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Interspecific hybrids between the homothallic *Phytophthora sojae* and *Phytophthora vignae*

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Abstract. An interspecific cross was attempted between two homothallic species of *Phytophthora*, *P. sojae* and *P. vignae*. From 1640 single-oospore cultures isolated, DNA was extracted from 800, and two interspecific F_1 hybrids ($F_1$1121 and $F_1$1426) were putatively identified using RAPD markers. The true hybrid nature of these F_1 hybrids was confirmed using additional AFLP analysis. Single-zoospore cultures were generated for each F_1 hybrid and one single-zoospore culture of each was used in pathogenicity and virulence tests. Both F_1 hybrids were pathogenic to soybean and cowpea, causing symptoms including lesions, wilting and death of susceptible soybean and cowpea cultivars. However, the aggressiveness of the F_1 hybrids was reduced and was substantially more variable when compared with that of the parental isolates on their respective hosts. The F_1 hybrids were reisolated from infected seedlings and their hybrid nature confirmed using RAPD and AFLP analysis. These results provide a basis for further research aimed at obtaining an increased understanding of the genetics of host specificity in the Oomycetes.

Additional keywords: genetics, host specificity, pathogenicity, Oomycetes, outbreeding.

Introduction

The genus *Phytophthora* comprises about 60 species, of which almost all are plant pathogens (Brasier and Hansen 1992; Erwin and Ribeiro 1996). Most are primary invaders of healthy plant tissue, with limited saprophytic ability. Approximately half of the 60 species are heterothallic (Brasier and Hansen 1992), exhibiting two mating types (A1 and A2) whereas the remaining species are homothallic (inbreeding). Some *Phytophthora* species are host specific, whereas others have wide host ranges which extend to numerous different plant species, often encompassing different plant families (Ribeiro 1978; Erwin and Ribeiro 1996). Both *P. sojae* and *P. vignae* are homothallic and host specific to soybean (Hildebrand 1959) and cowpea (Purss 1957), respectively. As well as exhibiting host specificity, both of these species show cultivar specificity and many different races have been identified (reviewed in Erwin and Ribeiro 1996). The molecular basis for cultivar specificity has been an interesting research area, and several pathogen elicitors and host resistance genes have been cloned, but their functional relationships are still largely unknown (Laugé and de Wit 1998). Although there has been considerable emphasis on the genetics of cultivar specificity, host specificity in pathogens has been much less studied. Since *P. sojae* and *P. vignae* are both homothallic, host specific and closely related based on ITS rDNA sequence data (Cooke *et al*. 2000), they may provide an ideal experimental model system to study the genetics of host specificity.

Simple inheritance of host range determinants may underlie the genetic basis of host specificity. Evidence for this involves *Phytophthora* species that produce low-molecular-weight proteins called elicitins, and which induce a defence response in species of *Nicotiana* (Kamoun *et al.* 1997). Elicitins appear to be involved in simple gene-for-gene relationships at the host species level (Ricci *et al.* 1992; Kamoun *et al.* 1994). Genes encoding elicitins have been isolated from *P. infestans* (*inf1*) (Kamoun *et al.* 1998) and *P. parasitica* (*para1*) (Kamoun *et al.* 1993*a*, 1993*b*). A defence response is elicited from *Nicotiana* species when inoculated with wild-type strains of *P. infestans* containing *inf1* (Kamoun *et al.* 1998). Molecular support for the role of elicitins in host specificity was obtained when *inf1* was interrupted and *P. infestans* was capable of performing its full infection cycle on *N. benthamiana* (Kamoun *et al.* 1998).

