Interaction of *Rhizoctonia* spp. with Peanut Lectin

Jane R. Leatherman and Steven G. Pueppke

Former graduate assistant and former associate professor, respectively, Department of Plant Pathology, University of Florida, Gainesville 32611. Present address of both authors: Department of Plant Pathology, University of Missouri, Columbia 65211.
Florida Agricultural Experiment Station Journal Series Paper 5780.
Accepted for publication 19 December 1984.

ABSTRACT

Leatherman, J. R., and Pueppke, S. G. 1985. Interaction of *Rhizoctonia* spp. with peanut lectin. Phytopathology 75:598-602.

Cotyledons of 9-day-old peanut seedlings were inoculated with *Rhizoctonia solani* and harvested at 3-day intervals until 18 days postinoculation. Protein was extracted from infected and control cotyledons in phosphate-buffered saline (PBS). Peanut lectin (PNL) subsequently was purified from the protein extracts by affinity chromatography. Each cotyledon contained about 18.7 mg of PBS-soluble protein and 145 μg of PNL at inoculation. Protein and PNL concentrations decreased at similar rates in infected and control cotyledons, such that by 18 days postinoculation, each cotyledon contained 4–5 mg of protein and 3–4 μg of PNL. The PNL preparations were subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, and isolectins were resolved by isoelectric focusing in polyacrylamide gels. Although basic isolectins disappeared most rapidly, infection did not substantially alter the subunit molecular weight of the lectin or isolectin profiles. Fluorescent-labeled PNL bound hapten-specifically to young hyphae of *R. solani* and *R. zeae* (nonpathogenic to peanut), but physiological concentrations of the lectin did not inhibit the in vitro radial growth or dry weight increase of either fungus. A new acidic isolectin appeared in liquid cultures of *R. solani* that had been amended with PNL, and the amount of basic isolectin 6 decreased. Alteration of PNL isolectins by liquid cultures of *R. zeae* was much less pronounced. After 6 days, recovery of PNL from liquid cultures was 40% (uninoculated controls), 5% (inoculated with *R. solani*), and 0.2% (inoculated with *R. zeae*) of the amount recovered at time 0. We conclude that PNL is of questionable significance in the interaction of *Rhizoctonia* spp. with peanut cotyledons.

Additional key word: *Arachis hypogaea*.

Lectins, proteins that recognize and bind to specific carbohydrates, are particularly abundant in legume seeds and may comprise 2–10% of the total protein (15). The in vivo significance of lectins is unclear, and they have been termed proteins in search of a function (19). In 1975, Mirelman et al (18) found that a seed lectin from wheat inhibits *Trichoderma viride*, and they hypothesized that such lectins may have a general function in protecting seeds and young seedlings from soilborne microorganisms. Seed lectins exert a number of effects on phytopathogenic fungi, including lysis of zoospores (8,10,23) and inhibition of spore germination and hyphal growth (2,3,11,18). In many bioassays, however, the fungi were exposed to relatively high nonphysiological concentrations of lectins from plants not normally infected by the fungi under investigation. The specificity of the fungal response to the lectin was not always established, because controls containing hapten sugars or inactivated lectin were omitted. In addition, the effect of fungal infection on the structure, concentration, and function of lectins in vivo has received little attention.

Here we describe the results of our investigation of the interaction of *Rhizoctonia* spp. with peanut lectin (PNL), a 110-kdalton seed lectin with binding specificity for galactose, lactose, and related saccharides (16). Our main objectives were to examine the influence of fungal infection on concentrations of endogenous PNL in peanut cotyledons, to assess the in vitro stability of PNL in the presence of the fungi, and to measure the growth responses of *R. solani* and *R. zeae* to concentrations of PNL found in peanut cotyledons.

MATERIALS AND METHODS

Growth of fungi and plants. *Rhizoctonia solani* Kuehn was provided by R. C. Ploetz (University of Florida, Gainesville). The isolate was recovered from soil that had been cropped with soybean. *R. zeae* Voorhees was provided by T. Schubert (Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville). The fungi were maintained on slants of potato-dextrose agar (PDA) at room temperature.

