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Induction of Systemic Resistance to *Albugo candida* in *Brassica juncea* by Pre- or Coinoculation with an Incompatible Isolate

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ABSTRACT

Singh, U. S., Doughty, K. J., Nashaat, N. I., Bennett, R. N., and Kolte, S. J. 1999. Induction of systemic resistance to *Albugo candida* in *Brassica juncea* by pre- or coinoculation with an incompatible isolate. Phytopathology 89:1226-1232.

In an investigation of the interaction between two isolates of *Albugo candida* that were compatible (CO) and incompatible (IN) on a *Brassica juncea* accession, the IN isolate induced both local and systemic protection of cotyledons and true leaves against the CO isolate. The extent of the protection was proportional to the zoosporangia concentration used in the inducing (IN) inoculation. Protection was greatest locally on cotyledons and least on true leaves (the most remote tissue from the point of the inducing inoculation). Protection occurred when the two isolates

were inoculated together but was greater when the interval between the IN and CO isolate inoculations was longer. The IN isolate induced only slight protection when it was inoculated after the CO isolate. No induced susceptibility to the IN isolate occurred with any treatment. There was some evidence of competition between CO and IN zoospores for infection sites (stomata). The occurrence of systemic protection and changes detected in phenylalanine ammonia lyase and total soluble peroxidase activities in inoculated cotyledons, particularly after the inducing (IN) inoculation, suggested that host-mediated factors also may be involved in the interaction between the two isolates.

Additional keywords: compatibility, incompatibility, induced resistance.

White rust, caused by the biotrophic oomycete pathogen *Albugo candida* (Pers. ex Hook.) Kunze, is an important disease of *Brassica juncea* (L.) Czernj. & Coss. and *B. rapa* L. The pathogen can infect all aboveground parts of the plant, producing characteristic white blisters (sori). Severe infection culminates in systemic "staghead" infection of the inflorescence (often in association with *Peronospora parasitica*), which is the main cause of yield loss in susceptible cultivars (2,3,16).

A. candida exhibits specialization on different cruciferous species and cultivars within species (16). The North American population of the pathogen has been categorized into races that cause the most severe disease on their respective homologous hosts, although some also are capable of infecting heterologous hosts (13,17).

In some host-pathogen systems, prior inoculation with an incompatible isolate can protect plants against subsequent infection by a compatible isolate. There has been much interest in this type of induced resistance, because it suggests the possibility of "immunizing" crops against disease (10,18). Little work has been done on the interactions among isolates of pathogens differing in virulence on *Brassica* hosts, although Mahuku et al. (12) recently demonstrated that highly and weakly virulent isolates of *Leptosphaeria maculans* can coexist in lesions on *B. napus* and described how isolates can interact during disease development. The aim of our study was to determine the interaction between incompatible (IN) and compatible (CO) isolates of *A. candida* on *B. juncea*. We measured isolate interaction in terms of symptom expression and did preliminary investigations of physical (competition for infection sites among zoospores) and host-mediated (defense-related enzymes) factors associated with the interaction.

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MATERIALS AND METHODS

Fungal isolates. Two single-pustule isolates of *A. candida*, originally collected by N. I. Nashaat at Pantnagar in northern India during January 1995 (MAFF import license PHF 1307C/1253/114), were used. The incompatible (with *B. juncea*) isolate (IA01A) was collected from toria (*B. rapa*); the compatible isolate (IA102A) was collected from mustard (*B. juncea*). The isolates were maintained separately on seedlings of accessions of the hosts from which they were originally collected: PT303 (*B. rapa*) and Kranti (*B. juncea*).

Plant material. A *B. juncea* accession (PPBJ-1) was used as the host throughout the experiments. Seedlings were raised from untreated seed, either in 8-cm-diameter plastic pots or in 5-cm^2 cardboard jiffy pots (Nursery Trades [Lee Valley] Ltd., Cheshunt, U.K.). Seeds were sown ≈1 cm deep in a soilless peat-based compost mix (Petersfield Products, Cosby, U.K.). Seedlings that emerged were thinned to seven per pot. Compost was kept moist by placing pots in propagator trays (35.5 × 21 × 18 cm), each containing a layer of water ≈1 cm deep. All plant material was raised in 1 × 2.5 × 1.3-m controlled-environment (CE) cabinets set at 18/15°C day/night temperatures and 16-h photoperiod, with a photosynthetic photon flux (measured at seedling height) ranging from 70 to 110 μmol quanta sec^{-1} m^{-2}. Seedlings were first inoculated 6 to 7 days after sowing, when cotyledons were fully expanded but true leaves were still developing (i.e., growth stage [GS] 1.0, as described by Sylvester-Bradley [21]). Inoculation of first and second true leaves usually was done 10 (GS 1.1) or 12 (GS 1.2) days after sowing. After inoculation, seedlings were returned to the incubation chamber under the same conditions, except for a transparent propagator lid that was placed over them to provide the high humidity required for successful infection.

Preparation of zoosporangia suspensions, inoculation, and disease assessment. Inoculum (zoosporangia suspensions) was prepared by shaking excised cotyledons supporting abundant sporulations in sterile distilled water (SDW) in a glass vial. Extraneous

matter was removed from the resulting suspension by filtering through several layers of muslin. Before inoculation, zoosporangial suspensions were adjusted to the required concentration with a hemacytometer slide and appropriate dilution with SDW. In preliminary tests, zoosporangial germination was high (>90%) and similar at each of the inoculum concentrations used in the experiments. Inoculation was done within 15 min of preparing zoosporangial suspensions.

