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# OZONE-INDUCED CHANGES IN HOST-PLANT SUITABILITY: INTERACTIONS of *Keiferia lycopersicella* AND *Lycopersicon esculentum*

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**Abstract**—Tomato pinworms, *Keiferia lycopersicella* (Walsingham), survived better and developed faster on tomato plants, *Lycopersicon esculentum* Mill., damaged by ozone than on plants not subjected to ozone fumigation. Other measures of fitness, including survival during pupation, sex ratio of adults, female longevity, and fecundity, were not affected. Analyses of ozonated foliage at zero, two, and seven days following fumigation demonstrated a transient but significant increase (18–24%) in soluble protein concentration. Although the concentration of the total free amino acids in ozonated foliage did not increase significantly, significant changes were observed in at least 10 specific amino acids, some of which are critical for either insect development or the production of plant defensive chemicals. A reduction in total nitrogen in ozonated foliage at seven days postfumigation indicated that nitrogen was being translocated to other portions of the plant. The implications of increases in assimilable forms of nitrogen in ozonated foliage, which lead to improved host-plant suitability for insect herbivores, are discussed both in relation to some current ecological theories and in regard to pest-management strategies.

**Key Words**—Ozone, nutrition, insect–plant interactions, nitrogen, secondary plant compounds, *Keiferia lycopersicella* Lepidoptera, Gellechiidae, tomato, *Lycopersicon esculentum.*

## INTRODUCTION

Ozone, a colorless, highly reactive gas formed from the interaction of the products of fossil fuel combustion and oxygen, causes more plant damage in the United States than any other air pollutant (EPA, 1978). Losses in southern California are reportedly in excess of 275 million dollars annually (Leung et al., 1982). Upon entering leaves through the stomata (Dugger et al., 1962), ozone reacts with plant membranes and cellular structures such as chloroplasts, resulting in structural damage and, in some cases, the onset of premature senescence (Mudd et al., 1969; Chang, 1971a; Heath, 1980). Such physiological changes reportedly decrease photosynthesis in several species (Ormrod et al., 1981; Reich and Amundson, 1985); both photosynthesis (Todd and Probst, 1963) and partitioning of photosynthates are affected in tomatoes (McCool and Menge, 1983).

An important action of ozone on foliar tissues is the apparent release of large amounts of soluble nitrogenous compounds from plant structural forms. Nitrogen in soluble form (soluble proteins and free amino acids) is apparently more easily digested by insects than many protein complexes in plants (Slansky and Feeny, 1977). Ozone has been shown to preferentially oxidize certain amino acids, specifically cysteine, methionine, tyrosine, histidine, and cystine (Chang, 1971b; Mudd and Freeman, 1977). This action breaks structural proteins into their component parts, causing membrane dissociation and producing significant increases in free amino acid concentrations (Menzel, 1971; Ting and Mukerji, 1971; Craker and Starbuck, 1972), and in some reports, soluble protein levels (Beckerson and Hofstra, 1979). However, these results cannot be generalized across all plant species, as some exceptions have been reported (Ting and Mukerji, 1971; Tingey et al., 1973).

The implications of increased concentrations of readily available forms of nitrogen for insect herbivore development are considerable (Mattson, 1980; White, 1984). Researchers have clearly demonstrated that nitrogen availability affects such basic life processes as growth rates, survival, and reproductive capacity (Onuf, 1978; Prestidge, 1982). White (1984) speculated that improved host quality resulting from an increase in free amino acid concentration following an episode of plant stress would greatly enhance survival of newly enclosed larvae. Although a variety of physiological changes have been reported for plants (primarily evergreens) stressed by $SO_2$ (Alstad et al., 1982), increased concentrations of free amino acids in foliage due to $SO_2$ injury have been suggested as the causal factors for improved survival in both aphids (Dohmen et al., 1984) and beetles (Jaeger et al., 1972). Since air-pollution incidents typically occur over broad areas of crop production (Perkins, 1974), the cumulative effects on herbivores at the population level may be quite significant. Thus, potential changes in plant physiology due to stress or direct injury resulting from air

pollutants may well have more serious consequences than surveys of direct economic losses have suggested.