Peanut (*Arachis hypogaea* L. 'Spanish') seeds were purchased from W. Atlee Burpee Co. (Warminster, PA). Seeds were removed

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

from the pods, surface disinfested by soaking them in 1% sodium hypochlorite for 3 min, and then given three 3-min rinses in sterile deionized water. The seeds were placed aseptically into petri dishes containing 1.5% water agar and incubated at 26 C for 48 hr. Germinated seeds were planted in vermiculite (10 seeds per 10-cm-diameter clay pot) and maintained in the greenhouse. Nine-day-old seedlings were inoculated by placing a 1-cm-diameter plug from the advancing edge of a PDA culture of *R. solani* in contact with each cotyledon. Fungus-free PDA plugs were placed on the cotyledons of control plants. Inoculated plants were placed in a plastic-covered moist chamber in a greenhouse to maintain high relative humidity. The infection experiment was repeated three times.

Purification of PNL. At least 30 cotyledons from control plants and 30 from infected plants were harvested at 3-day intervals from 12- to 27-day-old seedlings. Cotyledons of healthy 3-, 6-, and 9-day-old plants also were analyzed. The cotyledons were rinsed in tap water, blotted dry, weighed, and stored at −20 C. The frozen tissue was lyophilized, weighed, and powdered with a mortar and pestle. The powdered tissue was defatted with chloroform in a Soxhlet apparatus. After air-drying, the dry weight of each defatted sample was recorded. Defatted meal was stored at −20 C until analysis, and replicates of each sample were prepared.

The defatted meal was homogenized in phosphate-buffered saline (PBS), and proteins were extracted as described previously (20). Three-milliliter samples of each crude extract were stored at −20 C for analysis of PBS-soluble protein. The PNL from the remainder of each extract was purified by affinity chromatography (20) and stored at −10 C. Protein in the crude extracts and in the purified PNL preparations was measured by the Folin phenol method with bovine serum albumin as the standard (17). If PNL samples were dilute, they were concentrated with ultrafiltration membrane cones (CF25; Amicon Corp., Lexington, MA) prior to analysis.

The molecular weights (MW) of protein subunits were determined by electrophoresis in 10% polyacrylamide slab gels containing sodium dodecyl sulfate (13). Each well was loaded with 8–10 μg of protein, and PNL from seed meal was included as an MW standard. Gels were stained with Coomassie Brilliant Blue R-250 (20). PNL in each sample was resolved into isolectins by isoelectric focusing in cylindrical (0.5 cm diameter × 10 cm long) 7.5% polyacrylamide gels as described previously (20). The final concentration of ampholytes (Ampholine 3.5/9.5; LKB, Bromma, Sweden) was 0.05%, and a 30-μg sample was layered on the anodal end of each gel. The gels were subjected to 200 V for 20 hr at 12 C and then stained with Coomassie Brilliant Blue R-250 (20).

Binding of PNL to fungi. Affinity-purified PNL was labeled with rhodamine isothiocyanate (RITC; Pfaltz and Bauer, Stamford, CT) according to the method of Hapner and Hapner (12). The ratio of absorbance at 546 nm to that at 280 nm was 0.10, and the final protein concentration was about 1 mg/ml. Hyphal strands from the edges of actively growing colonies on PDA were placed in droplets containing 5 μl of RITC-PNL and 5 μl of PBS on microscope slides. To confirm that binding was sugar-specific, 300 mM lactose (a hapten inhibitor of PNL) was added to the PBS in some experiments. The RITC-PNL was omitted in other experiments to serve as a control for autofluorescence. The slides were examined at ×400 with a Zeiss standard microscope equipped with interference-contrast optics and incident fluorescence (BP546 exciter, FT580 chromatic beam splitter, LP590 barrier). The slides were photographed with Kodak Technical Pan 2415 film.

Fungal growth assays. Peanut lectin from seeds was dialyzed against the B5 growth medium of Gamborg et al (9), filter-sterilized, and protein concentration determined (17). Lectin concentration was adjusted to 0.34 mg/ml and 1.12 mg/ml, the concentrations present in cotyledons of ungerminated seeds and in those of 9-day-old seedlings, respectively. (Cotyledon volume was determined by water displacement; PNL content was measured as described above.) PNL for control experiments was denatured by incubation at 80 C for 3 min, a treatment that eliminated biological activity as measured by hemagglutination (20). Controls containing lactose were omitted, because effects of the sugar on fungal growth could not be controlled.

