Chemotactic Preferences and Strain Variation in the Response of *Phytophthora sojae* Zoospores to Host Isoflavones

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The zoospores of *Phytophthora sojae* are chemotactically attracted to the isoflavones genistein and daidzein that are released by soybean roots. In this study we have examined the response of *P. sojae* zoospores to a wide range of compounds having some structural similarity to genistein and daidzein, including isoflavones, flavones, chalcones, stilbenes, benzoin, benzoates, benzophenones, acetophenones, and coumarins. Of 59 compounds examined, 43 elicited some response. A comparison of the chemotactic responses elicited by the various compounds revealed a primary role for the phenolic 4'- and 7-hydroxyl groups on the isoflavone structure. A few compounds acted as repellents, notably methylated flavones with a hydrophobic B ring. The chemotactic response to many of the analogs was markedly different among different strains of *P. sojae*.

The exchange of signals represents the earliest step in a plant-microbe interaction (3). In a complex environment like the soil, detection of specific plant molecules by microbes may be critical to recognition and subsequent colonization of potential hosts. In *Rhizobium* and *Bradyrhizobium* species of bacteria, expression of nodulation (*nod*) genes is induced by flavonoids or isoflavonoids specific to the particular hosts via the action of the transcription factor NodD (1, 24). Induction of the *nod* genes leads to production of a lipopolysaccharide which triggers formation of the nodule structure by the plant (14, 24). The virulence (*vir*) genes of *Agrobacterium tumefaciens*, which mediate the transfer of DNA to the plant cells, are specifically induced by phenolic compounds, such as acetosyringone, which are released from wounded tissue (3, 24, 26).

The responses of bacteria such as *Rhizobium* and *Agrobacterium* species to plant signals also include chemotaxis, in which the bacteria are attracted to swim towards potential colonization sites (3, 26). The zoospores of plant-pathogenic oomycetes also exhibit chemotaxis in response to plant signals (5, 12, 16, 20, 21). Most oomycetes have a predominantly filamentous hyphal growth pattern. However, motile unicellular zoospores are generally released under conditions of flooding and nutrient deprivation (5). Zoospores are often the predominant means by which pathogenic oomycetes spread throughout the soil and infect plant roots (5).

Oomycete zoospores achieve chemotaxis by the same strategy as bacteria; that is, they swim more smoothly in the presence of an attractant and turn more frequently in the presence of a repellent (5), a strategy called klinokinesis. Zoospores of most *Phytophthora* species are attracted to a variety of sugars and amino acids, particularly aspartate, glutamate, arginine, and methionine (5). Most are also attracted by 0.2 to 20 mM ethanol, which may promote infection of flooded roots (5). Several oomycetes are attracted to specific plant signals. For example, isovaleraldehyde, valeraldehyde, and anti-isovaleraldehyde are chemoattractants of *Phytophthora palmivora* zoospores at concentrations as low as 1 μ M (4, 5). Prunetin (4',5-

dihydroxy-7-methoxyisoflavone) and related compounds are potent attractants (at concentrations as low as 10 nM) of *Aphanomyces euteiches* zoospores (20, 21), while the zoospores of *Aphanomyces cochlioides* are attracted to cochliophilin A [5-hydroxy-6,7-(methylenedioxy)flavone] from the roots of its host, the spinach plant, at concentrations as low as 1 nM (12).

The zoospores of the soybean pathogen *Phytophthora sojae* (syn. *P. megasperma* f. sp. *glycinea*) are highly sensitive to the isoflavones daidzein and genistein (16), which are present in soybean seeds and are exuded by the roots of that plant. *P. sojae* zoospores are attracted to these compounds at concentrations as low as 10 nM, but the zoospores from six other species of *Phytophthora* and one species of *Pythium* were not attracted even at 30 μ M (16). Therefore, Morris and Ward (16) suggested that the sensitive attraction of *P. sojae* zoospores to soybean isoflavones may be part of the mechanism which determines host range. In this study, we examined the response of zoospores from several *P. sojae* strains to a wide range of isoflavone analogs in order to assess the specificity with which the zoospores recognize plant signals and to examine the extent of genetic variability, if any, in their ability to recognize those signals.

MATERIALS AND METHODS

Strains. *P. sojae* P6497, P7076, and P7063 are of genotypes I, II, and IV, respectively (9).