Oospores form readily in pairings of different heterothallic *Phytophthora* species in culture and it may be

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Table 1. Race, origin and source of parental isolates of *Phytophthora sojae* and *P. vignae* used in this investigation

Isolate	Species	Host	Race	Origin	Year of isolation	Source
UQ1200	*P. sojae*	Soybean	25	Condobolin, NSW	1994	M. J. Ryley
UQ3035	*P. vignae*	Cowpea	1	Coomera, Qld	1974	G. S. Purss

possible to use any resultant interspecific hybrids to examine the genetics of host specificity for these organisms (Haasis and Nelson 1963; Savage *et al.* 1968; Boccas and Zentmyer 1976). Interspecific hybrids were obtained from a cross between *P. infestans* and *P. capsici* and, based on pathogenicity data, it was suggested that host specificity may be under simple genetic control (Vorobèva and Gridnev 1981, 1983). This has also been suggested by Goodwin and Fry (1994) who constructed a cross between *P. infestans* and *P. mirabilis* and identified a number of interspecific hybrids using neutral markers. When compared with the parental isolates, the interspecific hybrids exhibited reduced pathogenicity and aggressiveness such that none infected *Mirabilis jalapa* and they were considerably less aggressive on potato and tomato.

The formation of interspecific hybrids among homothallic species of *Phytophthora* for genetic studies concerning host specificity would be more problematic as they would not be expected to outcross readily. However, interspecific hybrids between homothallic parents would be expected to have the full complement of genetic information from both parents, as the parents are expected to be homozygous at a high percentage of their loci due to their homothallic nature (Liew *et al*. 1991; Tyler *et al*. 1995; Whisson *et al*. 1995). Therefore, the aims of the research reported in this paper were: (i) to construct an interspecific cross between the two homothallic species, *P. sojae* and *P. vignae,* and to unambiguously identify interspecific F_1 hybrids using neutral genetic markers; (ii) to conduct pathogenicity and virulence tests to examine the relative ability compared with the parents of the resultant interspecific F_1 hybrids to infect multiple hosts, soybean and cowpea; and (iii) to examine the morphology of the sexual structures of the interspecific F_1 hybrids and compare these with the parental species.

Methods

Fungal cultures, F_1 hybrid construction and oospore isolation

The *P. sojae* isolate, UQ 1200, and *P. vignae* isolate, UQ 3035, both generated from single zoospores, were used as parents (Table 1). Both come from the culture collection of the Cooperative Research Centre for Tropical Plant Protection, University of Queensland, Australia. Each isolate was grown on clarified V8 media in the dark at 25°C. Mycelial discs from both parental mating types were placed on opposite sides of five plates of carrot agar (Ribeiro 1978) that were incubated at 25°C in the dark for 30 days. Oospores were extracted from the cross in the following manner. A strip of media where the two cultures merged was excised and placed in a sterile blender with 180 mL of sterile distilled water and homogenised until liquid. The liquid was kept at 4°C at all times. The macerated culture was then strained through a 75 μM sieve and the resultant liquid collected in 10 mL centrifuge tubes. The samples were centrifuged at 2000 rpm for 2 min and the supernatant carefully discarded. To each tube, 5 mL of 0.05% $KMnO_4$ was added, mixed thoroughly and left to stand for 10 min. After 10 min, the tubes were centrifuged at 2000 rpm for 2 min and the supernatant discarded. The pellet was washed with sterile distilled water and centrifuged again under the above conditions. The washes were repeated until all visible traces of $KMnO_4$ were gone. The remaining oospores were resuspended in 150 μL of sterile distilled water, the concentration determined, and then spread thinly onto 1.5% water agar plates. Germinating oospores were transferred using microdissection and incubated in the dark at 25°C on V8 agar plates without antibiotics.

DNA extraction procedures

A rapid DNA extraction technique was used to extract DNA from 800 single oospore cultures. This method has been previously described by McDonald *et al.* (1994) with the following modifications. Extraction buffer (200 μL) (McDonald *et al.* 1994) was added to fungal tissue scraped from a solid V8 agar culture, which was then thoroughly macerated with a micropestle. A further 800 μL of the extraction buffer was then added and the tubes mixed on a vortex mixer. The fungal material was incubated at 60°C for 30 min, left to cool slightly, then 50 μL of equilibrated phenol was added to each tube and mixed thoroughly. All tubes were centrifuged for 2 min at 14000 rpm and the supernatant transferred to a new tube. Finally, when the DNA pellet was obtained, the supernatant was discarded, the pellet dried and resuspended in 50 μL of water.

