Defense Expression in Protected Tissues of Medicago sativa Is Enhanced During Compatible Interactions with Colletotrichum trifolii

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ABSTRACT


The defense response to anthracnose, induced in alfalfa by avirulent Colletotrichum trifolii race 1, was further enhanced following challenge by a virulent race of C. trifolii. Rapid, significant increases in the phytoalexins medicarpin, sativain, vestitol, and coumestrol were observed in cotyledons protected from anthracnose by inoculation with race 1 and challenge-inoculated 24 h later with race 2. Sativain and medicarpin levels were significantly greater than quantities accumulating in protected, non-challenged tissues or in tissues both protected and challenged by the incompatible race 1. Inoculation of nonprotected tissues with the compatible race did not induce protection, nor did the tissues accumulate significant quantities of phytoalexins. The rapid increase in phytoalexin accumulation following challenge-inoculation of protected tissues with a compatible race was correlated with increased hypersensitive browning and the absence of disease lesions, acervuli, and secondary spores. The degree of disease protection from challenge-inoculation by race 2 was dependent upon the spore density of race 1 inducing-inoculum. Full protection from race 2 infection required inducing race 1 inducing-inoculum densities ranging from 1 x 10^4 to 1 x 10^6 spores per milliliter. These spore densities resulted in significant phytoalexin accumulation following race 2 challenge. The most virulent isolates of race 2 caused the greatest increase in concentrations of medicarpin, sativain, vestitol, and coumestrol. These results suggest that protection may not be solely a result of defense activation in response to inoculation by an avirulent race. The induction phase may serve to facilitate an enhanced defense expression upon challenge by a virulent race. The protected plant responds rapidly and specifically to challenge by the compatible fungus.

Additional keywords: hypersensitive response, induced defense response, lucerne, race specificity, resistance mechanism.

Anthracnose, caused by Colletotrichum trifolii Bain & Essary, is a serious foliar, stem, and crown disease of Medicago sativa L. (2,41). The current principle disease-control strategy is the use of resistant cultivars (12). The disease interaction in alfalfa follows a typical gene-for-gene reaction for race-culturivar specificity, in which an incompatible resistance response results in biochemical recognition and the accumulation of phytoalexins (Table 1) (1,13,14,15,32,43). Although the biochemical determinants of race-culturivar specificity in the alfalfa/C. trifolii system are not yet known, it is clear that the synthesis of phytoalexins is one of the first defense responses taking place. As first suggested by Muller and Behr (29) in the Phytophthora infestans/potato system, there are two linked but separable defense reactions in plants: hypersensitive response (HR) and phytoalexin synthesis. The active defense reaction in alfalfa includes rapid, localized cell death (hypersensitive cell death), accumulation of phytoalexins, synthesis and deposition of phenolic compounds and proteins in the cell wall, and synthesis of pathogenesis-related (PR) proteins including the hydrolytic enzymes 1,3-β-glucanase and chitinase (10). During incompatible interactions by alfalfa plant tissues or elicited suspension cells, enzymes from the phenylpropanoid pathway are activated and antimicrobial compounds accumulate (1,6,33). The pterocarpan medicarpin is the most abundant of the major phytoalexins accumulating in elicited alfalfa plants, and sativain, vestitol, and coumestrol are also fungitoxic (8,9,22,37,40). A thorough review of the molecular biology and biochemistry of medicarpin biosynthesis in alfalfa suspension cultures has been published (10). The entire biosynthetic pathway to medicarpin is induced de novo in whole plants and cell cultures by biotic or abiotic elicitors (20,34,36,37).

Alfalfa exhibits a local acquired immunity, or induced resistance, during which tissues are protected from attack by virulent isolates (32,35). In these studies, we determined that race 1 virulence was associated with a greater capacity to induce protection in seedlings and that induced resistance was stable and not broken down by challenge with high race 2 inoculum pressure. The phenomenon of induced resistance needs to be investigated further to determine the basic mechanisms leading to disease control (38). Although induced resistance in alfalfa and other legumes is considered a localized reaction, the systemic induction of antifungal isoflavonoids has been reported in nematode-infected alfalfa (11). Results from this study suggest that isoflavonoid conjugates increased in root in response to a systemic signal from infected shoots.

Induction of plant resistance mechanisms by application of elicitors has previously been suggested as an alternative approach.

