Interaction of *Phoma terrestris* and Soil Moisture Level on Yield of Two Onion Cultivars Differentially Susceptible to Pink Root

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ABSTRACT

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The effect of *Phoma terrestris* on yield of the onion (*Allium cepa*) cultivars Kodiak (tolerant) and Paragon (susceptible), using onions grown outdoors in flats, was investigated. Similar experiments in the greenhouse included a third factor, drought stress. When onions were grown in organic field soil, the addition of *P. terrestris* increased average levels of pink root at harvest from 40% (control) to 52% (amended) in the greenhouse. However, yield did not decrease. When onions were grown in greenhouse mix, the addition of *P. terrestris* increased average levels of pink root at harvest from 0% (control) to 71% (amended) in the flats and from 0% (control) to 62% (amended) in the greenhouse. The yield of onions in the greenhouse mix was decreased in the amended treatments in both cases, but in the flats, the yield of Kodiak was decreased more than that of Paragon. In amended treatments in all three experiments, Paragon had more pink roots at harvest than Kodiak. However, the yield of Paragon was higher than that of Kodiak in two of the three experiments, and their yields were equal in one experiment. Drought stress decreased the yield in both greenhouse experiments, but no interaction between drought stress and inoculum levels occurred. These studies indicate that onion yield can be decreased at high levels of *P. terrestris*.

Phoma terrestris E. M. Hans., a fungus that causes pink root of onion (*Allium cepa* L.) and other plant species, is common in many onion-growing regions in the United States. The fungus invades the roots, causing them to turn pink, shrivel, and die, but it does not invade the basal plate (stem) or the fleshy scales of the bulb (3,5).

Yield losses were attributed to pink root as early as 1929 (3) in mineral soils in California. However, in many studies (3,7,8), the effects of *P. terrestris* were not separated from the effects of other soilborne pathogens. Pink root may not greatly reduce yield (7), because new roots initiated from the basal plate may allow onions to compensate for the loss of diseased roots. Since *P. terrestris* is a soilborne pathogen, the soil type and climate may significantly affect its survival as both a saprophyte and a parasite. Results from studies on the mineral soils in warm climates may not be relevant to the organic soils and temperate climates of New York. Amendment of organic soil with *P. terrestris* does not decrease bulb weight per hectare at harvest as compared to unamended but naturally infested field soil (6), but yield in the absence of pink root (noninfested soil) was not determined in this study. Pink root symptoms developed in onions from both amended and control soils because background levels of *P. terrestris* were present. In New York it is common to find the root systems of most onion plants infected with pink root at harvest, but yield losses due to pink root have not been quantified.

Efforts to control pink root of onion have centered on the use of resistant cultivars. Resistant short-day cultivars have been available since the 1950s in Texas (9), but long-day cultivars available for commercial use in New York are susceptible to pink root. Resistance has often been assessed at the seedling stage, as in the Wisconsin method, in which seedlings are rated for pink root approximately 30 days after seeds have been planted (2). This method is rapid and convenient, but it is merely a preliminary indication of resistance. When many onion cultivars and breeding lines were grown in the field in New York, the percentage of pink roots was often not correlated with yields (*unpublished*).

Since the primary effect of pink root is death of roots, reduced water uptake could be an important mechanism whereby yield is reduced. If this were the case, irrigation of dry soils might provide a way to reduce yield losses.

In the present study, onions were grown from seed to harvest in order to compare yield at high disease levels with yield at low or zero disease levels, to determine whether *P. terrestris* causes a loss in yield of onions under New York growing conditions. Two cultivars were used to determine whether a tolerant cultivar would exhibit less reduction in yield than a susceptible cultivar. In order to assess the effect of irrigation, the yield of drought-stressed plants was compared to that of adequately watered plants.

MATERIALS AND METHODS

Outdoor flats experiments. Experiments were arranged in a 2 × 2 factorial design to test the effects of *P. terrestris* and cultivar on onion growth. Both cultivars are yellow globe, storage-type onions, grown commercially on organic soils in New York. Kodiak, obtained from Harris Moran (Rochester, NY), is reported to be pink root-resistant, and Paragon, from Sunseeds (Eden Prairie, MN), is susceptible to pink root. These cultivars were also chosen because they mature at approximately the same time.

