Effects of Nitrogen Fertilization on Bacterial Soft Rot in Two Broccoli Cultivars, One Resistant and One Susceptible to the Disease

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ABSTRACT

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Sidedress applications of ammonium nitrate increased the incidence and severity of bacterial soft rot (caused by *Pseudomonas marginalis*) in a susceptible broccoli cultivar, Premium Crop, but had no effect on a resistant one, Shogun. Plots received N in amounts ranging from 19 to 152 kg/ha and were sprinkler-irrigated two to five times a day during head maturation to create conditions conducive to the disease. The length of time necessary for heads to reach harvest maturity decreased with increased N. Thus, in simulated once-over harvests of Shogun, a large range in head size (6–22 cm in diameter) resulted in apparent increases in both disease incidence and severity as the N rate increased, since these disease indices were positively correlated with head size. Among heads of similar size (10–16 cm in diameter) in all treatments, there were no significant effects of N on disease incidence or severity. In subsequent experiments involving multiple harvests of heads of similar size, disease incidence and severity in Shogun were not significantly affected by the N rate, but in Premium Crop they were significantly increased as the total N applied increased from 38 to 152 kg/ha. The marketable yield of Premium Crop decreased and that of Shogun increased with increased N.

Bacterial soft rot (head rot) of broccoli (*Brassica oleracea* L. var. *italica* Plenck) commonly plagues commercial plantings when head maturation coincides with periods of prolonged rainy, wet weather (2,3,8,14). The disease is caused by a complex of bacterial pathogens, of which *Pseudomonas marginalis* pv. *marginalis* (Brown) Stevens, *P. fluorescens* (Trevisan) Migula biovar II (Palleroni), and *P. fluorescens* biovar IV are considered the most virulent (8,14). *Erwinia carotovora* (Jones) Bergey et al, *P. fluorescens* biovar V, and *P. viridiflava* (Burkholder) Dowson have also been implicated (8), although infection of unwounded heads requires another bacterium that produces viscosin (8,9). This biosurfactant alters the membrane permeability of the waxy tissues of broccoli, resulting in increased solute leakage and an attack by soft-rot pathogens (9,11). Crop losses of 30–100% due to soft rot have been reported (8), and postharvest losses in storage and transit are common (1,4,13).

Control of the disease with commonly used fungicides and bactericides has proved largely unsuccessful (2; P. D. Hildebrand, *personal communication*). Control in Tennessee has focused on the use of cultivars with resistance to the disease (3) and certain management practices. Broccoli yields generally respond to fertilization with nitrogen at high rates (5,6,10,12), and one management practice of concern is the use of high rates of nitrogen to maximize yield. The objectives of this study were, therefore, to determine the effects of nitrogen fertilization on bacterial soft rot of broccoli and the concomitant effects on head quality and yield.

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MATERIALS AND METHODS

Field experiments. Plots were located at the West Tennessee Experiment Station in Jackson, on a Lexington silt loam (fine silty, mixed, thermic, typic Paleudalf). The soil contained phosphorus at 45–72 kg/ha, potassium at 224–247 kg/ha, calcium in excess of 1,120 kg/ha, magnesium in excess of 72 kg/ha, and 1.4–1.8% organic matter; a 1:1 mixture of soil and water had a pH of 6.5–6.8. Broccoli was seeded in the greenhouse in a commercial peat-vermiculite-perlite potting mix (Pro-Mix BX, Premier Brands, Stamford, CT) in 128-cell Todd planter flats (Speedling, Sun City, FL). The seedlings were fertilized every 7–10 days with a soluble 20-20-20 fertilizer and acclimatized in cold frames prior to transplanting. They were transplanted by hand in rows of 20–25 plants on raised beds 0.95 m apart, with the plants 30 cm apart within each row.

In the spring of 1989, fertilizer treatments were applied to single-row plots of Shogun, a broccoli cultivar with resistance to bacterial soft rot (3), beginning on 15 March, 14 days after the transplants were set. One to four sidedresses of ammonium nitrate fertilizer (34-0-0), providing N at 19 or 38 kg/ha, were applied by hand to the soil surface along rows. The total amounts of N provided, in applications made at 2-wk intervals, were 19, 38, 76, 114, and 152 kg/ha. The treatments were replicated four times in a randomized complete block design.

