Phenylalanine ammonia-lyase activity in soybean hypocotyls and leaves following infection with *Phytophthora megasperma* f.sp. *glycinea*

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Phenylalanine ammonia-lyase activity increased rapidly beginning 2 h after inoculation with *Phytophthora megasperma* (Drechs.) f.sp. *glycinea* (Hildebr.) Kuan & Erwin race 1 in unwounded hypocotyls of soybean cv. Harosoy 63 (resistant) but did not change significantly in cv. Harosoy (susceptible). Small increases in phenylalanine ammonia-lyase activity also were caused by wounding. Activity increased more slowly in hypocotyls (cv. Harosoy 63) wounded just before inoculation than in intact inoculated hypocotyls, but most activity developed in hypocotyls wounded 12 h before inoculation. There were comparable effects of wounding on symptom development. Trifoliate leaves of 14-day-old cv. Harosoy 63 plants are resistant, but trifoliate leaves of 12-day-old cv. Harosoy 63 plants and of 14-day-old cv. Harosoy plants are susceptible to race 1. Increases in phenylalanine ammonia-lyase activity following inoculation were demonstrated only in 14-day-old Harosoy 63 plants but not until 24–36 h after the inoculation. Significant accumulation of glyceollin occurred by 24 h. Susceptible trifoliate leaves of 12-day-old cv. Harosoy 63 plants produced only low levels of glyceollin following either infection or treatment with the abiotic elicitor AgNO₃, whereas trifoliate leaves of 14-day-old cv. Harosoy plants produced high levels of glyceollin in response to AgNO₃. It is concluded that trifoliate leaves of 12-day-old, as opposed to 14-day-old, cv. Harosoy 63 plants have not developed mechanisms that trigger responses to either infection or the abiotic elicitor or they are deficient in metabolic processes that support glyceollin biosynthesis or other defense-related responses.


L’activité de l’ammonium phénylalanine lyase augmente rapidement à partir de la 2e h après l’inoculation avec le *Phytophthora megasperma* (Drechs.) f.sp. *glycinea* (Hildebr.) Kuan & Erwin race 1 sur hypocotyle de soja non blessé du cv. Harosoy 63 (résistant); cependant, cette activité ne change pas significativement sur le cv. Harosoy (susceptible). La blessure occasionne également une faible augmentation de l’activité de l’ammonium phénylalanine lyase. L’activité augmente plus lentement chez les hypocotyles qui sont blessés juste avant l’inoculation (cv. Harosoy 63) que chez les hypocotyles intact au moment de l’inoculation, mais l’activité la plus élevée apparait chez les hypocotyles blessés 12 h avant l’inoculation. On retrouve des effets comparables de la blessure sur le développement des symptômes. Les feuilles du cv. Harosoy 63 agées de 14 jours sont résistantes mais celles de 12 jours de ce même cultivar ainsi que celles du cv. Harosoy agés de 14 jours sont susceptibles à la race 1. L’augmentation de l’activité de l’ammonium phénylalanine lyase suite à l’inoculation n’a été démontrée que chez les plants du cv. Harosoy 63 agés de 14 jours et pas avant 24–36 h. On note une augmentation significative de la glyceolline autour de 24 h. Les feuilles susceptibles agées de 12 jours du cv. Harosoy 63 ne produisent que de faibles quantités de glyceolline à la suite soit de l’infection soit d’un traitement avec l’élécuteur abiotique AgNO₃, alors que les feuilles de plants agées de 14 jours du cv. Harosoy produisent de grandes quantités de glyceolline lorsqu’elles sont traitées avec AgNO₃. Les auteurs concluent que les feuilles de plants du cv. Harosoy 63 agés de 12 jours, contrairement à celles de plants agés de 14 jours, ne possèdent pas les mécanismes qui déclenchent les réactions soit à l’infection soit à l’élécuteur abiotique, ou encore que ces plants ne possèdent pas les processus métaboliques qui supportent la biosynthèse de la glyceolline ou d’autres réactions relatives à la défense.