Onions were seeded in the greenhouse and transplanted into wooden flats, 0.6 × 0.6 × 0.3 m, with wire mesh bottoms. These flats were placed outdoors to simulate the conditions in a commercial onion field and to allow manipulation of disease levels. Rows of plants were 10 cm apart, with plants 5 cm apart within the rows, for a total of 62 onions per flat. There were 6 replications and 4 treatments, for a total of 24 flats, arranged in a completely randomized design.

To obtain inoculum for amending the growth media, *P. terrestris* was grown in potato-dextrose broth at room temperature for 2 wk. Broth was strained through cheesecloth, and the mycelium collected was added to distilled water and blended briefly at high speed. The same quantity of inoculum was added to each amended flat and was thoroughly mixed with soil or greenhouse mix of each flat on the day that onions were transplanted.

In 1988, the growth medium was a 50:50 mixture of organic soil and perlite. The organic soil had recently been drained and cleared but had never been in agricultural production. Onions were seeded in the greenhouse on 20 March. Seedlings with three true leaves were trimmed to a height of 6 cm and transplanted on 30 April. Plants were harvested on 21 August. In 1989, the growth medium was a 50:50 mixture of peat moss and perlite. Onion seeds were planted in the greenhouse on 12 April. Seedlings with two true leaves were trimmed to 6 cm and transplanted on 13 May. Onions

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were harvested on 7 September.

All plants in the flats were harvested and counted when tops fell and began to turn brown. Roots and bulbs were weighed fresh at harvest. Leaves were oven-dried at 45 C and weighed. Percentage of pink root was assessed with a pretransformed rating scale (see greenhouse experiments section, below). Ten bulbs in 1988 and five bulbs in 1989 from each flat were rated for percentage of pink root.

The *P. terrestris* culture utilized was isolated by N. Shishkoff from an onion grown on a commercial farm in New York. Pathogenicity of this isolate was confirmed by growing onion seedlings in soil and greenhouse mix amended with mycelium of the pathogen. After 28 days, all seedlings in the amended soil showed symptoms of pink root, but no symptoms were visible on seedlings in the unamended control group.

Greenhouse experiments. A similar experimental design was used in the greenhouse, except that a third factor, watering regime, was included, resulting in a 2 × 2 × 2 factorial. Main-effect comparisons were amendment with *P. terrestris* vs. no amendment, cultivars Kodiak vs. Paragon, and adequate soil moisture vs. drought stress. All two-way interactions also were tested.

In 1988, seedlings were transplanted at the two-leaf stage into the same organic soil used in the flats experiments, but without perlite. Each treatment consisted of four onions planted into a 20-cm clay pot and was replicated eight times. In 1989, seedlings were trimmed to 4 cm tall at the two-leaf stage and then transplanted into a 1:1 peat moss–vermiculite mix. Each treatment consisted of five onions planted into a 3-L plastic pot and was replicated 10 times. In both years, pots were arranged in a completely randomized design.

In 1988, onions were planted on 17 March, transplanted on 10 April, drought-stressed during 1–13 July and 22 July–1 August, and harvested on 4 August. In 1989, onions were planted on 15 February; transplanted on 10 March; drought-stressed during 3–17 June, 18 June–1 July, and 2–15 July; and harvested on 17 July.

Drought stress, which consisted of withholding water from "dry" pots while watering "moist" pots daily, was imposed after the onions started bulbing. The water was withheld for approximately 2 wk, after which all pots were thoroughly watered. There were two drought-stress periods in 1988 and three in 1989.

In an attempt to confirm that growth for both cultivars was the same at the time drought stress was begun, green leaves were counted and the length of each leaf was measured on 26 May 1988. Four parameters were measured: length of longest leaf, length of fourth living leaf, number of living leaves, and total length of all leaves.

In order to quantify the differences between the moist and dry treatments, soil and pots were weighed before the transplanting of onions and again at the end of drought-stress periods, just prior to the watering of the plants. Differences in weight were assumed to be due to differences in the moisture content of the growth medium.

To directly measure the effect of drought on the plants, the relative water content of the leaves was measured on 13 July 1988, at the end of the first drought-stress period. Pieces of leaf, 2 cm long, were cut in thirds longitudinally and weighed to obtain the fresh weight (*fw*). Leaf pieces were then floated on distilled water and allowed to absorb water until their weights stabilized in order to obtain turgid weight (*tw*). Leaf pieces were then oven-dried at 45 C to obtain dry weight (*dw*). Relative water content (RWC) is given by the following relationship: $RWC = 100(fw - dw)/(tw - dw)$.