DNA was also extracted using a large-scale extraction technique. Single-oospore cultures suspected of being interspecific hybrids as well as ten single-zoospore cultures of a putative interspecific hybrid were used for DNA extraction. Two different DNA extraction techniques were used. The first method has been described in Whisson *et al.* (1993) and the second was obtained from Drenth *et al.* (1993). The integrity of the DNA was checked using agarose gel electrophoresis and the DNA concentration measured using fluorometry.

Detection of interspecific hybrids using Random Amplified Polymorphic DNA (RAPD) analysis

Hybrids were detected using the decanucleotide primer OPW15 (Operon Technologies, Alameda, CA; www.operon.com/store/merkits.php) in the following manner. DNA was extracted from single-oospore cultures using the rapid extraction technique described above and diluted 100 times for the RAPD reaction. The RAPD reaction and agarose gel analysis has been previously described in Whisson *et al.* (1994). Any single-oospore culture possessing a combination of fragments amplified in both the *P. sojae* parent and the *P. vignae* parent was further tested with additional RAPD primers after DNA was extracted using the large-scale extraction technique described above. If the single-oospore cultures possessed all the bands amplified from both parents, they were retained as putative interspecific F_1 hybrids for additional testing with AFLPs. A total of ten single-zoospore cultures from each of the putative F_1 hybrids was obtained and used in further RAPD reactions to confirm the hybrid nature of the putative hybrids. The RAPD primers, OPC16, OPE09,

OPF03, OPF07, OPM07, OPW03 and OPW15, were used for further confirmation of the interspecific F_1 hybrids.

AFLP analysis of interspecific hybrids

AFLP markers were prepared and detected as described by Vos *et al.* (1995) using the GibcoBRL Life Technologies AFLP Core Reagent and Analysis System II that is modified for the small genomes of some plants as follows. Digestion of genomic DNA, ligation of adaptors and preamplification of prepared DNA template conformed to the protocol except for a modified thermocycling sequence: one cycle of 94°C for 15 s, 65°C for 15 s and 72°C for 30 s. The annealing temperature was lowered stepwise by 0.7°C for each step for the following 12 cycles (touchdown cycle). This was followed by 23 cycles of 94°C for 15 s, 56°C for 15 s and 72°C for 30 s. Selective amplification was also performed according to the manufacturer's instructions with one modification. The *Eco*R1 primer with two selective nucleotides was replaced with a primer with a single, selective nucleotide and the *Mse*1 primer was MCTA. The PCR amplification protocol was the same as that used in the preamplification reactions.

Pathogenicity testing

The known susceptible soybean cultivars Harosoy 1 (*rps*) and Ross (*rps*) and the susceptible cowpea cultivar, Poona, were used in pathogenicity testing to determine if the putative interspecific F_1 hybrids were pathogenic to both soybean and cowpea. The pathogenicity test performed was the standard hypocotyl inoculation test (Whisson *et al.* 1995). The *P. sojae* and *P. vignae* parents as well as the single-zoospore cultures F_1 1121-5 and F_1 1426-5 of each putative F_1 hybrid underwent pathogenicity testing. For each hybrid or parental isolate tested, an experimental unit was comprised of a 15-cm-diameter pot containing ten soybean or cowpea seedlings of each cultivar. Three replications of each experimental unit were inoculated with each of the single-zoospore and parental cultures. The experiment was repeated on three separate occasions.