Introduction

The association of phenylalanine ammonia-lyase (PAL) activity with the accumulation of phytoalexins was reported by Hadwiger (1967) for peas and by Rahe et al. (1969) for beans. The enzyme catalyzes the first step of the phenylpropanoid pathway and hence it would be expected that activity should be correlated with production of isoflavonoid phytoalexins in incompatible host—pathogen interactions. This has been found to the the case for elicitor-treated cell suspensions of beans and soybeans (e.g., Ebel et al. 1976; Dixon and Bendall 1978; Dixon and Lamb 1979; Lawton et al. 1983; Ebel et al. 1984; Cramer et al. 1985; Robbins et al. 1985). Results with whole plants have been less consistent. Recently PAL activity was correlated with glyceollin production in soybean (Glycine max (L.) Merr.) roots (Bonhoff et al. 1986); however Partridge and Keen (1977) concluded earlier that PAL activity was not related to glyceollin synthesis in soybean hypocotyls wounded and inoculated with *P. megasperma* (Drechs.) f.sp. *glycinea* (Hildebr.) Kuan & Erwin. They found little difference between PAL activity in wounded controls, in which glyceollin did not accumulate, and that in inoculated hypocotyls in which it did accumulate. Börner and Grisebach (1982), in a study of the same interaction, detected little PAL activity in wounds but reported a gradual increase in activity following inoculation. They also found that activity was similar in both compatible and incompatible interactions for the first 14 h. This was consistent with their data for glyceollin accumulation but not with the data of others (Yoshikawa et al. 1978, 1979), or with development of early differences in the extent of tissue correlation.

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colonization in compatible and incompatible interactions in soybean hypocotyls or roots in which glyceollin is presumed to play a causal role (Yoshikawa et al. 1978; Hahn et al. 1985). Furthermore, a recent report (Esnault et al. 1987) has provided evidence for gene transcription of PAL mRNA in etiolated soybean hypocotyls within 3 h of inoculation in the incompatible but not in the compatible interaction.

We reported recently that the Rps, gene for resistance to Phytophthora megasperma f.sp. glycinea race 1 carried by cv. Harosoy 63 is expressed in leaves in addition to hypocotyls (Bhattacharyya and Ward 1986). However, this was influenced greatly by the age of the plant and the maturity of the leaves. Thus, while leaves from 14-day-old cv. Harosoy 63 plants were resistant and accumulated glyceollin, leaves from 12-day-old plants of the same cultivar were susceptible. In this paper, therefore, we have examined PAL activity in leaves as well as in wounded and intact hypocotyls following inoculation with Phytophthora megasperma f.sp. glycinea.

Materials and methods

Host

Seeds of the near-isogenic soybean cultivars ‘Harosoy’ and ‘Harosoy 63’ were provided by R. I. Buzzell, Research Station, Agriculture Canada, Harrow, Ont. Etiolated seedlings were grown in trays of vermiculite for 6 days in the dark as described previously (Ward et al. 1979). Trifoliate leaves were obtained from 14-day-old cv. Harosoy and 12- and 14-day-old cv. Harosoy 63 plants grown in soil room at 95% RH with a daily 15-h light period (approximately 150 μE·m⁻²·s⁻¹ of photosynthetically active radiation) and daily temperatures of 23:16°C (light:dark).

Pathogen

Phytophthora megasperma f.sp. glycinea race 1 (Ward and Buzzell 1983) was maintained on V8-juice agar at 25°C. This race is virulent on cv. Harosoy (rps1) and avirulent on cv. Harosoy 63 (Rps1). Zoospores were produced as described previously (Ward et al. 1979). A 10-μL drop of zoospore suspension (10⁵/mL) was used as inoculum.

Elicitor

An abiotic elicitor of glyceollin, AgNO₃ (10⁻⁴ M) (Stössel 1982), was applied in 10-μL drops to the upper surface of leaves.

Inoculation and incubation

Etiolated hypocotyls from 6-day-old seedlings were arranged horizontally in glass trays as described previously (Ward et al. 1979) and two drops of zoospore suspension were placed on each hypocotyl about 2 cm below the cotyledons and about 0.7 cm apart. Drops of sterile distilled water were added to control hypocotyls. In hypocotyls that were wounded, two surface wounds (about 3 mm long and 0.5 mm deep) were made about 2 cm below the cotyledons and about 0.7 cm apart immediately prior to inoculation, except in one experiment, in which hypocotyls were wounded 12 h prior to inoculation. In that experiment sterile distilled water (10 μL) was placed in each wound to prevent desiccation during the 12-h period. Wounds were inoculated with 10 μL of zoospore suspension or sterile distilled water in controls. Ten hypocotyls (20 inoculated or control sites) were used for each treatment. After inoculation or wounding, hypocotyls were incubated in the dark at 25°C and 100% RH.