Bulbs, green leaves, and roots were separated at harvest. Bulbs and roots were weighed fresh. Roots were placed in an ice chest immediately after collection to avoid water loss. Fresh weights of leaves were obtained in 1988, whereas dry weights were obtained in 1989. The percentage of pink discoloration on each root system was assessed with the following pretransformed rating scale: 0 = no pink roots, 1 = 2.5% pink root tissue, 2 = 10%, 3 = 21%, 4 = 35%, 5 = 50%, 6 = 65%, 7 = 79%, 8 = 90%, 9 = 97.5%, and 10 = 100%. Data were back-transformed to obtain the percentage of pink roots, and these percentages were analyzed using analysis of variance with orthogonal contrasts. A regression line was fitted to the yield data if the main effect indicated significant differences in yield between amended and control treatments.

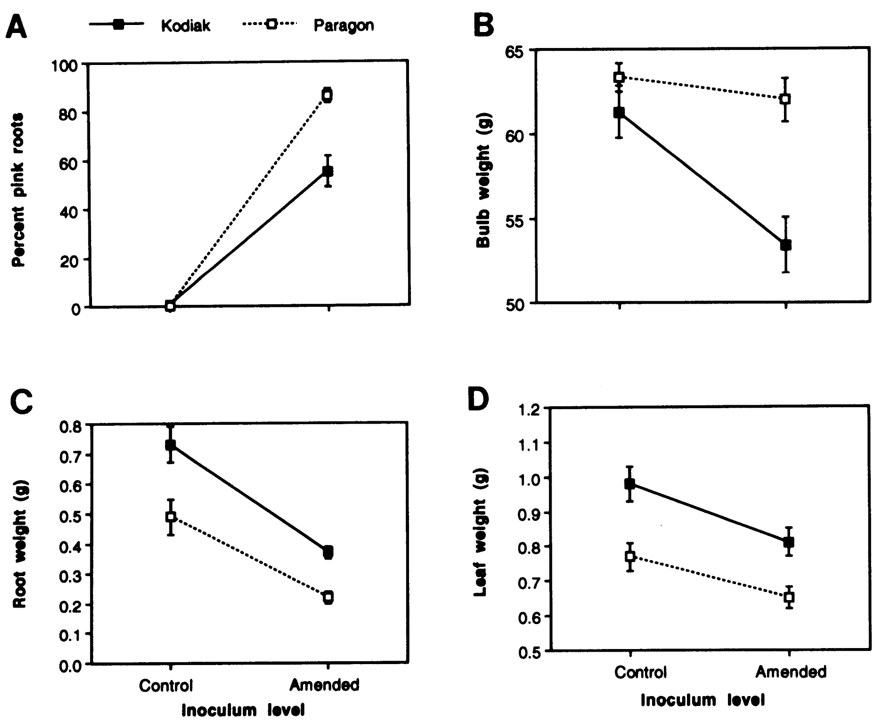

Fig. 1. Effect of inoculum amendment on **(A)** pink root severity, **(B)** bulb weight, **(C)** root weight, and **(D)** leaf weight in the outdoor flats experiment. Vertical bars represent standard errors.

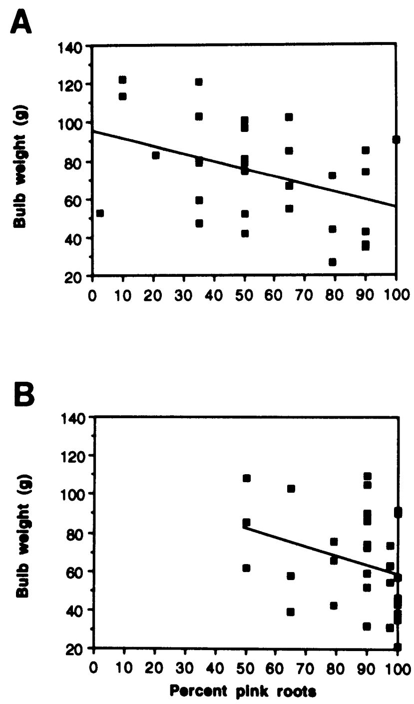

Fig. 2. Relationship between bulb weight and pink root severity for cultivars **(A)** Kodiak (the line is $Y = 95.8 - .4X$, with $R^2 = 0.173$) and **(B)** Paragon (the line is $Y = 106.4 - .5X$, with $R^2 = 0.094$) in the outdoor flats experiment.