In the fall of 1989 and spring of 1990, the main plot treatments were one to four sidedresses of ammonium nitrate, providing N at 38 kg/ha, applied at 10- to 17-day intervals, so that the total amounts of N applied were 38, 76, 114, and 152 kg/ha. Each plot was split into single-row subplots of either Shogun or Premium Crop, a cultivar highly susceptible to soft rot (3). Seedling production and transplanting were as described above, except that Premium Crop (a short-season cultivar) was seeded and subsequently transplanted 1–2 wk after Shogun, to ensure near-simultaneous maturity of both cultivars. The treatments were replicated four times.

In all experiments, two to four rows of a cultivar susceptible to soft rot, generally Packman or Green Comet, were planted along the borders of each test to minimize edge effects. Standard management practices for insect and weed control were used. Chemicals were not applied during head maturation, to avoid possible injury to the developing florets. Plots were sprinkler-irrigated three to five times daily, for 5–10 min each time, from the appearance of the first heads 2 cm in diameter (the "button stage") through the final harvest.

Harvest data. In the spring of 1989, heads were harvested without regard for differences in head size due to treatment. The first 10 plants in each row were harvested on 26 May, when numerous mature heads were noted in plots receiving N at the highest rate of application. The next eight plants in each row were harvested 5 days later. These two cuttings simulated once-over harvests 86 and 91 days after transplanting, respectively. In the fall of 1989 and spring of 1990, the crops were harvested several times. Mature heads (10–16 cm in diameter) were harvested every 2–4 days over a 2-wk period as they reached harvest maturity. Only terminal heads were harvested in all experiments.

Each head was trimmed to a length of 15 cm and weighed, its diameter was recorded, and it was examined for the presence of bacterial soft rot. Disease incidence and severity were recorded. Disease severity was rated on a 0–5 scale, in which 0 = no disease, 1 = 1% of the surface area of the head soft-rotted, 2 = 10%, 3 = 30%, 4 = 60%, and 5 = 100% of the head surface rotted (4). Heads exhibiting small areas of discolored or water-soaked florets with the characteristic scent of bacterial soft rot but without any apparent softening of floret tissues were presumed to be infected and were given a rating of 0.5. Only heads without any evidence of disease, i.e., those with a rating of 0, were considered marketable. Total yield estimates were based on a harvestable population of 31,500 plants per hectare (35,000 plants per hectare minus 10% for offtypes and stand loss).

An analysis of variance was conducted for each experiment with the Statistical Analysis System (SAS Institute, Cary, NC). Data on disease incidence, expressed as percentages, were arcsine-transformed. Regression analysis was used to determine the relationship between N rates and disease indices or yield estimates. Tests for both linear and quadratic fits were conducted. Data from the experiments in the fall of 1989 and spring of 1990 were combined. Each cultivar's response to the N rate was analyzed separately.

Inoculation of plots. Natural inoculum was supplemented with that from inoculated plants in the border rows. These plants were inoculated with strains tentatively identified as *P. marginalis* or as *E. carotovora*. The cultures were grown in Difco nutrient broth amended with glucose (2.5 g/L) or in neutralized sucrose-broccoli broth as previously described (3). Inflorescences were excised with a horizontal cut, and small depressions, or wells, 1–2 cm deep, were made in the freshly cut ends of the stems. Broth cultures were diluted with distilled water, and 2 ml of diluted inoculum, containing about 1×10^7 cfu/ml, was pipetted into the freshly made wells. Wells continued to collect water from frequent irrigation, and soon the ends of inoculated stems were severely rotted, ensuring abundant inoculum for spread by insects and rain- and irrigation-generated aerosols (7).

RESULTS

In the once-over harvests in the spring of 1989, differences in plant growth due to nitrogen treatment affected both disease incidence and disease severity ratings. As was previously observed by Hipp (10), plant maturation was accelerated with increasing rates of N. At the time of the first harvest, on 26 May, plants receiving N at the lowest rate (19 kg/ha) were obviously stunted and had slightly chlorotic foliage. These plants had small heads and an uneven appearance due to unequal growth of raceme peduncles. Plants receiving N at the highest rate (152 kg/ha) were significantly taller than those in most other treatments and had dark, blue-green foliage with significantly larger, domed heads. The head diameter ranged from 6 to 22 cm and was linearly correlated with soft rot incidence (Fig. 1) and severity ($r^2 = 0.69$ and $r^2 = 0.61$, respectively; $P = 0.001$). In plot means in this experiment calculated from data on all the heads harvested (6–22 cm in diameter in once-over harvests), soft rot incidence and severity increased with the N rate ($r^2 = 0.30$ and $r^2 = 0.40$, respectively; $P = 0.01$) (Fig. 2). However, in plot means calculated only from data on harvested heads 10–16 cm in diameter (about 75% of the total harvest, or 46–61 heads per treatment), the N rate did not affect disease incidence ($r^2 = 0.004$; $P = 0.78$) or severity ($r^2 = 0.07$; $P = 0.26$) (Fig. 2). Head diameter, weight, and quality and marketable yield also increased with N.

