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Altered phenotypic response to *Peronospora parasitica* in *Brassica juncea* seedlings following prior inoculation with an avirulent or virulent isolate of *Albugo candida*

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Abstract

A study was made of the biological interactions between an isolate of *Peronospora parasitica* compatible with *Brassica juncea* and two isolates of *Albugo candida* either incompatible or compatible with the host species. Prior inoculation with the incompatible isolate of *A. candida* induced resistance to subsequently inoculated *P. parasitica.* The degree of resistance was proportional to the zoosporangia concentration of the incompatible isolate and induced resistance was more marked in the cotyledon receiving the inducing inoculum compared to the opposite cotyledon and subsequently emerging true leaves that had not been pre-inoculated. Induction of resistance was also observed if the incompatible isolate of *A. candida* and *P. parasitica* were co-inoculated simultaneously. However, the effect was greater the longer the interval between inoculations, up to a period of 4 days. When the incompatible isolate of *A. candida* was inoculated 4 h after *P. parasitica*, there was no marked effect on resistance to the latter. In contrast, prior inoculation with the compatible isolate of *A. candida* increased susceptibility to *P. parasitica* inoculated subsequently. However, pre- or co-inoculation with *P. parasitica* suppressed the development of the compatible isolate of *A. candida*. A spectrum of responses was observed when one cotyledon was inoculated simultaneously with both the incompatible and compatible isolates of *A. candida* and followed subsequently with *P. parasitica* after different time intervals. In such combinations, a transition was observed in the host response to *P. parasitica* from induced resistance/reduced susceptibility, which increased up to 24 h following a simultaneous inoculation with incompatible + compatible isolates of *A. candida* to an almost neutral reaction after 72 h to induced susceptibility after 96 h. This range of altered responses appeared to reflect the outcome of the differing kinetics and counter-effects of resistance and susceptibility induction.

Abbreviations: IN – incompatible isolate of *A. candida*; CO – compatible isolate of *A. candida*.

Introduction

Peronospora parasitica Pers. Ex Fr. (downy mildew) and *Albugo candida* Pers. Ex Hook. (white rust) are two economically important biotrophic oomycete pathogens of *Brassica* and other cruciferous species. These pathogens infect all aerial parts of the plant and are often observed growing intimately together in the same host tissue. Combined infection by these pathogens has been recorded as the cause of 17–37% yield losses in rapeseed-mustard crops in India (Kolte, 1985).

Peronospora parasitica mainly affects young plants that may, in severe cases, be stunted or killed. Infection at a later stage can result in yield and quality reduction of the crop whether grown for seed or the vegetative parts. The fungus survives as oospores (or as latent mycelium) in decaying crop debris and soil. Soilborne oospores are often a primary source of new infections. Secondary spread of disease is by airborne conidia produced mainly on the lower surfaces of leaves. After germination, conidia produce appressoria from which infection hyphae develop. The pathogen occasionally enters the leaf through a stomatal pore but more usually it penetrates directly between the anticlinal wall of epidermal cells.The infection hypha grows initially in the middle lamella and following formation of haustoria in adjacent epidermal cells it colonises the leaf and stem parenchyma producing haustoria from intercellular mycelium. After a period of incubation, conidiophores are produced which emerge through stomata primarily on the lower leaf surface (Channon, 1981).

Albugo candida forms localised white pustules on the leaves and other organs of infected plants, and inflorescence galls, referred to as 'stagheads', may appear later in the growing season as a result of meristematic infection. Stagheads are often dually infected with *P. parasitica* and this symptom is the main cause of yield loss in turnip rape (*B. rapa*) and mustard (*B. juncea*). Survival of *A. candida* is by oospores. Primary infections result from zoospores originating from oospores; secondary spread is by airborne sporangia that release zoospores on host surfaces. The encysted zoospores produce germtubes, which invade the host through stomata and colonise intercellularly the leaf and stem parenchyma producing intracellular haustoria. Masses of hyaline sporangia are produced under the lower and upper epidermis appearing as white, shiny 'blisters' which subsequently rupture to release the dry, powdery spores. *Albugo candida* is most damaging when it infects flower buds, subsequently inducing systemic 'staghead' infections of racemes, particularly on *B. juncea* and *B. rapa* L. (Bisht et al., 1994; Kolte, 1985; Paul and Rawlinson, 1992; Saharan and Verma, 1992).

