progresses is clearly evident by the differences in the values for color in 1958–62 compared to those values for the 1963–66 period or to the annual values for 1966 (Table 7). Thus, a management practice, either beneficial or harmful, though not evident during the early years in the life of an orchard, may become evident as time progresses.

This work shows quite clearly the fallacy of striving for yield without considering treatment influence upon quality. The unfavorable influence of Mg application upon firmness and keeping quality must be considered against the increase in fruit yield and size resulting from Mg application. The marked decrease in quality which occurred with the second level of Mg was accompanied by a marked yield increase (4). Thus, it is apparent a proper balance of K and Mg is essential not only for optimum yield but also for acceptable quality.

The data indicate that treatments which increased firmness also increased shelf-life. Both N and Mg reduced firmness, shelf-life, and surface color; they tended also to reduce specific gravity. The application of K significantly increased firmness, shelf-life, and surface color; but K tended to reduce specific gravity.

The primary goal in fruit production is to obtain high yields of marketable fruit. The literature is replete with instances of increased yields from the application of N, K, and to a lesser extent Mg. However, when fruit quality is also considered, it is apparent that N and Mg, may severely decrease quality at or below the level needed for maximum yield. The use of K at or even slightly above that needed for optimum yield would tend to increase the quality factors measured. One effect of excessive K reported previously (5) has been that it resulted in a decrease in Mg levels in the trees and induced Mg deficiency.

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Stomate Density in Relation to Winter Hardiness of *Ilex opaca* Ait.[1]

G. N. Knecht[2] and E. R. Orton, Jr.,[3]
Rutgers University, New Brunswick, N.J.

Abstract. Stomate density was determined for 11 cultivars of *Ilex opaca* which had been subjectively ranked for winter hardiness as either very hardy, hardy, semi-hardy, or not hardy on the basis of field observations recorded over a 5-year period. Variance analysis revealed that the number of stomates for the cultivars rated very hardy was significantly lower than that of the cultivars rated not hardy. Stomate counts for the cultivars in the intermediate hardiness classes all fell within the range delimited by the counts of the very hardy and not hardy cultivars.

Ilex opaca, American holly, is a broad-leaved evergreen which generally retains its leaves for two or three years. Plants of borderline hardiness, however, often exhibit complete defoliation following a particularly cold winter. Much winter injury appears to result from desiccation of the tissues of the plant. Since most moisture loss from plants occurs through the stomates, this study was conducted to determine if there is a relationship between the number of stomates per area of leaf surface of plants of *I. opaca* and the winter hardiness of those plants.

According to Vasil'yev (4), plant roots have difficulty absorbing water when the temperature drops to 0°C, and, if the soil freezes, the process may stop altogether. Vasil'yev stated, however, that water evaporates from the aerial parts of the plants even during severe frosts. He further reported that herbaceous plants dry out in the winter due to intense evaporation on bright, windy days when the soil is frozen and concluded that desiccation of wintering plants may injure or kill them. In recent investigations of the movement of water in stems of *I. opaca* held at low temperatures, Winslow and Havis (5) found that water movement stops in the stem within a temperature range of −1.1° and −1.4°C. They suggested that rapid water loss from exposed plant parts may occur even when the stem becomes frozen.

Hirano (1) studied the relative abundance of stomata in leaves of various species of *Citrus* and related genera and observed some coincidence of hardiness with the density of stomata in the commercial cultivars. He concluded that the hardier cultivars and species of *Citrus* trees are characterized by a low stomatal density although a few marked exceptions were noted. More recently Salazar (2) reported that he was unable to show any relationship between stomatal density and cold tolerance in four cultivars of *Citrus*, or to their tolerance to freezing tests. However, this conclusion may be misleading since the work involved a comparison of four species of *Citrus* rather than four cultivars within a single species.

[1]Received for publication October 20, 1969. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Horticulture and Forestry.

[2]Present address: Environmental Research Laboratory, Institute of Atmospheric Physics, University of Arizona, Tucson.
[3]Department of Horticulture and Forestry, Rutgers University—The State University of New Jersey, New Brunswick.