Little research is available concerning the impact of ozone on insect–plant interactions. In the most detailed study to date, Endress and Post (1985) demonstrated that the Mexican bean beetle preferred to feed on excised soybean foliage damaged by ozone, but the authors only speculated as to the physiological mechanisms underlying the changes in foliage suitability. In a related study, Jeffords and Endress (1984) reported a preference by gypsy moth larvae for ozonated foliage excised from white oak. Hughes et al. (1981) showed a similar preference by the Mexican bean beetle for foliage damaged by $SO_2$.

Earlier research on the direct impact of ozone on insects suggested there were no significant effects on two species of cockroaches or on fire ants (Levy et al., 1974). Prolonged exposure to high ozone concentrations on housefly populations caused insignificant variations in growth and survival, but apparently stimulated oviposition (Beard, 1965). However, insects in these two latter studies were not fed living plant material, so any potential ozone-induced changes in nutritional quality of their food source would not occur.

The first objective of the study reported here was to evaluate the influence of ozone on interactions between *K. lycopersicella,* a specialist herbivore of economic significance in the United States, and its host plant, *L. esculentum.* Secondly, we wished to document some of the chemical and physiological changes in ozonated tomato plants which might alter the nutritional or defensive status of the foliage.

## METHODS AND MATERIALS

Tomato pinworm (TPW) larvae, *K. lycopersicella,* used in all tests were reared in the laboratory from a colony initiated in 1982 with insects collected from commercial tomatoes in Orange County, California. The culture was maintained at 26 ± 1°C in a photoperiod of 14L : 10D on tomato plants (variety VFN 7718). All tomatoes used in these studies (VFN 7718) were grown from the same seedlot in a glasshouse and were planted in one-gallon pots containing UC soil mixture (Matkin and Chandler, 1957). Fertilization was standardized within each test. All plants were eight weeks old (six true leaf stage) at the initiation of each experiment.

Ozone fumigation chambers utilized in these tests were either large (2.5 m diameter) outdoor chambers (Musselman et al., 1986) or smaller (1.37 m diameter) chambers located in a glasshouse (1.1 air exchanges/min, similar to Heck et al., 1978). Temperatures and relative humidities during fumigation ranged from 20 to 25.5°C and 35 to 42%, respectively. The outdoor chambers operated continuously, and plants received all ambient ozone present. Glass-

house fumigation chambers, which are suitable for precise metering of ozone, were utilized for developmental tests since temperature could be maintained near 26°C, the optimum for TPW (Lin and Trumble, 1985). Regardless of size, both multiple chamber facilities were equipped with electrical arc ozone generators and activated charcoal filters for filtered air (low ozone) fumigation. Ozone concentrations within individual chambers were recorded using Dasibi UV ozone monitors linked to data loggers.

*Insect Development, Survival, and Fitness:* TPW developmental rates and survival on ozonated plants, and the potential effects of direct exposure to ozone, were evaluated in glasshouse chambers using three treatments: (1) control, no ozone; (2) two ozone fumigations prior to TPW infestation of plants; and (3) two ozone fumigations prior to, and two fumigations after, TPW infestation. All fumigations lasted three hours and were two to three days apart; treated plants received 0.28 ppm ozone and control plants received filtered air. Such concentrations of ozone are not unrealistic for agroecosystems in the Los Angeles–Riverside area, as ozone levels usually exceed 0.20 ppm (first-stage alert) on at least 30 days per year, and levels of 0.35 ppm (second-stage alert) occur annually.

Five plants with 10 eggs per plant (i.e., 50 eggs/treatment) were exposed in each treatment. Age of the eggs was standardized by using eggs oviposited during a 2-hr period. Eggs were transferred to test plants prior to randomization for the three ozone treatments. Following the last fumigation, the tomato plants were moved to the laboratory (maintained at 26 $\pm$ 2°C) and placed on trays covered with sand. The pots were covered at this time to prevent larvae exiting the foliage from pupating in the soil.