Virulence tests

The same single-zoospore isolates used in the pathogenicity tests, $F_1$1121-5 and $F_1$1426-5, and the two parental isolates were then used in virulence tests on the soybean and cowpea differential sets. For soybean, these were Harosoy 12 (*Rps*1a), Harosoy 13 (*Rps*1b), Wells II (*Rps*1c), PI103091 (*Rps*1d), Kingwa (*Rps*1k), L83-570 (*Rps*3a), L88-1479 (*Rps*3b), X572-373 (*Rps*3c), L85-2352 (*Rps*4), L85-3059 (*Rps*5) and L89-1581 (*Rps*6) (Whisson *et al*. 1995) and for cowpea the differentials were Poona, Blackeye 5 and Bechuana White, as used to distinguish races of *P. vignae* by Purss (1972). The virulence tests were conducted using the same protocol as for the pathogenicity tests with two replications, each replicate comprising a pot of ten seedlings for each cultivar or line.

Morphological studies of interspecific hybrids

The morphology of the sexual structures of the two parental isolates and of the two interspecific F_1 hybrids isolated was studied. The diameter of 50 oogonia and oospores was measured as well as the dimensions of the antheridia; the type of antheridial attachment (amphigynous or paragynous) was also determined.

Results

Identification of interspecific hybrids

A total of 1640 germinating single-oospore cultures was harvested from the interspecific cross, and 800 of these were screened with RAPD primers to identify potential interspecific hybrids. The vast majority of the single oospores that germinated and produced a culture had RAPD banding patterns identical to that of the *P. sojae* parent, and none of the single-oospore cultures had banding patterns identical to the *P. vignae* parent. This reflects the lower germination rate of oospores of *P. vignae* (< 1%) compared with those of *P. sojae* (> 10%) (J. A. G. Irwin and M. Sterling, unpublished data). Two putative interspecific F_1 hybrids that possessed a combination of the RAPD bands amplified from the *P. sojae* and the *P. vignae* parents with primer OPW15 were obtained and designated $F_1$1121 and $F_1$1426 (Figs 1 and 2). DNA was isolated from ten single-zoospore cultures for each of the two putative F_1 hybrids and screened against a series of RAPD primers. The results indicated that the two putative F_1 hybrids were true hybrids, as the single-zoospore cultures possessed the same banding pattern as the original single-oospore F_1 culture (Figs 1 and 2). AFLP analysis also confirmed that $F_1$1121 and $F_1$1426 were interspecific F_1 hybrids (Fig. 3). Based on our experimental conditions *in vitro*, an outcrossing rate of 0.25% occurred between *P. sojae* and *P. vignae*.

Pathogenicity tests

In all of the pathogenicity tests, the *P. sojae* parent produced typical symptoms and killed all inoculated susceptible soybean seedlings without causing symptoms on the cowpea cultivar. The *P. vignae* parent produced typical symptoms on the cowpea cultivar killing 100% of inoculated seedlings and no symptoms on the soybean cultivar. Each single-zoospore culture from both F_1 hybrids showed variable levels of pathogenicity towards cowpea and soybean in our experiments (Table 2). Both F_1 isolates killed > 50% of soybean and > 30% of cowpea seedlings, indicating that they had acquired pathogenicity towards both hosts. However, there were varying levels of intermediate and resistant responses by both soybean and cowpea to the two hybrids, indicating their reduced aggressiveness (relative capacity to cause disease) compared with the parental isolates (Table 2).

Virulence tests

The *P. sojae* parent produced a virulence spectrum typical of race 25 (virulent on *Rps* 1a, 1b, 1c, 1k, 7) when inoculated on a differential set of soybean cultivars and the *P. vignae* parent produced the typical virulence spectrum of a race 1 isolate on the cowpea differential set (virulent on Poona only). Neither parent caused any symptoms on the non-host differential set. The hybrids $F_1$1121-5 and $F_1$1426-5 produced disease symptoms only on soybean and cowpea cultivars that were susceptible to the parental isolates.