Materials and Methods

Plant materials. Leaf samples were collected from 11 different cultivars of *I. opaca* growing at the New Jersey Agricultural Experiment Station, New Brunswick, N.J. The plants are growing in the holly orchard and arboretum and are between 16 and 20 years old. All of the plants sampled have been subjected to the same general cultural practices for the past 10 years.

The selection of the cultivars studied was based upon hardiness evaluation records compiled following the winters of 1959–1960, 1960–1961, 1961–1962, 1962–1963, and 1966–1967. The hardiness evaluation consisted of a numerical rating for the extent of defoliation and dieback observed each spring. The 11 cultivars selected for study were designated as 1) very hardy, 2) hardy, 3) semi-hardy, and 4) not hardy (Table 1).

Table 1. Eleven cultivars of *Ilex opaca* and their hardiness ratings.

Number	Cultivar	Hardiness
1	Judge Brown	Very hardy
2	Jersey Knight	Very hardy
3	Farage	Hardy
4	Ed Thomas	Hardy
5	Manig	Hardy
6	Laura Thomas	Semi-hardy
7	Wheeler #1	Semi-hardy
8	Margaret	Not hardy
9	Goldie	Not hardy
10	Brown #13	Hardy
11	Canary	Semi-hardy

Methods. Leaves from the midpoint of growth of the current season were collected at random from each plant studied. The stomate numbers were determined by the replica method of Stoddard (3). A thin film of cellulose acetate was painted directly onto the lower epidermis of the leaves. The cellulose acetate was allowed to dry at room temperature and was then peeled from the leaves. Sections were taken from three positions of each leaf: 1) the base, 2) the center, and 3) the apex. The sections extended from the midvein to a point midway between the midvein and the leaf margin. Since the stomate density was noticeably reduced in the area of major veins, care was taken to select sections between lateral veins. The apex, center, and base impressions from each leaf were mounted on a glass microscope slide and covered with a cover slip which was sealed with paraffin and balsam (40% paraffin, 60% balsam).

The prepared slides were coded and all stomate counts were made with the aid of a binocular microscope using a 15X ocular and a 43X objective. This provided a true field area of 0.057 mm². Stomates wholly or partially included within the field were counted. Fig. 1 is a photomicrograph of a cellulose acetate film replica of the lower surface of a leaf.

The experimental design included cultivars 1 through 9 (Table 1), each represented by two plants. Twenty leaves were collected from each plant and three leaf impressions (apex, center, and base) were taken from each leaf. Five stomate counts or observations, were made from each leaf position for a total of 15 observations per leaf. This provided 300 observations per plant and a total of 600 observations per cultivar. The data were subjected to an analysis of variance.

Leaves were available from just one plant of cultivars 10 and 11 (Table 1). Although these cultivars were not included in the analysis of variance, the mean stomate count was determined for each.

Cellulose acetate imprints of the upper surface of five leaves of each of the 11 cultivars studied were examined for the presence of stomata.