<table>
<thead>
<tr>
<th>C. trifolii</th>
<th>'Sarlac' (SS)</th>
<th>'Arc' (RS)</th>
<th>'Sarlac AR' (RR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race 1</td>
<td>Compatible</td>
<td>Incompatible</td>
<td>Incompatible</td>
</tr>
<tr>
<td>Race 2</td>
<td>Compatible</td>
<td>Compatible</td>
<td>Incompatible</td>
</tr>
</tbody>
</table>

a S and R indicate the absence or presence of either the An1 or An2 gene for race 1 or race 2 anthracnose resistance. S = susceptible and R = resistant.
for crop disease control (4). Recently, in two pathosystems, a plant defense response was reported to be potentiated by prior treatment with abiotic elicitors. Kauss et al. (23,24) found that pretreatment of suspension cells of parsley (*Petroselinum crispum*) with abiotic elicitors enhanced coumarin secretion during subsequent treatments with a fungal elicitor. Abiotic elicitor-induced potentiation of a defense system has also been reported in sugar beet (*Beta vulgaris*) against *Cercospora beticola* (30).

This study was undertaken to examine some of the basic biochemical and molecular mechanisms leading to acquired resistance and induction of the isoflavonoid branch pathway in alfalfa and, in particular, to examine responses to secondary challenges in alfalfa by compatible fungi. There is evidence suggesting that the quantity and toxicity of phytoalexins accumulating in resistant alfalfa plants responding only to initial infection may not be sufficient to repel a challenge attempt by virulent pathogens (3,33). We also have observed an increased degree of browning in induced resistant cotyledons responding to challenge-inoculations (N. O'Neill, unpublished data). These results prompted an investigation of defense responses occurring in induced resistant tissues responding to challenge-inoculations. The enhanced induced defense expression may play a critical role in disease resistance under field conditions in which plants are subject to variable disease pressure from numerous foliar pathogens. Preliminary results suggested that an as yet uncharacterized defense response occurs, in which there is an enhanced biochemical response to the challenge attempt by compatible fungi (31).

**MATERIALS AND METHODS**

**Fungal isolates.** Cultures of field isolates of *C. trifolii* race 1 (isolate 2sp2) and race 2 (isolates H-6-1, H-5-1, 5-1, SB-3, S-3, H-5-3, SB-1, SB-4, and SB-2) were obtained from different geographic locations in Maryland and North Carolina (32). Cultures were derived from single spores and stored in sterile soil or on silica gel crystals at 4°C. Spore suspension inoculum was prepared from isolates cultured on half-strength oatmeal agar (36 g of Difco oatmeal agar [Difco Laboratories, Detroit], 1 liter of distilled water, and 7.5 g of agar) in petri dishes for 7 days at 21°C under 12 h of fluorescent light. Relative isolate virulence and ability to induce resistance in interactions with alfalfa cultivars possessing specific genes for resistance were determined previously (32).

**Alfalfa cultivars.** Race-differential cultivars Saranac, Arc, and Saranac AR were used in these experiments (Table 1). ‘Saranac’ is susceptible to both races (SS phenotype). ‘Arc’ is resistant to race 1 and susceptible to race 2 (RS phenotype). ‘Saranac AR’ is resistant to both races (RR phenotype). Cotyledons appear to be ideal tissues to examine defense responses in alfalfa (33). Alfalfa is an autotetraploid and seed is heterogenous; therefore, only a portion of the seed of ‘Arc’ (60 to 70%) carries the anthracnose resistance gene. The number of seed actually exhibiting disease resistance was determined by evaluating resistance in one cotyledon from each seedling. The corresponding cotyledon was then used in biochemical defense response experiments, and the weight from cotyledons pooled for each treatment was adjusted for actual percent resistance.

**Inoculation.** Inoculum was prepared as described previously (33). Seeds were planted in sterile Pro-mix (Premier Horticulture Inc., Red Hill, PA) and grown at 21 to 22°C with a 16-h day/night photoperiod. After 14 days, cotyledons were detached, placed abaxial surface up onto moist filter paper in sterile, glass petri dishes, and inoculated with the appropriate spore suspension. Plates were sealed and placed in an incubation chamber at 23°C with a 16-h day/night photoperiod.