Morphological analysis

The morphology and dimensions of the sexual structures of the two F_1 hybrids were compared with those of the parental isolates. For $F_1$1121-5, the oogonial diameter and

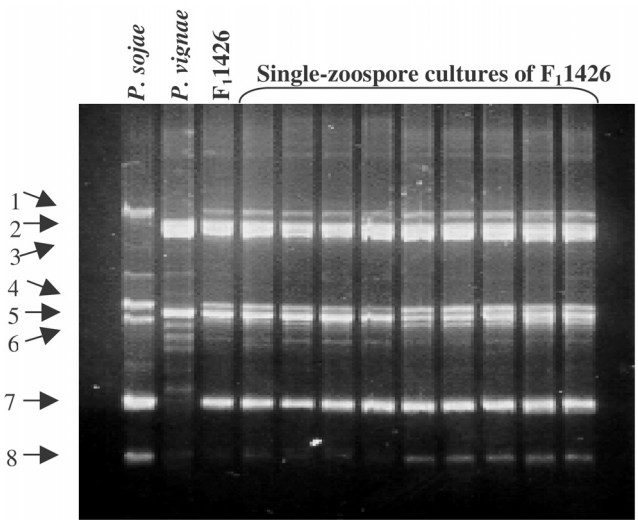

Fig. 1. Identification of interspecific F_1 hybrids using the RAPD primer OPW15. Bands 1, 4, 7 and 8 are present in the *Phytophthora sojae* parent and bands 2, 3, 5 and 6 are present in the *P. vignae* parent. All polymorphic bands are present in the F_1 hybrid and the single-zoospore cultures derived from that F_1 hybrid.

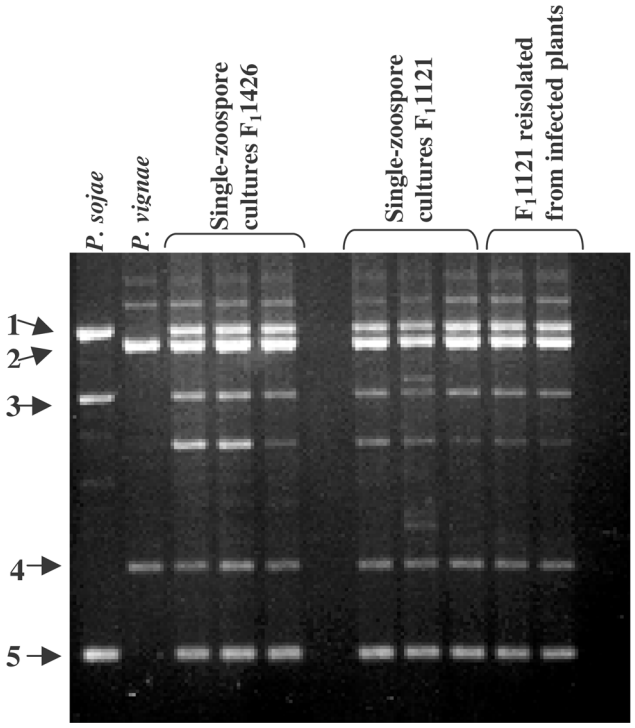

Fig. 2. Confirmation of interspecific F_1 hybrids using the RAPD primer OPM07. Bands 1, 3 and 5 are present in the *Phytophthora sojae* parent, and bands 2 and 4 are present in the *P. vignae* parent. All polymorphic bands are present in the single-zoospore cultures of $F_1$1121 and $F_1$1426 as well as re-isolations of $F_1$1121 obtained from infected seedlings.

