peaks in the OH/NH stretch region. The cotyledon extract gave no precise peak maxima matching the peaks from the known cucurbitacin B and E, or B or E. The derivation of either pure, or BZ cucurbitacins was not successful so identification of cucurbitacins by GLC was not possible.

Relative abundance of major positive fragments for cucurbitacin B, E, and for BZ cotyledon extracts are shown in Fig. 2. All major peaks in 300 to 500 *m/e* region for known cucurbitacin B also appeared in cotyledon extract, the largest (relative abundance 5%) in the high mass region was at 499 *m/e*. These data indicate that the cotyledons of 'Black Zucchini' contained cucurbitacin B.

We also compared a commercial source of cucurbitacin I with the cucurbitacin I standard. Although peaks obtained for the major fragment ions in the high mass region (300-540 *m/e*) were distinct for cucurbitacin I, additional peaks indicated the presence of B and E as an impurity.

Mass spectra of pure cucurbitacins were similar to those reported previously (1). Information obtained from the other analytical methods was less conclusive than that obtained by mass spectrometry, which, therefore, appears to be appropriate for characterizing unknown mixtures of cucurbitacins.

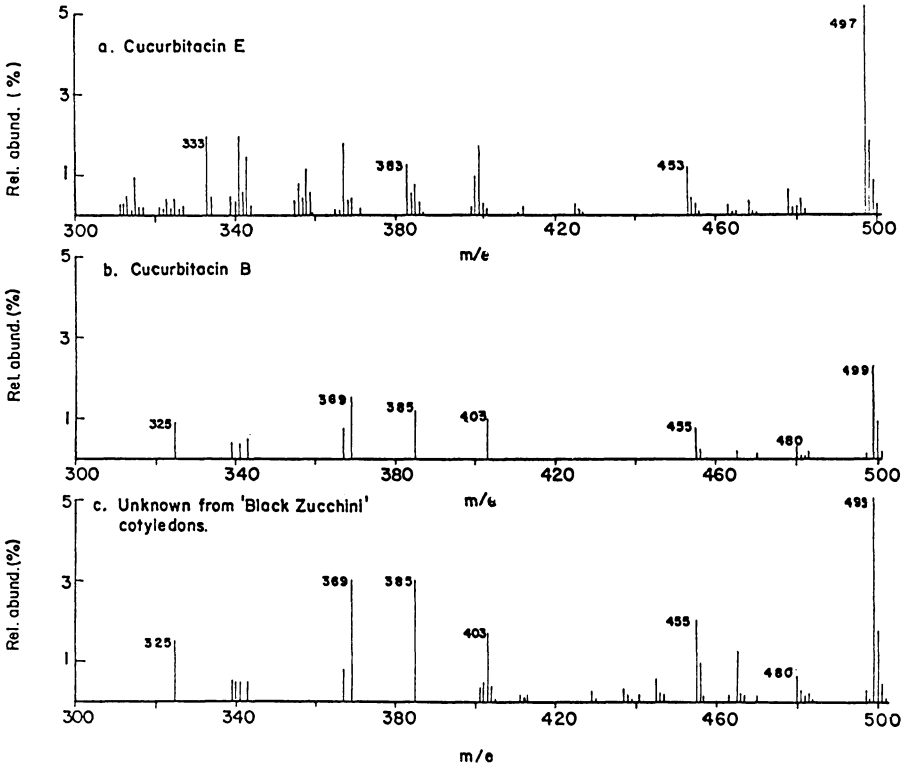

Fig. 2. Mass spectra (*m/e* 300-510) of cucurbitacin B, E and unknown from 'Black Zucchini' cotyledons. Relative abundance expressed as % of base peak.

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Interactions in Source of Nitrogen Fertilizer and Liming Procedure in the Control of Fusarium Wilt of Tomato[1]

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Abstract. **Symptoms of fusarium wilt of tomato were less severe in plants supplied with nitrate-N and greater in those supplied with ammonium-N. Liming with calcium hydroxide decreased disease severity but this effect was negated by high ammonium-low nitrate fertilization. The combination of high nitrate, low ammonium, and lime reduced disease development additively. *Fusarium oxysporum* f. sp. *lycopersici* race 2 was more virulent when grown in liquid culture with ammonium than with nitrate as the sole N source.**

Liming certain soils to a pH of about 7.0 to 8.0 reduced the severity of fusarium wilt of tomato (3, 4). Albert (1) using nutrient solution culture, showed that 2 mutually dependent factors, pH and nitrate-N, significantly increased fusarium wilt resistance and decreased wilt injury of cotton. This study evaluates the individual and interrelated effects of NO_3 versus NH_4-N, lime, and soil pH upon the severity of fusarium wilt of 'Manapal' tomato incited by *Fusarium oxysporum* f. sp. *lycopersici* race 2.

The pathogen was cultured in liquid media with NO_3 or NH_4 as the sole N source (5). Mycelial material was separated from solution cultures by filtration, and washed 3 times with deionized water. Inoculum was prepared by a brief blending of 5 mg of fresh mycelial material in each ml of deionized water. Virulence of inoculum was tested by root inoculation of 'Manapal' tomato seedlings. Fifteen seedlings were inoculated with each of the 24 cultures. Disease ratings indicated that inoculum grown on the ammonium form of N was more virulent than that grown on nitrate-N (Table 1). This observation on the direct effect of

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Table 1. Effect of N source in liquid cultures of *Fusarium oxysporum* f. sp. *lycopersici* race 2 on virulence to 'Manapal' tomato seedlings.