Albugo candida and *P. parasitica* exist as specialised pathotypes on different cruciferous species and on cultivars within species. In general, asexual reproduction is the greatest on host of origin (Mathur et al., 1995; Nashaat and Awasthi, 1995; Petrie, 1988; Pidskalny and Rimmer, 1985; Saharan and Verma, 1992; Sherriff and Lucas, 1987; Silué et al., 1996).

Under natural conditions, plants are often subjected simultaneously to a range of biotic and abiotic stresses. While much recent attention has been paid to the phenomenon of induced resistance (Bostock et al., 2001; Oostendorp et al., 2001), interactions between stresses, and different pathogens in particular, are not well characterised. *Peronospora parasitica* is often observed to colonise stagheads induced by *A. candida* and, depending on the sequence of infection by the two pathogens, *A. candida* may suppress host resistance (or induce resistance) to *P. parasitica* (Awasthi et al., 1997). The present study was conducted to investigate the interaction in *B. juncea* between a compatible isolate of *P. parasitica* and either an incompatible or a compatible isolate of *A. candida.*

Materials and methods

Fungal isolates

Two single-pustule isolates of *A. candida* were derived from field collections of the pathogens, originally made by N.I. Nashaat (N.I.N.) at Pantanagar, northern India, during January 1995. Isolate IA02A (hereafter referred to as 'CO') is compatible with *B. juncea* accession PPBJ-1 and was collected from cv. Kranti (*B. juncea*). Isolate IA01A (hereafter referred to as 'IN'), which is incompatible with *B. juncea* accession PPBJ-1 and was collected from toria (*B. rapa*) accession PT303. These isolates were maintained separately on accessions of the hosts from which they were originally derived. A single-spore isolate of *P. parasitica* IP04A, compatible with *B. juncea* accession PPBJ-1, was derived from a field isolate collected by N.I.N. from a staghead on a *B. juncea* plant in Delhi, India during April 1994. *Peronospora parasitica* was subsequently maintained on seedlings of PPBJ-1.

Isolates of both pathogens were maintained separately on 6-day-old cotyledons, following the same method. The only difference was that inoculum of *A. candida* consisted of zoosporangia suspension in sterilised distilled water (SDW), whereas *P. parasitica* consisted of conidia suspension in SDW (Vishunavat et al., 1998).

Plant material

Brassica juncea accession PPBJ-1 was used in all experiments. Seedlings were raised from untreated

seeds in 8 cm diameter plastic pots and seeds were sown 1 cm deep in a soilless peat-based compost mix (Petersfield Products, Cosby, UK). Seedlings that emerged were thinned to leave seven per pot. The compost was kept moist by placing the pots in plastic trays ($35.5 \times 21 \times 18\,cm^3$) containing a layer of water 1 cm deep. All plant material was raised in $1 \times 2.5 \times 1.3\,m^3$ controlled-environment (CE) cabinets set at 18/15 °C day/night temperature with a 16-h photoperiod and a photon flux density (measured at seedling height) ranging from 70 to 110 $\mu mol\,m^{-2}\,s^{-1}$. Seedlings were first inoculated 6–7 days after sowing, when cotyledons were fully expanded, but true leaves were still developing (i.e. growth stage (GS) of 1.0, as described by Sylvester-Bradley (1985). Inoculation of true leaves was usually carried out 12 days after sowing (GS 1.2). After inoculation, seedlings were returned to the incubation chamber under the same conditions, except that a transparent propagator lid was placed over them to provide the high humidity required for successful infection and sporulation.

Preparation of spore suspensions and inoculation

Inoculum of the pathogens was prepared as conidial (*P. parasitica*) or zoosporangial (*A. candida*) suspensions by shaking excised cotyledons supporting abundant sporulation in SDW in a glass vial. Extraneous debris was removed from the resulting suspension by filtration through several layers of muslin. Before inoculation, conidial or zoosporangial suspensions were adjusted to the required concentrations using a haemocytometer followed by dilution with SDW. Conidial and zoosporangial germination was consistently high (>90%) and similar at each of the inoculum concentrations used in the experiments. Inoculations were made within 15 min of preparing suspensions that were continually agitated prior to use.