Prior to inoculation, seedlings were sprayed with SDW to remove compost debris from their surfaces and left to dry for 30 min. Inoculum was applied either by pipetting inoculum droplets onto cotyledons or by spraying seedlings to runoff with an atomizer. When droplets were used, 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. Droplets of this size were used, rather than larger ones, because they were never observed to run off the inoculated cotyledon. To test the local interaction between the IN and CO isolates, the isolates were applied either together as mixed inoculum or separately in succession as close as possible to the same site on the cotyledons. To test systemic interactions, various combinations of isolates were applied to opposite cotyledons. True leaves each received a total of 25 μl of inoculum in similarly sized droplets pipetted onto their adaxial surfaces. Inoculum was agitated during application. Unless otherwise specified, inoculum concentrations of 1×10^5 (IN isolate) and 5×10^4 (CO isolate) zoosporangia per ml were used. In experiments that involved different treatments to opposite cotyledons, the position of each treatment was marked with a marker pen.

Disease reaction was assessed 7 days after inoculation, using a 0 to 9 scale (modified from Williams [26]) for both cotyledons and true leaves: 0 = no symptoms or signs of *A. candida* infection; 1 = pinpoint necrotic flecks at inoculation site, no sporulation; 2 = larger necrotic flecks at inoculation site, no sporulation; 3 = sparse sporulation, up to 5% of surface covered with pustules; 4 = 6 to 10% of leaf area covered with pustules; 5 = 11 to 20% leaf area covered with pustules; 6 = 21 to 30% leaf area covered with pustules; 7 = 31 to 50% leaf area covered with pustules; 8 = 51 to 75% leaf area covered with pustules; and 9 = >75% leaf area covered with pustules. At least seven seedlings were scored for each replication of a treatment combination.

All experiments involved three replications of each treatment, and propagator trays receiving different treatments were arranged randomly within CE cabinets. All experiments were repeated at least once. Mean disease severity was calculated for each tray, and the values were used in analysis of variance. *F* tests were used to assess the significance of treatment main effects and interactions. Treatment means were differentiated using Fisher's least significant difference test (LSD). All analyses were done using the Genstat statistical package (Lawes Agricultural Trust, Hertfordshire, U.K.).

Local and systemic induction of resistance. In a preliminary experiment, batches of seedlings were either spray-inoculated with the IN isolate or sprayed with SDW; 4 h later all seedlings were spray-inoculated with the CO isolate. In a second experiment, seedlings were droplet-inoculated on one cotyledon (treatment A) with SDW or the IN isolate followed by the CO isolate and then inoculated on the other cotyledon (treatment B) with either the CO or IN isolate. The possibility of systemic protection of true leaves was investigated in a third experiment: both cotyledons of a seedling were spray-inoculated with the IN isolate or with SDW as a control before the emergence of true leaves; 5 days later newly emerged true leaves were droplet-inoculated with the CO isolate.

Effect of IN inoculum concentration on CO infection. The CO isolate was droplet-inoculated either alone or in mixed suspension with increasing concentrations of the IN isolate (0, 5×10^3, 1×10^4, 5×10^4, 1×10^5, and 2×10^5 zoosporangia per ml) on one cotyledon per seedling from different seedling batches; the opposite cotyledons of all seedlings received the CO isolate alone

(1×10^5 zoosporangia per ml). Mixed suspensions were prepared in such a way that the inoculum concentration of the CO isolate remained constant.

Importance of timing and sequence of IN and CO inoculations. Both cotyledons of each seedling in a batch received droplet inoculations with both the IN and CO isolates, but the inoculations were staggered so there were different time intervals between the applications of each isolate. Both IN followed by CO and CO followed by IN combinations were tested in four experiments. (i) Intervals of 4 h between initial application of the CO or IN isolate or H_2O followed by application of the CO or IN isolate or H_2O compared with simultaneous application of the CO and IN isolates. (ii) Cotyledons were first inoculated with H_2O or the IN isolate and subsequently inoculated with the CO isolate 0, 1, 3, 5, or 7 days later. In similar experiments, true leaves 1 and 2 were inoculated with the CO isolate 5 or 7 days and 7 days, respectively, after initial inoculation of cotyledons with H_2O or the IN isolate. Results for cotyledons and true leaves 1 and 2 were analyzed separately. (iii) Cotyledon 1 was inoculated initially with H_2O or the IN isolate, and both cotyledons were inoculated with the CO isolate after 0, 1, or 3 days. (iv) Cotyledons were initially inoculated with the CO isolate and then with the IN isolate after 0, 1, 3, or 5 days. Cotyledons of control seedlings were inoculated a second time with H_2O. In all cases, disease reaction was scored 7 days after last inoculation.