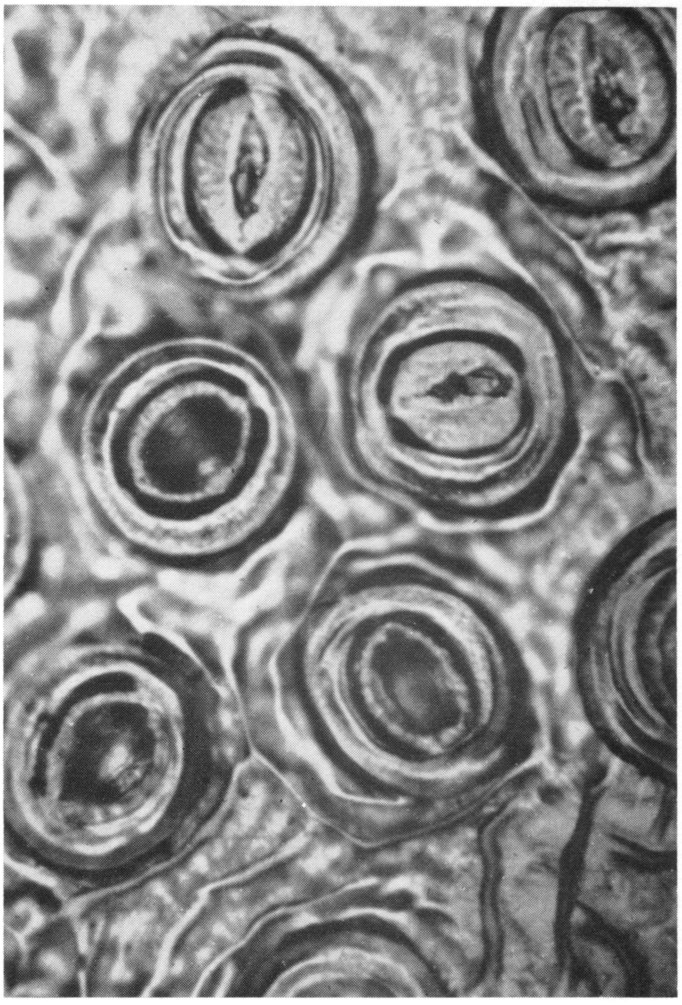

Fig. 1. Photomicrograph of a cellulose acetate film replica of the lower leaf surface of *Ilex opaca*.

Results

An analysis of variance of the data indicates highly significant differences for the cultivar, position, and position by cultivar sources of variation (Table 2).

Cultivar source of variation. A highly significant difference exists among the cultivars investigated (Fig. 2). Using a Tukey multiple comparison test (HSD), cultivars 1 and 2 (very hardy) were found to have a significantly lower stomate count per leaf area than cultivars 8 and 9 (not hardy). However, there is an overlapping of confidence intervals of the hardy and semi-hardy cultivars both with one another and with the very hardy and not

Table 2. Analysis of variance.

Source of variation	d f	MS	Calc F
Cultivar	8	4578.60	39.92**
Plant (C)	9	114.70	
Leaves	342	35.01	
Position	2	355.94	44.77**
Pos. × Cult.	16	18.85	2.37**
Pos. × Pl (C)	18	7.89	
Pos. × L[Pl(C)]	684	7.95	
Obs.	4320	3.88	

**Significant at probability level .01.

hardy cultivars. There is no significant difference at the 5% level between cultivars 1 and 2 (very hardy), but these two cultivars differ in their relationship to the cultivars in the hardy and semi-hardy classes. The same is true for the two not hardy cultivars: although not significantly different from one another, they differ in their relationship to the cultivars in the intermediate classes.

Leaf position source of variation. The importance of considering leaf position as an experimental variable when determining the mean stomate count per leaf area is evidenced by the data presented in Table 2. The difference between the apex and center leaf positions was not statistically significant (Fig. 3). However, the mean stomate counts for both the apex and center positions were significantly higher (α = .01) than the stomate counts for the base position of the leaves.

Position by cultivar interaction. The calculated F value of 2.37 for the position by cultivar interaction indicates a highly significant interaction between the cultivar and position main effects (Fig. 4). A comparison of the mean stomate number of the apex and center positions of each cultivar (Table 3) indicates that cultivar 6 is the only one in which this difference is statistically significant (α = .05). With cultivars 3,4,6 and 9, the mean stomate number is significantly greater (1% level) at the apex than at the base position but not significantly different for cultivars 1,2,5,7 and 8. The mean stomate counts for the center and base positions of 1,2,5 and 7 were not significantly different. However, these counts were significantly different (5% level) in the case of cultivars 6 and 8, and highly significant (1% level) for cultivars 3, 4 and 9.