Prior to inoculation, seedlings were sprayed with SDW to remove debris from their surfaces and left to dry for 30 min. Inoculum was applied by either pipetting inoculum droplets onto cotyledons or true leaves or by spraying seedlings to run-off with an atomiser. Unless otherwise stated, droplet inoculation was used in the majority of experiments; a total of 10 µl was applied per cotyledon as four ≈2.5-µl droplets, two each on the adaxial surface of each half of a cotyledon. True leaves each received a total of 25 µl inoculum in similarly sized droplets pipetted onto their adaxial surfaces. Droplets of this size were used because they were readily retained on cotyledons and leaves. To examine local effects, the IN, CO and *P. parasitica* isolates were applied either together as mixed inoculum or separately in succession as close as possible to the same site on the cotyledons. To examine effects remote from the site of inoculation (resulting from systemic or volatile signals), various combinations of isolates were applied to the opposite cotyledons. Unless otherwise specified, inoculum concentrations of 2×10^5 zoosporangia per ml (*A. candida* IN isolate), 5×10^4 zoosporangia per ml (*A. candida* CO isolate) and 5×10^4 conidia per ml (*P. parasitica* isolate) were used. The reason for using 2×10^5 zoosporangia per ml of the IN isolate of *A. candida* was because it provided the best protection against 5×10^4 zoosporangia per ml of the CO isolate (Singh et al., 1999). This concentration of the IN isolate of *A. candida* was also most effective against 5×10^4 conidia per ml of *P. parasitica* isolate, though it did not differ significantly from 1×10^5 zoosporangia per ml of the same isolate (Figure 2). Concentration of 5×10^4 zoosporangia (*A. candida* IN isolate) or conidia (*P. parasitica* isolate) per ml were used because preliminary work showed that this was the optimum concentration which caused the highest disease severity. In experiments that involved different treatments to opposite cotyledons, the position of each inoculation was marked with ink.

Disease assessments

Phenotypic responses to *P. parasitica* were assessed 4 days after inoculation, using a 0–9 scale modified from Nashaat and Rawlinson (1994) for both cotyledons and true leaves.

For cotyledons: 0 = no symptom of *P. parasitica* infection; 1 = minute to larger necrotic flecks under the inoculum drop and either a small amount or no necrosis on the lower cotyledon surface; 2 = large necrotic flecks at the inoculation site and no sporulation; 3 = very sparse sporulation with 3–10 conidiophores on the margins of the lower surface, possible presence of necrotic flecks or tissue necrosis; 4 = sparse sporulation on either or both cotyledon surfaces, heavy scattered tissue necrosis; 5 = moderate scattered sporulation on either or both cotyledon surfaces with associated tissue necrosis; 6 = abundant sporulation mainly on the lower surface with light sporulation on the upper surface and associated tissue necrosis; 7 = abundant to heavy sporulation, mainly on the lower surface and light to scattered sporulation on

the upper surface with associated tissue necrosis and chlorosis; 8 = heavy sporulation on both surfaces; 9 = heavy sporulation on both surfaces, associated with collapse of the cotyledons.

For true leaves: 0 = no symptoms; 1 = minute necrotic flecks and no sporulation; 2 = necrotic flecks and no sporulation; 3 = sparse sporulation (3–10 conidiophores); 4 = up to 5% of the leaf area covered with conidiophores; 5 = 6–10% of the leaf area covered with conidiophores; 6 = 11–20% of the leaf area covered with conidiophores; 7 = 21–30% of the leaf area covered with conidiophores; 8 = 31–50% of the leaf area covered with conidiophores; 9 = >50% of the leaf area covered with conidiophores and associated leaf collapse.

