APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1994, p. 1593–1598
0099-2240/94/$04.00+0

Vol. 60, No. 5

Potential Role of Elicitins in the Interaction between *Phytophthora* Species and Tobacco

SOPHIEN KAMOUN,[1] MARY YOUNG,[1] HELGA FÖRSTER,[2] MICHAEL D. COFFEY,[2] AND BRETT M. TYLER[1,3]*

Center for Engineering Plants for Resistance Against Pathogens[1] and Department of Plant Pathology,[3] University of California, Davis, Davis, California 95616 and Department of Plant Pathology, University of California, Riverside, Riverside, California 92521[2]

Received 22 November 1993/Accepted 1 March 1994

The potential role of extracellular elicitor proteins (elicitins) from *Phytophthora* species as avirulence factors in the interaction between *Phytophthora* and tobacco was examined. A survey of 85 *Phytophthora* isolates representing 14 species indicated that production of elicitin is almost ubiquitous except for isolates of *Phytophthora parasitica* from tobacco. The production of elicitins by isolates of *P. parasitica* correlated without exception with low or no virulence on tobacco. Genetic analysis was conducted by using a cross between two isolates of *P. parasitica*, segregating for production of elicitin and virulence on tobacco. Virulence assays of the progeny on tobacco confirmed the correlation between production of elicitin and low virulence.

Pathogens of the oomycete group, especially the order *Peronosporales*, cause destructive diseases of a wide range of plants, many of them of economic importance. Oomycetes include obligate biotrophic pathogens, such as the downy mildews and white rusts, and necrotrophic pathogens such as *Pythium* and *Phytophthora* species. The oomycetes classically have been included with the fungi by taxonomists. However, several biochemical characteristics such as cell wall composition, the pathway for lysine biosynthesis, and most conclusively the sequence of the 17S rRNA (5) have demonstrated that the oomycetes are more closely related to algae in the kingdom *Protoctista* (3), in particular to chrysophytes and diatoms, than to true fungi or to higher plants. Thus, oomycetes constitute a distinct group of eukaryotic plant pathogens with an independent evolutionary history. They may potentially have unexpected biochemical mechanisms for interacting with plants and unexpected genetic mechanisms for regulating those interactions and creating variation.

More than 43 species of *Phytophthora* have now been described. Members of this genus cause a wide variety of diseases on major food crops, forest, fruit, and nut trees, and many ornamental plants. The species *Phytophthora parasitica* causes root, stem, and fruit rot on more than 90 plant species, including conifers, citrus, tomato, and tobacco (13). Although *P. parasitica* as a species has a wide host range, individual strains usually have a very narrow host range (1, 6, 12). For example, isolates of *P. parasitica* from tobacco (sometimes referred to as *P. parasitica* var. nicotianae) are pathogenic only on that host (7).

Phytophthora species secrete small extracellular proteins collectively termed "elicitins." These proteins induce a vigorous defense response (hypersensitive response) locally and distally in certain plants of the families *Solanaceae* and *Cruciferae* (9). Among the *Solanaceae*, response to elicitins is genus specific, being restricted to *Nicotiana* species, whereas among the *Cruciferae*, the response is cultivar specific, being restricted to certain radish and turnip cultivars (9). The response to elicitins induces resistance against subsequent infection by *P. parasitica* (on tobacco) and by the bacterial pathogen *Xanthomonas campestris* pv. armoraciae (on radish) (9, 14, 15). Some isolates of *P. parasitica* that are virulent on tobacco do not produce elicitins, while many isolates of *P. parasitica* and other *Phytophthora* species that are nonpathogenic on tobacco secrete elicitins (14, 15). Therefore, elicitins have been proposed to act as avirulence factors in the interaction between *P. parasitica* and tobacco. That is, they may block or slow infection by the pathogen by triggering a defense response in the host (9, 14, 15). In this paper, evidence for this hypothesis is examined by determining elicitin production by a large collection of *Phytophthora* isolates representing 14 species. We also examine the virulence on tobacco of a collection of *P. parasitica* isolates which do or do not produce elicitin, and the virulence of progeny from a *P. parasitica* cross that segregates for elicitin production.