TABLE 1. Concentrations of soluble protein and peanut lectin (PNL) in control and *Rhizoctonia solani*-infected peanut cotyledons

Plant age (days)	Protein (mg ± SD/cotyledon)		PNL (μg ± SD/cotyledon)	
	Control	Infected[a]	Control	Infected[a]
3	34.1 ± 1.2	···	344 ± 65	···
6	33.8 ± 1.2	···	262 ± 30	···
9	18.7 ± 1.1	···	145 ± 35	···
12	12.6 ± 7.8	10.6 ± 0.7	110 ± 28	91 ± 42
15	7.4 ± 3.1	10.3 ± 3.0	40 ± 24	37 ± 18
18	3.3 ± 0.6	5.4 ± 2.5	16 ± 5	12 ± 9
21	6.4 ± 2.5	5.4 ± 1.3	15 ± 13	19 ± 19
24	3.5 ± 0.9	9.7 ± 0.4	5 ± 2	34 ± 22
27	4.7 ± 4.4	3.9 ± 3.1	4 ± 1	3 ± 5

[a] Cotyledons were inoculated with *R. solani* on day 9.

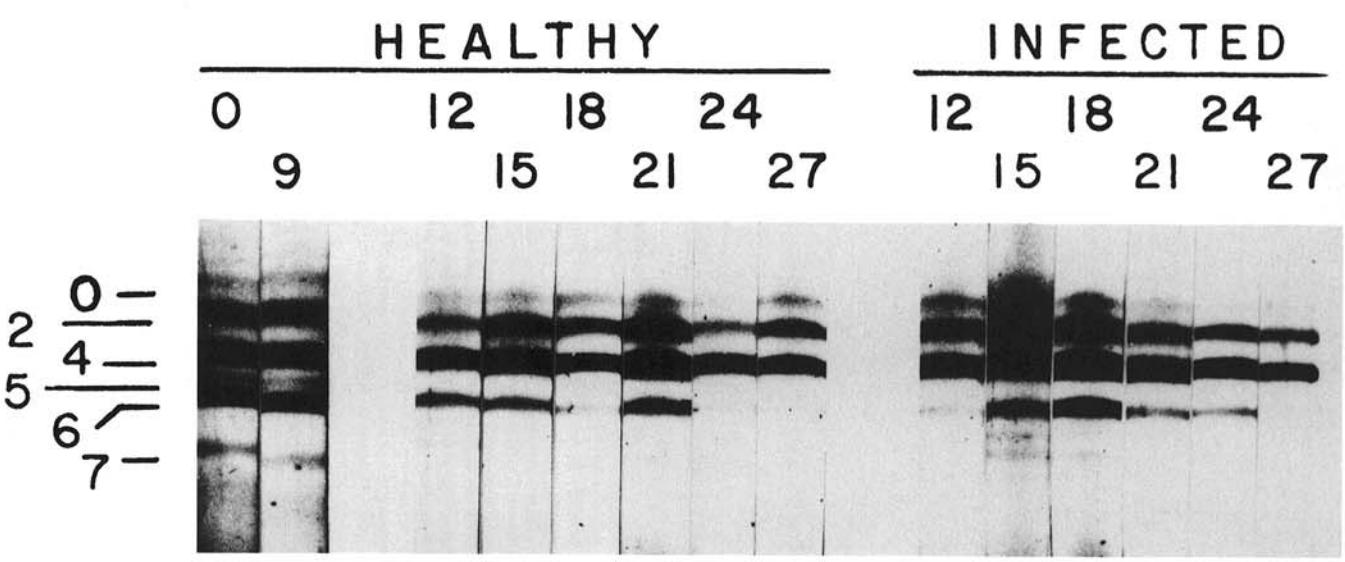

Fig. 1. Isolectin composition of peanut lectin (PNL) from control peanut cotyledons and cotyledons infected with *Rhizoctonia solani*. PNL was extracted, purified, and subjected to isoelectric focusing as described in the text. Cotyledons were 9 days old at the time of inoculation. The number above each gel indicates the age of the cotyledons in days at the time of harvest, and the numbers on the left identify the three major isolectins (pI 5.9, 6.2, and 6.5) and the three minor isolectins (pI 5.7, 6.3, and 6.7) (20). The photos have been trimmed to remove portions of the gels that lacked protein bands.

Equal volumes of molten 3% water agar and of double-strength B5 medium, active PNL in double-strength B5 medium, or denatured PNL in double-strength B5 medium were aseptically placed into 30-mm-diameter petri plates maintained at 60 C on a slide-warming tray. The final PNL concentrations (active or denatured) were 0, 0.34, 1.12, and 2.24 mg/ml. The final volume of medium in each plate was 1.5 ml. Each plate was swirled rapidly, sealed with Parafilm®, and allowed to cool. Each plate was inoculated with a 4-mm-diameter plug taken from the advancing edge of a colony of either *R. solani* or *R. zeae*. The plates were resealed and incubated in darkness at 29 C. Colony diameters were measured at regular intervals (*R. solani* at 6, 10, 14, and 18 hr; *R. zeae* at 4, 7.5, 11, and 14.5 hr). Radial growth was determined by dividing the sum of two perpendicular colony diameters, minus the diameter of the original plug, by four. Three to six replicates were measured for each treatment, and the experiment was repeated.