Table 1. *F* values for bulb weight, percentage of pink roots, leaf weight, and root weight in the 1988 and 1989 greenhouse experiments[a]

	1988				1989			
Contrasts	Bulb weight	Percentage of pink roots	Leaf weight	Root weight	Bulb weight	Percentage of pink roots	Leaf weight	Root weight
Main effects								
1. Amended × control soil	0.9	10.3*	0.2	0.3	14.8*	768.3*	9.7*	52.6*
2. Moist × dry soil	14.9*	0.1	2.7	4.3*	10.7*	1.3	8.8*	0.4
3. Paragon × Kodiak[b]	0.2	3.0	3.8	8.6*	5.1*	3.2	2.9	7.4*
Interactions								
1 × 2	0.7	0.1	0.8	1.7	1.6	1.3	3.6*	0.2
1 × 3	0.6	2.9*	4.0*	1.2	0.6	3.2*	0.1	2.4
2 × 3	0.2	1.3	1.3	0.1	3.7*	0.1	2.5	0.0
Overall	2.6*	2.5*	2.3*	2.3*	5.2*	111.1*	4.0*	9.1*

[a]Contrasts significant at the 5% level (main effects) or the 10% level (interactions) are followed by an asterisk.
[b]Susceptible (Paragon) × resistant (Kodiak) cultivar.

RESULTS

Outdoor flats experiments. The addition of inoculum to artificial mix in the outdoor flats significantly increased pink root at harvest from 0% (control) to 71% (amended) (Fig. 1A). Fresh weight of bulbs was affected by amendment with *P. terrestris* and by cultivar, and the interaction was significant (Fig. 1B). Fresh weight of roots and dry weight of leaves were decreased in the presence of *P. terrestris* and were lower in Paragon than Kodiak, but there was no interaction (Fig. 1C and D).

The weight of individual bulbs from the amended flats only was plotted against the percentage of pink root of each bulb (Fig. 2). In both cultivars, bulb weight decreased as pink root increased. The regression line was significant at $P = 0.05$ for Kodiak and $P = 0.10$ for Paragon.

Greenhouse experiments, 1988. At the conclusion of the first drought-stress period, the average weight of soil (± standard error) was 2,116 (± 34) g in the moist treatments and 1,001 (±24) g in dry treatments, which was a significant difference at $P < 0.01$. The RWC of leaves of plants in moist soils (0.774 ± 0.014) was significantly different from the RWC of leaves from plants in dry soils (0.682 ± 0.009) at $P < 0.01$. At the end of both drought-stress periods, soil in the dry treatments was dry to the touch and appeared to be too dry for optimal plant growth.

Growth, as measured before drought stress and analyzed with ANOVA, did not differ among the eight treatments at $P = 0.05$ ($F = 2.20$). Growth parameters measured were length of longest leaf ($F = 1.33$), length of fourth living leaf ($F = 0.68$), number of living leaves ($F = 1.45$), and total length of all leaves ($F = 0.63$).

Results from the greenhouse experiments with organic soil (1988) are summarized in Tables 1 and 2. The addition of inoculum increased the average percent pink root at harvest from 40% (control) to 52% (amended). The amendment vs. cultivar interaction was significant, indicating that, when grown in amended soil, pink root severity was increased more in Paragon than in Kodiak.

Table 2. Effect of soil moisture level and inoculum on weight of bulbs, leaves, and roots and percentage of pink roots for resistant (Kodiak) and susceptible (Paragon) onion cultivars in 1988 greenhouse experiment

		Weight (g)						Pink roots (%)	
		Bulb		Leaf		Root			
Soil moisture level	Cultivar	A[a]	U	A	U	A	U	A	U
Moist	Kodiak	51.4	47.5	5.9	4.9	2.4	2.3	43.0	37.0
	Paragon	45.5	48.9	2.8	5.1	1.2	1.7	60.0	41.0
Dry	Kodiak	41.2	36.7	4.3	4.0	3.2	2.4	48.0	43.0
	Paragon	40.8	36.7	3.8	3.7	2.2	2.0	55.0	40.0

[a]A = soil amended with *Phoma terrestris* ; U = unamended (control) soil.