MATERIALS AND METHODS

Fungal strains and culture conditions. The various *Phytophthora* isolates used in this study are summarized in Table 1. *Phytophthora* strains were grown in cleared or uncleared 20% vegetable juice (V8) medium supplemented with 1.5% agar at 24°C (13). For elicitin production, *Phytophthora* isolates were grown for 2 to 3 weeks at 25°C in still culture in medium containing, per liter, 0.5 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 1 g of asparagine, 1 mg of thiamine, 0.5 g of yeast extract, and 25 g of glucose (from Bonnet [1]).

SDS-PAGE. Proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (8). Following electrophoresis, gels were silver stained as described by the manufacturer (BioRad, Richmond, Calif.). Molecular weights were estimated by comparison with known molecular weight standards (Amersham, Arlington Heights, Ill.).

Purification of elicitins. Chromatographic purification of elicitins was conducted as described earlier (9).

Hypersensitivity and pathogenicity assays. Induction of hypersensitivity was determined by infiltration of sterile distilled water solutions of elicitins into attached leaves as described by Klement (10). Virulence of *P. parasitica* on Havana

* Corresponding author. Mailing address: Department of Plant Pathology, University of California, Davis, CA 95616. Phone: (916) 752-4771. Fax: (916) 752-5674. Electronic mail address: bmtyler@ucdavis.edu.

TABLE 1. Elicitin production by *Phytophthora* species

Strain	Host plant, geographical origin, and mating type	Elicitin production
P. cactorum		
F18	*Arbutus* sp.	+
P. capsici		
19		+
P. cinnamomi		
P2121	*Persea americana*, Madagascar, A1	+
P2428	*P. americana*, California, A2	+
P7153	*P. americana*, Israel, A2	+
P7154	*P. americana*, Israel	+
F7	Soil under *Juglans hindsii*	+
F34	*Rhododendron* sp.	+
P. citricola		
7A-19C	*Rhododendron* sp.	+
7G-13D	*Gardenia* sp.	+
5	*Juglans hindsii*, California	+
6	*J. hindsii*, California	+
P. citrophthora		
C1	*Citrus* sp.	+
W1	*Juglans hindsii*	+
P. cryptogea		
P1088	*Aster* sp., A1	+
P1810	*Prunus avium*, California	+
P2001	*Malus sylvestris*, Australia	+
P3198	*Abies nobilis*, Oregon	+
P3200	*Pinus lambertiana*, Oregon	+
P3320	*Prunus persica*, New York	+
F2	*Chrysanthemum* sp., California	+
F3	*Chrysanthemum* sp., California	+
F44-3A	*Carthamus tinctorius*, Arizona	+
10J-1B	*Juniperus* sp., California	+
9J-15A	*Juniperus* sp., California	+
F5	Unknown	+
11C-16C	*Cotoneaster* sp.	+
P1738	*Lycopersicum esculentum*, Ireland, A1	+
P1739	*L. esculentum*, New Zealand, A1	+
P3447	*L. esculentum*, Channel Is.	+
P6510	*L. esculentum*, Great Britain	+
7G-8E	*Gardenia* sp., California	−
P7113	*Brassica* sp., Australia	−
P. cryptogea f. sp. begoniae		
P3265	*Begonia elatior*, Germany, A2	+
P3104	*B. elatior*, Germany, A2	+
P. drechsleri		
P1087	*Beta vulgaris*, Idaho, A2	+
P1899	*B. vulgaris*, California, A1	+
P1741	*Lycopersicum esculentum*, A2	+
P. drechsleri f. sp. cajani		
P1798	*Cajanus cajan*, India, A1	+
P. infestans		
20		+
P. megasperma		
A8	*Malus pumila*	+
K1	*Actinidia deliciosa*	+
A12	*Prunus avium*	+
P3136	*Brassica napus*, Australia	+
P3162	*Brassica* sp., Great Britain	+
P6046	*Brassica* sp., Great Britain	+
P6471	*Brassica oleracea*, Oregon	+
P6820	*Brassica napus*, Australia	+
P. melonis		
P1746	*Cucumis sativa*, Taiwan	+
P. palmivora		
P6960	*Theobroma cacao*, Malaysia, A2	+
P7334	*Durio zibethinus*, Malaysia	+
P. parasitica (from various hosts)		
P3456	*Hibiscus* sp., Pakistan, A2	+
P7337	*Grevilla hookeriana*, Australia	+
P7339	*Eucalyptus* sp., Italy	+
P7346	*Choisya ternata*, Great Britain	+
P3455	*Piper nigrum*, Malaysia, A2	+
P6622	*Psidium guajava*, Taiwan, A1	+
P7236	Fluorophenylalanine-resistant mutant, P6622	+
6G-14A	*Gardenia* sp.	+
5-3A	*Lycopersicum esculentum*, California, A2	+
P1325	*Citrus* sp., California, A2	+
6H-11A	*Hibiscus* sp., California	+
P3550	*Dianthus* sp., France	+
P7341	*Lycopersicum esculentum*, Great Britain	+
P1964	*L. esculentum*, California, A2	+
P1965	*L. esculentum*, California, A2	+
P1979	*L. esculentum*, California, A2	+
P3118	*L. esculentum*, Australia, A2	+
F13	*L. esculentum*, California	+
P3461	*L. esculentum*, Great Britain, A1	−
P7336	*Chamaedorea* sp., Australia	−
P. parasitica (from *N. tabacum*)		
P1960	*N. tabacum*, South Africa, A1	+
P1751	*N. tabacum*, Australia, A1	+
P1752	*N. tabacum*, Australia, A1	+
P1352	*N. tabacum*, North Carolina, A1	+
P1495	*N. tabacum*, Australia, A2	+
P582	*N. tabacum*, Kentucky, A2	−
P1351	*N. tabacum*, North Carolina, A1	−
P1350	*N. tabacum*, North Carolina, A1	−
P1349	*N. tabacum*, North Carolina, A2	−
P3110	*N. tabacum*, Kentucky, A1	−
P1955	*N. tabacum*, South Africa, A1	−
P1956	*N. tabacum*, South Africa, A1	−
P. sojae		
R12A	*Glycine max*	+
Phytophthora sp.		
P3196	*Pseudotsuga menziesii*, Canada	+