Since the position by cultivar interaction is highly significant, it is important to consider the mean stomate counts of the nine cultivars at each of the leaf positions. The calculated H.S.D. (α = .01) for the position source of variation is 1.01. At the apex position (Table 3),

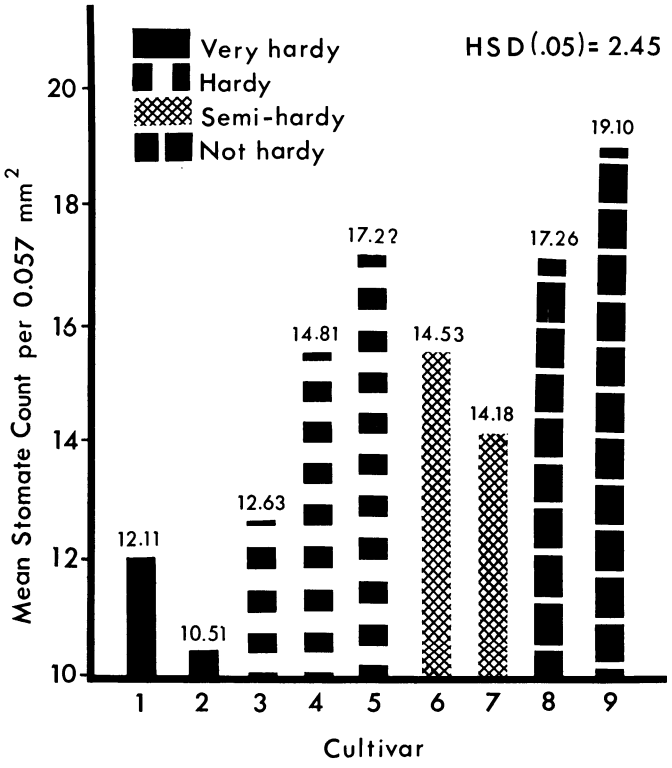

Fig. 2. Mean stomate counts of 9 cultivars of *Ilex opaca*.

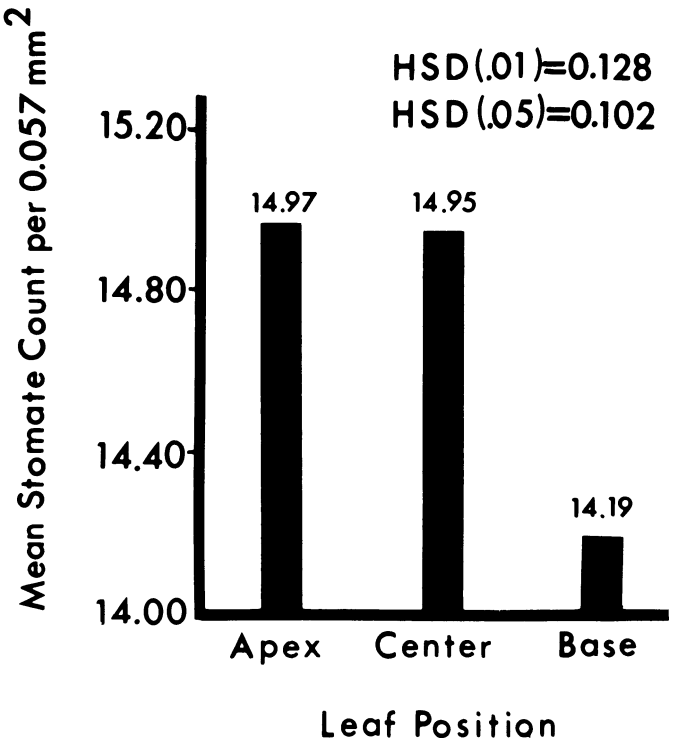

Fig. 3. Mean stomate counts for the apex, center, and base leaf positions averaged for 9 cultivars of *Ilex opaca*.

cultivars 1 and 2 (very hardy) have a significantly lower mean stomate number than any of the other cultivars with the exception that the count of cultivar 1 is not significantly lower than that of cultivar 3 (hardy). At the other extreme of the hardiness range, cultivars 8 and 9 (not hardy) yielded mean stomate counts significantly higher than that of any of the other cultivars with the exception of cultivar 5. Cultivar 3 was found to exhibit a significantly lower stomate count than the two hardy cultivars (nos. 4 and 5) as well as the semi-hardy and not hardy cultivars. The stomate count for cultivar 4 was significantly lower than the counts for the two cultivars classified not hardy (nos. 8 and 9), but it was significantly higher than that of cultivar 7 (semi-hardy). Cultivar 5 (hardy) had a significantly higher mean stomate count than the very hardy, the other two hardy cultivars, and the semi-hardy cultivars. The mean stomate count of cultivar 5 is significantly lower than that of the not hardy cultivar 9, but it does not differ significantly from that of cultivar 8.