The phenotypic response to inoculation with *A. candida* was assessed 7 days after inoculation, using 0–9 scale (Singh et al., 1999) for both cotyledons and leaves: 0 = no symptoms; 1 = minute necrotic flecks at inoculation site and no sporulation; 2 = large necrotic flecks at the inoculation site and no sporulation; 3 = sparse sporulation, with up to 5% of the leaf surface covered with pustules; 4 = 6–10% of the leaf surface covered with pustules; 5 = 11–20% of the leaf surface covered with pustules; 6 = 21–30% of the leaf surface covered with pustules; 7 = 31–50% of the leaf surface covered with pustules; 8 = 51–75% of the leaf surface covered with pustules; and 9 = >75% of the leaf surface covered with pustules.

Where both *A. candida* and *P. parasitica* were inoculated on the same cotyledon, phenotypes were assessed 4 days after inoculation with *P. parasitica*. If a cotyledon received both the CO isolate of *A. candida* and the *P. parasitica* isolate, and both pustules (*A. candida*) and conidiophores (*P. parasitica*) were present, assessments were made for both pathogens separately.

In all experiments, there were three replicates of each treatment, except in the experiment related to the effect of inoculum concentration of the IN isolate of *A. candida* on subsequent response to *P. parasitica* inoculation which had four replicates. Plants in propagator trays receiving different treatments were arranged randomly within CE cabinets. All experiments were conducted at least twice, but only data from the second set of experiments was presented. The first set of experiments was conducted to test and improve the methods used. The mean phenotypic response was calculated for each treatment and the data were subjected to analysis of variance. *F* tests were used to assess the significance of treatment main effects and interactions. The LSD was presented in all cases where there was significant interaction at $P < 0.05$. All analyses were carried out using the Genstat statistical package (Lawes Agricultural Trust, Hertfordshire, UK).

Local and systemic induction of resistance

In a preliminary experiment, seedlings were spray-inoculated with the IN isolate of *A. candida* or treated with SDW as a control. Four hours later, all seedlings were spray-inoculated with the *P. parasitica* isolate. In a second experiment, seedlings were inoculated by placing a droplet of spore suspension of the IN isolate of *A. candida* on one cotyledon (with SDW as a control) followed by the *P. parasitica* isolate on both cotyledons. The possibility of an altered response of true leaves following inoculation of a cotyledon was investigated in a third experiment. Both cotyledons of a seedling were inoculated with the IN isolate of *A. candida* (with SDW droplets as a control) before the emergence of true leaves. Five days later, newly emerged true leaves were inoculated with the *P. parasitica* isolate.

Effect of inoculum concentration of the IN isolate on subsequent response to P. parasitica *inoculation*

Both cotyledons of each seedling were inoculated with different concentrations of the IN isolate of *A. candida* (0.5×10^3, 1×10^4, 5×10^4, 1×10^5 and 2×10^5 zoosporangia per ml), and with SDW droplets as control. Four hours later, all seedlings were inoculated with *P. parasitica*.

Effect of inoculum sequence and timing between inoculations

Cotyledons of each seedling in a batch were inoculated with both isolates of *A. candida* and the *P. parasitica* isolate, but the inoculations were carried out sequentially resulting in different time intervals between the application of each isolate.

Local and remote effects of prior inoculation treatments

Three-way interactions between the two isolates of *A. candida* and *P. parasitica* was investigated, for

local and remote effects, as affected by the interval between inoculations. One cotyledon of a seedling was pre-inoculated with the IN and/or the CO isolate of *A. candida*, followed at various intervals after this by the *P. parasitica* isolate applied to both cotyledons. Mixed suspension of IN and CO isolates was prepared in such a way that concentration of each isolate was the same as when inoculated individually. Where a treatment involved successive inoculations with one or both of the two *A. candida* isolates and the *P. parasitica* isolate, separate assessments were made of the development of pustules and production of conidiophores, respectively.

Results

The IN isolate of *A. candida* did not produce pustules on cotyledons, irrespective of treatment. However, it sometimes caused perceptible necrosis, and a slight distortion of inoculated cotyledons.

Induction of resistance to P. parasitica *by the IN isolate of* A. candida

A preliminary experiment indicated that prior inoculation with the IN isolate of *A. candida* reduced the susceptibility of seedlings to *P. parasitica* (disease reaction = 5.26 *versus* 7.26 for control; $P < 0.001$). When one cotyledon of a seedling was inoculated with the IN isolate of *A. candida* followed at intervals of varying duration by the *P. parasitica* isolate, development of *P. parasitica* was always reduced significantly on the pre-inoculated cotyledon. However, the systemic effect on the opposite cotyledon was very weak and was only observed when the interval between inoculations was more than 96 h (Table 1).