or Xanthi-nc tobacco plants was determined by a stem assay described in detail by Kamoun et al. (9). Fully bolted plants were decapitated. Agar blocks infested with the pathogen were then placed on the cut surface of the attached stem (not the detached upper portion). Virulence was measured as the linear rate of progression of the lesion.

Fungal crosses. The oospore progeny used in this study resulted from a cross between *P. parasitica* P1751 (mating type

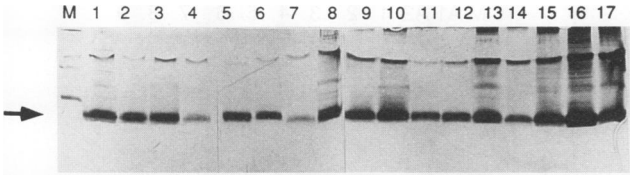

FIG. 1. Silver-stained tricine-SDS-PAGE of culture filtrates of *P. parasitica* P7236 (lane 1), 6G-14A (lane 2), 5-3A (lane 3), and P1323 (lane 4), *P. sojae* R12A (lane 5), *P. cryptogea* P7113 (lanes 6 and 15), *P. drechsleri* P1087 (lanes 7 and 11) and P1741 (lanes 8 and 12), *P. parasitica* P7339 (lane 9) and P7341 (lane 10), *P. megasperma* A8 (lane 13) and P6471 (lane 14), and *P. cryptogea* F3 (lane 16) and F44-3A (lane 17). Sigma low-molecular-weight protein standard is shown in lane M. The arrow indicates the position of the elicitin band.

A1) and P582 (mating type A2) described by Förster and Coffey (4).

Statistics. The correlation between growth rate in vitro and lesion rate in planta (see Fig. 5A) was determined by using the computer program Cricket Graph 1.3 (Cricket Software, Malvern, Pa). Since it is reasonable to expect that linear growth rate in vitro may contribute to the rate of linear lesion progression in planta, the statistical contribution of growth rate to lesion rate among the non-elicitin-producing progeny was estimated by linear regression, by using Cricket Graph. Insufficient data were available for regression analysis of the elicitin-producing progeny. However, because the elicitin-producing progeny have the same parents as the non-elicitin-producing progeny, we assumed that the regression analysis of the non-elicitin-producing progeny could be extrapolated to the elicitin-producing progeny. The residual lesion rate was calculated for all progeny by subtracting the contribution of growth rate as calculated from the regression function: lesion rate (in mm/day) = 0.82 + 0.30x (growth in mm after 5 days) (see Fig. 6B). The association of elicitin production with the residual lesion rate was then assessed by using the Mann-Whitney U test (11). This nonparametric test was also used to assess the association between elicitin production and growth rate in vitro.