Trifoliate leaves were arranged on wet Cellucotton in glass trays as described previously (Bhattacharyya and Ward 1986). Six or eight drops of zoospore suspension, AgNO₃ solution, or sterile distilled water were placed on adaxial surfaces of leaflets of 12-day-old plants; 20 drops (to give comparable surface distribution) were used on the larger leaflets of 14-day-old plants. Eighty inoculated sites were used for each treatment (10 leaflets from 12-day-old plants, 4 leaflets from 14-day-old plants). Trays were closed immediately with plastic film and left undisturbed on a laboratory bench for 3–4 h. Thereafter, they were incubated in a growth cabinet at 100% RH with a daily 16-h light period (fluorescent lamps; approximately 33 μE·m⁻²·s⁻¹, at 23:16°C (light:dark).

Determination of phenylalanine ammonia-lyase activity

For determinations of activity in hypocotyls, sections approximately 1.75 cm long containing the wounded and inoculated sites were used. For leaves, the tissues of 80 lesions for each treatment were excised carefully with a scalpel eliminating noninfected tissue; in controls and AgNO₃-treated leaves the tissues covered by the drops of water or AgNO₃ solution were excised. The tissues were weighed, immediately frozen in liquid nitrogen, and stored at −70°C. The tissues were ground with a mortar and pestle with 0.1 M sodium borate buffer, pH 8.8, containing 2 mM mercaptoethanol (Lamb et al. 1979). The slurry was centrifuged in a microcentrifuge at 15 000 rpm for 5 min. The supernatant was collected and, after its volume was recorded, immediately frozen in liquid nitrogen and stored at −70°C until required. PAL activity in the supernatant was determined by measuring spectrophotometrically the production of cinnamic acid from L-phenylalanine (Lamb et al. 1979). The reaction mixture contained 300 μM sodium borate, pH 8.8, 30 μM L-phenylalanine, and 0.5 (hypocotyl) or 1 mL (leaf) of supernatant in a total volume of 3 mL. After incubation for 1 h at 40°C the absorbance at 290 nm was read against an identical mixture in which D-phenylalanine was substituted for L-phenylalanine. The enzyme activity was expressed as nanomole cinnamic acid produced per minute per gram fresh weight of tissue.

Determination of glyceollin

Excised tissues from lesions in leaves inoculated with zoospores or treated with AgNO₃ solution (10⁻⁴ M) were extracted with 5 mL 95% ethanol by boiling for 2 min. The ethanol was decanted and combined with two ethanol rinses (2 mL) of the tissues. The tissues were dried and weighed. The ethanol was reduced nearly to dryness and the residue extracted three times with 2 mL of ethyl acetate. The ethyl acetate soluble fraction was dried and Redissolved in 100 μL ethyl acetate and together with two 200-μL rinses applied to a thin-layer chromatography plate (silica, Whatman LK6DF, 250 μm thick). The plates were developed in benzene–methanol (95:8, v/v) and glyceollin was detected by fluorescence quenching under ultraviolet light. Silica bands containing glyceollin were eluted with ethyl acetate. After evaporating the ethyl acetate and Redissolving the residue in ethanol, glyceollin (a mixture of three isomers) was determined from its absorption at 285 nm and the extinction coefficient (Ayers et al. 1976). Glyceollin concentrations are expressed as micrograms per gram fresh weight of tissue. Efficiency of the procedure was assessed by analyzing leaf tissue to which purified glyceollin I had been added. Recovery was from 83 to 87% of glyceollin I added in the concentration range of 250 to 3000 μg·g⁻¹ fresh weight. Data are presented without correction.

Determination of reducing sugars

Reducing sugars in the supernatant used in the assay for PAL activity were determined using the arseno-molybdate method of Nelson (1944).

Statistical analysis

Data were analyzed in a completely randomized block design (Little and Hills 1978).