The time of host abandonment, signaling the cessation of feeding and the onset of the prepupal stage, was used to determine developmental rate and survival of larvae. The sand, serving as a pupation medium for larvae exiting the leaves, was sifted at 8-hr intervals until all surviving larvae had exited the leaves. The numbers of larvae successfully pupating and emerging as adults were subsequently recorded.

A second experiment, designed to evaluate general fitness parameters of TPW, was conducted in the outdoor fumigation chambers. Three treatments were investigated: high ozone = 35.8 ppm-hours (ambient levels plus four, 4-hr fumigations with 0.28 ppm ozone over the course of 10 days); ambient ozone concentrations = 31.6 ppm-hours; and a filtered air control = 7.7 ppm-hours. Total dose is reported because the daily ozone dosage varies with ambient in field studies. The filtered air treatments contained background levels of ozone due to the inability of the activated carbon filters to completely remove ozone. Forty-five plants per treatment were arranged in a randomized complete block design within nine fumigation chambers which had been previously selected at random for treatments. Each plant was infested with five eggs, for a total of

225 eggs/treatment, prior to randomization. Following the fumigations, the plants were returned to the laboratory and prepared as in the previous test. The pupation medium was sifted at 24-hr intervals, and all pupae were segregated by treatment and held to adult emergence. The first time this experiment was conducted, only sex ratio data were collected. In a second trial, with the identical exposure and infestation schedule (high ozone = 38.8 ppm-hours; ambient = 34.2 ppm-hours; control = 12.4 ppm-hours), males and females emerging from within each treatment were paired and allowed to oviposit on an artificial substrate (Schuster and Burton, 1982). Total egg production by each female was recorded at 48-hr intervals. Longevity of the females was assessed by recording survival at 24 hr intervals. All data from these experiments were analyzed using analysis of variance followed by Duncan's (1955) multiple-range test (DMRT) as appropriate.

*Nitrogen, Free Amino Acid, and Chlorogenic Acid Analyses:* Eighteen tomato plants were exposed to four, 2-hr, 0.18 ppm ozone fumigations at three-day intervals in glasshouse chambers. Eighteen control plants received identical treatment but were fumigated with ozone-free air. Immediately following the last fumigation, individual leaves, consisting of seven leaflets each, from six randomly chosen plants per treatment were divided into roughly equal aliquots for analysis of total nitrogen, soluble proteins, free amino acids, and chlorogenic acid. Samples were weighed immediately after collection and frozen for later analysis. Enough foliage was available on a single plant to provide four pairs of samples for each of the variables analyzed. Leaves were chosen on the basis of position on the main stem, starting with the third fully expanded leaf from the top; above this level not all leaves had been exposed to all four fumigations. At 7 and 14 days postfumigation, foliage from six ozone-fumigated plants and six control plants were evaluated in an identical fashion, except that only soluble protein and chlorogenic acid concentrations were assessed at 14 days postfumigation.

In a related test, five tomato plants were exposed to two, 3-hr fumigations at 0.28 ppm of ozone. Fumigations were three days apart. As in the previous trial, five additional plants were fumigated for an equivalent time with ozone-free air. Two foliage samples per plant from control and treated plants were collected at two days postfumigation as described previously and analyzed for soluble protein content.

Total nitrogen was analyzed using the micro-Kjeldahl technique (McKenzie and Wallace, 1954). The technique was modified by replacing the mercuric oxide catalyst with copper sulfate and by utilizing bromocresol green in place of methylene blue as an indicator.