RESULTS

Most *Phytophthora* isolates produce elicitins. We surveyed 85 *Phytophthora* isolates representing 14 species for elicitin production by PAGE of crude culture filtrates (Table 1). The production of extracellular elicitins appeared to be a common phenotype in all tested species of *Phytophthora*, as judged by the presence of 10-kDa proteins comigrating with authentic elicitins (Fig. 1). (Of approximately 20 isolates examined more closely by two-dimensional electrophoresis, elicitin purification, and/or plant inoculation, none produced 10-kDa proteins that were not elicitins.) Fifty of the 52 tested *Phytophthora* isolates representing *P. cactorum*, *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. infestans*, *P. megasperma*, *P. melonis*, *P. palmivora*, *P. sojae*, and an unidentified *Phytophthora* sp. produced elicitins. However, for *P. parasitica* only 24 of the 31 (72%) tested isolates produced elicitins. This high incidence of elicitin-nonproducers was especially noticeable in strains isolated from tobacco, of which only 5 of 12 (42%) produced elicitins, in contrast to *P. parasitica* strains isolated from sources other than tobacco, of which 19 of 21 (90%) produced elicitins. In summary, production of elicitins is almost ubiquitous in *Phytophthora* spp. except for isolates of *P. parasitica* from tobacco.

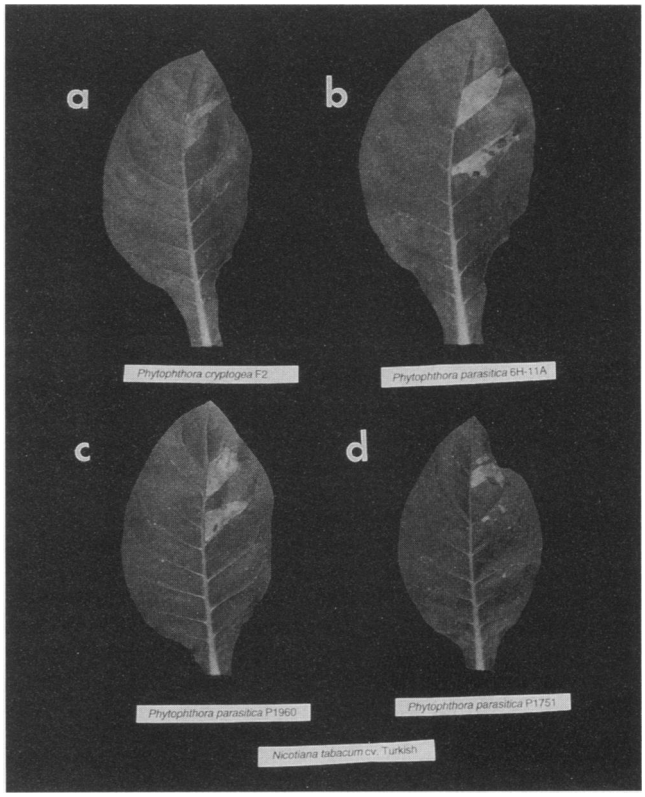

FIG. 2. Purified elicitins of *P. cryptogea* F2 (a), *P. parasitica* 6H-11A (b), P1960 (c), and P1751 (d) were infiltrated in panels of the right side of tobacco leaves at concentrations of 100 nM (top panel), 50 nM (middle panel), and 10 nM (bottom panel). The left side of each leaf was infiltrated with water.

Elicitins from tobacco isolates of *P. parasitica*. As shown in Table 1, five tobacco isolates of *P. parasitica* were found to produce elicitins. In order to test the biological activity of the proteins produced by these isolates, the elicitins of *P. parasitica* P1751 and P1960 were purified by chromatography (Materials and Methods). Leaves of tobacco (cv. Havana) were infiltrated with fractions containing 10, 50, and 100 nM of purified parasiticein from P1751 and P1960, along with another purified parasiticein (from *P. parasitica* isolate 6H-11A from *Hibiscus* sp.) and with a cryptogein (from *P. cryptogea* isolate F2 from a *Chrysanthemum* sp.) (Fig. 2). All proteins induced similar necrotic hypersensitive responses at 100 nM, and the minimal threshold for hypersensitive response induction varied between 10 and 50 nM. Therefore, the elicitins expressed by two tobacco *P. parasitica* isolates are as active as other elicitins in inducing a defense response in tobacco.