Results

Etiolated hypocotyls

In intact etiolated hypocotyls, symptoms following inoculation were consistent with those reported previously (Ward et al. 1979). In the incompatible interaction (cv. Harosoy 63) brown spots were visible after 5–6 h, while in the compatible interaction (cv. Harosoy), surfaces of inoculated sites developed a transparency that preceded the general water soaking.
tissues became brown 4 h after inoculation. Harosoy 63 until 8-9 h after inoculation. There also was a resistant responses in such hypocotyls of cv. Harosoy 63, not be detected in the resistant response in wounds in cv. reduction in the amount of browning in the compatible inter-

In intact etiolated hypocotyls PAL activity increased rapidly from 2 h after inoculation in the resistant response (cv. Harosoy 63) to P. megasperma f.sp. glycinea race 1 (Fig. 1a) but not in the susceptible response (cv. Harosoy). At 8 h after inoculation PAL activity in the resistant response was more than six times greater than that in the susceptible response or control hypocotyls. Wounding caused a small increase in PAL activity over that in intact hypocotyls (Figs. 1a, 1b). Significant differences in PAL activity between resistant and susceptible responses in wounded hypocotyls did not develop until after 6 h and stimulation in the resistant response (cv. Harosoy 63) was much smaller than in unwounded hypocotyls. In hypo-
cotyls wounded 12 h before inoculation the trends to increased PAL activity in the controls continued (Fig. 1c). There was also a rapid increase in activity in the resistant response, and this was significantly greater than that in intact hypocotyls. In hypocotyls wounded 12 h before inoculation there was also a significant stimulation of activity in the susceptible response at 8 h after inoculation above that in the control (Fig. 1c).

**Leaves**

The selection of leaves used for determination of PAL activity was based upon observations of symptoms reported previously (Bhattacharyya and Ward 1986). Trifoliolate leaves from 14-day-old cv. Harosoy plants were susceptible and developed pale brown lesions by 24 h, which started to spread by 36 h, with the development of soft rotten tissues. Trifoliolate leaves from 12-day-old cv. Harosoy 63 were susceptible also and lesions were similar to those in cv. Harosoy. However, by 14 days trifoliolate leaves of cv. Harosoy 63 were resistant. Brown spots appeared beneath inoculum drops 12 h after inoculation and conspicuous dark brown to reddish brown lesions, more or less restricted to the inoculated area, developed by 24 h.

There was no significant change in PAL activity in any of the infected leaves until 24 h after inoculation (Table 1). At 36 h after inoculation there was a major increase in activity in leaves of 14-day-old cv. Harosoy 63 plants (resistant) but not in leaves of other plants.

Following zoospore inoculation much more glyceollin accumulated in trifoliolate leaves from 14-day-old cv. Harosoy 63 plants than in those from either 12-day-old cv. Harosoy 63 plants or 14-day-old cv. Harosoy plants (Table 2). At 24 h, but not 36 h, after inoculation, glyceollin accumulation was significantly less in 12-day-old cv. Harosoy 63 trifoliolate leaves than in cv. Harosoy trifoliolate leaves. With AgNO₃ treatment (Table 2) amounts of glyceollin produced in trifoliolate leaves of 14-day-old plants of both cultivars were similar but only about one-fifth as much was produced in trifoliolate leaves of 12-day-old plants of cv. Harosoy 63.

No differences in reducing sugar levels were found among the three groups of trifoliolate leaves (Table 3).

**Discussion**

The rapid increase in PAL activity in the resistant response but not in the susceptible response of intact etiolated soybean hypocotyls demonstrated here (Fig. 1a) is comparable with that in intact roots of soybean seedlings reported by Bonhoff et al. (1986). It is consistent also with evidence for early increases in gene transcription for mRNA for PAL in resistant but not in susceptible reactions reported by Esnault et al. (1987) and with our unpublished evidence that rates of biosynthesis and accumulation of glyceollin I are higher in resistant than in susceptible inoculated hypocotyls.