Compounds. Most of the isoflavones, flavones, and coumarins used in this study were purchased from Indofine Chemical Company (Somerville, N.J.), most of the stilbenes were obtained from Apin Chemicals Ltd. (Abingdon, United Kingdom) or Research Plus, Inc. (Bayonne, N.J.), and most of the other chemicals were obtained from Aldrich Chemical Company (Milwaukee, Wis.). A few compounds were obtained from Sigma Chemical Company (St. Louis, Mo.). Isoformononetin was previously synthesized by P. F. Morris (16). Each solution was prepared by taking an approximately 1-mg sample of the dry compound, weighing it accurately on a five-place balance, and then dissolving it directly in 500 ml of water. Great care was taken to ensure that all the chemical had dissolved. Aliquots of 10 ml were then stored at -20°C for later use. Fresh dilutions were prepared for each day's experiment.

Preparation of zoospores and chemotaxis assays. Strains were grown on cleared V8 agar (17). Zoospores were prepared by repeated washing of plates when colonies covered two-thirds of the plate surface, as described previously (16). Each compound was diluted in 10-fold steps down to 10 nM (or lower for the most potent compounds). Each dilution of each compound was then assayed 5 to 10 times in direct comparison with a water control as follows (16). A 1- μ l glass capillary tube filled with the dilution of the chemical and a 1- μ l capillary tube filled with water were inserted into the same drop (500 μ l) of zoospores ($2 \times 10^5/\text{ml}$). After 15 min at 15°C , the numbers of zoospores in each tube were counted without fixation. The minimum attractive concentration was the lowest concentration at which the number of zoospores in the tube containing the test

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compound exceeded the number of zoospores in the water control tube in a statistically significant proportion of the comparisons (sign test; $P < 0.05$). In the case of repellents, the minimal repellent concentration was the lowest at which the control tube zoospore number exceeded the test zoospore number in a statistically significant proportion of the comparisons. All compounds were tested with at least two different preparations of zoospores of the same strain. All comparisons of P6497 and P7063 were carried out simultaneously with the same solutions and with zoospores of the same age.

RESULTS

Response of *P. sojae* zoospores to isoflavone analogs. For 59 isoflavone analogs, we measured the minimum concentration that could attract a statistically significant number of *P. sojae* zoospores in a capillary assay (16). The compounds were chosen on the basis of their structural similarity to different parts of the isoflavone structure and, in some cases, on the basis of their effect on nodulation gene induction in the bacterium *Bradyrhizobium japonicum* (6, 13). A diverse range of analogs was examined, including isoflavones, flavones, chalcones, stilbenes, benzoin, benzoates, benzophenones, acetophenones, and coumarins. The minimum attractive concentration for each compound is shown in Table 1. All compounds were tested with strain P6497, and then a subset of these chemicals was further tested with strain P7063. P6497 and P7063 represent two of the four major genotypes of *P. sojae* (9). Altogether, 43 of the compounds elicited some reaction from P6497 or P7063 at a level of 10 to 15 μM or lower. However, the only compounds to attract P6497 zoospores at concentrations below 1 μM were 7 of the 10 isoflavonoids tested and the compounds 4,4'-dihydroxystilbene and 4',6-dihydroxyflavone. Only isoflavones with a 4'-hydroxyl group and a 7-hydroxyl or 7-methoxyl group attracted P6497 zoospores at concentrations below 20 nM.

Several compounds acted as repellents of P6497 zoospores. In the presence of these compounds, significantly fewer ($P < 0.05$) zoospores entered the capillary tubes compared with the water control. The most potent repellents of P6497 zoospores were 7-hydroxy-5-methylflavone and 7-hydroxy-3-methylflavone, which caused significant repulsion at concentrations as low as 80 μM . No compounds with inhibitor activity were identified out of 17 compounds screened, which included repellents, weak attractants, and some compounds with neither activity.