**Phytoalexin extraction and high-pressure liquid chromatography (HPLC) analysis.** An extraction method was developed to reduce the chlorophyll content of samples and facilitate consistency in handling large sample numbers (1). Extraction efficiency was 85 to 92% for medicarpin; sample phytoalexin concentrations were not corrected for this value. Cotyledons for each treatment were combined, weighed (approximately 0.3 g/sample of 30 cotyledons), and ground to a fine powder in liquid nitrogen with a mortar and pestle. Four and one-half milliliters of 80% acetonitrile in 1 mM acetic acid was added, and samples were stored at −70°C. Samples were filtered to remove particulates, rinsed with 0.5 mL of 80% acetonitrile, and reduced to 25% acetonitrile by the addition of 1 mM acetic acid. The solution was poured into a 75-mL reservoir above a 6-mL Baker-10 SPE octadeicyl (C18) disposable extraction column (J. T. Baker Inc., Phillipsburg, NJ) that had been preconditioned with methanol followed by two 3-mL volumes of 25% acetonitrile. The eluate was discarded, and sample compounds adhering to the column were eluted with 3.5 mL of 100% acetonitrile. The samples were evaporated under nitrogen, resuspended in 60 μL of acetonitrile, and stored at −70°C. Compounds were separated by HPLC on a reverse phase C18 column, 25 × 4.6-cm diameter with 5-μm beads. The mobile phase was 50% acetonitrile in 1 mM acetic acid, increasing to 10% over 20 min, with a flow rate of 1 mL/min. The effluent was monitored for absorbance at 210 nm with a UV detector, and phytoalexins were quantified as peak area units (mV s⁻¹) by Baseline Chromatography software (Waters Corporation, Milford, MA) with pure medicarpin as a standard.

The analysis of extracts from experiments with different race 2 isolates was slightly modified with a standard of vestitol and a mobile phase of 20% acetonitrile in 1 mM acetic acid, increasing...
to 60% over 55 min, and 98% over an additional 5 min. The flow rate was 0.8 ml per minute, and the effluent was monitored for absorbance at 210 nm with a photodiode array detector and analysis by Millennium Chromatography software (Waters Corporation).

Pure phytoalexin standards were kindly provided by the following: medicarpin by D. Gustine, sativam by G. Spencer, and vestitol by N. Paiva. Purity of compounds was confirmed by gas chromatography-mass spectrophotometry analysis. Coumestrol was purchased from Eastman Kodak Co., Rochester, NY.

**Defense response in induced and challenged cotyledons.** The temporal accumulation of sativam and medicarpin was determined in race 1-resistant 'Arc' cotyledons protected by inoculation with *C. trifolii* race 1 and challenge-inoculated 24 h later with race 2. Treatments consisted of three replications of 33 cotyledons each, spray-inoculated with 2 x 10^6 spores per ml of race 1 isolate 2sp2.

In the challenge treatments, cotyledons were spray-inoculated with 2 x 10^6 spores per ml of race 1 isolate 2sp2, and then challenge-inoculated with 2 x 10^6 spores per ml of race 2 isolate SB-1 or race 1 isolate 2sp2. Plates for all treatments were incubated at 23°C for 24, 48, 72, 96, and 120 h. Control treatments consisted of plants inoculated with 2 x 10^6 spores per ml of race 2 isolate SB-1 and with water alone.

**Effect of inducing-inoculum concentration on accumulation of phytoalexins in induced and challenged cotyledons.** Changes in accumulation of medicarpin and sativam were evaluated in 'Arc' cotyledons inoculated with different spore concentrations of race 1 and challenge-inoculated with race 2. Treatments consisted of three replications of 33 cotyledons per petri plate spray-inoculated with spore concentrations ranging from 1 x 10^5 to 1 x 10^9 spores per ml of race 1 isolate 2sp2. Control treatments consisted of plates of cotyledons inoculated with distilled water and 'Saranac' cotyledons inoculated with race 1 (compatible interaction). Plates were incubated at 23°C for 24 h, and then challenge-inoculated with 2 x 10^6 spores per ml of race 2 isolate SB-1. Plates were incubated for an additional 72 h, and cotyledons were harvested, extracted, and analyzed for phytoalexins.

**Effect of inducing-inoculum concentration on protection from challenge-inoculation.** Cotyledons were treated as above except that they were evaluated for macroscopic signs and symptoms of disease 7, 10, and 14 days after inoculation. For each evaluation period, a cotyledon was considered resistant or pro-

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**Fig. 2.** The effect of race 1 inducing-inoculum concentration on the accumulation of phytoalexins A, medicarpin and B, sativam in 'Arc' cotyledons challenged by race 2. Treatments consisted of 'Arc' inoculated with race 1 alone and 'Arc' inoculated with race 1 and then challenge-inoculated 24 h later with race 2. Cotyledons were harvested 96 h after inoculation with race 1. Vertical bars indicate the standard error of the mean of three replicates.