Virulence of *P. parasitica* on tobacco. To determine the virulence of the various isolates of *P. parasitica* on tobacco, whole-plant stem assays were conducted. Isolates of *P. parasitica* representing elicitin nonproducers (seven isolates from tobacco) and elicitin producers (five isolates from tobacco, two isolates from tomato, and one isolate from a *Hibiscus* sp.) were inoculated onto decapitated stems of *Nicotiana tabacum* cv. Havana. Disease lesion progression was monitored regularly, and the rate of lesion progression was typically constant over time (Fig. 3). The virulence of 16 isolates was estimated from the average lesion progression rate in mm per day (Fig. 4). Lesion rates ranged from 0 mm/day (completely avirulent) to 12.2 mm/day (strongly virulent). Interestingly, tobacco isolates

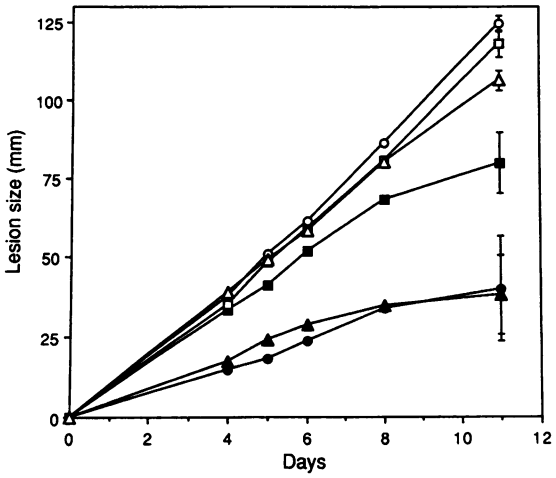

FIG. 3. Time course of the invasion by *P. parasitica* P582 (open circles), P1350 (open squares), P1349 (open triangles), P1752 (filled squares), P1495 (filled circles), and P1352 (filled triangles) of tobacco cv. Havana after stem inoculation. Open symbols refer to elicitin nonproducer isolates; filled symbols refer to elicitin producers. Bars represent standard errors.

of *P. parasitica* that produce elicitins were not completely avirulent but infected tobacco with lesion rates of 3.0 to 6.7 mm/day. Typically, tobacco isolates that do not produce elicitin showed lesion rates of 8.5 to 12.2 mm/day, except for one isolate, P1956, which was weakly virulent (3.4 mm/day). One *P. parasitica* isolate (P3461) from tomato does not produce elicitin and in fact lacks any elicitin genes at all (8). This isolate is completely nonpathogenic on tobacco (Fig. 4) or tomato and fails to produce zoospores or to mate. The three non-elicitin-producing isolates from sources other than tobacco (two *P. cryptogea* and one *P. parasitica*) have not been tested for virulence on tobacco. Elicitin-producing isolates of *P. parasitica* from tomato and a *Hibiscus* sp. ranged from avirulent to weakly virulent (0 to 3.1 mm/day) on tobacco. In summary, elicitin nonproducer isolates were generally more virulent than

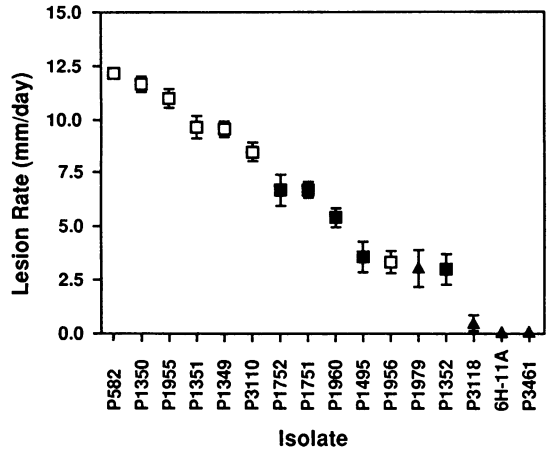

FIG. 4. Mean lesion rate (virulence) of *P. parasitica* isolates on tobacco cv. Havana. Open squares refer to *P. parasitica* isolates from tobacco that do not produce elicitins, filled squares refer to isolates from tobacco that produce elicitins, and filled triangles refer to isolates from host plants other than tobacco. Bars represent standard errors.