In hypocotyls wounded just prior to inoculation, symptom development was delayed compared with that in intact hypo-
cotyls. Similarly, PAL activity in the resistant response in

**TABLE 1. Phenylalanine ammonia-lyase activity (nmol cinnamic acid·min⁻¹·g fresh wt.⁻¹) in trifoliolate leaves of soybean inoculated with Phytophthora megasperma f.sp. glycinea**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Plant age (days)</th>
<th>Inoculated*</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h†</td>
<td>36 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Harosoy</td>
<td>14</td>
<td>20.6±9.7†</td>
<td>9.4±3.7</td>
</tr>
<tr>
<td>Harosoy 63</td>
<td>12</td>
<td>10.5±6.3</td>
<td>16.3±12.2</td>
</tr>
<tr>
<td>Harosoy 63</td>
<td>14</td>
<td>9.9±3.5</td>
<td>84.2±13.3</td>
</tr>
</tbody>
</table>

*Trifoliolate leaves were inoculated by placing 10μL drops of zoospore suspension (10⁹/mL) of Phytophthora megasperma f.sp. glycinea race 1 on the adaxial surface. Drops of water (10 μL) were applied to controls.
†Incubation period following inoculation until analysis.
‡Data are the means and standard errors from 2 replicate determinations. Data were analyzed in a completely randomized block design treating each experiment as a replicate; the LSD value (p = 0.05) was calculated to be 18.7.

**TABLE 2. Accumulation of glyceollin (µg·g fresh wt.⁻¹) in leaves of soybeans following inoculation with Phytophthora megasperma f.sp. glycinea race 1 or AgNO₃,*

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Age (day)</th>
<th>Zoospores</th>
<th>AgNO₃, 62 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h†</td>
<td>36 h</td>
<td></td>
</tr>
<tr>
<td>Harosoy</td>
<td>14</td>
<td>593±45‡</td>
<td>400±4</td>
</tr>
<tr>
<td>Harosoy 63</td>
<td>12</td>
<td>205±4</td>
<td>336±151</td>
</tr>
<tr>
<td>Harosoy 63</td>
<td>14</td>
<td>928±128</td>
<td>1097±94</td>
</tr>
</tbody>
</table>

*Trifoliolate leaves were inoculated by placing 10-μL drops of a zoospore suspension (10⁹/mL or a AgNO₃ solution (10⁻⁴ M) on the adaxial surface of detached leaves.
†Incubation period following inoculation or AgNO₃ treatment until analysis.
‡Data are the means and standard errors from 2 replicate determinations. Data were analyzed in a completely randomized block design; the LSD value (p = 0.05) was calculated to be 240. No glyceollin was detected in leaves that received water drops only.
Incubation period

FIG. 1. Phenylalanine ammonia-lyase activity in etiolated soybean hypocotyls unwounded and wounded and inoculated with Phytophthora megasperma f.sp. glycinea race 1. (a) Unwounded inoculated hypocotyls of cv. Harosoy (○) and cv. Harosoy 63 (□) and water-treated controls (cv. Harosoy, ▲; cv. Harosoy 63, ○). (b) Hypocotyls of cv. Harosoy (■) and cv. Harosoy 63 (□) wounded and inoculated immediately (0 h) and water-treated wounded controls (cv. Harosoy, ▲; cv. Harosoy 63, △). (c) Hypocotyls of soybean cv. Harosoy (■) and cv. Harosoy 63 (□) wounded 12 h prior to inoculation at 0 h and water-treated controls (cv. Harosoy, ▲; cv. Harosoy 63, △). Points on the graphs are the means of determinations from two experiments.

Data from each incubation period were analyzed in a completely randomized block design considering each experiment as a replication. LSD values (p = 0.05) calculated were 1.4, 3.8, 4.1, 9.6, and 6.7 for 0-, 2-, 4-, 6-, and 8-h incubation periods, respectively.

wounded hypocotyls increased at a lower rate than in intact hypocotyls. These differences were due evidently to the removal of the epidermal layer and a few cortical cell layers by wounding. This suggests that the epidermal cell layer is important for early and rapid stimulation of PAL activity in response to infection.