In the experiments conducted in the fall of 1989 and spring of 1990, the multiple harvesting of heads 10–16 cm in diameter minimized the effects of head diameter on the disease indices. The incidence of bacterial soft rot increased with the N rate in Premium Crop ($r^2 = 0.27$; $P = 0.01$) but not in Shogun ($r^2 < 0.001$; $P = 0.99$) (Fig. 3A). Similarly, disease severity increased with the N rate in Premium Crop ($r^2 = 0.29$; $P = 0.001$), but these variables were unrelated in Shogun ($r^2 = 0.01$; $P = 0.62$) (Fig. 3B).

The total yields of both Premium Crop and Shogun increased with increased N fertilization (Fig. 4A). A significant quadratic response was observed in

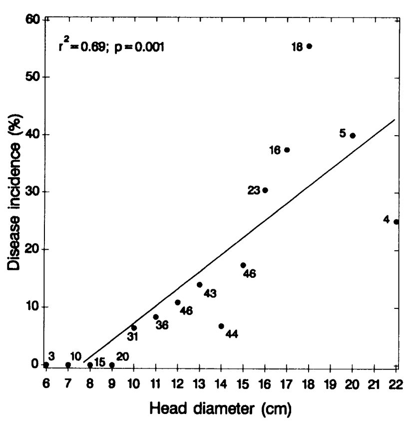

Fig. 1. Regression of the incidence of bacterial soft rot on the head diameter of the resistant broccoli cultivar Shogun. The value beside each point is the number of heads used to calculate the average disease incidence for that diameter.

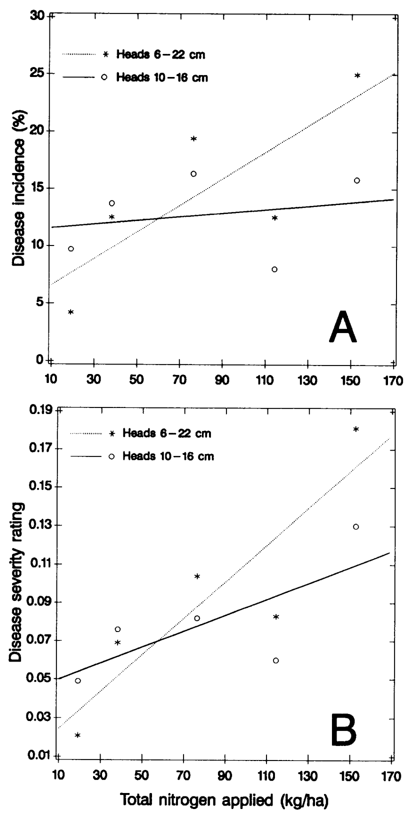

Fig. 2. Regression of (**A**) the incidence of bacterial soft rot in the resistant broccoli cultivar Shogun and (**B**) the disease severity rating on total nitrogen applied, in relation to ranges in head diameter. Each point is the mean of four replicates in an experiment conducted in the spring of 1989.

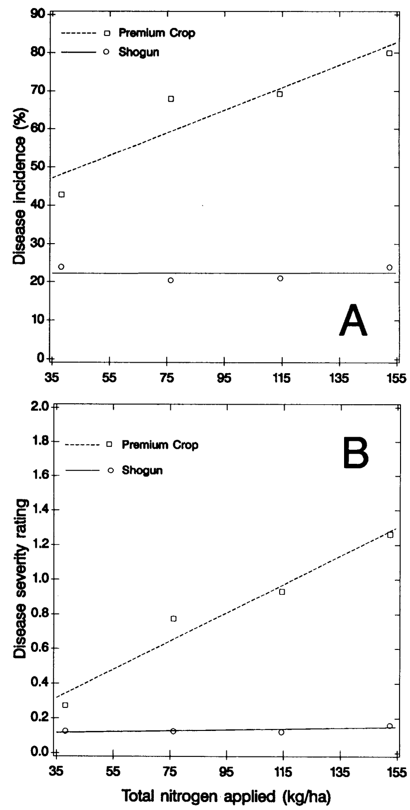

Fig. 3. Regression of (**A**) the incidence of bacterial soft rot in the susceptible broccoli cultivar Premium Crop and the resistant cultivar Shogun and (**B**) the disease severity rating on total nitrogen applied. Each point is the mean of eight replicates combining data from experiments conducted in the fall of 1989 and spring of 1990.