**Fig. 3.** The effect of race 1 inducing-inoculum concentration on disease protection in 'Arc' cotyledons challenged by race 2. Percent resistance was assessed A, 7; B, 10; and C, 14 days after inoculations with race 1. Treatments consisted of 'Arc' cotyledons inoculated with race 1 (induced resistance) and then challenged 24 h later with race 2, 'Saranac' cotyledons inoculated with race alone (susceptible interaction), and 'Arc' cotyledons inoculated with race 1 alone (resistant interaction). Vertical bars indicate the standard error of the mean of three replicates.
ected if no acervuli or secondary spores were observed and the tissue remained green.

Phytoalexin accumulation following challenge by different isolates of race 2. Enhancement of defense expression during challenge interactions by different race 2 isolates was determined in induced, resistant cotyledons. ‘Arc’ cotyledons were grown as described above, placed in petri dishes, and inoculated with race 1 isolate 2sp2 at 1 x 10^6 spores/ml. After 24 h incubation at 25°C, half the plates were inoculated with different isolates of race 2 at a concentration of 2 x 10^8 spores/ml. Plates were incubated for an additional 72 h, harvested, extracted, and analyzed for phytoalexin content. This experiment included three replications of 30 cotyledons per treatment. Quantity of phytoalexins was expressed as µg/g of fresh weight of tissue and, in this experiment, was not adjusted for percent ‘Arc’ seed susceptible to anthracnose. All experiments were repeated at least once with similar results.

RESULTS

Defense response in induced and challenged cotyledons. The accumulation of sativan and medicarpin was determined in race 1-resistant ‘Arc’ cotyledons protected by inoculation with C. trifolii race 1 and challenged with race 2 (Fig. 1). Sativan and medicarpin accumulation was detected between 24 and 48 h after inoculation with race 1. The accumulation of these phytoalexins increased more rapidly following challenge-inoculation with race 2. In both treatments, maximum accumulations were attained 96 h after inoculation. The challenge-inoculation with race 2 boosted both the medicarpin and sativan levels to more than twice that in cotyledons not challenged with race 2. A second inoculation with race 1 isolate 2sp2 also resulted in elevated phytoalexin levels, but the accumulation was significantly less than that resulting from a challenge with race 2. Thus, the plant is responding specifically to challenge by a compatible, rather than incompatible, interacting pathogen (Table 1). Inoculations with race 2 alone (compatible interaction) resulted in little or no accumulation of medicarpin or sativan. By 120 h, susceptible cotyledons exhibited chlorosis, acervuli, secondary spores, and tissue collapse.

Effect of race 1 inducing-inoculum concentration on the accumulation of phytoalexins in ‘Arc’-cotedledons challenged by race 2. A significant dosage effect was observed between race 1 inducing-inoculum concentration and the accumulation of the major phytoalexins sativan and medicarpin (Fig. 2). The phytoalexin response could be detected with 2 x 10^6 spores/ml of race 1, reaching maximum response at 1 x 10^4 to 1 x 10^6 spores/ml.

Cotyledons inoculated with race 1 and then challenged with race 2 exhibited similar accumulation patterns up to 1 x 10^4 spores/ml, but exceeded controls by nearly 100% at 1 x 10^8 spores/ml.

Race 1 inoculum pressure and disease protection following challenge by race 2. Protection from disease was determined in cotyledons subjected to a range of inducing-inoculum concentrations and challenged with race 2 (Fig. 3). Protection in ‘Arc’ cotyledons challenge-inoculated with race 2 was detected with race 1 inducing-inoculum concentrations of 1 x 10^8 spores/ml and reached a maximum degree of protection between 1 x 10^6 and 1 x 10^8 spores/ml. ‘Arc’ inoculated with race 1 alone exhibited 60 to 70% resistance after 1 week, which is typical for this cultivar. The susceptible portion of ‘Arc’ seed exhibited disease symptoms when race 1 inoculum concentrations reached 1 x 10^6 or higher. Fifty percent of ‘Arc’ cotyledons inoculated with race 2 alone (data not shown) exhibited susceptible symptoms within 7 days, progressing to 93% susceptibility by 14 days. By 10 days, the degree of protection from race 2 exhibited in ‘Arc’ was the same as that when inoculated with race 1 alone (approximately 70% resistant) at inoculum concentrations of 1 x 10^6 to 1 x 10^8 spores/ml. After 10 days, ‘Arc’ cotyledons not protected from race 2 by prior inoculation with race 1 exhibited 17% resistance.