Genetic variation in chemotaxis preferences. Förster et al. (9) identified four major genotypes of *P. sojae*. P6497 is representative of the most common genotype (genotype I). In order to look for genetic differences in chemotaxis preferences which might enable us to examine the genetics of chemotaxis, we screened 34 of the isoflavones and their analogs with strain P7063, which is representative of the second most common genotype, genotype IV (Table 1). The compounds examined included all of the major classes of compounds tested with P6497. Surprisingly, P6497 and P7063 zoospores reacted differently with all but nine of the compounds tested (summarized in Table 2). Most commonly (10 compounds; group A in Table 2), P7063 zoospores were more sensitive than P6497 zoospores (approximately 10-fold), showing significant attraction to 10-fold-lower concentrations of the compounds. Most of these compounds were isoflavones or other compounds which were good attractants, such as 4,4'-dihydroxystilbene. In addition, five compounds elicited a weak response from P7063 zoospores but no response at all from P6497 zoospores (groups B and C). In contrast, one compound, 4',7-dihydroxyisoflavan (equol; group D), was attractive to P7063 zoospores at concentrations 1,000-fold lower than for P6497 zoospores. Interestingly, all but two of the compounds which repelled P6497

zoospores were actually weak attractants for P7063 zoospores (group E). The exceptions were 6,7-dihydroxycoumarin (esculetin; group J), which repelled P7063 zoospores, and quercitrin (group H), which elicited no response. Only two compounds, 6,7-dimethoxy-2,2-dimethylchromanone and puerarin (groups F and G), were more attractive to P6497 than to P7063. Only three compounds were equally attractive to both strains (group I), and one was equally repulsive (6,7-dihydroxycoumarin). Five compounds elicited no response from either strain (group K).

In order to examine strain variation further, strain P7076 of genotype III was tested with five representative compounds. Its responses to 4',7-dihydroxyisoflavone (daidzein), 4',7-dihydroxyisoflavan (equol), and 6,7-dimethoxy-2,2-dimethyl-4-chromanone were identical to those of P7063. In the case of 7-hydroxy-5-methylflavone and 7-hydroxy-3-methylflavone, which repelled P6497 but attracted P7063 zoospores, the zoospores of P7076 were either insensitive or only very weakly attracted (at 8 μM), respectively. Only one strain representative of genotype II was available, and that strain produced insufficient zoospores for testing.

DISCUSSION

Structural requirements for attraction and repulsion. On the basis of the results of our survey, a number of generalizations can be made about the structural requirements for chemotaxis by *P. sojae* zoospores. These conclusions extend those initially noted by Morris and Ward (16). It should be noted that if there are several different receptors responsible for the responses to isoflavones and their analogs (see below), then some of the following generalizations may pertain to different receptors. The most striking generalization is the requirement for strong attractants (minimum attractive concentration of $<0.5 \mu\text{M}$ for P6497 and of $<50 \text{ nM}$ for P7063) to have phenolic hydroxyl groups at positions precisely matching the 4' and 7 positions of the isoflavone molecule. For example, 4,4'-dihydroxystilbene was an excellent attractant for both strains (Fig. 1). The relative positioning of the hydroxyl groups was very important. For example, 4',6-dihydroxyflavone was 100-fold more attractive to P7063 zoospores than was 4',7-dihydroxyflavone (Fig. 1), and 4,4'-dihydroxystilbene was more than 100-fold more attractive to P6497 than was 3',4,5'-trihydroxystilbene (Fig. 1). Methylation of the isoflavone 4'-hydroxyl group (to form biochanin A) reduced attractiveness 15- to 40-fold, while methylation of both hydroxyl groups (as in 4',6,7-trimethoxyisoflavone) reduced attractiveness 4,000-fold (Fig. 1). Methylation of the 7-hydroxyl group alone produced a more complex result; the attractiveness of 4',5-dihydroxy-7-methoxyisoflavone (prunetin) was reduced 10- to 30-fold compared with that of 4',5,7-trihydroxyisoflavone (genistein), but the attractiveness of 4'-hydroxy-7-methoxyisoflavone (isofomononetin) was comparable to that of 4',7-dihydroxyisoflavone (daidzein). In 4,4'-dihydroxystilbene, replacement of one hydroxyl group with an amino group reduced attractiveness 10- to 100-fold while replacement of both hydroxyl groups with amino groups eliminated attractiveness altogether (Fig. 1); aminated and nonhydroxylated isoflavones were unavailable commercially for testing. The spacing and orientation of the hydroxyl groups was also important; 3',4',6-trihydroxyaurone (sulfuretin), 2,6-dihydroxyanthraquinone, and 2,6-dihydroxynaphthalene were all very poor attractants compared with daidzein and 4,4'-dihydroxystilbene (Fig. 1). Interestingly, when methyl groups were arranged to precisely match the 4' and 7 positions of isoflavone, some attraction could be elicited. For example, 4,4'-dimethylbenzoin and 4,4'-dimethoxybiphe-