Microscope observation of zoospore distribution. A subsample of four cotyledons was collected 4 h after droplet-inoculation with the IN isolate alone. Cotyledons were washed thoroughly three times in SDW in a glass vial and mounted in a 0.1% aqueous solution of Calcofluor on microscope slides. The location of zoospores in relation to stomata was investigated with a fluorescence microscope (Leitz, Wetzlar, Germany) fitted with a 390- to 440-nm exciter filter and a 475-nm barrier filter (22). The extent of zoospore lodging over stomata was estimated by counting the number of zoospores over, or away from, 100 stomata under the site of the inoculum droplet on each replicate cotyledon.

Phenylalanine ammonia lyase and total soluble peroxidase assays. Subsamples of cotyledons were collected at the time of droplet-inoculation with the IN or CO isolate and 1, 3, and 5 days after inoculation. SDW-treated cotyledons were sampled at the same times for the control. For each treatment combination, a 2-g sample was homogenized in 20 ml of homogenization buffer (0.1 M potassium phosphate, 50 mM sodium metabisulphite, 1 mM phenylmethylsulphonyl fluoride, and 250 mM sucrose, pH 7.0). The homogenate was mixed with 2.5 g of polyvinylpolypyrrolidone (standard grade, Sigma Chemical Co., St. Louis) and 1.25 g of Amberlite XAD-4 (standard grade, Sigma) ion-exchange resin for 2 min, filtered through two layers of muslin cloth, and centrifuged at $100,000 \times g$ at 4°C for 30 min. A 1-ml aliquot of supernatant was purified further and desalted by centrifugation through a 2-ml Sepharose G25 mini-column (3,000 rpm for 5 min at 4°C). The G25 column removes excess salt and simple organic acids that might interfere with both the phenylalanine ammonia lyase (PAL) and total soluble peroxidase (POX) assays. PAL and POX activities were assayed by procedures modified from those reported by Strack and Mock (20). For PAL, 50 μl of supernatant was mixed with 950 μl of substrate buffer (10 mM L-phenylalanine in 100 mM potassium borate, pH 8.8), and the formation of *trans*-cinnamic acid was measured at 290 nm after 2 h of incubation at 30°C. PAL activity was expressed as nanomoles *trans*-cinnamic acid per gram fresh weight per hour. For POX, 50 μl of supernatant was mixed with 950 μl of substrate buffer (4.5 mM guaiacol [50 μl], 2.2 mM hydrogen peroxide [25 μl of 30% stock], and 200 mM potassium phosphate, pH 5.8). The formation of tetraguaiacol was recorded spectrophotometrically at 30°C for 2 min, and a rate was determined. POX activity was expressed as nanomoles tetraguaiacol formed per gram fresh weight per hour. Six assays for both PAL and POX were performed for each sample.

RESULTS

Local and systemic induction of resistance. A preliminary experiment indicated that pretreatment by spraying with the IN isolate protected seedlings from the CO isolate (disease reaction = 3.92 versus 7.06 for the control; standard error of difference between means = 0.326; F significant at $P = 0.0006$). The relative virulence of each isolate on *B. juncea* accession PPBJ-1 and the local and systemic induction of resistance to the CO isolate by the IN isolate are shown in Figure 1. Within the time (6 to 7 days) it took the CO isolate to produce abundant white pustules on the lower surfaces of cotyledons (Fig. 1A), the IN isolate produced few symptoms. Occasionally, IN-inoculated cotyledons became slightly curled, but they had a disease reaction of only 0 or 1. Coinoculation with the IN and CO isolates simultaneously on one cotyledon decreased disease reaction on the cotyledon, as well as on the opposite cotyledon inoculated with the CO isolate alone (Fig. 1B).
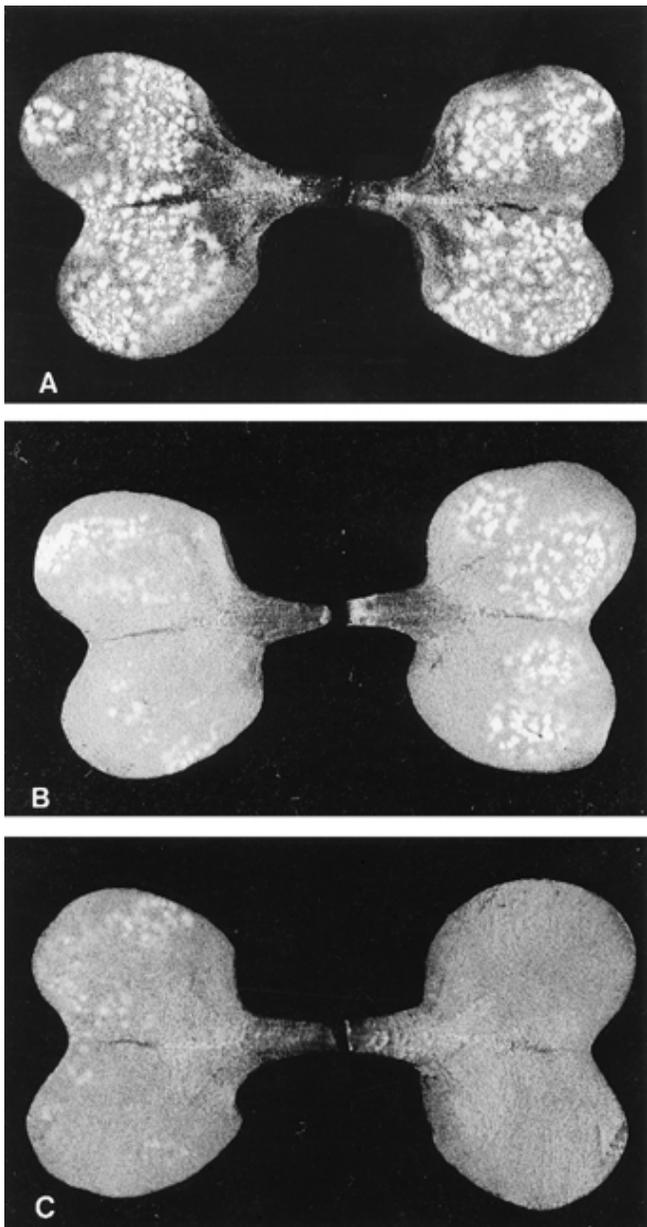