The development of *P. parasitica* on all inoculated tissues was decreased compared with the control ($P < 0.001$) when both cotyledons of a seedling were spray-inoculated with the IN isolate of *A. candida* and, 5 days later, these cotyledons and the newly emerged first and second true leaves were subsequently inoculated with the *P. parasitica* isolate (Figure 1). Induction of resistance or reduced susceptibility was more pronounced when the IN isolate of *A. candida* was droplet-inoculated (Figure 3) as compared to spray-inoculation (Figure 1), particularly when *P. parasitica* was inoculated 5 days later.

Effect of inoculum concentration

When the two cotyledons of a seedling were inoculated first with the IN isolate of *A. candida* and then 4 h later with *P. parasitica*, there was a significant increase in resistance to the latter ($P < 0.001$). The resistance induced by the IN isolate increased in proportion to applied inoculum concentration up to 1×10^5 zoospores per ml (Figure 2).

Effect of timing of inoculation

Pre-inoculation with the IN isolate of *A. candida* decreased the subsequent development of symptoms produced by *P. parasitica*, even if the interval between inoculations was as long as 120 h ($P < 0.001$). There was no consistent difference in the extent of the reduced susceptibility to *P. parasitica* as the interval between inoculations was changed, despite the fact that control cotyledons became increasingly susceptible as they became older (Figure 3).

Importance of sequence of inoculation to the induction of resistance

The sequence of inoculations with the IN isolate of *A. candida* and the *P. parasitica* affected the outcome. Inoculation with the IN isolate of *A. candida* 4 h before the *P. parasitica* isolate increased resistance to the latter ($P < 0.001$). Simultaneous inoculation of the two isolates also increased resistance to *P. parasitica* but to a lesser extent. No increase in resistance was observed when *P. parasitica* was inoculated 4 h prior to the IN isolate of *A. candida* (Figure 4).

Local and remote effects of prior inoculation treatments

Pre-inoculating a cotyledon with the IN isolate of *A. candida* alone suppressed the development of *P. parasitica* locally and when there was a greater duration between inoculations, this effect was also observed on the opposite cotyledon (Table 1). Pre-inoculation with the CO isolate of *A. candida* alone enhanced the development of *P. parasitica* locally ($P < 0.001$), but there was no consistent effect on the opposite cotyledon where the development of *P. parasitica* was increased after 4 h ($P < 0.05$), no significant

Table 1. Effect of pre-inoculation of one cotyledon (I) with an incompatible (IN) or a compatible (CO) isolate of *Albugo candida*, alone or in combination, on development of *Peronospora parasitica* (*PP*) on the same (I) and the opposite (II) cotyledons of *Brassica juncea* seedlings

Interval between treatments A and B (h)	Cotyledon I				Cotyledon II		
	Treatment A	Treatment B	Disease severity		Treatment A	Treatment B	Disease severity (*P. parasitica*)
			P. parasitica	*A. candida*			
4	H_2O	*PP*	6.86	NA	—	*PP*	6.99
	IN	*PP*	4.52	0	—	*PP*	6.62
	CO	*PP*	7.75	0.11	—	*PP*	7.67
	CO/IN	*PP*	5.47	0	—	*PP*	6.76
	CO	H_2O	NA	4.27	—	—	NA
	IN	H_2O	NA	0	—	—	NA
	CO/IN	H_2O	NA	2.07	—	—	NA
24	H_2O		6.45	NA	—		6.65
	IN	*PP*	2.88	0	—	*PP*	6.29
	CO	*PP*	7.37	2.83	—	*PP*	6.76
	CO/IN	*PP*	3.55	3.16	—	*PP*	6.02
	CO	H_2O	NA	6.89	—	—	NA
	IN	H_2O	NA	0	—	—	NA
	CO/IN	H_2O	NA	4.74	—	—	NA
72	H_2O	*PP*	7.21	NA	—	*PP*	7.11
	IN	*PP*	3.26	0	—	*PP*	6.39
	CO	*PP*	8.49	5.46	—	*PP*	6.99
	CO/IN	*PP*	6.52	5.34	—	*PP*	7.14
	CO	H_2O	NA	7.43	—	—	NA
	IN	H_2O	NA	0	—	—	NA
	CO/IN	H_2O	NA	4.91	—	—	NA
96	H_2O	*PP*	6.71	NA	—	*PP*	6.75
	IN	*PP*	3.00	0	—	*PP*	6.00
	CO	*PP*	8.57	7.06	—	*PP*	6.33
	CO/IN	*PP*	7.98	5.91	—	*PP*	7.23
	CO	H_2O	NA	8.37	—	—	NA
	IN	H_2O	NA	0	—	—	NA
	CO/IN	H_2O	NA	5.43	—	—	NA
$LSD_{(P=0.05,\ df=32)}$			0.611	0.761			0.540