The same clonal differences were found at the center and base leaf positions (Table 3) as at the apex position with the one exception that the stomate count of cultivar

Table 3. Mean stomate count for apex, center, and base leaf positions for 9 cultivars of *Ilex opaca*.

Cultivar	Apex	Center	Base
1	12.35	12.19	11.79
2	10.58	10.79	10.18
3	12.91	12.95	12.03
4	15.49	14.95	14.01
5	17.15	17.45	17.08
6	15.23	14.53	13.83
7	14.28	14.31	13.94
8	17.42	17.59	16.78
9	19.35	19.82	18.12

Position by cultivar comparisons HSD (.01) = 0.82
HSD (.05) = 0.66

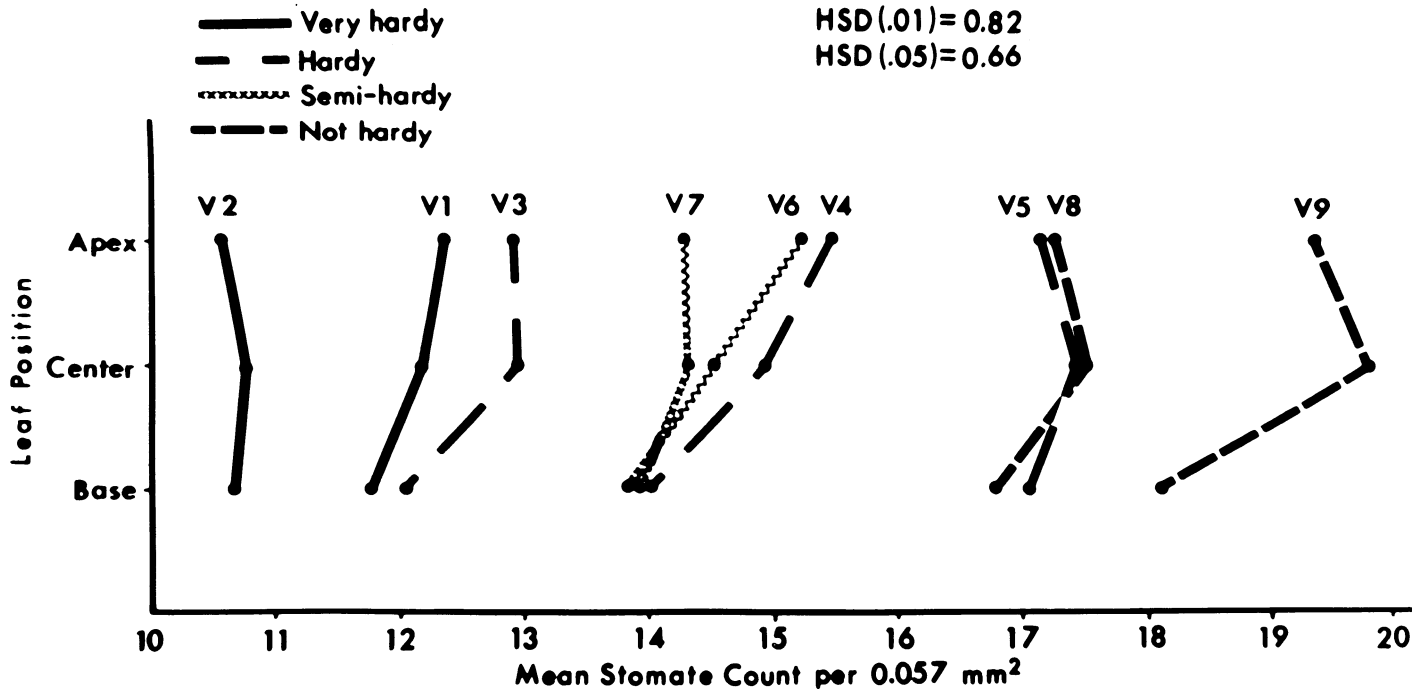

Fig. 4. Mean stomate count for the apex, center, and base leaf positions of 9 cultivars of *Ilex opaca*.