Fig. 1. Symptom development on cotyledons of *Brassica juncea* accession PPBJ-1 7 days after inoculation with incompatible (IN) and compatible (CO) isolates of *Albugo candida*. **A,** CO isolate inoculated on both cotyledons; **B,** CO and IN isolates inoculated together on left cotyledon; CO isolate applied alone to right cotyledon; **C,** CO and IN isolates inoculated together on left cotyledon; IN isolate applied alone to right cotyledon
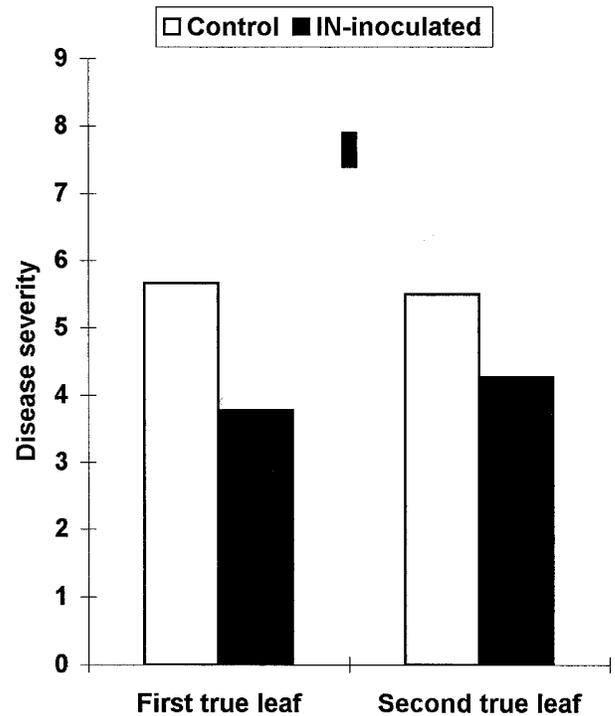

Fig. 2. White rust severity on first and second true leaves of *Brassica juncea* when the compatible *Albugo candida* isolate was applied 5 days after both cotyledons had been sprayed either with sterile distilled water (control) or inoculated with the incompatible (IN) isolate. Bar represents $LSD_{(P=0.05, df=8)}$.
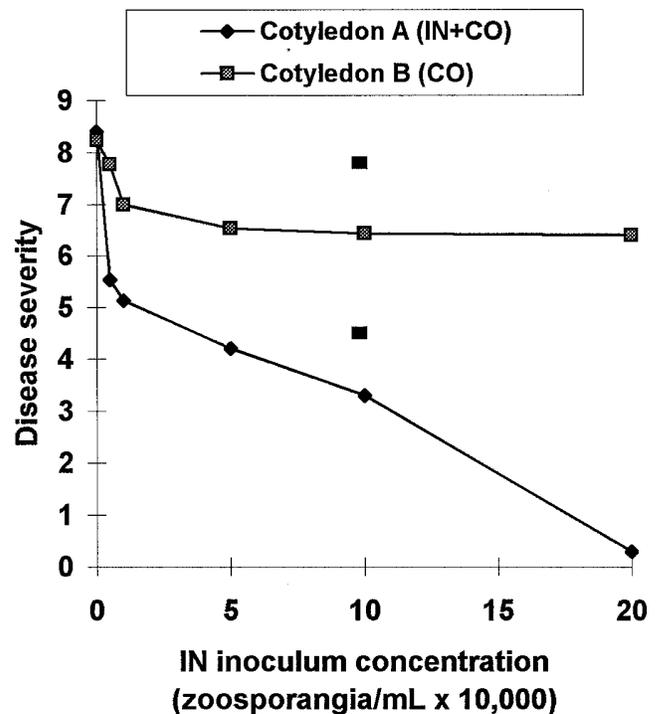

Fig. 3. Effect of incompatible (IN) isolate (*Albugo candida*) inoculum concentration on disease severity on *Brassica juncea* after inoculation with the compatible (CO) isolate. On each seedling, cotyledon A was inoculated with both the IN and CO isolates; at the same time, cotyledon B was inoculated with the CO isolate alone. The top bar represents $LSD_{(P=0.05, df=24)}$ for comparisons at different inoculum concentrations. The bar between the lines represents $LSD_{(P=0.05, df=12)}$ for comparisons of disease reaction on the two cotyledons at a particular IN inoculum concentration.