TABLE 1. Responses of *P. sojae* zoospores to isoflavones and analogs

Class ^a	Compound	Response of Strains ^b	
		P6497	P7063
Isoflavonoids	4',5,7-trihydroxy-isoflavone [genistein] ^c	A 10 nM	A 0.25 nM
	4',7-dihydroxy-isoflavone [daidzein]	A 10 nM	A 2 nM
	5,7-dihydroxy-4'-methoxy-isoflavone [biochanin A]	A 150 nM	A 10 nM
	4',5-dihydroxy-7-methoxy-isoflavone [prunetin]	A 75 nM	A 7.5 nM
	4'-hydroxy-7-methoxy-isoflavone [isoformononetin]	A 7 nM	A 0.7 nM
	4',7-dihydroxy-isoflavan [2,3-dihydro-4-deoxy-daidzein; equol]	A 30 nM	A 30 pM
	4',6,7-trimethoxy-isoflavone	I (7.5 μM)	A 1 μM
	4',6,7-trihydroxy-isoflavone	A 10 nM	n.t.
	daidzein-8-glucoside [puerarin]	A 1 μM	A 6 μM
	rotenone ^d	A 5 μM	A 0.5 μM
Flavonoids	7-hydroxy-flavone	I (8 μM)	n.t.
	7-hydroxy-5-methyl-flavone	R 80 nM	A 800 nM
	7-hydroxy-3-methyl-flavone	R 80 nM	A 800 nM
	4',5,7-trimethoxy-flavone	R 8 μM	n.t.
	5,7-dimethoxy-flavone	R 8 μM	n.t.
	4',7-dihydroxy-flavone	I (10 μM)	A 10 μM
	4',6-dihydroxy-flavone	A 850 nM	A 100 nM
	3,4',7-trihydroxy-flavone [5-deoxy-kaempferol]	I (7 μM)	n.t.
	4',5,7-trihydroxy-flavanone [naringenin]	A 8 μM	n.t.
	3,3',4',5,7-pentahydroxy-flavanone [taxifolin]	I (8 μM)	n.t.
Chalcones	4'-hydroxy-chalcone	I (12 μM)	A 12 μM
	2',4,4',6'-tetrahydroxy-7,8-dihydro-chalcone [phloretin]	R 7.5 μM	n.t.
Stilbenes & Benzoin ^e	4,4'-dihydroxy-stilbene	A 75 nM	A 7.5 nM
	4-hydroxy-4'-amino-stilbene	A 1 μM	A 1 μM
	4,4'-diamino-stilbene	I (7.5 μM)	n.t.
	4,4'-dinitro-stilbene	I (7.5 μM)	n.t.
	3,4',5-trihydroxy-stilbene [resveratrol]	A 10 μM	R 10 μM
	2,2'-dimethyl-4,4'-diamino-7,8-dihydro-stilbene	I (9 μM)	A 9 μM
	4,4'-dihydroxy-7,8-diethyl-stilbene [diethylstilbestrol]	n.t.	A 100 nM
	4,4'-dihydroxy-7,8-trans-diethenyl-7,8-dihydro-stilbene [dienestrol]	A 4 μM	A 1 μM
	4,4'-dihydroxy-7,8-diethyl-7,8-dihydro-stilbene [hexestrol]	A 10 μM	n.t.
	4,4'-dimethoxy-benzoin [anisoin]	I (7 μM)	n.t.
	4,4'-dimethyl-benzoin	A 11 μM	n.t.
	4'-hydroxy-8-deoxybenzoin	A 1.5 μM	A 150 nM
Benzoates	(4'-hydroxyphenyl) benzoate	I (11 μM)	A 100 nM
	hexyl 4-hydroxy-3-methoxy-benzoate [hexyl vanillate]	I (6 μM)	n.t.
Benzophenones	4-hydroxy-benzophenone	A 1.5 μM	A 150 nM
	2,4,4'-trihydroxy-benzophenone	A 14 μM	n.t.
	2,2',4',4'-tetrahydroxy-benzophenone	A 13 μM	n.t.
Acetophenones	2',4',6'-trihydroxy-acetophenone	I (14 μM)	R 14 μM
	2',4'-dimethoxy-acetophenone	A 14 μM	n.t.
	2',4'-dihydroxy-hexanophenone	I (14 μM)	n.t.
Biphenyls	4,4'-dimethoxy-biphenyl	A 10 μM	A 10 μM
	4,4'-bis(hydroxymethyl)-biphenyl	I (10 μM)	I (10 μM)
Coumarins	7-hydroxy-coumarin [umbelliferone]	I (16 μM)	n.t.
	7-hydroxy-4-methyl-coumarin [4-methyl-umbelliferone]	I (15 μM)	n.t.
	6,7-dihydroxy-coumarin [esculetin]	R 12 μM	R 12 μM
	coumestrol ^d	A 7.5 μM	A 7.5 μM
Other Bicyclic	6,7-dimethoxy-2,2-dimethyl-4-chromanone	A 8 μM	I (8 μM)
	6-methoxy-1-tetralone	A 3 μM	A 750 nM
	2-hydroxy-5,6,7,8-tetrahydro-naphthalene	I (10 μM)	A 10 μM
	2,6-dihydroxy-naphthalene	I (16 μM)	n.t.
	3,4-methylenedioxy-phenol [sesamol] ^d	I (15 μM)	I (15 μM)
Other ^f	3',4,6-trihydroxy-aurone [sulfuretin]	R 7.5 μM	A 10 μM
	tetrahydro-papaveroline	I (5 μM)	I (5 μM)
	2-(3-methoxy-phenyl)-1-cyclohexanone	I (10 μM)	n.t.
	2,6-dihydroxy-anthraquinone [anthraflavic acid]	A 11 μM	n.t.