For dry weight increase assays, 4-mm-diameter hyphal mats were cut from the advancing edges of fungal colonies growing on sterile 0.45-μm Gelman Metricel membranes on PDA. Vials containing 3 ml of filter-sterilized liquid B5 medium each received two mats. The concentration of PNL (native or denatured) was 0, 0.34, or 1.12 mg/ml. The vials were incubated in darkness on a rotary shaker at 125 rpm and 30 C. Cultures were harvested after 2, 4, and 6 days. The mycelium from each vial was filtered with suction onto tared Whatman #50 filter paper, dried for 5 days at 75 C, and then weighed. The mean dry weight of 10 mats similar to those used as inocula was used as the time 0 weight. At least three replicates were determined per treatment, and the experiment was repeated.

Effect of fungi on PNL in vitro. Filter-sterilized PNL in liquid B5 medium was placed in autoclaved vials (1.12 mg of PNL/ml, 3 ml total). Vials then were inoculated either with *R. solani* or *R. zeae*, or left uninoculated. The vials were incubated for 2, 4, and 6 days, and the mycelia were collected on filter paper. The mycelia were rinsed with PBS, and the combined culture media and rinse solutions were dialyzed against PBS for 6–12 hr at 4 C. PNL was recovered by affinity chromatography (20) and stored at −20 C prior to analysis. Three replicates were used per treatment, and the experiment was repeated. Duplicate assays were used for measurement of the pH of the culture media.

Statistical analyses. The fungal growth data were subjected to linear regression analysis and orthogonal contrasts comparing control to all other treatments, heat-denatured PNL to active PNL, concentrations of active PNL, linear effect of protein concentration, and (where applicable) quadratic effect of protein concentration.

RESULTS

Pathogenicity tests. Three days after inoculation with *R. solani*, cotyledons contained small chlorotic areas and sunken, brown