Soluble protein was quantified using methods modified from Hare (1983, Jones, Hare, and Compton, unpublished). Frozen samples (1–2 g fresh weight) were ground and extracted with 10 ml 0.1 M NaOH for 30 min at room tem-

perature. Leaf tissue was removed by centrifugation (11,500*g* for 10 min) and the supernatant was brought to 10 ml. Protein concentration of the NaOH solution was measured with the Bradford (1976) reagent using ribulose 1,5-biphosphate carboxylase (RuBPCase) (Sigma Chemical Co.) as the standard. Samples were diluted when necessary to avoid deviations from linearity due to NaOH at high protein concentrations. Duplicate readings were made on each extraction. Values are reported as milligrams RuBPCase equivalent protein per gram (fresh weight) of foliage.

Free amino acids were qualitatively and quantitatively evaluated by extraction from additional foliage samples (1–2 g, fresh weight) with 10 ml 0.2 M trichloroacetic acid (TCA) for 30 min at room temperature. Samples were gravity-filtered through Whatman No. 1 filter paper to remove leaf tissue and then centrifuged at 11,500*g* for 10 min to remove small particles and precipitated protein. The filtrate was subsequently brought to a volume (in ml) 10 times the original weight of the leaf tissue, diluted 1:4 with additional TCA, and analyzed by automated ion-exchange chromatography at the Biotechnological Instrumentation Facility at the University of California, Riverside. Specific amino acid concentrations are reported as micrograms of amino acid per gram (fresh weight) of foliage. Total amino acid concentration was calculated as the sum of the individual amino acids per sample.

Chlorogenic acid content of foliage was quantified using high-pressure liquid chromatography (after Elliger et al., 1981).

Chemical data were analyzed by nested analysis of variance (ANOVA). The variation between treatments was tested over the variation among plants within treatments, and the variation among plants was tested over the variation between samples within plants.

Pearson product-moment correlation coefficients were calculated between plant means of percent nitrogen, soluble protein concentration, and total free amino acid concentration in order to determine if these three parameters varied independently.

## RESULTS AND DISCUSSION

*Insect Survival, Development, and Fitness.* Survival of larvae developing on ozonated foliage was significantly greater than larvae fed control plants (Table 1). Survival was more than doubled for TPW fed plants exposed to two fumigations. A significant decrease in survival was observed for TPW developing on foliage exposed to four fumigations, as opposed to two fumigations. Whether such an effect was due to contact toxicity, irritation, or some other cause, warrants additional investigation. Upon entering the tentiform leaf-mining stage (instars 3 and 4), adverse effects of ozone were apparently lessened, as counts of mines on the foliage equaled numbers of larvae exiting from leaves.

TABLE 1. DEVELOPMENTAL RESPONSE OF *K. lycopersicella* TO OZONATED AND CONTROL *L. esculentum*[a]

| Treatment | % Completing larval development | % Successfully pupating[b] | % Adult emergence[b] |
|---|---|---|---|
| Control | 28 a | 93 a | 100 a |
| Two $O_3$ fumigations | 62 c | 94 a | 95 a |
| Four $O_3$ fumigations | 46 b | 100 a | 94 a |

[a] Five replicates of 10 larvae per treatment; numbers in column followed by the same letter are not significantly different ($P \leq 0.05$), arcsine transformation, DNMRT.
[b] Percentage of those individuals completing the previous stage.

No significant differences were detected in either percent pupation or successful adult emergence from pupal cases (Table 1). Although TPW larvae feeding on ozonated plants survived better, no corresponding improvement in other fitness parameters was observed; significant differences were not detected for ratios of males to females, female longevity, or fecundity (Table 2).

Developmental rates for TPW larvae feeding on ozonated tomato plants were faster by more than 32 hr than larvae feeding on control plants (Figure 1). Mean times for emergence from the plants (setting the first emergence as time zero) were 75.9 hr for larvae fed control foliage, 55.5 hr for TPW fed on plants exposed to two ozone fumigations, and 49.4 hr for larvae fed tomatoes fumigated four times. These values represent increases in overall larval development