Continued on following page

TABLE 1—Continued

^a On the generic structures, R₁, R₅, and R_{4'} (etc.) indicate various groups bonded to carbons at the 1, 5, and 4' positions, respectively. R_O indicates a group bonded to an oxygen. Dashed double bonds or aromatic rings indicate that some compounds in the class have unsaturated bonds at those positions and some do not.

^b A, attraction; R, repulsion; I, insensitive; n.t., not tested (in a couple of cases, n.t. also includes compounds tested but giving unreliable results). Concentrations given are minimum attractive concentration, minimum repellent concentration, or maximum concentration tried (in parentheses), respectively.

^c Commonly used trivial names are given in brackets.

^d Rotenone, coumestrol, and sesamol, while chemically related to the class of compounds with which they are grouped, have structures more complex than the generic ones shown in the table.

^e In benzoin, R₇ is =O, R₈ is —OH, and C₇—C₈ is saturated.

^f The structures of 3',4,6-trihydroxyaurone and 2,6-dihydroxyanthraquinone are shown in Fig. 1.

nyl were weakly attractive to P6497 zoospores, whereas 4,4'-dimethoxybenzoin and 4',6,7-trimethoxyisoflavone were completely unattractive (Fig. 1). Methoxyl groups also permitted some attraction when located on smaller bicyclic molecules, such as 6-methoxytetralone and 6,7-dimethoxy-2,2-dimethylchromanone (Fig. 1). Surprisingly, the aliphatic hydroxyl groups of 4,4'-bis(hydroxymethyl)biphenyl were less attractive than the methoxyl groups of 4,4'-dimethoxybiphenyl (Fig. 1). Glycosylation of 4',7-dihydroxyisoflavone at either the 7 position (to form daidzin [16]) or the 8 position (to form puerarin) reduced attractiveness at least 100-fold (Table 1), presumably because of steric interference in the recognition of the hydroxyl groups.

Other microbes are also sensitive to hydroxylation of flavone and isoflavone signal molecules. The nodulation response of *B. japonicum* to isoflavones also exhibited a strong requirement for a 7-hydroxyl group on the isoflavone molecule plus a free hydroxyl, methoxyl, or halogen four to six carbons distal to the carbonyl group (e.g., at the 4' position) (6, 10, 13, 18). Zoospores of the pea and alfalfa oomycete pathogen *Aphanomyces euteiches* also recognize nanomolar levels of isoflavones (20, 21). For this species, a hydroxyl group at the 4' position and a hydroxyl or methoxyl group at the 7 position also were

important. However, the most important substituent in this case was a 5-hydroxyl group (20, 21). The 4'-hydroxyl group of acetosyringone and related compounds is also critical for their recognition by *Agrobacterium tumefaciens* (11).

