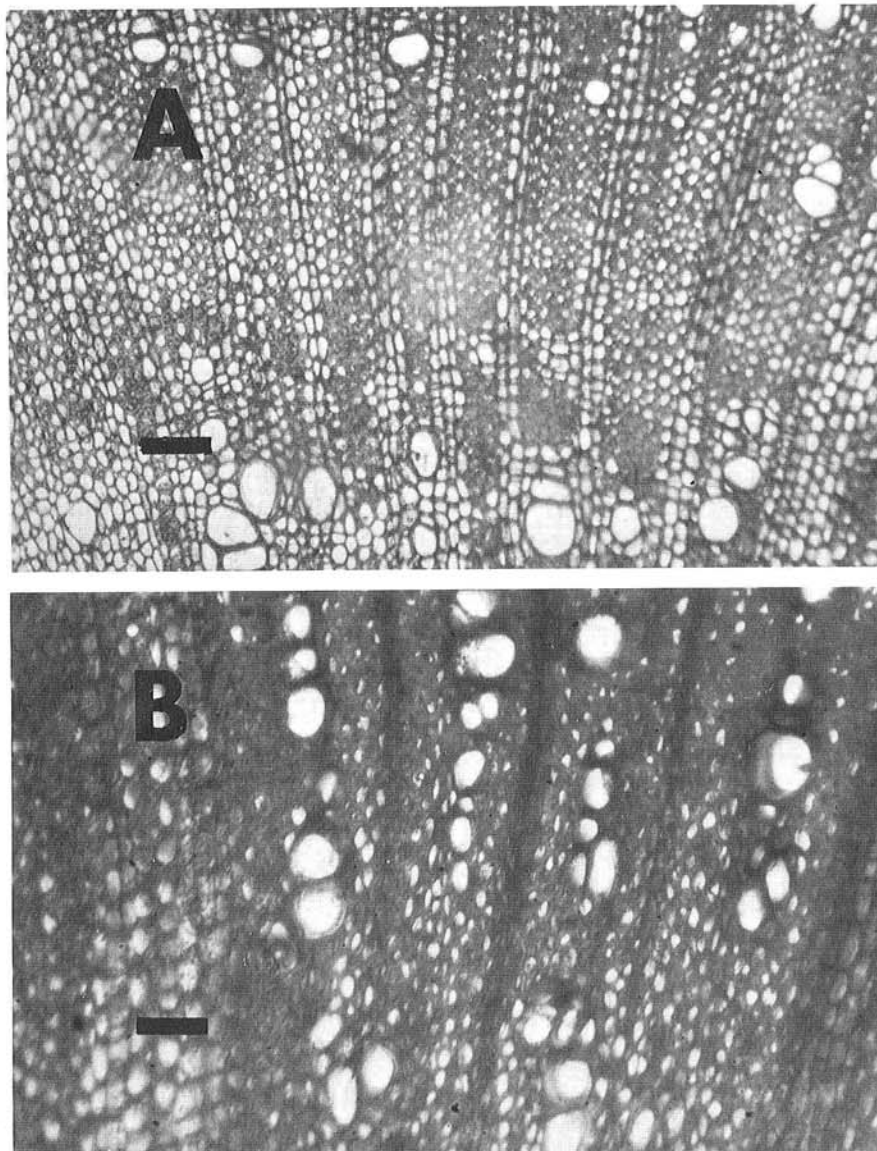
TIBA treatment of Amsoy 71 plants (but not Wayne plants) was associated with a changed pattern of vessel elements. Vessel elements were produced only during the early and late season growth but not during the midseason (Fig. 1A).

**Table 1.** Effect of 2,3,5-triiodobenzoic acid on *Macrophomina phaseolina* microsclerotial development on Amsoy 71 and Wayne soybeans

	Percent microsclerotial development <sup>y</sup>			
	Amsoy 71		Wayne	
	Untreated	Treated <sup>z</sup>	Untreated	Treated
<i>M. phaseolina</i>	33 ± 12 a	18 ± 16 b	21 ± 16 a	25 ± 22 a
Uninoculated control	0	0	0	0

<sup>y</sup> For each cultivar, numbers in a row followed by the same letter are not significantly different from each other at the  $P=0.05$  level. Each value is an average of 10 observations.

<sup>z</sup> 70 g/ha.



**Fig. 1.** Pattern of vessel elements in cross sections of soybean stems: (A) treated with 2,3,5-triiodobenzoic acid or (B) untreated. Scale bars represent 0.14 mm.

This change in TIBA-treated stems resulted in a significant decrease in the number of vessel elements per micrometer in the second radial division for the Amsoy 71 plants. In untreated and in TIBA-treated Wayne plants, vessel elements were evenly distributed within the radii because of their continuous production (Fig. 1B).

TIBA at 100  $\mu\text{g}/\text{ml}$  of CDB caused an 82% (significant at  $P = 0.05$ ) reduction in the growth of *M. phaseolina* compared with growth on the control medium. TIBA at 10  $\mu\text{g}/\text{ml}$  of CDB had no effect on the growth of the organism.

Growth of *M. phaseolina* was not significantly altered on the media made from TIBA-treated stem or root tissues or on the Tween 20-treated stem or root tissue media amended with TIBA.

## DISCUSSION

The resulting decrease in colonization of TIBA-treated Amsoy 71 by *M. phaseolina* was consistent with the results from an investigation by Oswald and Wyllie (7). The lack of control of *M. phaseolina* on TIBA-treated Wayne may be explained by a decrease in TIBA activity, because TIBA is less effective on plants with more than 10% flower emergence (4). Approximately 60% of the flowers on Wayne plants had emerged at the time of treatment.

The results of this investigation indicate that fungal colonization in Amsoy 71 may be impeded by a change in the pattern of vessel elements. Similarly, the unaltered stem anatomy in the Wayne plants may explain why TIBA was not effective in controlling the extent of microsclerotial development in that cultivar. This reasoning is supported by Pomerleau's (8) observation (of *Ceratocystis ulmi* colonization of elm) that lack of adjacent vessels impeded fungal movement within vascular cylinders. Similarly, plant species with a diffuse porous anatomy and adjacent vessels are more susceptible to vessel-colonizing fungi than are plant species with a ring porous anatomy (2). The altered arrangement of elements in Amsoy 71 caused by TIBA treatment simulates a ring porous anatomy.

There is no indication that *M. phaseolina* colonization is controlled by a direct or induced fungitoxic response. TIBA was fungitoxic in the in vitro study but only at the relatively high concentration of 100  $\mu\text{g}/\text{ml}$ . In addition, no reduction in growth was noted when *M. phaseolina* was grown on TIBA-treated or TIBA-amended untreated stem and root tissue media even though TIBA was applied or added, respectively, at a rate comparable to that which provided disease control in the field.

Even though *M. phaseolina* colonization was impeded by TIBA application, it is questionable whether its use would be feasible under agricultural conditions. Nevertheless, this investigation provides insight on the possibility of controlling vascular colonizing fungi by use of this class of growth regulating compound.

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