NA = Not applicable.

differences were shown after 24 and 72 h, and then significantly decreased after 96 h ($P < 0.05$) (Table 1).

Mixed pre-inoculation with the IN and CO isolates of *A. candida* 4 h before the *P. parasitica* isolate, decreased the development of *P. parasitica* locally, but significantly less so, than pre-inoculation with the IN isolate of *A. candida* alone ($P < 0.001$). However, as the interval between inoculation of the IN + CO isolates of *A. candida* and *P. parasitica* increased, the severity of disease caused by *P. parasitica* altered over the interval range of 24–96 h. When there was a 24-h interval between inoculations, the development of *P. parasitica* decreased significantly compared to the control. This was less marked when the interval was 72 h and disease severity significantly exceeded the control when the interval was 96 h. Mixed pre-inoculation with the IN and CO isolates of *A. candida* did not influence development of the *P. parasitica* isolate on the opposite cotyledon (Table 1).

Subsequent inoculation with the *P. parasitica* isolate inhibited the development of the CO isolate of *A. candida* compared to the control and this was most evident when the interval between inoculations with the CO isolate of *A. candida* and *P. parasitica* was 4 h. The longer the interval between inoculations, the less was the inhibition of *A. candida* symptom development (Table 1).

Discussion

Infection of *B. juncea* cotyledons by an incompatible isolate of *A. candida* gave partial protection to seedlings against subsequent challenge by a compatible

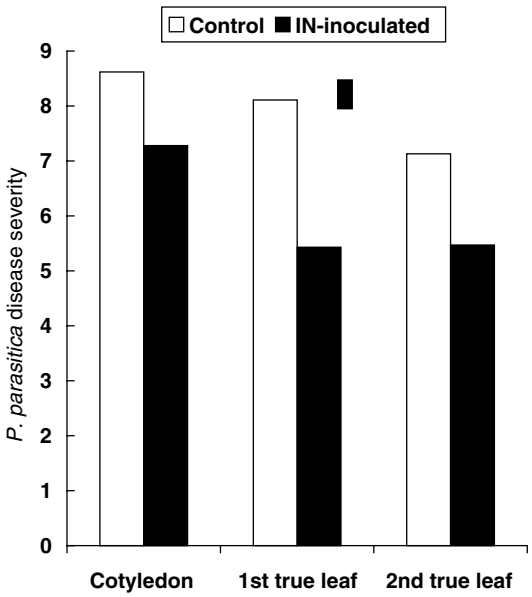

Figure 1. Severity of *Peronospora parasitica* on cotyledons and first and second true leaves of *Brassica juncea* when a compatible *P. parasitica* isolate was applied 5 days after both cotyledons had been sprayed with sterile distilled water (control) or spray-inoculated with an incompatible (IN) *Albugo candida* isolate. Bar represents $LSD_{(P=0.05,\ df=12)}$.