The carbonyl group at position 4 on isoflavone was not essential for attraction of the zoospores, since 4',7-dihydroxyisoflavan (equol) and 4,4'-dihydroxystilbene were excellent attractants. On the other hand, on small bicyclic compounds, the carbonyl group seemed to be important: 6-methoxytetralone and 6,7-dimethoxy-2,2-dimethylchromanone, which contain carbonyl groups at an analogous position, were weak attractants, whereas 4-methylumbelliferone, 5,6,7,8-tetrahydro-2-hydroxynaphthalene, and sesamol, which lack the carbonyl group, were completely unattractive (Fig. 1). In fact, most molecules having a phenolic hydroxyl group and a carbonyl group matching the 7 and 4 positions, respectively, of isoflavone showed some attractiveness. The attractiveness of 4,4'-dihydroxystilbene also shows that the heterocyclic oxygen is not essential, but the 10-fold-greater attractiveness of 4',7-dihydroxyisoflavan (equol) (Fig. 1) suggests that this oxygen, or the ring itself, does make some contribution.

The structural features required for repulsion are still somewhat unclear. Methoxylated or methylated flavones consistently repelled P6497. The position of the substitution did not seem to matter very much, since 7-hydroxy-3-methylflavone and 7-hydroxy-5-methylflavone were equally good repellents but 7-hydroxyflavone, with no methyl substitution at all, was inactive. There also appeared to be no requirement for the presence or absence of an unsubstituted hydroxyl group, since 5,7-dimethoxyflavone and 4',5,7-trimethoxyflavone also were good repellents. Testing of further analogs may uncover more consistent patterns. Intriguingly, one of the two strongest repellents of *P. sojae* zoospores, 7-hydroxy-5-methylflavone, was also the most potent inhibitor of the nodulation response of several genotypes of *B. japonicum* (6). In addition to the flavones, a few other compounds with no obviously related features showed weak repulsion, including phloretin, quercitrin, and sulfuretin for P6497, esculetin for both strains, and resveratrol and 2,4,6-trihydroxyacetophenone for P7063. For *P. sojae*, it is not yet clear whether repulsion is fundamentally different than attraction. In organisms that use klinokinesis as a chemotaxis strategy, such as *Phytophthora* species and many bacteria, attraction and repulsion are part of a continuum of response: attraction results from a lower-than-average frequency of turning, while repulsion results from a higher-than-average frequency of turning (5, 15). In *Escherichia coli*, the same receptors and the same signal transduction pathways mediate both attraction and repulsion (15). Therefore, in *P. sojae*, the same signal transduction pathway, if not the same receptor, probably mediates both responses (see below).

While *P. sojae* zoospores responded to a wide range of analogs at micromolar concentrations, they were attracted to very few of the compounds at nanomolar to picomolar concentrations, at which they respond to the native soybean isofla-

TABLE 2. Types of strain variation in preferences for isoflavones and their analogs

Group ^a	Number of compounds tested	General response of strain (genotype) ^b :	
		P6497 (I)	P7063 (IV) (relative to P6497)
A	10	Attracted	Attracted (1/10 as high)
B	3	Insensitive	Weakly attracted
C	2	Insensitive	Weakly repelled
D	1	Attracted	Attracted (1/1,000 as high)
E	6	Repelled	Attracted
F	1	Attracted	Attracted (6-fold higher)
G	1	Weakly attracted	Insensitive
H	1	Weakly repelled	Insensitive
I	3	Weakly attracted	Weakly attracted (same)
J	1	Weakly repelled	Weakly repelled (same)
K	5	Insensitive	Insensitive

^a Compounds are grouped not by structure but based on similar (though not identical) differentials in responses of P6497 and P7063 zoospores. The differentials for groups A, B, and C, groups F, G, and H, and groups I and J are very similar and may represent the same phenomenon.

^b Genotypes are according to Förster et al. (9); 1/10 as high, 1,000 as high, and 6-fold higher indicate that for all compounds in this group, P7063 zoospores were attracted at approximately 10-fold lower, 1,000-fold lower, and 6-fold higher concentrations, respectively, than were P6497 zoospores; "weakly" indicates that a response occurred only at a compound concentration of 5 μ M or higher.