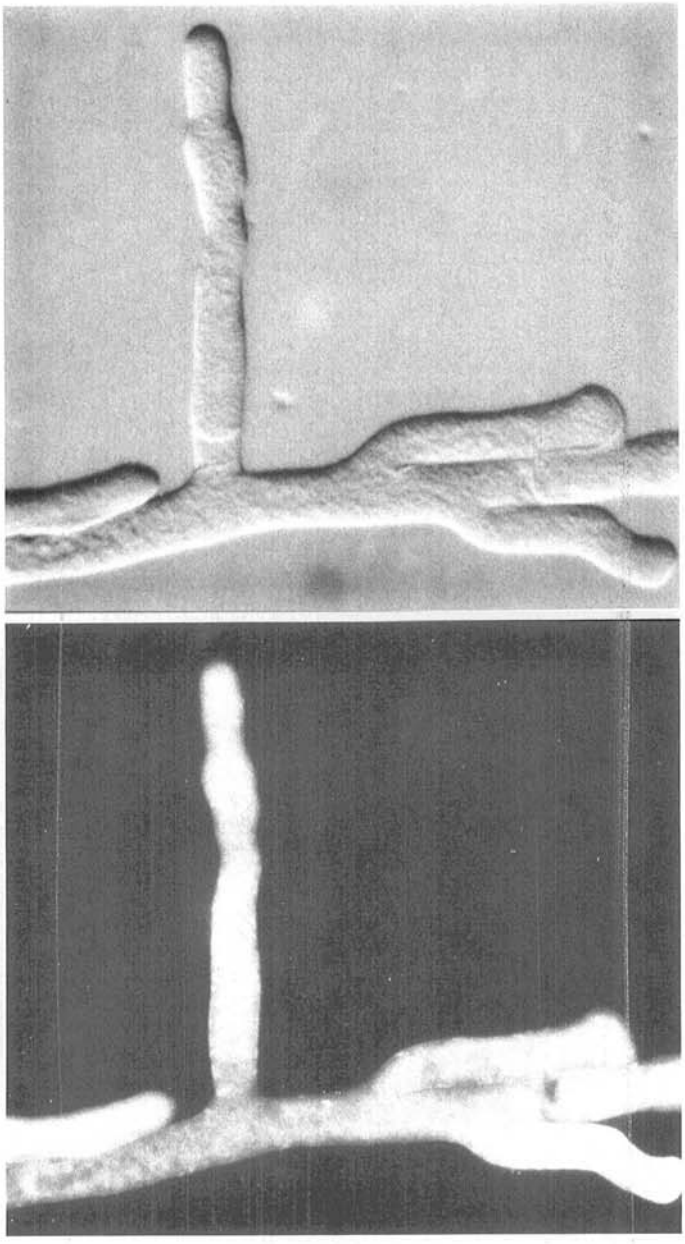

Fig. 2. Binding of rhodamine-labeled peanut lectin to young hyphae of *Rhizoctonia solani*. Top: interference-contrast optics. Bottom: fluorescence optics. (×800).

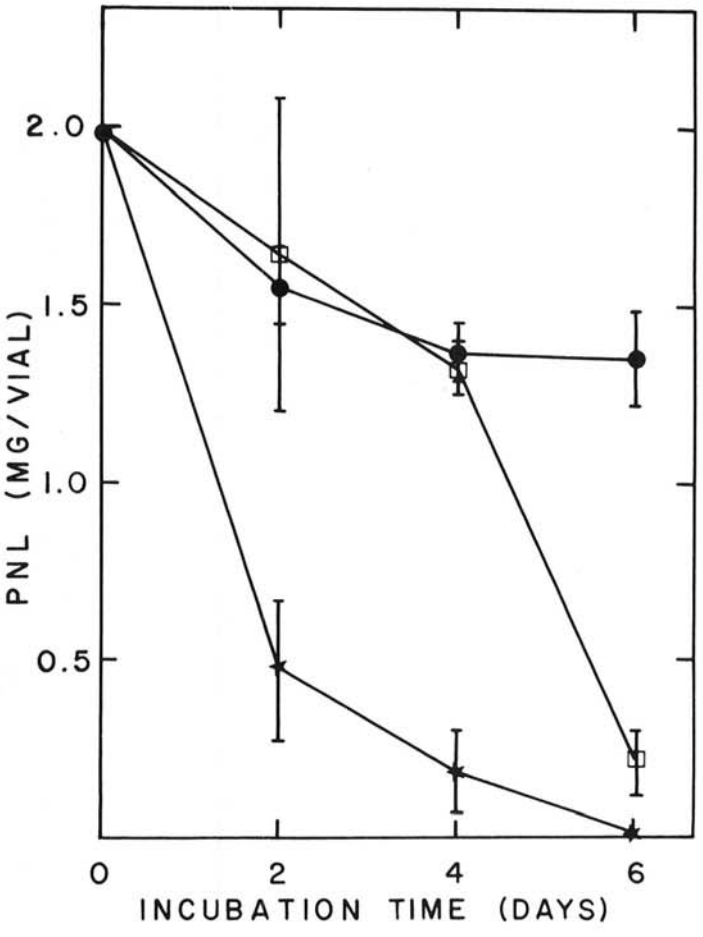

Fig. 3. Depletion of peanut lectin (PNL) from liquid cultures of *Rhizoctonia* spp. Each vial contained 3.36 mg of PNL at time 0. O = uninoculated controls; □ = *R. solani*; * = *R. zeae*. The bars mark standard deviations.

lesions. The number of lesions increased with time, as did their surface area and depth. Infected and control cotyledons began to senesce by day 18 (9 days postinoculation). Although approximately half of the tissue in infected cotyledons was necrotic by 18 days postinoculation, the only changes in control cotyledons were those associated with normal senescence. *Rhizoctonia zeae* did not produce lesions in peanut cotyledons.

Concentration of PBS-soluble protein and PNL. Cotyledons of 3-day-old plants contained about 34 mg of PBS-soluble protein and 344 μg of PNL (Table 1). The PBS-soluble protein concentration was unchanged at day 6, but it declined thereafter. PNL concentrations declined continuously after day 3, such that each cotyledon contained about 150 μg of PNL at inoculation. The PBS-soluble protein concentrations in 15- to 27-day-old control and infected cotyledons were similar, ~10 mg or less per cotyledon. Although the loss of PNL from infected cotyledons paralleled that from control cotyledons, there was more fluctuation in the data from 21- and 24-day-old infected plants. These differences may reflect disease-associated variation in the rate of cotyledon senescence.