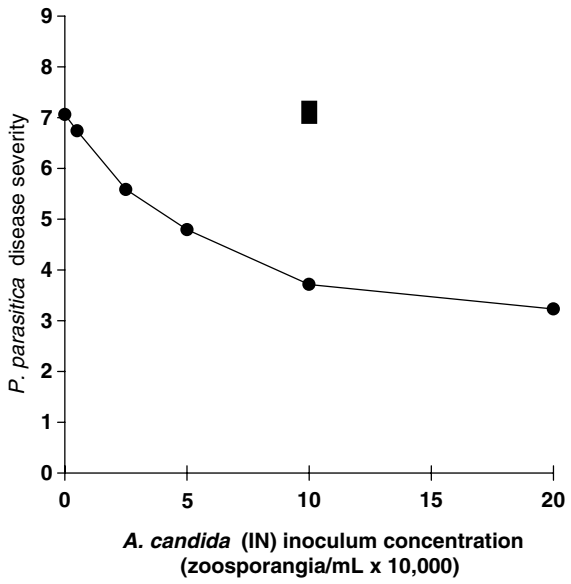

Figure 2. Effect of inoculum concentration on the reduction of susceptibility to *Peronospora parasitica* on *Brassica juncea* cotyledons following pre-inoculation 4 h earlier with an incompatible isolate of *Albugo candida* (IN). Bar represents $LSD_{(P=0.05,\ df=18)}$.

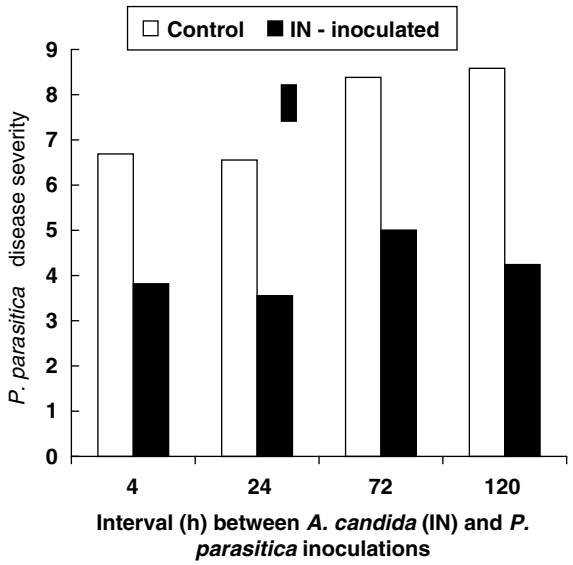

Figure 3. Severity of *Peronospora parasitica* on cotyledons of *Brassica juncea* when an incompatible isolate of *Albugo candida* (IN) was inoculated 4, 24, 72 and 120 h prior to inoculation with a compatible isolate of *Peronospora parasitica*. Bar represents $LSD_{(P=0.05,\ df=16)}$.

isolate of both *A. candida* and *P. parasitica*. This interaction between incompatible and compatible isolates of *A. candida* is the subject of a previous study (Singh et al., 1999). The ability of the IN isolate of *A. candida* to influence locally the response of *B. juncea* cotyledons to subsequent inoculation by *P. parasitica* was marked. The effect was not so evident in tissues remote from the point of inoculation with the IN isolate. The induction of the more resistant state lasted for a period of at least 120 h and the magnitude of the effect was inoculum concentration dependent up to 1×10^5 zoosporangia per ml. Although no direct evidence was obtained, it is likely that the concentration effect relates to the numbers of host cells responding to challenge by the incompatible isolate. Furthermore, *A. candida* invariably penetrates through stomata (Saharan and Verma, 1992); therefore competition for sites of penetration may also be a contributing factor for the locally reduced susceptibility in *B. juncea* to the CO isolate of *A. candida* when pre-inoculated with the IN isolate. However, this seems to be of little importance against *P. parasitica* since penetration of this pathogen is via the anticlinal wall, though infection hyphae may occasionally enter through stomata (Channon, 1981).

Brassica species are known to be capable of a number of inducible, putative defence responses