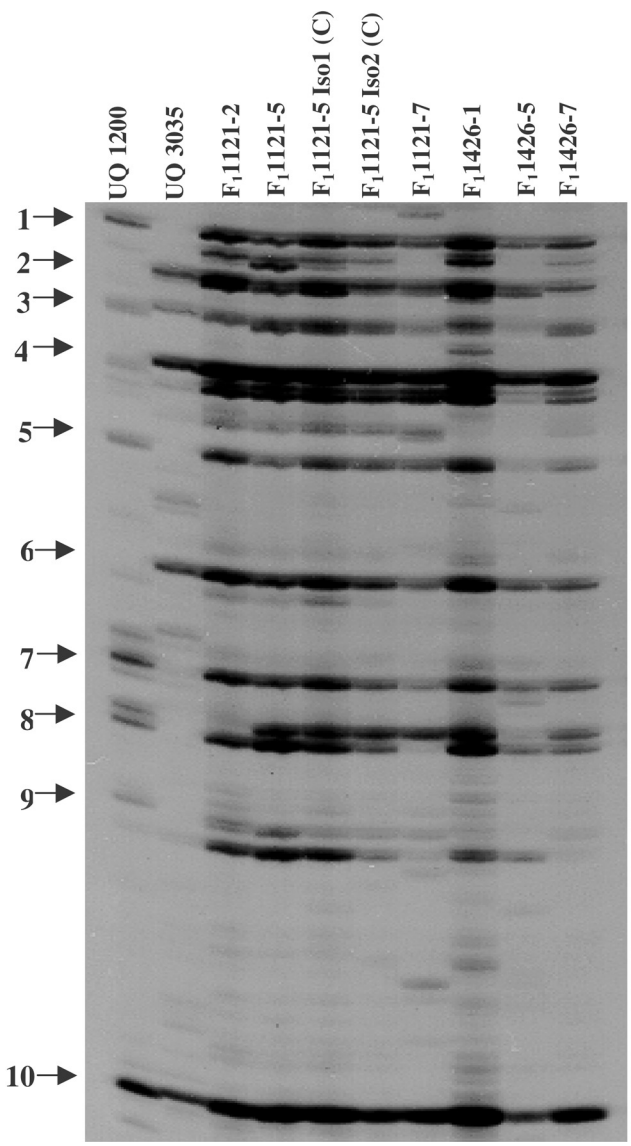

Fig. 3. Confirmation of the hybrid nature of the interspecific F_1 hybrids between *Phytophthora sojae* and *P. vignae* using the AFLP primers, E02 and MCTA. Arrows indicate polymorphic markers.

oospore wall thickness were similar to the *P. vignae* parent, whereas the oospore diameter was significantly larger than the *P. vignae* parental isolate (Table 3). The oogonial diameter of $F_1$1426-5 was similar to the *P. sojae* parent but the oospore dimensions and the wall thickness differed significantly from the *P. sojae* parent (Table 3). Antheridial dimensions were significantly different between the parents and both F1 hybrids whereas the hybrids had dimensions similar to each other compared with the parental isolates. Only amphigynous attachment of the antheridia to the oogonia was observed for both F_1 hybrids, this being a characteristic of the *P. vignae* parent. In contrast, antheridial attachment for the *P. sojae* parent is predominantly paragynous with a small percentage amphigynous, and it is

Table 2. Percentage infection of soybean and cowpea inoculated with *Phytophthora sojae* and *P. vignae* parents and their F_1 hybrids

Phenotype	*P. sojae*		*P. vignae*		$F_1$1121-5		$F_1$1426-5	
	Soybean	Cowpea	Soybean	Cowpea	Soybean	Cowpea	Soybean	Cowpea
S[A]	100	0	0	100	65	43	50	30
R	0	100	100	0	15	50	30	40
I	0	0	0	0	20	7	20	30

[A]S – susceptible; R – resistant; I – intermediate showing either a restricted lesion and/or wilting.

Table 3. Dimensions of sex organs (± standard deviation) and type of antheridial attachment for the parental isolates and F_1 hybrids

	Oogonium diameter (µm)	Oospore dimensions (µm)	Oospore wall thickness (µm)	% Amphigynous	Antheridial dimensions (µm)
P. sojae	34.1 ± 5.0 a[A]	27.8 ± 4.1 a	2.7 ± 0.6 a	26	11.8 ± 2.7 a × 11.3 ± 2.8 a
P. vignae	29.5 ± 3.6 b	24.2 ± 2.8 b	1.9 ± 0.5 b	100	21.2 ± 5.6 b × 15.6 ± 2.1 b
$F_1$1121-5	30.9 ± 3.3 b	25.6 ± 3.0 c	1.9 ± 0.5 b	100	14.0 ± 3.0 c × 13.7 ± 1.6 c
$F_1$1426-5	35.5 ± 4.0 a	30.1 ± 3.4 d	2.4 ± 0.8 c	100	14.7 ± 2.9 d × 14.1 ± 1.7 c

