Interaction of *Uromyces phaseoli* and *Meloidogyne incognita* on Bean[1]

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Abstract: Uromyces phaseoli, the causal agent of bean rust, suppressed shoot and root growth of three bean cultivars, reducing root weight more than shoot weight. The greatest suppression of root weight was on the cultivar that appeared most susceptible by visual ratings of shoot symptoms. *Meloidogyne incognita* suppressed shoot and root growth of all test cultivars; root weight reductions differed among cultivars identical in susceptibility to this pathogen in root-gall rating tests. Infection of plants with both pathogens suppressed plant weights significantly more than did infection by either pathogen alone, evidencing an additive effect. *U. phaseoli* and *M. incognita* on the same plant influenced the reproduction of one another, presumably through effects on the host. Fungal uredia were reduced in size and sporulation capacity; *M. incognita* produced fewer root galls, and fewer eggs per egg mass. *Key Words:* bean rust, root knot, *Phaseolus vulgaris.*

Although phytoparasitic nematodes affect their hosts directly, their major importance to plant disease development may be as partners with fungi, bacteria, and viruses (2). Nematodes participate in disease complexes by (i) creating portals of entry for other pathogens; (ii) vectoring bacteria, virus particles, and fungal spores; (iii) modifying the rhizoshere of the host, facilitating growth of other pathogens; and (iv) inducing physiologic changes in the host that alter its susceptibility to attack by other pathogens (2).

In most reported plant disease complexes, the interacting pathogens affect the same host organ(s) (14). There is evidence, however, that root-infecting nematodes can alter the susceptibility of the host to infection by foliar pathogens. For example, infection by *Meloidogyne* spp. increased the susceptibility of tomato plants to *Corynebacterium michiganense* (9) and to *Alternaria solani* (1), and of cotton seedlings to *Alternaria tenuis* (3). *Vigna sinensis* plants infected by *M. incognita* were less susceptible to infection by cowpea mosaic virus than were control plants (11). Such effects are expectable, since nematode infection has been shown to induce systemic responses in host plants (10).

We examined the interaction between *Meloidogyne incognita* (Kofoid and White) Chitwood and the obligate foliar parasite, *Uromyces phaseoli* (Pers.) Wint., on *Phaseolus vulgaris* L. to determine (i) the effect of this interaction on host growth and symptomatology and (ii) the effects of each pathogen on the growth and reproduction of the other. *P. vulgaris* was chosen as the test host because it is an important source of protein in tropical areas (4). A limited genetic base and lack of resistance in most cultivars (7) render the species vulnerable to both *U. phaseoli* and *M. incognita.* Both pathogens are responsible for significant yield losses in susceptible cultivars (4,5).

MATERIALS AND METHODS

Host cultivation: Seeds of three cultivars of common bean—Pinto 111 (P), Rustproof Golden Wax (R), and Harvester (H)—were planted singly in 10-cm plastic pots containing an aerated steam-pasteurized 1:1:1 mixture of Hagerstown silty clay loam, peat, and perlite. The plants were grown under unmodified greenhouse conditions; liquid 20:20:20 NPK fertilizer (2.4 g per liter) was applied weekly to each pot.

At harvest, plants to be weighed were washed free of soil, divided into shoot and root portions, blotted dry, and immediately weighed. Preliminary tests showed that fresh weights of plant materials were highly correlated ($r = 0.999$) with weights after oven drying.