However, systemic protection (on the opposite cotyledon) was not as great as local protection. If the opposite cotyledon was inoculated instead with the IN isolate after the first simultaneous IN and CO inoculation, there was no change in disease reaction on the cotyledon (Fig. 1C). There was no evidence that coinoculation with the two isolates encouraged infection by the IN isolate.

Inoculating cotyledons with the IN isolate also protected subsequently emerging true leaves against the CO isolate ($P < 0.001$). The first and second true leaves were protected to a similar extent ($P = 0.064$ for the F test for leaf-isolate interaction; Fig. 2).

Effect of IN inoculum concentration on CO infection. The degrees of both local and systemic protection of cotyledons against the CO isolate increased as the concentration of the IN isolate used in mixed inoculations increased ($P < 0.001$). Local protection was greater than systemic protection ($P < 0.001$; Fig. 3). The pattern of protection differed between local and systemic ($P < 0.001$). Systemic protection was greatest at $\approx 5 \times 10^4$ zoosporangia per ml (Fig. 3, cotyledon B), whereas local protection continued to increase up to the highest concentration of IN isolate applied (2×10^5 zoosporangia per ml; Fig. 3, cotyledon A).

Importance of timing and sequence of IN and CO inoculations. The local protection given by inoculation with the IN isolate increased when there was a delay of as little as 4 h before

challenge inoculation with the CO isolate (Fig. 4). Disease severity was reduced compared with cotyledons inoculated with the CO isolate after 4 h of inoculation with H_2O, whether inoculation with the IN isolate took place before or after inoculation with the CO isolate ($P < 0.001$). However, inoculation with the IN isolate 4 h before the CO isolate gave the greatest protection; protection was weaker with simultaneous inoculation with the CO and IN isolates and weakest with inoculation with the IN isolate 4 h after inoculation with the CO isolate ($P < 0.001$; Fig. 4).

There was clear evidence of both local protection of cotyledons and systemic protection of true leaves induced by initial inoculation of cotyledons with the IN isolate ($P < 0.001$; Fig. 5). Local protection of cotyledons and systemic protection of leaves increased as the delay until inoculation with the CO isolate increased ($P < 0.001$), but there appeared to be a decline in the systemic protection of true leaves, relative to control, when the interval was extended from 5 to 7 days. However, the difference in disease severity between the control and IN inoculation decreased as the delay until inoculation with the CO isolate increased ($P = 0.001$).

There also was evidence of both local and systemic protection of cotyledons induced by the IN inoculation ($P < 0.001$), but sys-
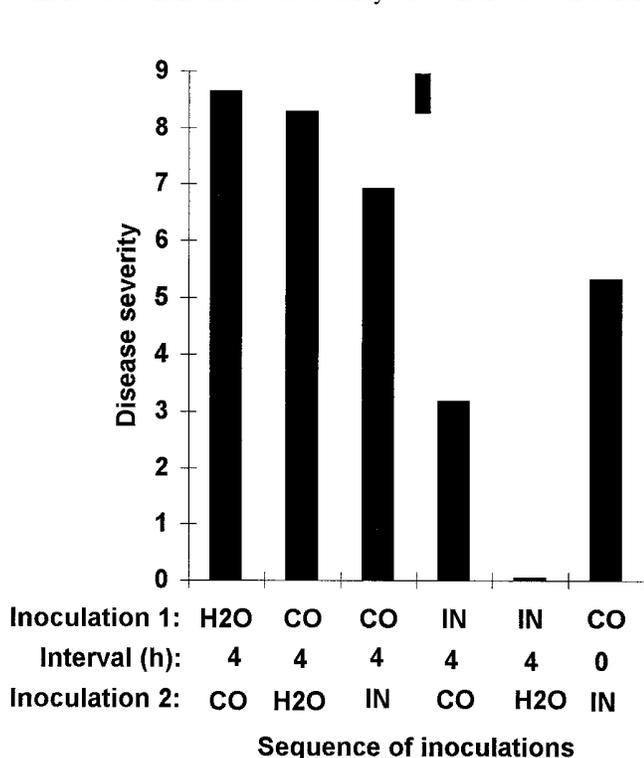

Fig. 4. Effect of sequence of inoculation of *Brassica juncea* cotyledons with the incompatible (IN) and compatible (CO) isolates of *Albugo candida* on local protection against the CO isolate. The bar represents $LSD_{(P=0.05, df=10)}$ for all means, except that of the IN/4h/H_2O treatment, which was excluded from the analysis.
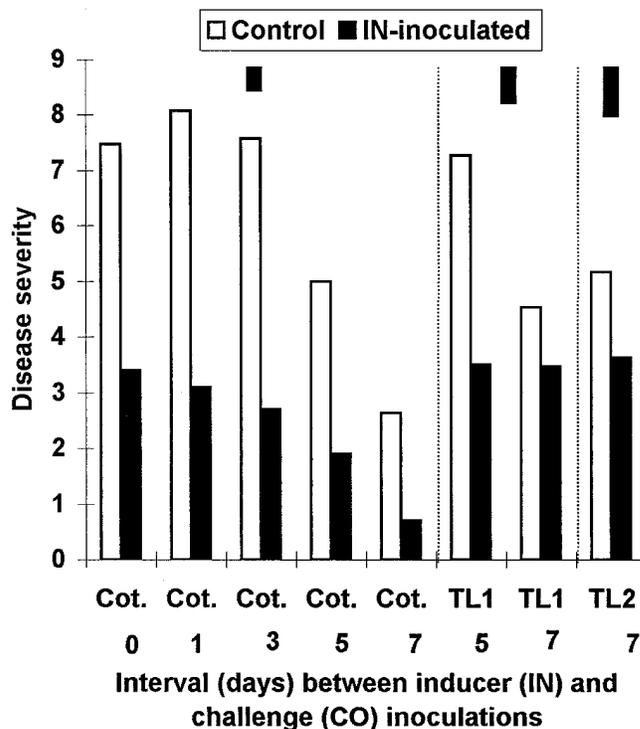