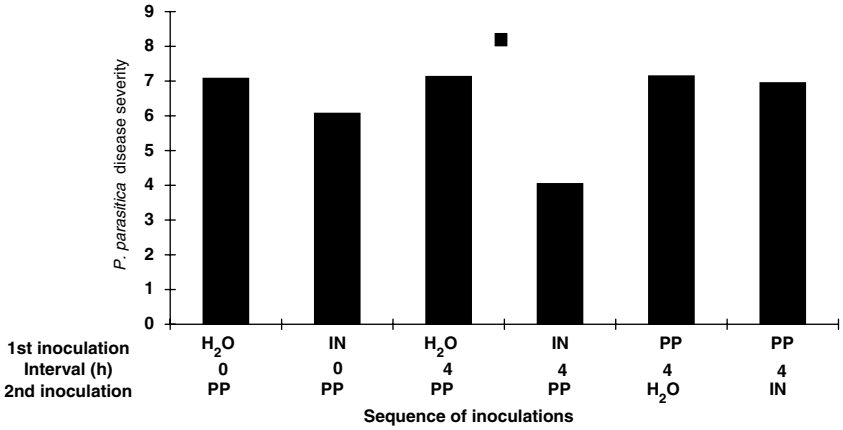

Figure 4. Effect of sequence of inoculation on local protection of *Brassica juncea* cotyledons following inoculation with an incompatible isolate of *Albugo candida* (IN) and compatible isolate of *Peronospora parasitica* (*PP*) against the later pathogen isolate. Bar represents $LSD_{(P=0.05,\ df=12)}$ for all means.

(Dahiya and Woods, 1987; Dixelius, 1994; Doughty et al., 1991; Rouxel et al., 1995) that may participate in bringing about the observed phenomenon. Mauch-Mani and Slusarenko (1994) have shown in *Arabidopsis thaliana* that pre-inoculation with *Fusarium oxysporum* leads to an induction of systemic resistance to *P. parasitica* associated with a systemic accumulation of transcripts for pathogenesis-related proteins. Cross-species induction of resistance has also been demonstrated in other host species (Arase and Fujita, 1992; Jørgensen et al., 1996; Manandhar, 1998).

The compatible *A. candida* isolate increased susceptibility to *P. parasitica*, in agreement with the earlier findings of Awasthi et al. (1997) for *B. juncea*, and of Holub et al. (1993) for *Arabidopsis thaliana.* The phenomenon of induced susceptibility has been recorded previously in a number of host–pathogen combinations (Adhikari and McIntosh, 1998) and can occur in combinations of isolates of species from the same genus (Kochman and Brown, 1975; Moseman et al., 1965), or, as in this example (Ouchi et al., 1974), in combinations represented by species from related genera that share a common pathogenic habit. This phenomenon warrants a full study at the cellular and transcriptional level.

Our results confirm the ability of compatible *A. candida* isolates to render *B. juncea* more susceptible to *P. parasitica*, and show, for the first time, the ability of an incompatible *A. candida* isolate to induce resistance to *P. parasitica* in this host. The results also describe for the first time the outcome of three-way interactions between IN and CO isolates of *A. candida* and a compatible isolate of *P. parasitica*. The experiments reported here suggest that processes with quite distinct kinetics are involved in the induction of resistance and susceptibility to *P. parasitica* by *A. candida*. Following dual inoculation with the two *A. candida* isolates, there was early evidence of increased resistance to *P. parasitica*. This effect declined with duration between the subsequent inoculation up to 72 h, and when the interval was 96 h an increase in susceptibility to *P. parasitica* was observed. These outcomes from complex interactions between one host and several pathogen isolates warrant more detailed characterisation at the histological and molecular level as a means to elucidate the pathways leading to the two induced states of increased resistance and increased susceptibility.

Infection of *B. juncea* with *P. parasitica* inhibited or adversely affected the development of the CO isolate of *A. candida*. This was particularly evident from the present study when *B. juncea* was simultaneously co-inoculated with both isolates (Table 1). This may be attributed to nutritional exploitation of host tissues by *P. parasitica*, which appeared to develop at faster rate than *A. candida.*

In crops of *B. juncea*, it is likely that both incompatible and compatible *A. candida* isolates as well as compatible *P. parasitica* isolates occur together. Hence, there may be practical relevance to studies of interaction complexes of the type studied here. Induction of resistance to *P. parasitica* in *B. juncea* crops would depend on the availability of significant quantities of incompatible *A. candida* inoculum (an unlikely event).

However, the predisposition of *B. juncea* to infection by *P. parasitica* as a consequence of the presence of compatible *A. candida* inoculum is more likely and from the observations reported in this study, the sequence of arrival of inoculum of the two pathogens may critically determine the outcome.

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