Subunits of PNL obtained from healthy and infected cotyledons of all ages comigrated with subunits of PNL from seed meal (*unpublished*). PNL from defatted seed meal and from cotyledons of 9-day-old seedlings was resolved into six isolectins by isoelectric focusing (Fig. 1). The major isolectins have been designated isolectins 2, 4, and 6, and the minor isolectins designated 0, 5, and 7 (20). The major time-dependent change in the isolectin profile of healthy cotyledons was the disappearance of minor isolectins 5 and 7 (Fig. 1). Intensely staining bands 2 and 4 were present in all samples. Although major isolectin 6 was not detected in 27-day-old control and infected cotyledons, for unknown reasons the staining intensity of this isolectin fluctuated substantially from sample to sample.

Interaction of PNL and *Rhizoctonia* spp. in vitro. Although autofluorescence prevented the examination of the binding of PNL to older hyphae, RITC-PNL bound to young hyphae of both *R. zeae* and *R. solani* (Fig. 2). Young hyphae did not bind RITC-PNL in the presence of lactose, a hapten inhibitor of PNL, and they did not autofluoresce. Physiological concentrations of active and denatured PNL failed to significantly alter the radial growth rates of *R. solani* and *R. zeae*. Radial growth rates were linear (correlation coefficients for the linear regressions were 0.96 to 0.99). The radial growth rate of *R. solani* was 0.7 to 0.8 mm/hr, regardless of treatment. The corresponding rate for *R. zeae* varied from 0.7 to 1.0 mm/hr. The dry weight increase of the fungi during a 6-day incubation period in liquid media similarly was unaffected by active and denatured PNL. Dry weight increase was linear (correlation coefficients for the linear regressions were 0.78 to 0.99). The dry weight increases for *R. solani* and *R. zeae* were 4.0 to 6.2 mg/day and 0.3 to 1.0 mg/day, respectively.

PNL was relatively stable during a 6-day incubation in uninoculated B5 medium at 30 C (Fig. 3). Approximately 60% of the lectin was recovered at time 0, and the value decreased to 40% after 6 days. The pH of the medium in these flasks varied from 4.9 to 5.0 (initial pH was 5.5) and the staining intensity of isolectin 0 gradually increased with incubation time (Fig. 4). Although recovery of PNL from young cultures of *R. solani* was similar to that from the controls, only 5% of the lectin could be retrieved from 6-day-old cultures (Fig. 3). The pH of the culture medium containing *R. solani* increased to 8.1, and the isolectin profile was altered such that relative staining intensity of basic isolectin 6 decreased somewhat and that of acidic isolectin 0 increased substantially (Fig. 4). PNL preparations from 4- and 6-day-old cultures also contained a new isolectin that was more acidic than isolectin 0 and not found in PNL samples from seeds or cotyledons.

Recoverable peanut lectin was depleted very rapidly in cultures of *R. zeae* (Fig. 3). Affinity chromatography recovered only 15% of the lectin after 2 days, and by 6 days the residual lectin was below the level detectable with the Lowry protein assay. Traces of PNL activity, nevertheless, were detected by hemagglutination (20). Although *R. zeae* lowered the pH of the medium to 3.0 within 2 days, it failed to significantly influence the isolectin composition of PNL (Fig. 4). The absence of the darkly staining acidic bands in PNL exposed to *R. zeae* is particularly striking in comparison to those exposed to *R. solani*. Because altered pH conditions could influence the biological activity and solubility of the lectin, we retested the binding of RITC-PNL to hyphae at pH 3 and 8. RITC-PNL retained its ability to bind hyphae of *R. zeae* under these conditions, and it was not precipitated from solution.

DISCUSSION

Despite extensive colonization of peanut cotyledons by *R. solani*, neither the concentration nor the isolectin composition of