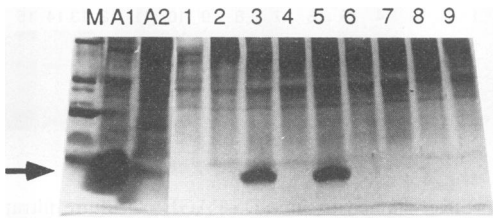

FIG. 5. Silver-stained SDS-PAGE of culture filtrates of parent strains of *P. parasitica* P1751 (elicitin producer, lane A1), P582 (elicitin nonproducer, lane A2), and selected oospore progeny (lanes 1 to 9). Progeny that produce elicitins are shown in lanes 3 and 5. Sigma low-molecular-weight protein standard is shown in lane M. The arrow indicates the position of the 10,000-molecular-weight elicitin band.

elicitin producers. Some of the isolates of *P. parasitica* that produce elicitins were still capable of infecting tobacco but were only weakly virulent.

Genetic analysis of elicitin production. To examine the genetic control of elicitin production and further evaluate the correlation between production of elicitin and weak virulence in *P. parasitica*, 21 oospore progeny of a cross between P582 (elicitin nonproducer, mating type A2) and P1751 (elicitin producer, mating type A1) were examined for elicitin production and virulence on tobacco. With PAGE analysis of crude culture filtrates, only 4 out of the 21 progeny were found to produce elicitins, suggesting a complex genetic control of elicitin production in *P. parasitica* (Fig. 5). To evaluate the virulence of the progeny, stem assays were conducted on tobacco (cv. Havana). A wide range of virulence was also observed, with lesion rates ranging from 0 to 11.3 mm/day. The nonproducing parent, P582, showed a lesion rate of 12.2 mm/day, whereas the producing parent, P1751, infected tobacco at 5.8 mm/day (Fig. 6). The four elicitin-producing progeny showed similar virulence, ranging from 5.1 to 7.7 mm/day. However, the 17 elicitin nonproducers showed variable lesion rates ranging from 0 to 12.2 mm/day.

Förster et al. previously showed that there was a high degree of variability in the morphology and growth rates of these progeny. Figure 6A shows that there is a strong correlation between linear growth rate in vitro and linear lesion progression in planta among non-elicitin-producing progeny ($r = 0.60$, $P < 0.01$). This suggests that there are significant genetic differences among the progeny that determine growth rate per se both in vitro and in planta. There also is a strong association between elicitin production and high growth rate in vitro ($P < 0.005$ by the Mann-Whitney U test), raising the possibility that elicitin is a fitness factor.

In order to examine the association between elicitin production and virulence, separate from the association with growth rate, the apparent contribution of growth rate to lesion rate was calculated by regression analysis (Fig. 6A). The regression equation was then used to calculate the residual lesion rate, i.e., the actual lesion rate less the lesion rate predicted from the in vitro growth rate. Figure 6B shows that there is a strong association between elicitin production and low virulence when the effects of growth rate are accounted for ($P < 0.025$ by the Mann-Whitney U test), supporting the hypothesis that lack of elicitin production is required for high virulence.

DISCUSSION

In this paper, we show that the production of elicitins, a small family of extracellular elicitors, is almost ubiquitous among *Phytophthora* species (87% of tested isolates). The