Fig. 5. Duration of local protection of *Brassica juncea* cotyledons (Cot.) and systemic protection of true leaves (TL) against challenge inoculation with the *Albugo candida* compatible (CO) isolate after inducing inoculation of cotyledons with the incompatible (IN) isolate. Both cotyledons of a seedling were treated identically. Bars represent $LSD_{(P=0.05)}$ of disease severity on cotyledons (df = 20), first true leaves (df = 8), and second true leaves (df = 4).

TABLE 1. Importance of the interval between inoculation with incompatible (IN) and compatible (CO) isolates of *Albugo candida* in relation to induced local (cotyledon 1) and systemic (cotyledon 2) protection against white rust in *Brassica juncea*[a]

| Interval (days) | Cotyledon 1[a] | | | Cotyledon 2[a] | | | Cotyledon 1 – cotyledon 2 |
	Treatment A	Treatment B	Disease reaction	Treatment A	Treatment B	Disease reaction	Disease reaction
0	H_2O	CO	8.13	–	CO	8.00	+0.13
	IN	CO	5.00	–	CO	6.76	−1.76
1	H_2O	CO	7.80	–	CO	7.73	+0.07
	IN	CO	5.00	–	CO	6.67	−1.67
3	H_2O	CO	6.47	–	CO	6.33	+0.14
	IN	CO	3.57	–	CO	4.83	−1.26

[a] $LSD_{(P=0.05, df=24)} = 0.44$.

temic protection was lower ($P < 0.001$; Table 1). Disease severity in general decreased as the delay until inoculation with the CO isolate increased, but there was no evidence of any change in this pattern as the length of the delay increased ($P > 0.35$ for all interactions with delayed inoculation with the CO isolate).

In another experiment, disease severity was reduced by ≈50% when cotyledons were simultaneously inoculated with the CO and IN isolates compared with those inoculated simultaneously with the CO isolate and H_2O, but the difference became minimal as the delay until inoculation with the IN isolate increased to 5 days (Fig. 6).

Zoospore distribution on inoculated cotyledon surfaces. At the zoosporangia concentration used for the IN isolate (1×10^5 zoosporangia per ml), almost all (>97%) stomata under the inoculum droplet were occupied by encysted zoospores.

PAL and POX activities in inoculated tissues. PAL and POX activities declined in uninoculated cotyledons during the course of the assays, but inoculated cotyledons showed net increases in the activities of both enzymes, and the difference from the control increased with time after inoculation ($P < 0.001$). The activities of both enzymes were consistently higher in IN- than in CO-inoculated cotyledons; the rate of increase in POX activity was similar for the two isolates, but the rate of increase in PAL activity was greater with the IN isolate (Fig. 7A and B). Cotyledons and true leaves that had been coinoculated with IN and CO isolates simultaneously were not assessed.

DISCUSSION

Attempted infection of cotyledons by an IN isolate of *A. candida* provided various degrees of protection of *B. juncea* seedlings, both local and systemic, against an CO isolate. The degree of protection depended on the zoosporangia concentration of IN isolate applied, sequence of inoculations, and interval between inoculations with the IN and CO isolates. However, the CO isolate only produced symptoms comparable with an IN reaction when a very high conc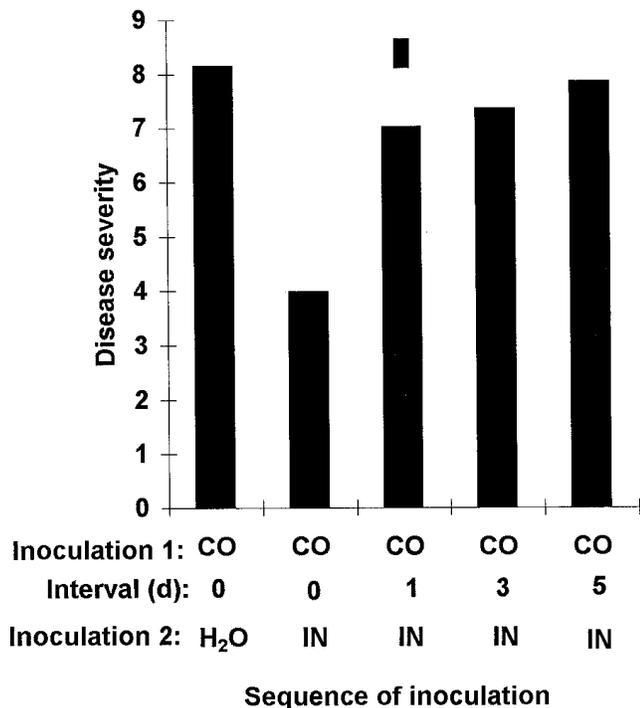entration of inducing (IN) inoculum was used. The protective effect was also greatest locally and appeared to decline in tissues remote from the point of IN isolate inoculation.