FIG. 1. Structural features of *P. sojae* chemoattractants. SA, strong attractant; the minimum attractive concentrations for P6497 and P7063 zoospores are <500 nM and <50 nM, respectively. A, attractant; the minimum attractive concentrations for P6497 and P7063 zoospores are between 500 nM and 5 μ M and between 50 nM and 0.5 μ M, respectively. WA, weak attractant; the minimum attractive concentrations for P6497 and P7063 zoospores are >5 μ M and >0.5 μ M, respectively. I, insensitive; P6497 zoospores are insensitive to the maximum tested concentration (usually 10 μ M), and P7063 zoospores are insensitive to the maximum tested concentration (usually 10 μ M) or attracted only at >5 μ M. See Table 1 for minimum attractive concentrations of individual compounds.

vones genistein and daidzein. Therefore, it appears that *P. sojae* has evolved to respond very sensitively to isoflavones characteristic of the soybean. Since no other *Phytophthora* species tested could detect these isoflavones as sensitively as *P. sojae* (16), the ability to recognize isoflavones closely matching genistein and daidzein in structure may be an important mechanism of host recognition in this pathogen. Moreover, although many legume species other than soybean exude isoflavones, those isoflavones are dominated by coumestrol and/or by isoflavones methylated at the 4' position (formononetin and biochanin A) or the 7 position (prunetin) (2, 7, 8, 20, 22, 23), substitutions which reduce the attractiveness of the compounds to *P. sojae* zoospores by a factor of 10 to 100. Relatively small amounts of daidzein and genistein are released. The recognition of potential host plants or sites of potential infection by *P. sojae* zoospores may be further refined by their attraction to or repulsion from higher concentrations of a wider range of flavonoids, coumarins, and other phenolic compounds. There is also the possibility that *P. sojae* zoospores may respond synergistically to very specific combinations of these or other root exudate compounds, similar to the synergistic response of *Agrobacterium tumefaciens* virulence genes to a combination of acetosyringone, sugars, and low pH (25).

P. sojae is one of several recent examples of plant pathogens having spores that respond to low levels of compounds exuded by their preferred hosts. As noted above, zoospores of *Aphanomyces euteiches* are chemotactically attracted to as little as 10 nM prunetin, which is exuded from the roots of its host, the pea plant (20, 21). Similarly, cochliophilin A [5-hydroxy-6,7(methylenedioxy)flavone] was isolated from the roots of spinach as a potent attractant (<1 nM minimum attractive concentration) of zoospores of the spinach pathogen *Aphanomyces cochlioides* (12). In the fungus *Nectria haematococca*, which is a pathogen of peas and beans, host flavonoids and pterocarpin isoflavonoids stimulate spore germination and the induction of phytoalexin-detoxifying enzymes (19).

Genetic variation in chemotaxis specificity. A surprising observation during this study was the extensive difference in chemotaxis specificity displayed by strains P6497 and P7063. Of 29 compounds which elicited a response from either strain, only four produced the same response (summarized in Table 2). At the DNA sequence level, these two strains exhibit only about 8% variation among randomly amplified polymorphic DNA and restriction fragment length polymorphism markers (9), and both are excellent pathogens of soybean, so the extensive variation in chemotaxis specificity was unexpected. These differences suggest the possibility that the two strains, and possibly the genotypes to which they belong, are adapted to or have coevolved with different plant communities, which may or may not include soybean. Since we did not test a wide range of strains of each genotype, we do not know yet whether the differences observed are specifically correlated with particular genotypes.

Other plant pathogens and symbionts show strain differences in their responses to plant signals. For example, in the fungus *N. haematococca*, apigenin (4',5,7-trihydroxyflavone), eriodictyol (3',4',5,7-tetrahydroxyflavanone), and pisatin (6 α -hydroxy-3-methoxy-8,9-methylenedioxypterocarpin) strongly stimulated spore germination in pea-pathogenic strains but not in bean-pathogenic strains, whereas genistein (4',5,7-trihydroxyisoflavone) and maackiain (3-hydroxy-8,9-methylenedioxypterocarpin) strongly stimulated spore germination in the bean strains but not in the pea strains (19). The six major genotypes of the bacterium *B. japonicum* also exhibit substantial variation in the responses of their nodulation genes to different isoflavone analogs (6, 13).

Our *P. sojae* strains displayed several different classes of differential responses (Table 2). This raises the possibility that there may be several different receptors for the different classes of responses. Preliminary genetic analysis suggests that at least three genes may be responsible for the differences. Genetic mapping of the genes responsible for the differences between P6497 and P7063 may enable us to clone genes encoding the receptors and possibly the signal transduction proteins responsible for chemotaxis from *P. sojae* and eventually from other oomycetes. Ultimately, knowing the structures and functions of key chemotaxis receptors may enable novel disease control strategies.

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