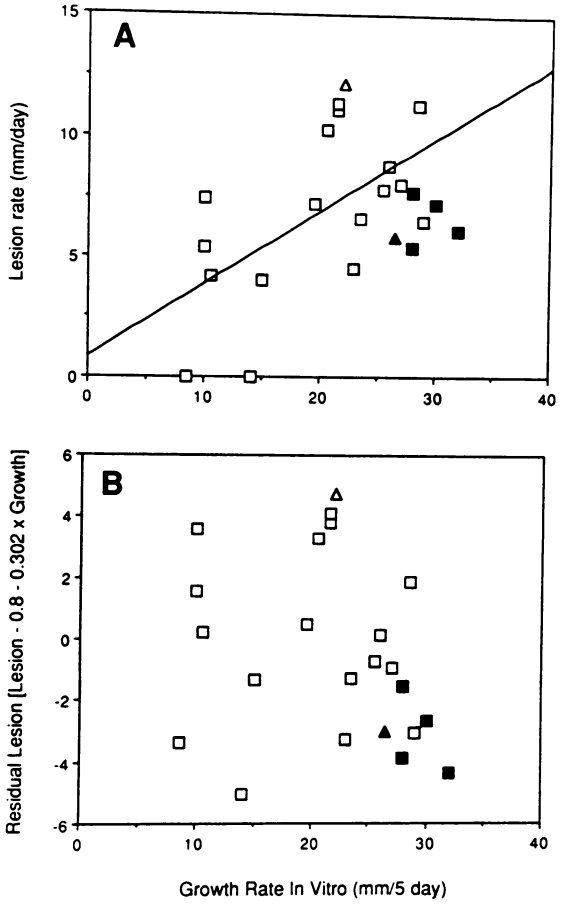

FIG. 6. Virulence on tobacco and growth in vitro of *P. parasitica* isolates P582 (open triangle), P1751 (filled triangle), and their progeny (elicitin nonproducers, open squares; elicitin producers, filled squares). (A) Correlation of lesion size with growth rate was determined by regression analysis to be $y = 0.82 + 0.30x$ ($r = 0.604$; $P < 0.01$). (B) Relationship of growth and residual lesion to the production of elicitins. Residual lesion rate is the actual lesion rate less the rate predicted from the growth rate in vitro. Thus, negative and positive values indicate lesion rates less or greater than the average for a particular growth rate.

absence of elicitin production was mostly constrained to field isolates of *P. parasitica* from tobacco. Ricci et al. examined fifteen isolates of *P. parasitica* and similarly found that only tobacco isolates (nine) failed to produce elicitins (15). We have shown previously that elicitins are host-specific elicitors, which induce a hypersensitive response only in tobacco and some radish and turnip cultivars, and that prior inoculation of tobacco plants with elicitin reduces subsequent infection by *P. parasitica* (9). Therefore, the production in planta of elicitins by *Phytophthora* spp. could interfere with the infection of responsive plants by triggering defense responses. The observation that many tobacco isolates fail to produce elicitins is consistent with the hypothesis that elicitins may act as avirulence factors in the *P. parasitica-Nicotiana* interaction.

As shown in Table 1, not all *P. parasitica* tobacco isolates examined were unable to produce elicitins. Five isolates from tobacco still produced elicitins, of which three were capable of infecting tobacco. In the two cases tested, the level of production and the biological activity of the elicitins were similar to those of nontobacco isolates. However, quantitative analysis of virulence on tobacco of the various isolates indicated that elicitin nonproducers are generally more virulent than elicitin producers. This was supported by the results of the genetic cross between a high-virulence elicitin nonproducer and a low-virulence elicitin producer. Among the progeny, the elicitin producers were significantly less virulent than the elicitin nonproducers (Fig. 6). Two interpretations can be made. (i) Elicitins confer only a quantitative decrease in virulence rather than complete avirulence. (ii) The tobacco isolates that produce elicitins and show intermediary virulence have evolved mutations that suppress the effect of elicitins. With the recent cloning of *parA1*, a gene from *P. parasitica* that encodes elicitin (8), it should now be possible to directly test these hypotheses by transformation of a *P. parasitica* elicitin-nonproducer isolate with the elicitin gene.

The production of large amounts of elicitins by almost all isolates from 14 different species of *Phytophthora* suggests that these extracellular proteins could have an important biological function in the genus *Phytophthora*. Because elicitins are produced in planta, one possibility for a potential role of elicitins lies in the interaction of *Phytophthora* spp. with host plants during compatible interactions or even during growth in vitro. It is interesting in this regard that the elicitin-nonproducer progeny had a lower growth rate than the elicitin producers. Additionally, an isolate of *P. parasitica* from tomato (P3461), which completely lacks elicitin genes (8), is completely nonpathogenic on tomato and tobacco and does not sporulate sexually or asexually (1a). Elicitins have also been shown to move systematically in nonhost tobacco plants, where they can translocate to mesophyll tissue to induce necrosis distally from the inoculation point (2, 17). It will be interesting to determine whether these proteins are capable of movement within host plants. Recently harpin, a protein of the bacterial phytopathogen *Erwinia amylovora* that induces a hypersensitive response on tobacco, was also shown to be required for pathogenicity on host plants (16), suggesting an overlap between the mechanisms leading to the elicitation of a defense and of a susceptible response. Further analysis of the physiology of elicitins on host plants of *Phytophthora* spp. should help answer these questions.