This interaction probably was not due to direct antagonism between zoospores of the two isolates. A more likely explanation is a combination of two effects: competition between the isolates for infection sites and inhibition of the CO isolate by host resistance responses induced by the IN isolate.
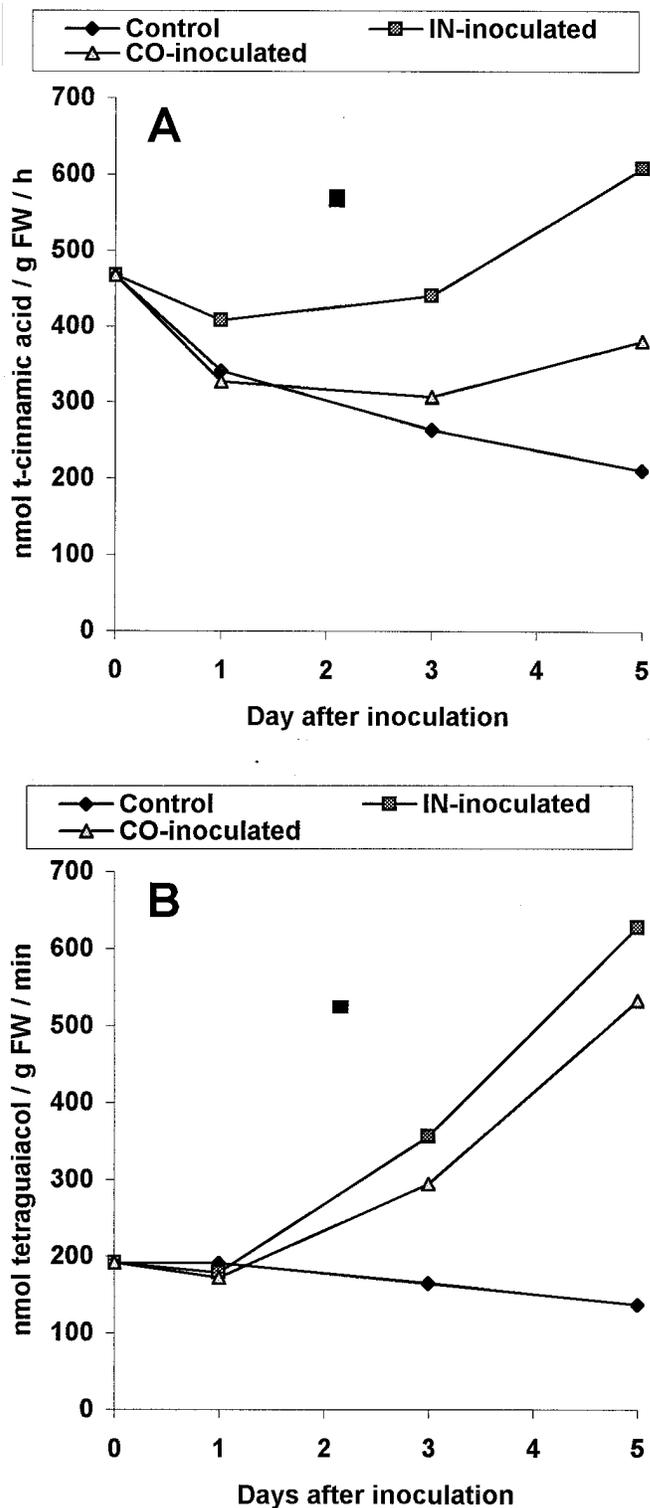

Fig. 6. Effect of inoculation with the incompatible (IN) *Albugo candida* isolate on disease severity on *Brassica juncea* when applied at different intervals after inoculation with the compatible (CO) isolate. Both cotyledons of a seedling were treated identically. The bar represents the $LSD_{(P=0.05, df=10)}$.

Fig. 7. A, Phenylalanine ammonia lyase (PAL) and **B,** total soluble peroxidase (POX) activities in *Brassica juncea* cotyledons after inoculation of seedlings (both cotyledons simultaneously) with either the compatible (CO) or incompatible (IN) *Albugo candida* isolates. The bars represent the $LSD_{(P=0.05)}$ when df = 44 and 22 for **A** and **B**, respectively.

Competition for infection sites is likely to have contributed to local protection conferred by the IN isolate, at least in treatments involving IN isolate inoculation before the CO isolate. *A. candida* zoospores must lodge over a stoma to initiate infection, and they must do so in an orientation that allows the emerging germ tube to grow directly into the stomatal chamber. Zoospores of the two isolates used in our study germinate from zoosporangia, encyst, and send germ tubes into stomatal chambers at a similar rate. More than one zoospore can lodge over a stoma, but once the stoma is occupied, the presence of one zoospore appears to reduce the likelihood that subsequent zoospores will lodge and penetrate successfully (U. S. Singh, K. J. Doughty, N. I. Nashaat, G. Ross, and S. J. Kolte, *unpublished data*). When the IN and CO isolates were inoculated together, the IN isolate is likely to have prevented a significant proportion of the CO isolate zoospores from penetrating successfully, particularly when a high IN zoosporangia concentration was applied. The fact that local protection was greater when the IN isolate inoculum preceded the CO isolate inoculum by as little as 4 h and less when the IN isolate followed the CO isolate than when the two isolates were inoculated together is consistent with this hypothesis. Four hours between successive inoculations with the IN and CO isolates is long enough to allow zoospores of the IN isolate to deny the CO isolate a proportion of stomatal infection sites by blocking them. Also, increasing the delay between inducing (IN) and challenge (CO) inoculations did not greatly increase the extent of local protection, and there was no significant "curative" effect when the IN isolate was applied more than 1 day after the CO isolate.

