# Effect of Temperature and Soybean Cultivar on Metalaxyl Efficacy Against *Phytophthora megasperma* f. sp. *glycinea*

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**ABSTRACT**

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Four soybean cultivars, treated or not treated with metalaxyl, were evaluated at 24, 28, and 32 C for reaction to two isolates of race 3 of *Phytophthora megasperma* f. sp. *glycinea*. In addition, metalaxyl was evaluated at rates of 0, 0.05, 0.25, 0.60, and 1.20 μg/ml at 24, 28, and 32 C. Internal stem discoloration was measured acropetally from the point of inoculation and was positively correlated ($r = 0.90$) with plant mortality. Lesions were longest at 32 C and shortest at 24 C for all cultivars and rates of metalaxyl. Regression analysis of metalaxyl concentration vs. lesion length showed a difference in intercepts, but not slopes, with the highest intercept at 32 C and the lowest at 24 C. Metalaxyl was effective in reducing lesion length at all temperatures, in all cultivars, and with both pathogen isolates. However, greater amounts of metalaxyl were needed to reduce disease severity at 32 C than at 24 and 28 C. The $Rps_6$ resistance gene expressed an incompatible reaction at 24 C but a compatible reaction at 32 C.

Additional keywords: disease evaluation, disease resistance, *Glycine max*, Phytophthora root and stem rot

Phytophthora root and stem rot of soybean (*Glycine max* (L.) Merr.), caused by *Phytophthora megasperma* Drechs. f. sp. *glycinea* Kuan & Erwin, is a disease of international importance (7). Host resistance is the foundation for management programs to control Phytophthora root and stem rot (4,7). However, the expression of resistance genes can be modified by races of *P. m.* f. sp. *glycinea* (7) and by temperature (2,4,5,14). Several genes that confer resistance to specific races of *P. m.* f. sp. *glycinea* at 24 C are defeated by these same races at 32 C (4,5,13,14). High concentrations of the phytoalexin glyceollin produced in soybean tissue are associated with incompatible reactions to *P. m.* f. sp. *glycinea* (16), and lower concentrations of glyceollin at 32 C are associated with changes from incompatible to compatible reactions to *P. m.* f. sp. *glycinea* (2,14).

The systemic fungicide metalaxyl, used as a seed dressing or soil application, is effective in reducing losses of stand and yield caused by Phytophthora root and stem rot (1,4,7). Metalaxyl has a direct fungistatic effect on members of the Peronosporales by inhibiting RNA and protein synthesis (3). Ward et al (15) reported higher glyceollin concentrations in soybean plants treated with metalaxyl and inoculated with compatible isolates of *P. m.* f. sp. *glycinea*. Glyphosate treatment of soybean hypocotyls resulted in less protection against *P. m.* f. sp. *glycinea* by metalaxyl and lower glyceollin levels by interfering with its synthesis (12). These findings suggest that metalaxyl acts as a fungistat and as an elicitor of host defense mechanisms at marginally inhibitory metalaxyl concentrations (12).

The effect of temperature on the efficacy of metalaxyl against *P. m.* f. sp. *glycinea* has not been reported. The objectives of this study were to determine the effect of temperature on the expression of resistance genes against *P. m.* f. sp. *glycinea* in the presence or absence of metalaxyl and to determine the relationship between temperature and metalaxyl concentration for the control of *P. m.* f. sp. *glycinea*.

## MATERIALS AND METHODS

**Cultivars and planting.** Soybean cultivars were selected on the basis of genes that confer resistance to specific races of *P. m.* f. sp. *glycinea* and levels of rate-reducing resistance (tolerance) expressed in field environments (11). Cultivars (resistance gene) selected for study were Agripro AP200 ($Rps_1{}^a$), Spansoy SP324 ($Rps_1{}^a$), Century 84 ($Rps_1{}^k$ and $Rps_1{}^h$), and Land-O-Lakes LL1771 ($Rps_6$). Previous field obser-

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vations indicated that SP324 had greater rate-reducing resistance (tolerance) to *P. m.* f. sp. *glycinea* than did AP200. Seed were of commercial quality and obtained directly from seed companies or, for Century 84, as Wisconsin certified seed. Vigorous 2-day-old germlings with 1- to 3-cm radicles were planted in potting mix (Jiffy-Mix) in 11.5-cm-diameter plastic pots, four germlings per pot. The pots were then placed in a controlled-environment chamber at 24, 28, or 32 C with 12 hr of alternating dark and light (275 $\mu E \cdot m^{-2} \cdot s^{-1}$) produced by fluorescent and incandescent light and 50% relative humidity. Plants were watered daily without leaching by adding water to containers below each pot; no visible water stress occurred.