TABLE 2. INFLUENCE OF OZONATED AND CONTROL PLANTS ON SELECTED BIOLOGICAL PARAMETERS OF *K. lycopersicella*

| Treatment | Test[a] | Sex ratio (male–female) | Mean longevity per female (days)[b] | Mean No. eggs per female ($N$)[c] |
|---|---|---|---|---|
| High ozone[d] | 1 | 0.92 | — | — |
| | 2 | 0.81 | 6.1 | 15.9 (18) |
| Ambient | 1 | 0.85 | — | — |
| | 2 | 0.67 | 5.5 | 19.4 (14) |
| Filtered air | 1 | 0.76 | — | — |
| | 2 | 1.12 | 5.6 | 16.8 (25) |

[a] Test 1 = 3 replicates of 75 TPW per treatment; test 2 = 3 replicates of 50 larvae per treatment.
[b] Based on 3 replicates of 11–36 females/treatment monitored daily.
[c] $N$ = paired males + females; oviposition evaluated on artificial substrate, see text for details.
[d] See text for fumigation, ambient and filtered air dosages of ozone.

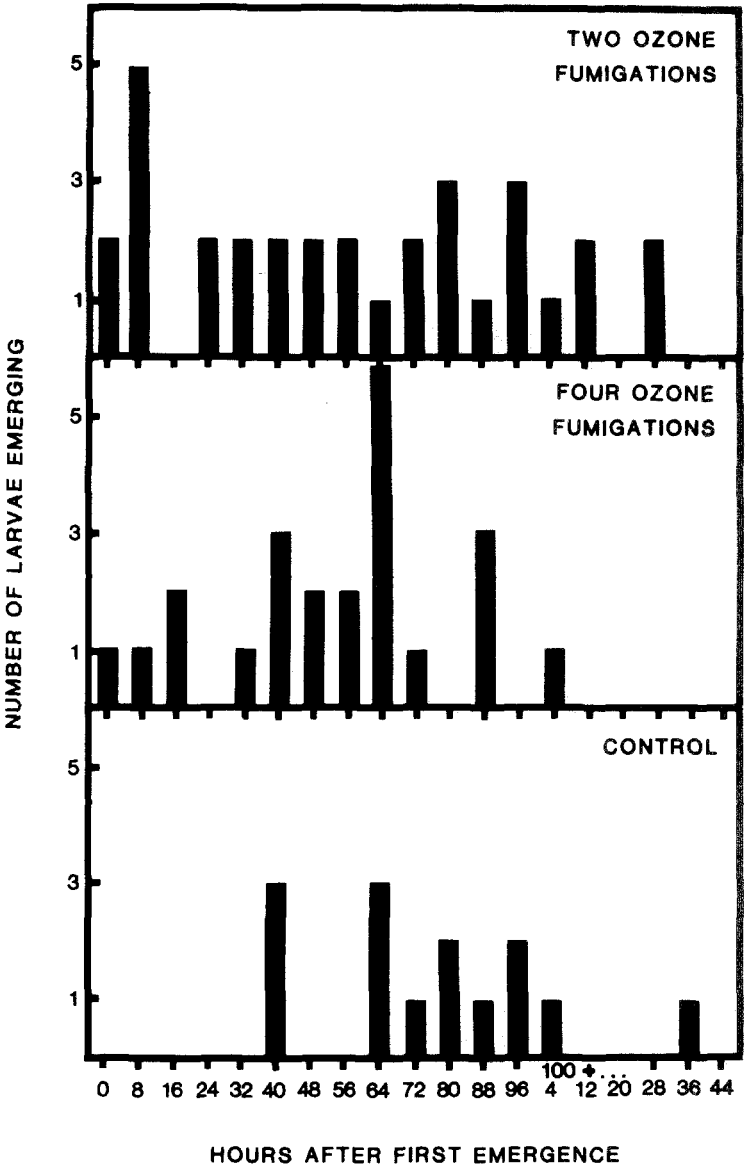


FIG. 1. Effects of ozone fumigation on time of emergence for larvae exiting ozonated and control foliage. Ozone fumigations in glasshouse chambers; dosage for each fumigation was 0.28 ppm.

rates of 1.3%, 7.1%, and 10.6%, respectively, as compared to expected larval developmental time predicted at 26°C by Lin and Trumble (1985). Although the observed increase in developmental rate may have occurred in response to ozone-induced stomatal closure in tomato foliage (which would elevate temperature through a decrease in evaporative cooling), Dugger et al. (1962) reported that ozone fumigation resulted in either no change or an increase in stomatal opening in beans.