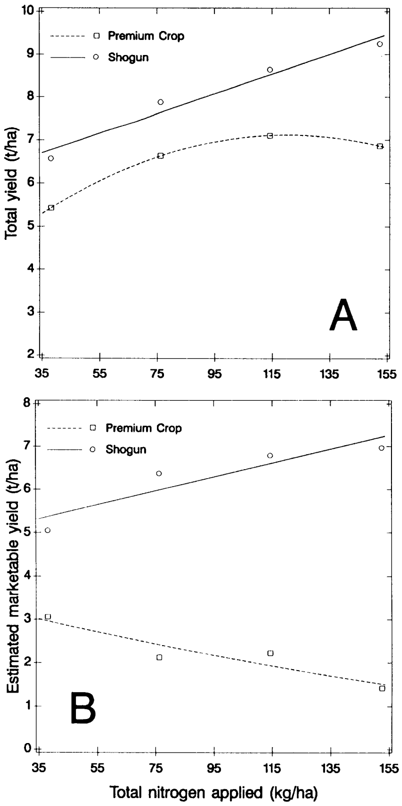

Fig. 4. Regression of (**A**) the total yield of a broccoli cultivar susceptible to bacterial soft rot (Premium Crop) and a resistant cultivar (Shogun) and (**B**) the estimated marketable yield on total nitrogen applied. Each point is the mean of eight replicates combining data from experiments conducted in the fall of 1989 and spring of 1990.

Premium Crop, with an estimated maximum yield response when the total N applied is 120 kg/ha. Since any sign of bacterial soft rot is sufficient to make a head unmarketable, the increase in disease incidence in Premium Crop with increased N led to a decrease in the estimated marketable yield (Fig. 4B). In contrast, the marketable yield of Shogun increased with the N rate ($P = 0.05$).

DISCUSSION

The incidence and severity of bacterial soft rot in Premium Crop, a susceptible cultivar, appear likely to increase with increased nitrogen under conditions conducive to the disease. In contrast, the rate of nitrogen fertilization has a negligible effect on soft rot incidence and severity in Shogun, a cultivar with some resistance. These observations have important implications for disease and crop management, and additional studies should be conducted to confirm whether these decidedly different trends hold true for all susceptible and resistant cultivars.

These observations are based on harvesting heads of similar size, since both the incidence and the severity of bacterial soft rot increase with head size. Disease incidence also increased with head diameter in broccoli variety trials in which nitrogen was not a variable (C. H. Canaday, *unpublished*). A change in harvest protocol to a once-over harvest, in which the variation in head size is greater, could significantly affect the results, as was observed in the initial experiment in this study.

An effect of nitrogen fertilization on disease response led to significant differences in the marketable yields of the cultivars in this study. The marketable yield of a susceptible cultivar decreased with increased nitrogen, while that of a resistant cultivar increased. However, the effect of nitrogen fertilization on marketable yield was not confined to disease response. Both head quality and the incidence of hollow stem (the latter reducing head marketability) increased with the N rate in these experiments, corroborating the observations of other researchers (5,6,10,12). Thus, the overall net effect of increased nitrogen on marketable yield depends on the relative response to several variables.

The frequent irrigation in these experiments, which was intended to create conditions conducive to the disease, probably affected the response of the plants to nitrogen, since increased leaching and denitrification could be expected under these conditions. Under slightly drier growing conditions, the yields of both cultivars could be substantially different. The conditions imposed in this study, however, are probably not significantly different from those that prevail when bacterial soft rot becomes a problem. Although this study should not be used to predict the yield of broccoli cultivars in response to increasing rates of nitrogen fertilization under ideal field conditions, it does demonstrate the potential for substantial yield loss when a susceptible cultivar is fertilized with nitrogen at a high rate and conditions become conducive for bacterial soft rot.

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