**Metalaxyl treatment.** For the cultivar study, metalaxyl was applied as a soil drench at planting to give a 1.2 $\mu$g/ml concentration based on soil volume. This approximates a banded application rate of 0.28 kg/ha of metalaxyl (currently labeled for field use in soybeans) to a volume of soil 10 cm wide by 12 cm deep along rows on 76-cm centers. Metalaxyl concentrations of 0, 0.05, 0.25, 0.60, and 1.20 $\mu$g/ml were used for the rate experiment.

**Inoculum and inoculation procedures.** Two race 3 isolates of *P. m.* f. sp. *glycinea* (isolates 1 and 2) were used in all experiments. To produce zoospores, *P. m.* f. sp. *glycinea* isolates were grown on V-8 juice agar for 8–10 days at room temperature. Twenty 5-mm-diameter disks were cut with a No. 2 cork borer from the colony margin, placed in 25 ml of sterile 1% soil extract (1 g of soil in 100 ml of $H_2O$) in 9-cm-diameter petri dishes, and incubated at 21 C for 18 hr (9). Zoospore concentration was adjusted to $1.5 \times 10^4$ spores per milliliter using a soil extract suspension. A technique similar to that described by Schwenk et al (8) was used to inoculate soybean plants at the V1 stage (one unfolded trifoliate leaf, 7–10 days after planting) using a Hamilton micropipet (Reno, NV) with a 22-gauge needle. The needle was punctured through the hypocotyl 1 cm below the cotyledon attachment. One 5-$\mu$l drop of zoospore suspension was deposited at both wound surfaces as the needle was pulled back through the hypocotyl to deliver 150 zoospores per plant. Three plants per pot were inoculated, the fourth was punctured with a sterile needle for comparison, and all were returned to a controlled environment chamber set at 24, 28, or 32 C.

**Disease assessment.** Plants were assayed for disease reaction 7–9 days after inoculation, at the V3 stage (three unfolded trifoliate leaves). The three inoculated plants per pot were rated for mortality, and their stems were split to measure brown discoloration inside the stem acropetally from the point of inoculation. (Basipetal measurement was not used because of cultivar difference in point of cotyledon attachment, which could vary the amount of potentially affected tissue.) The uninoculated plant in each pot was assayed for comparison, but no discoloration was noted except for injury at the point of inoculation.

**Statistics.** Four replicate pots were used per experiment and experiments were repeated once. Similarity in results between experiments allowed combining data for analyses of variance and regression. Treatment means were compared using Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

## RESULTS AND DISCUSSION

**Cultivars and temperature.** Lesion length was significantly greater at 32 C than at 24 C for all treatments except for Century 84 treated with metalaxyl (Table 1). Lesion length at 28 C was usually greater than that at 24 C but was not statistically significant except in the case of SP324 treated with metalaxyl and inoculated with isolate 1. The greatest difference in lesion length among temperatures occurred for intermediate reactions, because lesion length was not limited by plant death. Differences among soybean cultivars for reaction to *P. m.* f. sp. *glycinea* were more evident at 24 and 28 C than at 32 C. These results agree with those of Keeling (5), who noted that the greatest increase of susceptible plants occurred between 27 and 32 C, and Grau (4), who found more cultivars changed from a resistant to a susceptible phenotype when temperature was increased from 28 to 32 C than when it was increased from 24 to 28 C. Plant mortality was 100% for AP200 and SP324 inoculated with isolate 1 at all temperatures. On the basis of plant mortality, the *Rps*$_6$ resistance gene present in LL1771 was defeated by isolate 1 at 32 C. A similar trend was observed for isolate 2, but seedling mortality was only 38% at 32 C. These findings agree with those of Ward and Lazarovits (14) and Keeling (5).

**Metalaxyl, temperature, and cultivar.** Lesion length was significantly reduced by metalaxyl treatment for all cultivars and temperatures if at least 12 mm of lesion developed for plants not treated with metalaxyl (Table 1). Although metalaxyl suppressed lesion length at all temperatures, lesion length was still greatest at 32 C for all cultivars and both *P. m.* f. sp. *glycinea* isolates. The proportional reduction of lesion length by metalaxyl was 90, 84, and 52% for AP200 and 96, 73, and 61% for SP324 incubated at 24, 28, and 32 C, respectively. In contrast, lesion length was reduced 88, 99, and 81% for LL1771 and 100, 93, and 98% for Century 84 at 24, 28, and 32 C, respectively. A similar response was observed for plant mortality for each of these cultivars. Metalaxyl was more effective in reducing lesion length at 32 C when applied to cultivars that expressed a resistant reaction at 24 C. Across all temperatures, lesion lengths for metalaxyl-treated AP200 and SP324 (susceptible cultivars) were similar to lesion