If the observed effect of increased survival and developmental rate on ozonated plants proves applicable to other agricultural systems, two interesting implications for pest management programs become evident. First, programs dependent on developmental models driven by temperature will underestimate both stage of development and population size. To our knowledge, this effect has not yet been reported, but this manuscript reports the first results suggesting that the exposure of host plants to ozone is an important developmental factor for phytophagous insects. Also, those programs dependent on monitoring fruit and/or foliage for determination of threshold levels may require more frequent sampling (examples for the TPW include Wellik et al., 1979; and Wolfenbarger et al., 1975). Second, increased developmental rates may affect the success of biological control agents. For example, even though increased density of TPW may allow increases in the most common parasite species (Oatman et al., 1979), the time period for most successful parasitization would be shortened to less than the reported 48-hr period (2- to 3-day-old TPW at 26°C; Cardona and Oatman, 1971), thus decreasing the opportunity for parasites to utilize the most effective "window" for oviposition.

*Nitrogen, Free Amino Acid, and Chlorogenic Acid Analyses.* Since the biological effects of feeding on ozonated foliage were more evident for larvae than adults, we speculated that either the nutritional suitability of the foliage was improved for the neonate larvae as suggested for stressed plants by White (1984), or the plant's "defensive" system was being adversely affected. Analyses of total nitrogen content suggested that ozone-damaged foliage should be less nutritious than untreated leaves by one week following exposure to ozone fumigations (Table 3). However, total nitrogen analysis with the Kjeldahl method also includes nitrogen from structural proteins, nonprotein amino acids, and nitrogen incorporated in defensive compounds (Hare, 1983). Significant enhancement of soluble protein concentrations for up to two days postfumigation provided an increase in readily assimilable nitrogen available for the larvae, which suggests a plausible explanation for the observed improvement in larval survival. Similar increases in soluble protein concentrations have been documented for beans exposed to ozone (Beckerson and Hofstra, 1979), but reports in the literature have not been consistent (Ting and Mukerji, 1971).

Ten of the 20 amino acids quantified from ozonated foliage varied significantly from concentrations in control leaves (Table 4). Reductions in methionine and tyrosine immediately following fumigation were anticipated from ear-

TABLE 3. ANALYSES OF SOLUBLE PROTEINS AND TOTAL NITROGEN IN OZONATED AND CONTROL PLANTS AT SELECTED INTERVALS POSTFUMIGATION

| Days post fumigation | No. of fumigations | Fumigation interval (days) | Ozone (ppm) concentration | Soluble protein | | % Total nitrogen | |
|---|---|---|---|---|---|---|---|
| | | | | Ozone | Control | Ozone | Control |
| 0 | 4 | 3 | 0.18 | 14.699[a] | 12.025 | 5.483[b] | 5.190 |
| 2 | 2 | 3 | 0.28 | 6.638[c] | 6.592 | — | — |
| 7 | 4 | 3 | 0.18 | 7.766[b] | 7.352 | 5.481[d] | 5.957 |
| 14 | 4 | 3 | 0.18 | 4.701[b] | 4.835 | — | — |

[a] Significantly different from control plants at $P \leq 0.08$, $F_{(1, 10)} = 3.44$, based on two samples per plant and six plants per treatment.
[b] Concentrations were not significantly different from control plants.
[c] Significantly different from control plants at $P \leq 0.03$, $F_{(1, 8)} = 6.65$, based on two samples per plant and five plants per treatment; these data from a separate test, see text for details.
[d] Significantly different from control plants at $P \leq 0.03$, $F_{(1, 10)} = 6.66$, based on two samples per plant and six plants per treatment.