Phytoalexin accumulation following challenge by different isolates of race 2. Race 2 isolates were compared for differences in enhancement of defense expression during challenge interactions on induced resistant cotyledons (Table 2). Medicarpin, sativan, vestitol, and coumestrol accumulation increased significantly in induced cotyledons following challenge by the most virulent race 2 isolates. The total increase in the four phytoalexins ranged from no increase to 24.4 µg/g of fresh tissue weight, depending on the race 2 isolate. Race 2 isolate SB-1 was found to be the most virulent isolate among 12 isolates tested for virulence to ‘Saracen AR’, a cultivar resistant to this race (32). This isolate also induced the greatest quantity of total phytoalexins, 75.2 µg/g of fresh weight (Table 2). Races S2-4 and S2-3 are also highly virulent (32) and were effective elicitors of a challenge defense response. These three isolates induced the greatest increase in total phytoalexins in response to challenge-inoculations compared with other race 2 isolates. Two race 2 isolates, H6-1 and H5-1, did not induce further accumulations of phytoalexins following challenge treatment. These two isolates are also reported to be the least virulent of 12 isolates tested for virulence to ‘Saracen AR’, resulting in 68.0 and 53.5% survival in inoculated seedlings, respectively (32). The total phytoalexin accumulation following challenge with race 2 isolates appears to be associated with their relative virulence (Table 2).

<table>
<thead>
<tr>
<th>Race 2 isolate</th>
<th>Virulence (%)</th>
<th>Medicarpin</th>
<th>Sativan</th>
<th>Vestitol</th>
<th>Coumestrol</th>
<th>Total phytoalexins</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>C</td>
<td>I</td>
<td>C</td>
<td>I</td>
<td>C</td>
<td>I</td>
</tr>
<tr>
<td>SB-1</td>
<td>11.7</td>
<td>6.5</td>
<td>11.4</td>
<td>11.4</td>
<td>15.2</td>
<td>12.4</td>
<td>18.1</td>
</tr>
<tr>
<td>S2-3</td>
<td>32.6</td>
<td>6.6</td>
<td>9.1</td>
<td>7.1</td>
<td>10.8</td>
<td>9.0</td>
<td>12.1</td>
</tr>
<tr>
<td>S2-4</td>
<td>32.7</td>
<td>6.0</td>
<td>9.4</td>
<td>6.9</td>
<td>11.9</td>
<td>7.1</td>
<td>13.4</td>
</tr>
<tr>
<td>SB-2</td>
<td>41.0</td>
<td>4.3</td>
<td>6.3</td>
<td>10.0</td>
<td>10.0</td>
<td>10.5</td>
<td>17.1</td>
</tr>
<tr>
<td>H5-3</td>
<td>41.7</td>
<td>3.7</td>
<td>5.0</td>
<td>10.5</td>
<td>8.9</td>
<td>6.7</td>
<td>14.6</td>
</tr>
<tr>
<td>S1-1-1</td>
<td>45.0</td>
<td>3.7</td>
<td>5.5</td>
<td>6.2</td>
<td>8.0</td>
<td>7.3</td>
<td>12.0</td>
</tr>
<tr>
<td>SB-3</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
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</tr>
<tr>
<td>H6-1</td>
<td>53.5</td>
<td>3.7</td>
<td>4.0</td>
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<td>5.2</td>
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<tr>
<td>H5-1</td>
<td>68.0</td>
<td>3.7</td>
<td>3.8</td>
<td>4.5</td>
<td>5.9</td>
<td>5.2</td>
<td>6.3</td>
</tr>
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</table>

LSD: 2.7, 4.5, 3.8, 11.5

* Three replications of 30 cotyledons per treatment were harvested 96 h after inoculation with race 1 (incompatible interaction) or race 1 followed by an isolate of race 2 (compatible interaction).

b Virulence is expressed as percent ‘Saracen AR’ seedlings surviving 7 days after inoculation. ‘Saracen AR’ (RR phenotype) is resistant to race 2 (32).

c (induced) was inoculated with race 1; C (challenged) was inoculated with race 1 and then race 2 after 24 h.

d Not determined.