Fungus: Uromyces phaseoli var. *typica,* race 32, was cultured on primary leaves of *P. vulgaris.* When expanded 1/3 to 1/2, leaves were inoculated with suspensions of about 20,000 uredospores per ml in a solution of 125 mg/L of Ivory Snow surfactant (Proctor and Gamble Co., Cincinnati, Ohio)

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in distilled water. Two ml-aliquots of this suspension were atomized onto groups of five seedlings growing in flats. Inoculated plants were incubated in a darkened moist chamber for 24 h at 18–20 C, and then transferred to the greenhouse. Uredia began to sporulate about 10 to 14 d after inoculation, and uredospores were harvested once every 2 wk as follows: Leaves removed from plants were immediately cut into small sections and placed on a sieve with 150 μm openings. The sieve was shaken on a rotary shaker for 2 to 6 h, depending on the quantity of leaves. Uredospores were collected on paper and stored in glass vials at −12 C for no longer than 2 wk. The viability of uredospores to be used as inoculum was determined by the technique of Schein (15). Uredospore collections with less than 90% germination were discarded.

Numbers of uredia arising on infected leaves were measured as an indicator of vegetative growth of the fungus. Uredial diameters were used to estimate relative sporulation capacity per lesion. To ensure uniform distribution of *U. phaseoli* inoculum on target leaves, a quantitative applicator similar to that of Schein (16) was constructed. Leaves were inoculated by spraying aliquots of a suspension of 20,000 uredospores per ml of an aqueous solution of 125 mg/L onto the leaf surface from a distance of 30 cm in a single application lasting about 0.25 s at 25 psi. Inoculated plants were incubated in a darkened moist chamber at 18–20 C for 24 h and then transferred to the greenhouse.

In four experiments the response of bean foliage to *U. phaseoli* infection was determined by visual rating of uredial diameter using the 5-point scale of Davison and Vaughn (8): 1 = no infection; 2 = necrotic flecking; 3 = uredia present but less than 300μm in diameter; 4 = uredial diameter between 300 and 500 μm; 5 = uredial diameter greater than 500 μm. To facilitate observation of uredia free of interfering spores, a stream of pressurized air was played over leaves before scoring.

Nematodes: Meloidogyne incognita was maintained in the greenhouse on Bonny Best and Rutgers tomato plants. Eggs were extracted by the method of Hussey and Barker (13), suspended in distilled water, and diluted to 1,000 eggs per ml. Each pot was infested with 10 ml of this suspension pipetted into three holes in the soil around the seed or seedling within the pot. The inoculum level used in these tests was determined in preliminary studies to be the minimum concentration resulting in significant weight reductions in treated plants. Soil surrounding seeds was infested 48 h after planting. The response of host plants to *M. incognita* infection was measured by determining the number of root galls formed per gram of root tissue.

Host responses to single pathogens: Primary leaves of test plants were inoculated with *U. phaseoli* spore suspensions, or with suspending fluid alone (as a control), as described above. When active sporulation of uredia began, plants were harvested, and fresh weights of roots and shoots were determined. Fifteen plants of each cultivar per treatment were tested. The experiment was performed twice.

The number of spores produced per uredium was determined in order to measure the relationship between uredial size and sporulation capacity. Primary leaves of P, R, and H were inoculated with *U. phaseoli* or sprayed with suspending fluid alone, incubated as described above, and transferred to a vented 10 × 0.5 × 0.5 meter Plexiglas (Rohm and Haas Corporation, Bristol, Pa.) box to reduce air movement around developing uredia. Fifteen days after inoculation leaves bearing sporulating uredia were harvested, 5-cm^2 discs were cut from the centers of the leaves (one per leaf), numbers of uredia per disc were counted, and the number of spores present on each disc was determined by the method of Yarwood (21). Fifteen discs bearing uredia corresponding to each of ratings 3, 4, and 5, and containing between 50 and 100 uredia (8) were utilized. The experiment was conducted three times. Preliminary tests showed that discs cut from any of the test cultivars, and corresponding to the same rating score, did not differ significantly in numbers of spores per uredium.

Changes in shoot and root weights of plants infected by *M. incognita* were measured. Growth, inoculation, harvest, and weighing were conducted as described above. Distilled water served as a control treatment in these tests. Ten plants per cultivar per treatment were tested; the ex-

periment was conducted twice.