Addition of *P. terrestris* to the organic soil did not affect bulb weight. Drought stress was the only factor that decreased bulb weight. There was no disease vs. soil interaction, which indicates that pink root did not affect yield differently in moist, as opposed to dry, soil. There was a slight increase in pink root severity due to the amendment of soil with *P. terrestris*, but this did not affect bulb, leaf, or root weight.

Greenhouse experiments, 1989. After drought-stress cycles, the weight of the pots (including pots, plants, greenhouse mix, and water held in the growth medium) was significantly higher in moist than in dry treatments. Average weights (± standard error) in moist vs. dry treatments was 4,577 (± 56) g vs. 2,349 (± 44) g on 17 June; 4,136 (±110) vs. 2,542 (± 83) on 1 July; and 4,513 (±44) vs. 2,723 (± 76) on 15 July. The greenhouse mix in the dry treatments was dry to the touch and appeared to be too dry for optimal plant growth.

Results from greenhouse experiments using peat-based growth medium (1989) are summarized in Table 1 and Figure 3. There was an average of 62% pink root in the onions grown in the amended greenhouse mix, compared with none in those grown in unamended mix. Although pink root in amended treatments was not much higher than the 1988 level of 52%, the difference between amended and control soils was much greater. Disease vs. cultivar interaction was significant, since Kodiak had fewer pink roots than Paragon in the amended treatments.

All three factors (cultivar, inoculum level, and watering regime) influenced bulb weight. Cultivar vs. soil interaction was significant, which indicates that when both cultivars were under drought stress, the yield of Paragon decreased more than that of Kodiak.

The dry weight of leaves was decreased in amended treatments and in dry soils. When plants were drought-stressed, leaf weights were the same in amended and control treatments. When plants were given adequate moisture, leaf weights from amended treatments were lower than leaf weights from controls. The average weight of roots was lower in amended than in control treatments and was lower in Paragon than in Kodiak.

To determine whether a linear relationship existed between disease severity and yield, the average weight of all five bulbs in each pot was plotted against the average percentage of pink roots of all five plants in the pot (Fig. 4). For Paragon, the regression line was significant at $P = 0.05$. The relationship for Kodiak, which had been expected to be pink root–resistant, was not linear. In this experiment, yield of the resistant Kodiak was not affected by pink root, but yield of the susceptible Paragon was affected.

DISCUSSION

Although addition of inoculum increased the pink root level by a small amount in organic soil, it did not cause a measurable difference in onion yield, because roots in the unamended treatments were also affected by pink root. This soil was used as collected from the