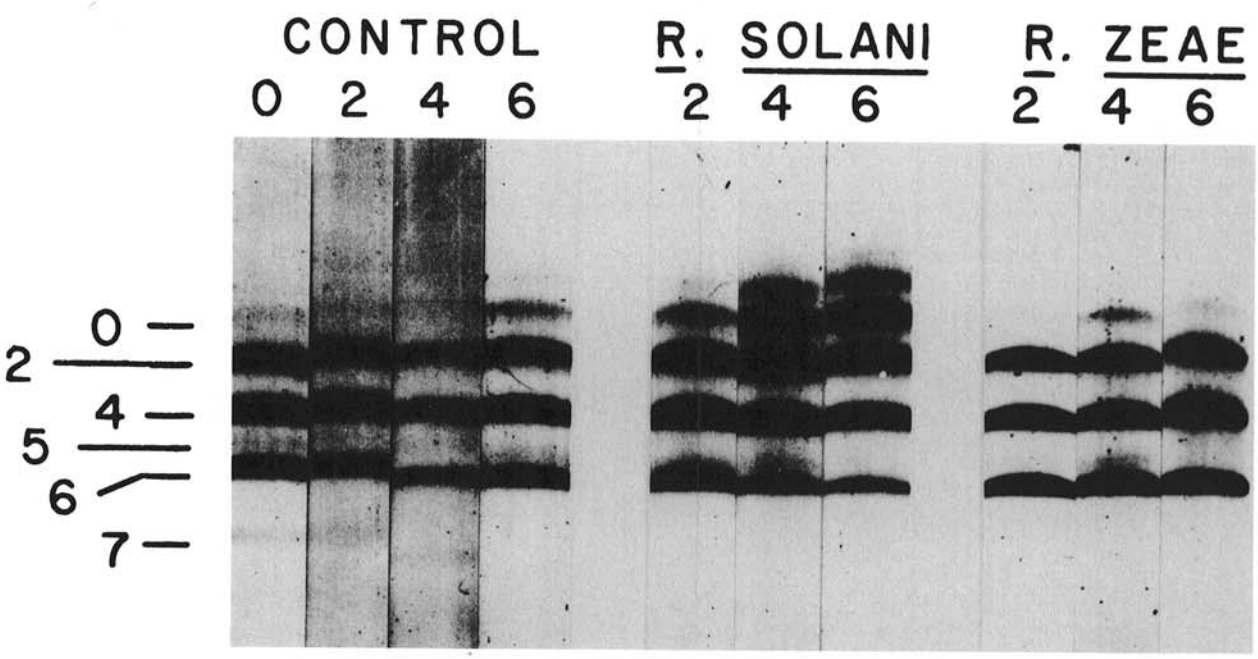

Fig. 4. Isolectin composition of peanut lectin incubated in the presence of *Rhizoctonia* spp. The numbers on the left mark the positions of the three major isolectins (pI 5.9, 6.2, and 6.5) and the three minor isolectins (pI 5.7, 6.3, and 6.7) (20). The number above each gel indicates the incubation time in days. Controls were uninoculated. The photos have been trimmed to remove portions of the gels that lacked protein bands.

PNL was substantially altered. This was unexpected. The recovery and isolectin composition of PNL were influenced by the fungus in vitro, and our extraction and analytical methods are sufficiently sensitive to detect these and other genetic and developmental alterations of PNL (20,21). *R. solani* produces extracellular proteolytic enzymes in diseased tissues (24), and colonization of peanut seeds by fungi significantly decreases the concentration of soluble proteins and modifies the electrophoretic behavior of seed polypeptides (5,6). Seed lectins, however, are very resistant to inactivation by proteases and other agents (4,14). Such inherent stability of PNL may explain its longevity in vivo. An alternative hypothesis, that PNL remains sequestered and inaccessible to the fungus, is less appealing. Legume seed lectins are stored in protein bodies (7,22). These structures, which are abundantly distributed in peanut cotyledons, are degraded upon germination and eventually disintegrate (1). The ready accessibility of PNL to simple aqueous buffers also argues against the hypothesis that PNL is sequestered in seedling cotyledons.

The pronounced time-dependent decrease in the recovery of soluble PNL from liquid cultures of *Rhizoctonia* sp., particularly those of *R. zeae*, is striking in comparison to the in vivo data. The dry weight of *R. zeae* hyphae from 6-day-old cultures, about 6 mg/ml, was not influenced by the presence of PNL (2 mg/ml). Thus, it is unlikely that 6 mg of hyphae simply bound and thereby rendered insoluble 2 mg of PNL. Although the rapid acidification of the medium by *R. zeae* could have denatured the lectin, PNL also disappeared from cultures of *R. solani*, the pH of which rose to near neutrality. An alternative hypothesis, that PNL was metabolized in liquid cultures of *Rhizoctonia* sp., thus warrants consideration.

Seed lectins may inhibit the growth of fungi, and there are suggestions that such lectins function to defend plants against fungal pathogens (2,3,18). Neither *R. solani* nor *R. zeae* was inhibited by physiological concentrations of PNL in the linear mycelial growth or dry weight increase assays. Although depletion of the lectin complicates the general interpretation of the dry weight data, *R. solani* grew normally for 4 days in the presence of relatively high concentrations of PNL (Fig. 2), and it did not degrade the lectin in vivo (Table 1). This suggests that PNL may not be an important factor in the interaction of peanut cotyledons with this pathogen.