Pathogen interactions: Shoot and root weights of host plants were measured as responses to the following treatments: (i) suspending solutions without pathogens, (ii) *M. incognita* alone, (iii) *U. phaseoli* alone, and (iv) both pathogens. Inoculum concentrations and inoculation techniques were as described above. *M. incognita* was applied after seeding, and *U. phaseoli* was applied to partially expanded primary leaves.

To facilitate observation of the effects of the disease interaction on growth and reproduction of the pathogens, a series of three experiments was performed: (i) simultaneous inoculation with both pathogens to plants with partially expanded primary leaves; (ii) inoculation with *U. phaseoli,* followed 8 d later by inoculation with *M. incognita;* and (iii) inoculation with *M. incognita* 48 h after planting, followed 15 d later by inoculation with *U. phaseoli* to primary leaves. In preliminary studies, these intervals maximized host responses to infection by each pathogen. Plants were harvested 45 d after planting, divided, and weighed as described above. Ten plants per cultivar per treatment were tested; each experiment was performed twice.

Nematode-induced changes in the growth of *U. phaseoli* were measured (i) as changes in counts of uredia in central 5-cm^2 discs from infected leaves and (ii) as visual ratings of uredial diameters, which served as estimates of uredial sporulation capacity.

Fungus-induced changes in *M. incognita* growth and development were measured as changes in the number of root galls produced per gram of root tissue of plants receiving both pathogens. Changes in nematode reproduction were determined as follows: 10 egg masses from each of the 10 plants in each treatment were disinfested by soaking for 2 min in 0.53% NaOCl, rinsed four times with sterile distilled water, and incubated in sterile distilled water in darkness at 25 C in hatching chambers described by Hamlen et al. (12). Hatched larvae were counted after 21 d of immersion. The remaining matrix material was dissolved by soaking in 1.05% NaOCl for 2 h, and the unhatched eggs were counted. Observations were recorded as numbers of hatchlings and eggs per mass. Total egg count and percentage hatch per mass were calculated. Each experiment was repeated twice.

Analyses of variance were performed for each repetition of each experiment, then for pooled data using pooled variances. Duncan's modified (Bayesian) least-significant-difference was used to determine significant differences among treatment means, with k = 100 (P = 0.05). Linear correlation coefficients were calculated for uredial sporulation-host-response scores.

RESULTS AND DISCUSSION

Infection by *U. phaseoli* suppressed shoot fresh weight of P, R, and H (Table 1) by 17%, 41%, and 38%, respectively (compared with controls, as is assumed with later comparisons herein). Mean root weight reductions of these cultivars were 69%, 42%, and 52%, respectively. Shoot weight reduction was smallest and root weight reductions were greatest on the cultivar (P) rated most susceptible to *U. phaseoli* by visual scoring of shoot symptoms in preliminary tests. That the fungus had a greater effect on root weight than shoot weight in cultivars P and H is consistent with findings of So and Thrower (17) that *U. phaseoli* inhibits the translocation of photosynthates from infected leaves, resulting in nutrient deprivation of roots.

Uredospore production was correlated with uredial diameter (r = 0.998). Mean spore yield of No. 4 lesions (2,471 spores per lesion) was 39% smaller than that of larger

TABLE 1. Effects of *Uromyces phaseoli* infection on fresh weights of seedlings of three cultivars of *Phaseolus vulgaris,* measured 15 days after inoculations.*

Cultivar	Treatment	Shoot weight (g)†	Root weight (g)†
Pinto '111'	control	12.9 a	5.3 a
	infected	10.7 b	1.6 e
Rustproof Golden Wax	control	9.8 c	3.4 b
	infected	5.8 e	2.0 d
Harvester	control	7.9 d	2.6 c
	infected	4.9 f	1.2 f

*Values are means of 30 observations, pooled from two experiments.

†Means followed by the same letter are not significantly different according to Duncan's LSD test (k = 100 approximates P of 0.05).