4 was not significantly higher than that of cultivar 7 at either the center or base positions.

Variance components. The characterization of the variability of this investigation as denoted by the variance components (Table 4) indicates a large degree of variability for the leaves from each plant and for the individual observations within each leaf position. The variance component of 0.27 for the plants-within-cultivars source of variation would indicate a very small amount of variability for this effect.

Additional cultivar means. Stomate counts were also determined for a single plant of each of two cultivars. These data were not included in the analysis of variance. One cultivar had been subjectively rated as hardy and the other cultivar as semi-hardy (Cultivars 10 and 11, Table 1). The mean stomate counts of 300 observations for each cultivar were 12.89 per leaf area for the hardy cultivar and 15.21 per leaf area for the semi-hardy cultivar. These means are in accord with the results for the intermediate cultivars previously discussed.

Upper epidermis. An investigation of the upper epidermal layer of all 11 cultivars utilized in this study substantiated previous reports that stomates are present only on the lower epidermis of leaves of *I. opaca*.

Discussion and Conclusion

The results of this investigation demonstrate that there is a relationship between the stomate density per area of leaf surface and the resistance to winter injury of the eleven cultivars of *I. opaca* selected for study. As would be expected, this relationship is most pronounced among those cultivars which lie at the extremes of the hardiness range (very hardy and not hardy classes). Those cultivars rated as hardy and semi-hardy have mean stomate counts which generally fall within the range of the counts of the cultivars in the two extreme hardiness classes. There is some overlapping of means of the cultivars in the two intermediate hardiness classes with that of the cultivars in the two extreme classes (very hardy and not hardy); however, one would not necessarily expect a direct positive relationship between a subjective hardiness rating and an objective measure of a quantitative characteristic such as stomate density. Nevertheless, it is suggested that stomate density may provide an index for the evaluation of winter hardiness of plants of *I. opaca*. Prior to utilizing such an index it will be necessary to determine what effect such environmental factors as light, temperature, soil moisture, atmospheric humidity, and plant juvenility may have on stomate density in order to assess the relative constancy of stomate number from area to area and from season to season for plants of different ages.

Table 4. Sources of variation and their variance components.

Source of variation	Variance component
Plants (cultivar)	0.27
Leaves [plants (cultivar)]	2.08
Observations	3.88

A highly significant position by cultivar interaction was obtained in this study. However, examination of the clonal differences at each of the three leaf positions revealed the same pattern of significant differences at each position with the exception that the stomate density of cultivar 4 was significantly higher than that of cultivar 7 at the apex position, but was not significantly higher at either the center or base position. The direction of this difference, however, was the same at all three leaf positions. Thus, it seems justifiable to restrict observations of stomate number to the apex position in future studies of this nature with *I. opaca*. By eliminating samples from the base and center positions, the number of leaves sampled could be tripled without increasing the total number of observations. Since leaves-within-cultivars was found to be a major source of variation, such an increase in the number of leaves sampled would be desirable.

The results of this study do not reveal a cause-effect relationship between stomate density and winter hardi-

ness, but the demonstrated relationship between these two factors may well constitute a basis for screening plants of *I. opaca* for winter hardiness.