**Table 1.** Soybean lesion length and plant mortality resulting from hypocotyl inoculation with two isolates of *Phytophthora megasperma* f. sp. *glycinea* (race 3) on four cultivars at three temperatures, with and without metalaxyl

| | *P. m.* f. sp. *glycinea* isolate 1 | | | | *P. m.* f. sp. *glycinea* isolate 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | No metalaxyl | | Metalaxyl, 1.2 $\mu$g/ml[w] | | No metalaxyl | | Metalaxyl, 1.2 $\mu$g/ml | |
| Temp. (C) | Lesion[x] (mm) | Mortality[y] (%) | Lesion (mm) | Mortality (%) | Lesion (mm) | Mortality (%) | Lesion (mm) | Mortality (%) |
| | | | | AP200 | | | | |
| 24 | 57 b[z] | 100 a | 6 d | 0 c | 31 b | 54 b | 3 e | 0 d |
| 28 | 62 ab | 100 a | 10 d | 0 c | 22 cb | 33 c | 5 de | 0 d |
| 32 | 73 a | 100 a | 35 c | 33 b | 59 a | 100 a | 18 cd | 0 d |
| | | | | SP324 | | | | |
| 24 | 53 b | 100 a | 2 e | 0 c | 21 bc | 38 b | 1 d | 0 c |
| 28 | 61 b | 100 a | 14 d | 0 c | 29 b | 83 a | 4 d | 0 c |
| 32 | 75 a | 100 a | 29 c | 13 b | 60 a | 92 a | 19 c | 0 c |
| | | | | LL1771 | | | | |
| 24 | 17 b | 0 c | 2 c | 0 c | 3 bc | 0 b | 2 bc | 0 b |
| 28 | 16 b | 25 b | 1 c | 0 c | 3 bc | 0 b | 1 bc | 0 b |
| 32 | 67 a | 96 a | 13 b | 0 c | 24 a | 38 a | 6 b | 0 b |
| | | | | Century 84 | | | | |
| 24 | 12 b | 0 b | 0 c | 0 b | 0 b | 0 a | 0 b | 0 a |
| 28 | 15 b | 0 b | 1 c | 0 b | 0 b | 0 a | 0 b | 0 a |
| 32 | 43 a | 13 a | 1 c | 0 b | 12 a | 0 a | 1 b | 0 a |

[w] Concentration of metalaxyl (a.i.) applied to constant potting mix volume.
[x] Length of internal stem discoloration measured acropetally from point of inoculation; observations are mean values from 24 plants.
[y] Reactions of 24 plants.
[z] Observations followed by the same letter within a cultivar and *P. m.* f. sp. *glycinea* isolate group are not different for each disease measurement according to Fisher's least significant difference ($P \leq 0.05$).

lengths for Century 84 and LL1771 (resistant cultivars) without metalaxyl. This indicates that 1.2 μg/ml of metalaxyl can provide protection similar to the resistant genes in Century 84 and LL1771 9 days after inoculation under these experimental conditions. These results agree with field results where high metalaxyl rates were needed for highly susceptible cultivars to have control equivalent to that with low rates on tolerant cultivars (1,10).

**Metalaxyl concentration.** As metalaxyl concentration increased, stem lesion length decreased for cultivar AP200 (Fig. 1). Multiple regression comparisons between temperature and metalaxyl concentration resulted in different intercepts, but not slopes ($P = 0.001$). Thus, temperature modified disease severity caused by *P. m.* f. sp. *glycinea* but did not influence the efficacy of metalaxyl. Regression comparisons between isolates also resulted in a difference between intercepts, but not slopes ($P = 0.001$). Coefficients of determination for all regression lines were significant ($P = 0.01$), and averages across temperature were $r^2 = 0.97$ and $r^2 = 0.92$ for isolates 1 and 2, respectively. These results indicate that over this concentration range, each increment of metalaxyl produced a corresponding increment of reduction in lesion length. We conclude from these data, however, that because of a reduced host response to *P. m.* f. sp. *glycinea*, a greater amount of metalaxyl is required to restrict lesion length at 32 C. The need for more metalaxyl at 32 C may be the result of less glyceollin production at this temperature (2,14).