Systemic protection against the CO isolate conferred by the IN isolate was host-mediated. Local protection conferred by the IN isolate probably also was host-mediated, at least in part. For example, treatments that involved inoculating a cotyledon with the CO isolate and then the IN isolate (precluding the possibility of denial of infection sites by the IN isolate) also decreased the extent of local symptom development. However, local symptom development was much less when the isolates were inoculated at the same time or when the IN isolate was inoculated first. It is difficult to estimate the relative contributions of competition for infection sites and induced resistance to the local interaction between the two isolates.

Induced (IN) responses were likely to be relatively rapid ones, coinciding perhaps with inhibition of haustorial development in the mesophyll, which appears to be the first indication of an IN interaction (11,23). The IN isolate probably was recognized by the host relatively early, and its attempt to infect may have led to the release of elicitors that condition the expression of plant resistance genes—the products of which may inhibit infection by the CO isolate. This is consistent with the earlier and greater increase in PAL activity and the slightly greater increase in POX activity after inoculation with the IN isolate than with the CO isolate. Visible symptoms of hypersensitive reactions to fungal infection commonly occur between 3 and 5 days after inoculation. However, there are clearly other defense-related events that occur much earlier in resistant and hypersensitive plants, including insolubilization of cell wall proteins and induction of various enzymes and crucifer indole phytoalexins (15,19). Both PAL and POX are associated with induced resistance in various species and have various functions, including control of key stages in biosynthesis of lignin precursors (PAL), strengthening of cell walls against pathogen invasion (PAL and POX), and biosynthesis of indolylglucosinolates and indole phytoalexins (POX) (4,7,25). Dahiya and Woods (5) reported that infection by *A. candida* induced production of fungitoxic phytoalexins in rapeseed (species unspecified). It is possible that increased PAL and POX activities may contribute to protection of *B. juncea* against the CO isolate conferred by the IN isolate. However, *Brassica* species also are capable of other biochemical responses to infection that are not measured here, including production of pathogenesis-related proteins and glucosinolates (6,8,19). Further

studies need to be completed before the precise biochemical mechanism(s) of the IN isolate-induced resistance can be identified.

The greater local and systemic protection resulting from increased inducing (IN) inoculum corresponds with other studies (14). However, when increasing inoculum concentrations of the IN isolate were applied to one cotyledon, there appeared to be a limit to the extent of protection of opposite cotyledons inoculated with the CO isolate, such that the response (and presumably the signaling mechanism that invokes it) were greatest at relatively low inducing zoosporangia concentrations.

The pattern of interactions between the CO and IN isolates of *A. candida* on *B. juncea* corresponded, to some extent, to those found between highly virulent (HV) and weakly virulent (WV) isolates of *Leptosphaeria maculans* on *B. napus* (12). However, in contrast to *A. candida*, the optimum interval between WV and HV *L. maculans* inoculations was 24 h, and it was possible to achieve a curative effect up to 48 h after HV inoculation.

Our controlled-environment studies suggest there are likely interactions in the field among pathotypes of *A. candida* expressing different host specificities. In similar work, Voorrips (24) concluded that induction of host resistance was a better explanation for interactions between *Plasmodiophora brassicae* isolates differing in virulence on *B. oleracea* than competition for infection sites. In the *A. candida-B. juncea* system, both competition and induced resistance may be involved, although the relative importance of each in nature is determined by the amount of avirulent inoculum present. In western Canada, where both *B. rapa* and *B. juncea* are grown, there is a predominance of isolates that infect only *B. rapa* (13), and these might be expected to interfere with infection by isolates specific to *B. juncea*. The scale and significance of these interactions in crops depends not only on the virulence composition of the local pathogen population, but also on the relative timing and proximity of cultivation of respective hosts and on the likelihood that *A. candida* races differing in virulence coexist at infection sites, as is the case for the *L. maculans-B. napus* (12) and other (1) systems. In our study, the optimum local protection that occurred after preinoculation with the IN isolate alone approached a response comparable to an IN interaction when a high zoosporangia concentration (2×10^5 ml^{-1}) was used. The importance of the timing and sequence of IN and CO inoculations to the induction of resistance to *A. candida* in *B. juncea* suggests that major effects in the field are less likely than for *L. maculans*, for which induced resistance appears to be more flexible (12).

Based on the results presented in our paper, biological control of *A. candida* based on intervention with IN isolates appears difficult to achieve. The use of antagonistic or resistance-inducing bacterial inoculants for control may be a more promising approach (9). However, the *A. candida-B. juncea* interactions described here may provide a useful model for identifying the systemic biochemical responses that determine incompatibility.

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