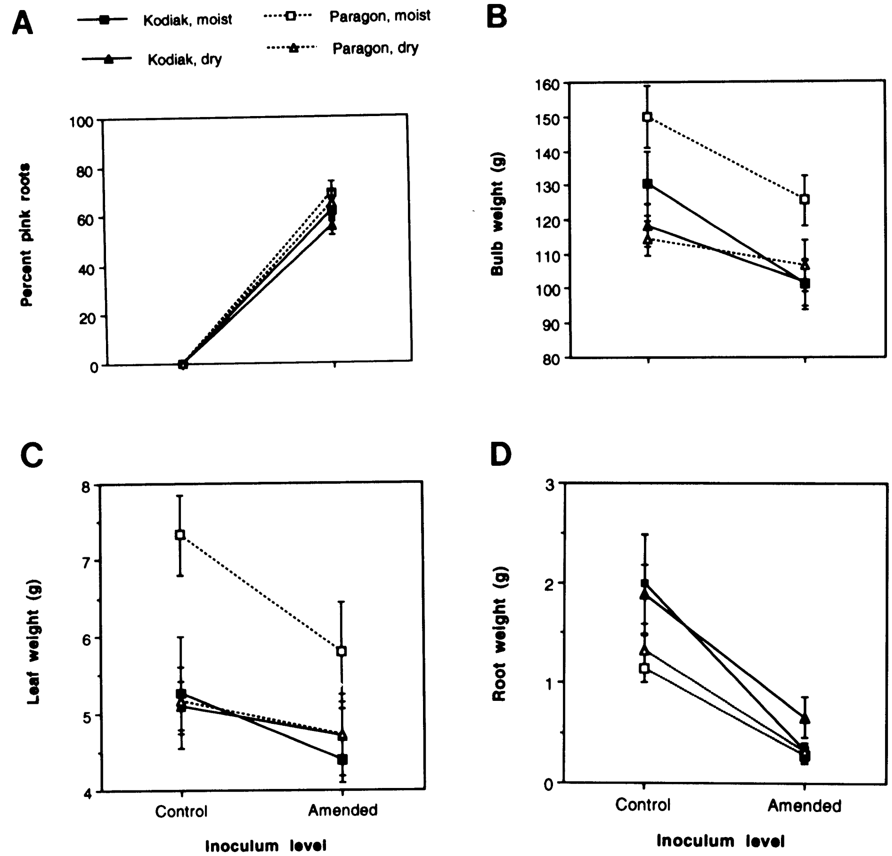

Fig. 3. Effect of inoculum amendment on **(A)** pink root severity, **(B)** bulb weight, **(C)** leaf weight, and **(D)** root weight in the 1989 greenhouse experiments. Vertical bars represent standard errors.

field, without sterilization, because it is the type of soil on which onions are grown commercially. Since the soil had never been in agricultural production, it was presumed to contain no *P. terrestris*. However, it may have been naturally infested even before crops had been grown on it, because *P. terrestris* is able to grow as a saprophyte or as a parasite on a wide variety of plants (1,3,5,10,11,12).

In 1989, in contrast, the growing medium was based on peat moss, so it was not naturally infested with *P. terrestris* and the other microorganisms found in organic soil. Amendment of this artificial mix with *P. terrestris* caused a large increase in pink root and a decrease in yield. Hess (4) found that *P. terrestris* causes a greater yield reduction in autoclaved than in nonautoclaved soil. These results could be due in part to effects of soil organisms on the growth of *P. terrestris*. Addition of an organism to soil is easy, but ensuring that it becomes established in the soil environment can be difficult. Establishment of *P. terrestris* is likely to be more difficult in natural soil with a complex microbial community than in artificial mix.

It was initially postulated that the effects of pink root could be ameliorated by supplying onion plants with sufficient moisture to compensate for lost potential for water and nutrient uptake due to diseased roots. This was not supported, since the amendment vs. watering regime interaction was not significant in either year. Optimizing soil moisture increased yields, but this occurred equally in both amended and unamended treatments.

Kodiak is reported to be resistant, and Paragon susceptible, to pink root. This is supported by the observation that Paragon consistently had more pink roots than Kodiak. Resistant cultivars often have a more extensive root system than susceptible cultivars (7), and the weights of Kodiak's roots were consistently greater than those of Paragon's.

One often assumes that all diseases cause yield losses, but there was no measurable yield loss in 1988, when pink root symptoms were 12% higher in the amended treatments than in the controls. In 1989, when differences in pink root symptoms were 62 and 71%, there was a measurable decrease in yield due to *P. terrestris*. Onion plants in commercial fields in New York may have over 90% pink root near harvest. Therefore, it is likely that some growers experience yield reductions due to the pink root disease.

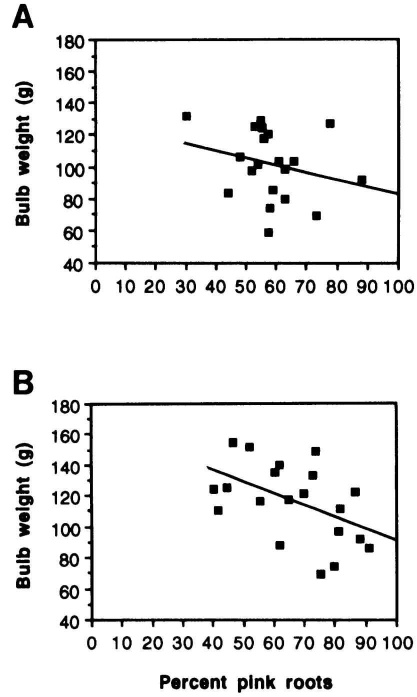

Fig. 4. Relationships between bulb weight and pink root severity for the cultivars **(A)** Kodiak (the line is $Y = 128.3 - 0.5X$, with $R^2 = 0.067$) and **(B)** Paragon (the line is $Y = 167.6 - 0.8X$, with $R^2 = 0.246$) in the 1989 greenhouse experiments.

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