PAL activity in wounded tissues treated with water only was slightly higher than that of intact hypocotyls (Figs. 1a, 1b). Börner and Grisebach (1982) also observed only a low level of PAL activity, but Partridge and Keen (1977) reported a high level of PAL activity in wounded green hypocotyls of soybeans treated with water. Neither of the two studies, however, observed any difference in PAL activity between resistant and susceptible responses during early stages following inoculation. This contrasts with the present study, in which PAL activity in wounded inoculated hypocotyls was significantly lower in the susceptible response than in the resistant response. Both studies used fragmented mycelium for inoculum, which may have contained nonspecific elicitors that caused similar responses in both resistant and susceptible tissues. They also used green hypocotyls and it is possible that, as we observed for leaves, green tissues have a sufficient basal level of PAL to support biosynthesis of glyceollin during the first few hours of the host-pathogen interaction.

Wounding of hypocotyls 12 h prior to inoculation (Fig. 1c) enhanced the development of symptoms and PAL activity significantly compared with wounding immediately before inoculation. This appears to be similar to the effects of wounding on the development of hypersensitivity in potatoes reported by Tomiyama (1960). There was a gradual increase in PAL activity due to wounding alone and by the time of inoculation this was appreciably higher in hypocotyls wounded 12 h previously than in unwounded hypocotyls. Following inoculation, PAL activity reached a higher level in the hypocotyls wounded 12 h before inoculation than in the unwounded hypocotyls. In these hypocotyls also we have observed accelerated production of glyceollin (M. K. Bhattacharyya and E. W. B. Ward, unpublished data). Possibly, the wound response involves the priming of cellular mechanisms for synthesis of PAL and other enzymes that are rapidly mobilized on infection. Similar conclusions were reached by Inoue et al. (1984) for enhanced activity of enzymes for furanoterpeneoid biosynthesis in wounded sweet potato roots. As they suggested, the enhanced response may increase resistance to wound-invading microorganisms.

In leaves, PAL activity increased after 24 h only in the resistant response of 14-day-old trifoliolate leaves of cv. Harosoy 63. Therefore, accumulation of glyceollin that was demonstrated at 24 h occurred without stimulation of PAL activity over background levels. This indicates that background levels of PAL are sufficient for initial accumulations of glyceollin and suggests that the control of pathways after PAL, leading to other phenylpropanoids, may differ in leaves from that in etiolated hypocotyls, e.g., there may be more demands for precursors for lignification in hypocotyls than in leaves. It suggests also that the Rps gene for resistance in cv. Harosoy 63 does not directly control glyceollin biosynthesis at the PAL step, but that changes in PAL activity occur secondarily in
response to demand and may be under allosteric control. In contrast to the resistant response of trifoliolate leaves of 14-day-old plants of cv. Harosoy 63 to race 1, trifoliolate leaves of 12-day-old plants of this cultivar were susceptible and developed PAL activity comparable with that in 14-day-old trifoliolate leaves of the susceptible cultivar, Harosoy. The relatively small differential in glyceollin concentrations between resistant and susceptible responses in leaves has been discussed elsewhere (Bhattacharyya and Ward 1986). Glyceollin concentrations in trifoliolate leaves of 12-day-old cv. Harosoy 63 plants were reduced also at both 24 and 36 h after elicitor or they are deficient in metabolic processes to support these mechanisms for response to either infection or the abiotic stimulus. Elicitor or they are deficient in metabolic processes to support mechanisms for response to either infection or the abiotic stimulus.

**Table 3.** Total reducing sugar concentrations (mg·g fresh wt.−1) in soybean leaves following inoculation with *Phytophthora megasperma* f.sp. *glycinea* race 1*

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Plant age (days)</th>
<th>Inoculated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>36 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Harosoy</td>
<td>14.87 ± 0.91</td>
<td>4.47 ± 0.11</td>
<td>3.85 ± 0.31</td>
</tr>
<tr>
<td>Harosoy 63</td>
<td>12.45 ± 0.23</td>
<td>3.85 ± 0.31</td>
<td>3.33 ± 0.54</td>
</tr>
<tr>
<td>Harosoy 63</td>
<td>14.87 ± 0.91</td>
<td>4.47 ± 0.11</td>
<td>3.85 ± 0.31</td>
</tr>
</tbody>
</table>

*Trifoliolate leaves were inoculated by placing 10-μL drops of a zoospore suspension (10⁷ mL⁻¹) of *Phytophthora megasperma* f.sp. *glycinea* race 1 on the adaxial surface. Drops of water (10 μL) were applied to controls.

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