No. 5 lesions (4,058 per lesion); the smallest uredia, No. 3, produced 75% fewer spores (1,016) than did No. 5 uredia; these differences were significant according to Duncan's LSD test with k = 100. Leaves with similar numbers of uredia (no significant differences) were tested, since Yarwood (21) found that sporulation capacity decreased with increasing lesion number.

Effects of M. incognita on plant weights: Nematode infection reduced shoot fresh weights of P, R, and H by 14%, 10%, and 13%, respectively (Table 2). Mean root fresh weights were reduced by 20%, 21%, and 10%, respectively. That shoot growth of infected plants was reduced was consistent with observations of Townsend and Ruehle on plants in the field (18).

The observed differential reductions in root weights among test cultivars was unexpected, since all showed identical susceptibility to *M. incognita* in preliminary tests, as measured by a semi-quantitative visual root rating scale similar to that of Winstead and Sasser (20), or by counts of galls per gram of root tissue. This suggests that tests currently used to measure resistance to root knot nematodes, which generally rely on gall counts or visual scores (20), may give incomplete information and may in fact fail to detect certain resistance or tolerance phenomena in "susceptible" bean lines.

Effects of disease interaction on plant weight: Infection by single pathogens suppressed shoot growth of all tested cultivars (Table 3.A.); weight was reduced by *U. phaseoli* by 7%, and by *M. incognita* by 9%. Infection by both pathogens suppressed shoot weights by 15%, suggesting that nematode and fungus act additively. Differences between cultivars were not significant. The mean shoot weight of plants receiving pathogens simultaneously was greater than the mean shoot weight of plants receiving either pathogen prior to the other (Table 3.B.). Differences between cultivars were not significant.

The smaller responses of plants to infection in these tests than in earlier experiments (Tables 1, 2) may be related to environmental variations; this series was performed during July and August, while the earlier tests were performed in April and May, suggesting effects of photoperiod and temperature on host-pathogen interaction.

Root weights of bean plants were reduced by individual pathogens (Table 3.A.) by 8% (*U. phaseoli*) and 16% (*M. incog-*

TABLE 2. Effect of an inoculum of 10,000 *Meloidogyne incognita* eggs fresh weights of three *Phaseolus vulgaris cultivars* harvested 40 days after inoculation.*

Cultivar	Treatment	Shoot weight (g)†	Root weight (g)†
Pinto '111'	control	63.4 a	12.0 b
	infected	55.4 b	9.6 d
Rustproof Golden Wax	control	44.5 e	11.3 bc
	infected	39.9 f	8.9 e
Harvester	control	53.1 c	12.9 a
	infected	45.9 d	11.6 b

*Values are means of 20 observations, pooled from two experiments.

†Means followed by the same letter are not significantly different according to Duncan's LSD test (k = 100 approximates P of 0.05).

TABLE 3. Effects of the interaction of *Uromyces phaseoli* and *Meloidogyne incognita* on shoot and root fresh weights of three *Phaseolus vulgaris* cultivars. A) Main effects of pathogens.* B) Effects of sequential pathogen application.†

Pathogen(s)‡	Application sequence§	Shoot weight (g)‖	Root weight (g)‖
A			
C	—	76.5 a	14.6 a
M	—	71.5 b	12.3 c
U	—	69.8 c	13.5 b
U, M	—	65.1 d	11.2 d
B			
U, M	S	73.6 a	—
U, M	M→U	69.9 b	—
U, M	U→M	68.7 c	—

*Values are means of 180 observations pooled from two experiments, and averaged among three cultivars.

†Values are means of 60 observations pooled from two experiments, and averaged among three cultivars.

‡Pathogens: U = fungus, M = nematode, U, M = both, C = control.

§Sequences: S = simultaneous application, U→M = fungus applied 8 days before nematode, M→U = nematode applied 15 days before fungus (see text for explanation of sequences).