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Studies on the Ammonium Tolerance of Some Cultivated Solanaceae[1,2]

Herman E. Hohlt,[3] Donald N. Maynard, and Allen V. Barker
University of Massachusetts, Amherst

Abstract. Tomato, tobacco, pepper, petunia, and eggplant were screened for their tolerance to continuous $(NH_4)_2SO_4$ applications. Stem lesions, analogous to those which appear on tomato during ammonium toxicity, were formed on eggplant but not on the other species. Of the plants tested, tobacco was the most tolerant to applications of $(NH_4)_2SO_4$. Potassium applications increased the ammonium concentration of tobacco tissues but lowered the ammonium concentration of tomato tissues. Diamine concentrations were increased by the application of $(NH_4)_2SO_4$. The application of KCl decreased the putrescine concentration of tobacco and increased the concentration of cadaverine in tomato and tobacco. The application of putrescine·2HCl in aqueous solution to cut stem ends of axillary shoots of tomato and tobacco induced the formation of stem lesions analogous to those formed by the $(NH_4)_2SO_4$ fertilization of tomato. It is postulated that the tolerance of tobacco to stem lesion formation is related to putrescine utilization in nicotine synthesis.

MANY higher plant species manifest a variety of toxicity symptoms under prolonged ammonium nutrition. An example of this effect was reported by Maynard et al. (7); tomato plants developed dark brown lesions on the stem surface with $(NH_4)_2SO_4$ fertilization. Thirty-six tomato lines were screened for varietal susceptibility and found to vary in their response to ammonium-N (8); however, none was completely resistant to lesion formation. Barker et al. (2,3) studied the role of K^+ in relation to the tomato stem lesion symptom and described the anatomical characteristics of the lesion area. Potassium was found to lessen the effect of NH^+_4, and the lesions, which were confined to the epidermal and cortical regions, contained an appreciable amount of unknown crystalline-like substances. However, a more direct relationship to the cause of the lesion symptom, other than the ammonium:potassium ratio (3), has remained obscure.

These studies were initiated using several genera within the Solanaceae to determine the direct cause of the stem lesion symptom and link it to plant metabolism in a susceptible, and a tolerant species.

MATERIALS AND METHODS

Plant material. Five economically important species of Solanaceae were grown in the greenhouse in soil culture. Tomato cv. 'Heinz 1350', sweet pepper cv. 'Staddon's Select', tobacco cv. 'Havanna Leaf K-1', petunia cv. 'Maytime', and eggplant cv. 'Black Beauty' were seeded at intervals so that all plant material was of a similar physiological age when tested. Treatments were begun after the plants were established in 6-inch clay pots containing a greenhouse soil mixture (7). The plant age, measured from transplanting of seedlings, was tomato, 3 weeks, eggplant, 4 weeks, and tobacco, petunia, and pepper, 7 weeks.

At the start of a treatment period the plants, within a species, were selected for uniformity and arranged in a randomized complete block design with five single-pot replications. Treatment solutions were made fresh daily by dilution of 0.5 *M* $(NH_4)_2SO_4$ and KCl stock solutions to the treatment levels noted in the individual experiments. Each plant received a total of 150 ml of treatment solutions daily for a 3-week period.

Upon termination of the treatment period, the plants were harvested, and the fresh weight of the shoot recorded. A random, 10 g sample of leaves was obtained from non-necrotic plant tissue, wrapped in aluminum foil, and stored at −20° C until analyzed for nitrogenous constituents. The remainder of the plant shoot was dried in a forced draft oven at 70° C.

Chemical analyses. Ammonium and amide-N samples were prepared and assayed by a modified Kjeldahl procedure (4). Total N was determined from the dried plant material (16).

The technique for the isolation of the diamines, putrescine and cadaverine, was modified slightly from that of Sinclair (10). The resin (Dowex 50W × 4, 200 to 400 mesh) was stored in distilled water rather than in 80% ethanol. The use of water gave higher flow rates through the resin and eliminated the need of suction or pressure applications. The residue remaining after the evaporation of a 70% ethanol plant extract from frozen plant material was dissolved in chloroform and water. The residue was readily soluble in chloroform

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[2]Research supported in part by a grant from the Massachusetts Society for Promoting Agriculture.

[3]Present address: Virginia Truck Experiment Station, P. O. Box 133, Painter, Virginia. Formerly Graduate Research Assistant, Department of Plant and Soil Sciences, University of Massachusetts.