TABLE 4. COMPARISONS OF FREE AMINO ACID CONCENTRATIONS AT ZERO AND SEVEN DAYS POSTFUMIGATION IN OZONATED AND CONTROL PLANTS

| | 0 days postfumigation | | 7 days postfumigation | |
|---|---|---|---|---|
| Amino acid[a] | Ozone | Control | Ozone | Control |
| Alanine | 103.78 ** | 80.78 | 157.51 ** | 133.23 |
| Aspartic acid | 272.35 NS | 221.79 | 344.53 * | 305.95 |
| Glutamine | 39.58 ** | 92.79 | 383.84 NS | 353.24 |
| Glycine | 16.77 ** | 13.28 | 35.66 * | 27.80 |
| Histidine | 7.99 NS | 7.29 | 85.55 * | 68.72 |
| Methionine | 5.89 ** | 9.46 | 4.52 NS | 7.49 |
| Proline | 67.01 ** | 107.20 | 96.11 ** | 178.18 |
| Serine | 141.61 ** | 113.12 | 919.23 * | 442.88 |
| Tyrosine | 9.98 ** | 16.63 | 39.91 ** | 77.74 |
| Valine | 68.89 ** | 54.83 | 246.30 NS | 238.83 |

[a] Includes only those amino acids with substantial changes in concentration, amino acid concentration in $\mu$g/g. Horizontal comparisons within 0 or 7 days postfumigation are not significant (NS), significant at the $P \leq 0.1$ level (*), or significant at the $P \leq 0.05$ level (**) as noted.

lier reports (Chang, 1971b; Mudd and Freeman, 1977), but significant reductions in glutamine (57%) and proline (37%) had not been previously noted. Of the other amino acids which reportedly react with ozone, cysteine and cystine were only nominally present in tomato foliage, and histidine was not significantly affected. Unlike previous studies (Menzel, 1971; Craker and Starbuck, 1972), significant increases in the total pool of free amino acids were not detected. However, a trend toward higher concentrations of total free amino acids in ozone-treated plants was evident (ozonated plants = 1020.1 and 3310.5 $\mu$g/g; control plants = 968.6 and 2601.3 $\mu$g/g at 0 and 7 days postfumigation, respectively).

Comparisons between amino acid concentrations immediately following fumigation and concentrations at seven days postfumigation suggest an explanation for the variable reports of amino acid fluctuations in the literature. While proportions of some amino acids remained approximately the same on both sampling dates, the relative amounts of others, specifically serine, glutamine, histidine, and valine, increased considerably. Whether the tomato plant is stimulated to compensate for ozone-induced reductions in certain amino acids such as glutamine, or if the leaves are entering a premature senescence causing nitrogenous compounds to be converted to soluble forms available for reallocation, should be the focus for additional research. The general increase in free amino acid concentration in both ozonated and control plants over the course of the experiment probably reflects a general mobilization of energy reserves from the leaves to flowers or immature fruit (Ting, 1982). Regardless of the

cause of amino acid concentration differences between ozonated and control plants, time elapsed between ozone fumigation and leaf sampling can affect the quantities of amino acids, soluble protein, and total nitrogen measured.

The implications of ozone-induced changes in amino acid concentrations are potentially quite significant for insect populations. Of the 10 rat-essential amino acids generally considered necessary for insect growth (Dadd, 1973), only methionine and valine differed significantly in ozonated foliage. Since growth of TPW was not inhibited by the transient reduction in methionine concentration, and valine concentrations increased, changes in these amino acids were not limiting. Vanderzant (1966) reported that even relatively small changes in concentrations of free amino acids can have significant and negative effects on insect development if the amino acids compete for sites on the absorption system. House and Barlow (1964) documented the negative effects of increasing the amounts of amino acids in an artificial diet, and thereby altering the osmotic balance of the diet. However, since our data indicated: (1) the total pool of free amino acids was not significantly increased at either 0 or 7 days following ozone fumigation, (2) the increase in total free amino acids between day 0 and day 7 was greater than the increase between ozonated and control foliage, and (3) the concentrations of free amino acids in ozonated and control plants were similar to those used in successful artificial diets for other phytophagous lepidopterans (Vanderzant, 1957; Arai and Ito, 1964), neither competition for absorption sites nor changes in osmotic balance of the host would be likely to exert a negative effect in our system.