**Comparison of disease measurements.** Evaluating soybean reaction to *P. m.* f. sp. *glycinea* inoculation by measuring the acropetal extent in internal stem discoloration (lesion length) correlated ($r = 0.90$) with plant mortality ($P = 0.001$) (Table 1). The smallest mean lesion length (15 mm) associated with dead plants was at 28 C for LL1771 with *P. m.* f. sp. *glycinea* isolate 1. All other mortality values matched with correspondingly greater lesion lengths except for Century 84 at 32 C with isolate 1, which had only 13% mortality and 42.7 mm of lesion length. This might indicate that Century 84 was tolerant to infection, as is reported in field observations (4). Resistance and susceptibility have traditionally been evaluated by plant mortality. The measurement of internal stem symptoms made a more accurate disease assessment possible in this study. The measurement of internal stem lesion length caused by *P. m.* f. sp. *glycinea* can be useful for breeding for resistance, race identification, and fungicide efficacy studies.

From these studies we conclude that: 1) high temperature (32 C) increased disease severity regardless of resistant genes, *P. m.* f. sp. *glycinea* isolate, or metalaxyl treatment; 2) metalaxyl reduced plant mortality and the extent of internal stem necrosis at all temperatures and for all cultivars used; and 3) reduction in disease severity was positively correlated with metalaxyl concentration.

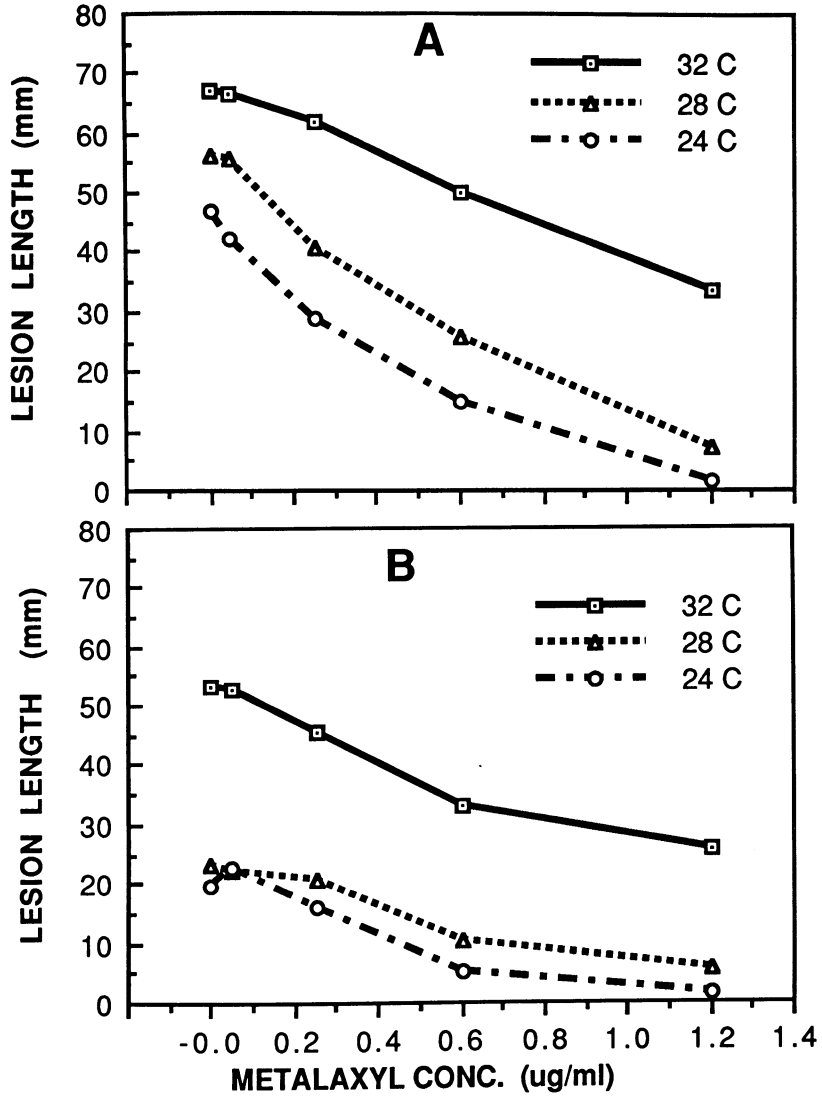


**Fig. 1.** Effect of metalaxyl concentration on internal lesion length and temperature of soybean stems (cv. Agripro AP200) inoculated with **(A)** isolate 1 and **(B)** isolate 2 of *Phytophthora megasperma* f. sp. *glycinea* (race 3). Regression lines (not shown) are different for intercept, but not for slope, for temperature and isolate comparisons ($P = 0.05$).

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