Liquid culture	No. healthy plants: N-source in culture $NaNO_3$	No. healthy plants: N-source in culture $(NH_4)_2SO_4$
Stationary	67	28
Shaken	43	13
Total	110	41
LSD 1% for N source = 43		

[z]180 plants total.

N nutrition on the pathogen led to an experiment with varied NO_3:NH_4 ratios in culture of 'Manapal' plants that were subsequently inoculated with inoculum produced on potato-dextrose agar.

The experiment was carried out in a greenhouse with daily maxima of 33 ± 2°C and minima of 25 ± 2°C. Nine-day old seedlings were transplanted into 15 cm plastic pots containing virgin Leon fine sand previously amended with either 1 g $CaCO_3$/kg soil or a combination of 1 g $CaCO_3$ + 2 g $Ca(OH)_2$/kg soil to establish a differential in soil pH. Ten plants were established in each pot, arranged in 2 rows so that inoculation could be conveniently accomplished by cutting the roots 2.5 cm to one side of stems to a depth of 5 cm, and pouring a spore suspension into the trenches thus produced. Plants were watered once and later twice weekly with 5 different nutrient solutions containing various combinations of $NaNO_3$ and $(NH_4)_2SO_4$ as N sources. Other nutrient compounds in the solutions were $NaH_2PO_4 \cdot H_2O$, KCl and $MgSO \cdot 7H_2O$. Nutrient element concn used, mg/l, were: N, 500; P, 200; K, 500; and Mg, 20. Thirty ml applications of nutrient solution were made once weekly during the 1st and 2nd weeks of the experiment and twice weekly during the 3rd, 4th and 5th weeks. Following the 5th week, two 50 ml applications were made weekly until the end of the experiment. High levels of plant nutrition were avoided in order that the plants would not be unduly damaged by certain NO_3:NH_4 ratios that were varied over a wide range without regard for optimum balance for plant growth. Plants were grown 21 days to permit response to the various nutrient treatments and an equilibration of the soil with the various N fertilization regimes. Roots were then cut as described and 16.5 million microconidia suspended in 60 ml water were poured into the trenches in 4 replicate pots per treatment. Control plants were similarly treated except that 60 ml deionized water was poured into the trenches made by cutting the roots.

Plants were grown for 28 days after inoculation. During this time disease ratings were made weekly beginning with the first appearance of symptoms. Plants were harvested at the end of the period and the fresh wt of the shoots and washed root systems determined. Stems were cut 1-2 cm above the cotyledonary node and the no. of macroscopically visible discolored vascular bundles recorded (Table 2). Soil pH was determined for each replicate pot because of the significance of this parameter of the soil environment in the development of Fusarium wilt.

Comparisons were made of the various methods used to evaluate disease incidence and severity. Good agreement was found among the following parameters: shoot and root wt, disease index ratings based on external symptoms, and no. of vascular bundles showing discoloration according to the evaluation methods of Dimond, et al. (2). Vascular discoloration (browning) was chosen for this report because of the agreement with other categories of data as well as the objective nature of the procedure.

$Ca(OH)_2$ plus $CaCO_3$ and the resultant elevated pH values resulted in less disease. As the proportion of NO_3-N was increased, pH increased and disease was less. Conversely, increasing NH_4-N caused decreased pH values and increased disease. Apparently NO_3 and NH_4-N each have positive effects that work in opposite directions. Other experiments not reported here indicated that high rates of NO_3-N are more inhibitory to disease development than low rates, while higher levels of NH_4-N are more effective in increasing disease. Our findings are in general agreement with those of Albert (1). However, the findings for one host-parasite relationship do not necessarily apply to other *Fusarium oxysporum* wilt diseases.

Of significance is the fact that NH_4-N, when substituted for NO_3-N was effective in overcoming the protective effect of liming even though the soil pH remained at 7.5 and above. The possible localized effect of N source in the rhizosphere should be evaluated in further work. Evidence has been presented (3, 4) that high soil pH produced by lime application may reduce virulence of race 2 by interfering with the heavy metal nutrition of the fungus. The dependence of *Fusarium* on micronutrients for growth and virulence has been clearly demonstrated (5). As reported here the virulence of the pathogen was increased by NH_4-N, which may account for the development of wilt at high soil pH values in some instances when a degree of control was expected.

It is readily apparent from the data presented, together with previously published information (1, 3, 4, 5), that fusarium wilt development is affected by inorganic nutrients in the soil environment and by the many factors affecting their availability to the host and pathogen. Methodology for the study of this disease and for the development of predictably effective control procedures therefore must be based on a comprehensive understanding and consideration of the effects of inorganic nutrition on the pathogen, the host, and pathogenesis.

Table 2. Effect of N fertilizer source and liming procedure upon soil pH and development of Fusarium wilt of 'Manapal' tomato seedlings incited by *Fusarium oxysporum* f. sp. *lycopersici* race 2.

N source[z]		Soil pH: Lime level[y]		Mean no. of discolored vascular bundles per stem: Lime level[y]	
% NO_3	% NH_4	Low	High	Low	High
100	0	7.5	8.3	2.0	0.8
80	20	7.5	7.9	4.0	2.5
50	50	6.9	7.8	4.5	3.1
20	80	6.1	7.7	6.0	4.3
0	100	5.3	7.6	4.1	3.1
LSD 5%		0.4	0.4	1.9	1.9

[z]NO_3 furnished as $NaNO_3$; NH_4 as $(NH_4)_2SO_4$.
[y]Low = 1 g $CaCO_3$/kg soil; high = 1 g $CaCO_3$ plus 2 g $Ca(OH)_2$/kg soil.

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