‖Means followed by the same letter are not significantly different according to Duncan's LSD test (k = 111 approximates P of 0.05).

nita). Infection by both pathogens reduced root weights by 23%, again suggesting that the pathogens acted additively. No significant differences resulted from pathogen application sequence variation.

Effects of disease interaction on behavior of U. phaseoli: Nematode infection did not reduce the number of uredia observed on leaf discs, suggesting that germination and establishment of *U. phaseoli* was apparently not affected. Uredial sporulation could not be measured directly, because of spore loss resulting from air circulation over infected plants in the greenhouse. Nematode infection significantly reduced uredial diameters (Table 4), which reflected a lower sporulation capacity of uredia on leaves of nematode-infected plants. The mechanism by which *M. incognita* caused this response was not determined, but it may be related to the suppression of shoot growth caused by this pathogen. Others have shown that the lower weight of root knot-infected tomato plants is correlated with retarded photosynthetic rate (19). Suppressed host photosynthesis reduced sporulation of *U. phaseoli* on bean (6) and could be involved in the observed fungal response to nematode infection. Simultaneous application of pathogens resulted in greater lesion development than did either of the other application sequences (Table 4), suggesting that the sequence allowing maximum host growth also allowed maximum *U. phaseoli* sporulation. No significant differences were found among cultivars in these tests.

TABLE 4. Effects of disease interaction on development of *Uromyces phaseoli* on foliage, and on *Meloidogyne incognita* root gall counts on infected plants of three *Phaseolus vulgaris* cultivars.*

Pathogen treaments†	Pathogen sequences‡	Rust severity rating§ ‖	Number of nematode galls per gram of root tissue‖
U	—	4.11 a	92.15 a
M	—	—	
U, M	S	3.78 b	82.33 b
U, M	U→M	3.45 c	77.82 b
U, M	U→M	3.33 c	92.87 a

*Values are means of 60 observations pooled from two experiments, averaged among three cultivars.

†Pathogens: U = fungus, M = nematode, U, M = both.

‡Sequences: S = simultaneous application, U→M = fungus applied 8 days before nematode, M→U = nematode applied 15 days before fungus (see text for explanation of sequences).

§Ratings: 3 = uredial diameter < 300 μm, 4 = uredial diameter between 300 and 500 μm, 5 = uredial diameter > 500 μm.

‖Means followed by the same letter are not significantly different according to Duncan's LSD test (k = 100 approximates P of 0.05).

Effects of disease interaction on behavior of M. incognita: The mean number of root galls per gram of root was reduced by *U. phaseoli* infection when both pathogens were applied simultaneously and when the fungus was applied first (Table 4). Gall counts were not reduced when nematodes were applied first. The time required for fungus-induced effects on root growth (which reached maximum levels 8 d after infection, in preliminary tests) and attractiveness to *M. incognita* was apparently greater than that required for hatch of, infection by, and establishment of *M. incognita* juveniles.

There were 62% fewer *M. incognita* eggs per egg mass in rusted plants than in nonrusted plants (788 per mass and 2,048 per mass, respectively). This response may be related to suppression of translocation of photosynthates to roots of *U. phaseoli*-infected plants (17), resulting in reduced root growth. Such retarded root growth apparently resulted in less egg production by *M. incognita* in these tests.

While fungus infection reduced the numbers of eggs produced per egg mass below those of unrusted plants, the percentage hatch (60% and 58%, respectively) was not reduced. Egg production by *M. incognita* females was affected by stress, while egg viability was held constant under experimental conditions.

There is a disease interaction between the root knot nematode *M. incognita* and the foliar parasite *U. phaseoli* on their common host. The pathogens elicit an additive effect on the host plant and interact with each other through their influence on the host plant. This interaction may be due to changes in the nutritional status of the infected host and/or some more direct mechanisms, e.g., antibiosis.

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