[A]Mean values within columns followed by the same letter are not significantly different at $P = 0.05$ ($n = 50$).

possible that amphigynous attachment may be epistatic to paragynous attachment. Many of the oospores of the F_1 hybrids that were observed appeared to have aborted leaving either empty oogonia or large globular and refractile structures within the oogonium. No germination of oospores formed by the F_1 hybrids was observed at any time.

Discussion

The interspecific F_1 hybrids produced here are the first known interspecific hybrids to be generated from homothallic *Phytophthora* species. The two interspecific F_1 hybrids, $F_1$1121 and $F_1$1426, were obtained from a cross between *P. sojae* and *P. vignae* after 800 single-oospore cultures obtained from a pairing of the two *Phytophthora* species on agar plates were screened using RAPD primers. Both RAPD and AFLP data confirmed that $F_1$1121 and $F_1$1426 were interspecific hybrids. An outcrossing rate of 0.25% between *P. sojae* and *P. vignae* was observed under our experimental conditions. As outcrossing has occurred, it is evident that the two species have not as yet diverged sufficiently for evolving genetic barriers to prevent the exchange of genetic material.

P. vignae was first reported in Australia on cowpea by Purss (1957). It has also been observed in Japan on adzuki bean (Kitazawa *et al.* 1979) and on *Vigna sesquipedalis* (yard-long bean) in China (Hwang and Qi 1984). However, molecular markers have shown that there is a greater level of diversity within isolates obtained from adzuki bean when compared with isolates obtained from cowpea. This provides evidence for the hypothesis that *P. vignae* was introduced to Australia either from Japan or China. As *P. sojae* is thought to have originated from China, it can be speculated that *P. sojae* and *P. vignae* might have evolved from a common Oomycete ancestor in this region. Host specialisation would have been dependent on, and may even have been driven by, the availability of potential hosts, and on the evolution of necessary genes that allowed specificity on the new host. Very little is known about the evolution of *Phytophthora* species from a common ancestor with the exception of *P. infestans* and *P. mirabilis* both of which appear to have originated from Central Mexico. These two heterothallic species are closely related and *P. mirabilis* was once considered to be a variety of *P. infestans* (*P. infestans* var. *mirabilis*) until small morphological differences were elucidated, resulting in their separation into two species (Stamps *et al*. 1990). It has been suggested that these two heterothallic species shared a common ancestor and that a speciation event resulted in the change of host specificity (Goodwin and Fry 1994). There are other reports providing support for the hypothesis that new species in *Phytophthora* may arise through hybridisation (Sansome *et al*. 1991; Man in't Veld 1998; Brasier *et al*. 1999).

The interspecific F_1 hybrids from our cross were pathogenic on both soybean and cowpea, but appeared to exhibit reduced aggressiveness to both plant species. A reduction in aggressiveness for interspecific hybrids of heterothallic *Phytophthora* species when compared with the parental isolates has also been observed by Vorobèva and Gridnev (1983), Goodwin and Fry (1994) and Ersek *et al.* (1995). It has been suggested that the reduction in fitness of the interspecific hybrids may be due to the re-assortment of chromosomes during meiosis and the loss of genes through aneuploidy necessary for conditioning pathogenicity equal to that of the parental isolates. However, the interspecific hybrids of homothallic parents are presumed to possess the full set of genes from both parents within the hybrid genome.