ACKNOWLEDGMENTS

Kevin Klucher, Howard Judelson, and the members of the Cellular Pathogens and Resistance Genes Groups (CEPRAP) provided much helpful intellectual interaction. We thank Harald Sherm and Ariena van Bruggen for statistical advice, Jim McDonald for several *Phytophthora* isolates, Bella Chun for technical assistance, and Jeff Hall for photography.

This work was supported by NSF cooperative agreement BIR-8920216 to CEPRAP, an NSF Science and Technology Center, and by CEPRAP corporate associates Calgene, Inc., Ciba Geigy Biotechnology Corporation, Sandoz Seeds, and Zeneca Seeds.

REFERENCES

1. **Bonnet, P.** 1985. Reactions differentielles du tabac à 9 espèces de *Phytophthora*. Agronomie **5**:54–60.
1a. **Coffey, M.** Personal communication.
2. **Devergne, J.-C., P. Bonnet, F. Panabieres, J.-P. Blein, and P. Ricci.** 1992. Migration of the fungal protein within tobacco plants. Plant Physiol (Bethesda) **99**:843–847.
3. **Dick, M. W.** 1990. Phylum Oomycota, p. 661. *In* M. Margulis, J. O. Corliss, M. Melkonian, and D. J. Chapman (ed.), Handbook of Protoctista. Jones and Bartlett, Boston.
4. **Förster, H., and M. D. Coffey.** 1990. Mating behavior of *Phytophthora parasitica*: evidence for sexual recombination in oospores using DNA restriction fragment length polymorphisms as genetic markers. Exp. Mycol. **14**:351–359.
5. **Förster, H., M. D. Coffey, H. Elwood, and M. L. Sogin.** 1990.

Sequence analysis of the small subunit ribosomal RNAs of three zoosporic fungi and implications for fungal evolution. Mycologia **82:**306–312.

6. **Hine, R. B., and M. Aragaki.** 1963. Pathogenicity, vitamin nutrition, and cultural characteristics of isolates of *Phytophthora parasitica* from carnation and other hosts in Hawaii. Phytopathology **53:**1194–1197.
7. **Ho, H. H.** 1981. Synoptic keys to the species of Phytophthora. Mycologia **73:**705–714.
8. **Kamoun, S., K. M. Klucher, M. D. Coffey, and B. M. Tyler.** 1993. A gene encoding a host-specific elicitor protein of *Phytophthora parasitica*. Mol. Plant-Microbe Interact. **6:**573–581.
9. **Kamoun, S., M. Young, C. B. Glascock, and B. M. Tyler.** 1993. Extracellular protein elicitors from *Phytophthora*: host-specificity and induction of resistance to bacterial and fungal phytopathogens. Mol. Plant-Microbe Interact. **6:**15–25.
10. **Klement, Z.** 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology **54:**474–477.
11. **Little, T. M., and F. J. Hills.** 1978. Agricultural experimentation: design and analysis. John Wiley & Sons, Inc., New York.
12. **Matheron, M. E., and J. C. Matjeka.** 1990. Differential virulence of *Phytophthora parasitica* recovered from citrus and other plants to rough lemon and tomato. Plant Dis. **74:**138–140.
13. **Ribeiro, O. K.** 1978. A source book of the genus *Phytophthora*. J. Cramer, Vaduz, Liechtenstein.
14. **Ricci, P., P. Bonnet, J.-C. Huet, M. Sallantin, F. Beauvais-Cante, M. Bruneteau, V. Billard, G. Michel, and J.-C. Pernollet.** 1989. Structure and activity of proteins from pathogenic fungi *Phytophthora* eliciting necrosis and acquired resistance in tobacco. Eur. J. Biochem. **183:**555–563.
15. **Ricci, P., F. Trentin, P. Bonnet, P. Venard, F. Mouton-Perronnet, and M. Bruneteau.** 1992. Differential production of parasiticein, an elicitor of necrosis and resistance in tobacco, by isolates of *Phytophthora parasitica*. Plant Pathol. **41:**298–307.
16. **Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S. Y. He, A. Collmer, and S. V. Beer.** 1992. Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science **257:**85–88.
17. **Zanetti, A., F. Beauvais, J. C. Huet, and J. C. Pernollet.** 1992. Movement of elicitins, necrosis-inducing proteins secreted by *Phytophthora* sp., in tobacco. Planta **187:**163–170.