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**DISCUSSION**

Induced resistance has been described in many host-pathogen systems (27,39). Investigations of mechanisms of induced resistance may provide new strategies for crop protection through manipulation of key regulatory genes expressing components of defense or by the application of specific signals that sensitize or condition plants for protection upon challenge. In this paper, we describe a localized, enhanced defense response in alfalfa activated by challenge with a compatible pathogen.

Phytoalexin accumulation is increased during previously compatible interactions on plants induced to the resistant state by prior inoculation with an incompatible race. The rapidity of this accumulation in comparison with the nonprotected tissues suggests that a portion of the defense response is preactivated.

There are several possible general mechanisms for the increased response to challenge-inoculation. It is possible that sensitized or primed tissues accumulate untranslated mRNAs encoding defense proteins so that, following a second challenge infection, the mRNAs can be translated and result in a more rapid accumulation of phytoalexins. Preinduction of elicitor-releasing enzymes such as glucanases or chitinase, or up-regulation of signal transduction components may be occurring. Perhaps further molecular analysis using translation and transcription inhibitors will show whether there is de novo mRNA or protein synthesis following challenge-inoculations. Further dynamic variables that may affect inhibition of fungal development during challenge interactions may be the role of isoflavonoid conjugates. Preformed phytoalexins in the form of isoflavone glycosides may be present and released upon signals from the challenge pathogen. Such conjugates have been reported in alfalfa roots and suspension cells (11,25,42). This would also account for the rapid appearance of these compounds. Such a mechanism has been described in wounded soybean cotyledons, in which proximal cells responded with accumulations of isoflavonoids and distal cells responded with an increase of isoflavone conjugates (17,18,19). Another variable that would affect in vivo phytoalexin levels is phytoalexin tolerance or metabolic degradation by the pathogen (7,21,26,28).

The enhanced defense response during the secondary phase was associated with high virulence of the challenge pathogen. The basis for virulence differences within a race has not been investigated. We reported earlier that the degree of protection from challenge appeared independent of challenge-inoculum concentration, in which protection from race 2 was effective at spore densities ranging from $2.5 \times 10^5$ to $4 \times 10^6$ spores/ml (32). Phytoalexin accumulation was not determined in that study. These results suggest that, in protected tissues, an enhanced defense response is specifically triggered by a very low challenge pressure by a virulent pathogen, perhaps a very efficient way for the plant to protect itself. It appears that the fungal component eliciting the response to challenge is also a virulence factor. It is possible that the more virulent isolates either colonize more tissue or result in more overall fungal biomass and, thus, elicitor release. This would increase the potential to produce more phytoalexin than the less virulent isolates.

The results from this and earlier studies suggest that there are multiple phases to the disease defense response. In the induction phase, resistant alfalfa plants exhibit hypersensitive cell death, followed by moderate accumulation of phytoalexins and the cessation of fungal development at the initial infection site (5,33). In the challenge phase, inoculation boosts the defense response, resulting in higher levels of phytoalexins and increased hypersensitive browning. This response is not observed in compatible interactions with unprotected tissues. The response to challenge is dependent on inoculum density during the induction phase (Fig. 2), which correlates with the inoculum required for effective induced resistance (Fig. 3). The total accumulation of medicarpin and sativin increase proportionally to the degree of protection as inducing-inoculum spore concentrations levels are increased to $5 \times 10^6$ spores/ml. This dosage-dependent correlation provides evidence that phytoalexins play a role in localized induced disease resistance to anthracnose.

Induced resistance has great potential as a broad-spectrum, alternative crop protectant to fungicides. Practical application of induced resistance for disease control mechanisms will be facilitated by further studies of signaling mechanisms, biochemical expression of defense response to challenge, and investigations of the spatial and temporal expression of defense related genes. Further studies are also needed to determine whether alfalfa exhibits systemic acquired resistance in response to biotic or abiotic elicitors.

In this paper, we provide evidence for the enhancement of localized, induced defense expression during challenge by virulent fungi. This additional defense component may play a critical role in disease resistance under field conditions in which plants are subject to variable disease pressure from numerous foliar pathogens. We propose that the acquired immune state serves to prime or sensitize the plant for subsequent rapid defense activation. Plants, thus, expend little energy unnecessarily as the metabolic alterations occur only when and where needed to counter the potential invader.

**LITERATURE CITED**