This would be expected as the parents are predicted to be homozygous at a large percentage of loci due to their homothallic reproductive cycle. As the interspecific F_1 hybrids of our cross should have the full complement of genes from both diploid parents, the reduced pathogenicity could be due to cytological imbalances, if chromosome numbers and/or ploidy levels are different between the parents. The karyotype of either parent has not been reliably determined. In addition, conflicting signals from the different parental components within the F_1 hybrids may impair their ability to infect the host plant after hypocotyl inoculation. Nevertheless, our results show the ability of the F_1 hybrids to infect both soybean and cowpea and establish some form of basic compatibility as defined by Ellingboe (1976), albeit with reduced aggressiveness.

Although interspecific hybrids between homothallic *Phytophthora* species have never been isolated from nature, the ability of *P. sojae* and *P. vignae* to hybridise *in vitro* suggests that interspecific hybridisation cannot be completely discounted in natural populations of these two pathogens. The two species are closely related, hypothesised to have evolved in the same geographic region, both infect leguminous plants, and chances of possible hybridisation may occur in case they are able to infect the same host plant.

However, the hybrid oospores are unlikely to be competitive as observed by their reduced aggressiveness in our and other studies. When observed *in vitro*, the majority of the oospores produced by these interspecific F_1 hybrids appeared to have aborted, thus, greatly reducing the number of viable oospores and depriving naturally occurring F_1 hybrids of a survival structure. This may force any interspecific hybrid to rely on vegetative propagation for perpetuation, further reducing the chances of the season-to-season survival of such hybrids in native and agricultural systems.

Over time, interspecific hybrids such as we observed may compete successfully with both *P. sojae and P. vignae* following a combination of extensive asexual reproduction and inbreeding under selection over many generations. There is also the potential for them to come into contact with, and exploit, a new host. Such a scenario was proposed by Brasier *et al.* (1999), who examined a naturally occurring interspecific hybrid complex of *Phytophthora* that caused the death of riparian alder (*Alnus* spp.). They determined that the two most likely parents of this new *Phytophthora* complex were *P. cambivora* and *P. fragariae* (or an as yet unidentified species of *Phytophthora* closely related to *P. fragariae*). Neither is pathogenic on *Alnus* species and specificity for alders may have arisen through the hybridisation event and reassortment of genes.

Future research with our hybrids would involve the production of F_2 populations and backcrosses. Both F_1 hybrids produced copious numbers of oospores that may be capable of germination even though a very high proportion of these oospores have an irregular appearance and would appear to have aborted. F_2 populations and backcrosses would provide useful information, especially from segregation ratios obtained from pathogenicity tests, on the genetics of host specificity for these pathogens. The interspecific hybrids could also be tested on a greater range of related and unrelated plant species to determine if they exhibit preferences for new hosts as the reassortment of genes could have resulted in a change of host preference for the new interspecific hybrid (Brasier *et al.* 1999).

Host specificity may be controlled by a series of gene-for-gene-like interactions between signal molecules or proteins of host or pathogen origin that interact with a receptor molecule and initiate complex plant defence responses that could lead to fungal arrest. Evidence for this theory involves elicitins and other elicitors from *Phytophthora,* which suggest they confer only a quantitative decrease in the virulence of the pathogen rather than complete avirulence (Parker *et al.* 1991; Kamoun *et al.* 1994; Sacks *et al.* 1995; Baillieul *et al.* 1996). This would suggest that these elicitors act as host range determinants interacting with a receptor triggering one set of defence responses and act in concert with other elicitors that switch on other defence mechanisms. If this is true, then genes whose products are elicitors and receptors involved in pathogen/host specificity and nonspecific host defences may also be useful in plant breeding and engineered resistance against potential pathogens. Factors involved in host specificity and nonhost resistance may be more effective in engineered resistance as they are active against a broad spectrum of pathogens and may be more durable than resistance based on gene-for-gene systems as are found in many cultivar specificity genes. While no conclusions regarding the genetics of host specificity can be made from this study, it shows that interspecific hybrids can be generated and have potential in studies of the genetics of host specificity which would greatly contribute to our understanding of host–pathogen interactions.

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