Potentially critical changes in free amino acid concentrations occurred in the key supplementary amino acids (terminology after Dadd, 1973), all of which increased significantly in ozonated foliage. Alanine, glycine, aspartic acid, and serine have been reported as important growth factors for the silkworm (Ito and Arai, 1966, 1967), while proline was documented as "semi-essential" for the silkworm (Ito and Arai, 1965) and critical to the development of many dipterans (Friend, 1968; Gingrich, 1964). Thus, since changes in concentrations of these amino acids were relatively major (up to 207% increase for serine), increased availability of supplementary amino acids probably accounts for at least some of the improved survival demonstrated for TPW fed on ozonated tomato foliage. None of the correlation coefficients between percent nitrogen, soluble protein, or total free amino acid concentration were significantly different from zero in either data set, suggesting that exposure to ozone affected these three aspects of plant nitrogen metabolism independently.

Since insect survival could also have been affected by the concentration of plant defensive chemicals, we considered which compounds would be most likely to be affected by ozone. Of the secondary plant compounds documented in commercial tomatoes which have proven antibiotic properties against lepidopterans, only chlorogenic acid has an "exposed" double bond susceptible to rapid oxidation by ozone (J. Kumamoto, personal communication). Ozone-in-

duced reductions in tyrosine would not effectively inhibit productivity of chlorogenic acid in tomatoes, since this amino acid is only an important precursor in monocotyledonous plants (Rhodes and Wooltorton, 1978). Since chlorogenic acid production can be an induced response, occurring after stress from either disease (Carrasco et al., 1978) or insects (Elliger et al., 1981), oxidation should prevent chlorogenic acid levels from increasing as long as contact with ozone is frequent. We found that chlorogenic acid levels did not differ significantly from controls either immediately following the last fumigation or at seven days postfumigation. However, by 14 days after the last fumigation, significant increases in chlorogenic acid concentration were observed in ozonated plants (ozone-treated plants = 31.1 $\mu$g/g fresh weight, control plants = 16.3 $\mu$g/g, ANOVA, $P \leq 0.05$). Therefore, although stress generated by ozone may induce chlorogenic acid production in tomato plants, benefits relating to insect control may be minimized by repetitive exposures and subsequent oxidation. Nonetheless, the increase in developmental rates and survival of TPW on ozonated foliage cannot be explained by suppression of chlorogenic acid production in our experiments.

## CONCLUSIONS

The observed increase in survival and developmental rates of TPW larvae feeding on ozonated tomato foliage was due to a complex of factors, the most important of which were nutritional. Concentrations of readily assimilable nitrogenous compounds such as soluble protein and important supplementary amino acids proved to be considerably better indicators of host suitability than total nitrogen analysis. Although chlorogenic acid production in tomatoes may be induced by exposure to ozone, the plant response occurred too late to effect the enhanced growth and survival of TPW and could therefore be eliminated as a primary cause for the observed effects.

Although our data show an increase in soluble nitrogenous compounds as predicted by White's (1984) hypothesis, this apparent agreement may be an artifact. White proposed that the breakdown of insoluble proteins to smaller, more soluble compounds was a general response to plant stress and adaptive to the extent that the increase in free amino acid concentration stimulated seed production during stressful periods. While this may be true for some stresses, (e.g., drought), we do not believe that our results necessarily reflect an adaptive response of plants to ozone damage. Rather, the increases in concentration of soluble nitrogen following ozone exposure are more likely to be simply the direct consequence of the chemical reactivity of ozone with plant proteins and amino acids (Craker and Starbuck, 1972; Mudd and Freeman, 1977).

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