LITERATURE CITED

1. Bagley, B. W., Cherry, J. H., Rollins, M. L., and Altschul, A. M. 1963. A study of protein bodies during germination of peanut (*Arachis hypogaea*) seed. Am. J. Bot. 50:523-532.
2. Barkai-Golan, R., Mirelman, D., and Sharon, N. 1978. Studies on growth inhibition by lectins of penicillia and aspergilli. Arch. Microbiol. 116:119-124.
3. Barraqueta-Egea, P., and Schauz, K. 1983. The influence of phytolectins on spore germination of *Tilletia caries*, *Puccinia graminis* and *Aspergillus flavus*. J. Plant Dis. Prot. 90:488-495.
4. Causse, H., and Rouge, P. 1983. Lectin release from imbibed soybean seed and its possible function. Pages 559-572 in: Lectins. Vol. III. T. C. BøgHansen and G. A. Spengler, eds. Walter de Gruyter & Co., Berlin.
5. Cherry, J. P., Beuchat, L. R., and Koehler, P. E. 1978. Soluble proteins and enzymes as indicators of change in peanuts infected with *Aspergillus flavus*. Agric. Food Chem. 26:242-245.
6. Cherry, J. P., Mayne, R. Y., and Ory, R. L. 1974. Proteins and enzymes from seeds of *Arachis hypogaea* L. IX. Electrophoretically detected changes in 15 peanut cultivars grown in different areas after inoculation with *Aspergillus parasiticus*. Physiol. Plant Pathol. 4:425-434.
7. Chrispeels, M. J. 1984. Biosynthesis, processing and transport of storage proteins and lectins in cotyledons of developing legume seeds. Philos. Trans. R. Soc. Lond. B Biol. Sci. 304:309-322.
8. Furuichi, N., Tomiyama, K., and Doke, N. 1980. The role of potato lectin in the binding of germ tubes of *Phytophthora infestans* to potato cell membrane. Physiol. Plant Pathol. 16:249-256.
9. Gamborg, O. L., Miller, R. A., and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158.
10. Garas, N. A., and Kuc, J. 1981. Potato lectin lyses zoospores of *Phytophthora infestans* and precipitates elicitors of terpenoid accumulation produced by the fungus. Physiol. Plant Pathol. 18:227-237.
11. Gibson, D. M., Stack, S., Krell, K., and House, J. 1982. A comparison of soybean agglutinin in cultivars resistant and susceptible to *Phytophthora megasperma* var. *sojae* (Race 1). Plant Physiol. 70:560-566.
12. Hapner, S. J., and Hapner, K. D. 1978. Rhodamine immunohistofluorescence applied to plant tissue. J. Histochem. Cytochem. 26:478-482.
13. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.
14. Liener, I. E. 1958. Inactivation studies on the soybean hemagglutinin. J. Biol. Chem. 233:401-405.
15. Liener, I. E. 1976. Phytohemagglutinins (phytolectins). Annu. Rev. Plant Physiol. 27:291-319.
16. Lotan, R., Skutelsky, E., Danon, D., and Sharon, N. 1975. The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). J. Biol. Chem. 250:8518-8523.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
18. Mirelman, D., Galun, E., Sharon, N., and Lotan, R. 1975. Inhibition of fungal growth by wheat germ agglutinin. Nature 256:414-416.
19. Mishkind, M., Keegstra, K., and Palevitz, B. A. 1980. Distribution of wheat germ agglutinin in young wheat plants. Plant Physiol. 66:950-955.
20. Pueppke, S. G. 1979. Distribution of lectins in the Jumbo Virginia and Spanish varieties of the peanut, *Arachis hypogaea* L. Plant Physiol. 64:575-580.
21. Pueppke, S. G. 1981. Multiple molecular forms of peanut lectin: Classification of isolectins and isolectin distribution among genotypes of the genus *Arachis*. Arch. Biochem. Biophys. 212:254-261.
22. Pusztai, A., Croy, R. R. D., Grant, G., and Stewart, J. C. 1983. Seed lectins: Distribution, location and biological role. Pages 53-82 in: Seed Proteins. J. Daussant, J. Mosse, and J. Vaughan, eds. Academic Press, London.
23. Sing, V. O., and Bartnicki-Garcia, S. 1975. Lysis of zoospores of *Phytophthora palmivora* induced by concanavalin A. Experientia 31:643-644.
24. VanEtten, H. D., and Bateman, D. F. 1965. Proteolytic activity in extracts of Rhizoctonia-infected bean hypocotyls. (Abstr.) Phytopathology 55:1285.