The Role of Ethylene in the Development of Constant-light Injury of Potato and Tomato

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ABSTRACT. The role of ethylene in the development of constant-light injury of potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) was investigated. In one study, silver thiosulfate (STS) was applied to the foliage of four potato cultivars growing under constant light. Leaf area and shoot dry mass of 'Kennebec' and 'Superior', cultivars normally injured by constant light, were greater ($P < 0.05$) than those of control plants given foliar applications of distilled water. Examination of STS-treated 'Kennebec' leaflets revealed significantly less injury (necrotic spotting and reduced starch content) than the water-treated controls. 'Norland' and 'Denali', cultivars tolerant of constant light, exhibited no differences in growth between treatments. In a second study, injury (necrotic spotting and reduced starch content) was induced in leaflets of 'Denali' when exposed to spray applications of $0.5 \text{ mmol} \cdot \text{L}^{-1}$ ethephon or air containing 0.5 to $0.8 \mu\text{L} \cdot \text{L}^{-1}$ ethylene. In a third study, three genotypes of 'Ailsa Craig' tomato were grown under constant light. Leaves of the normal 'Ailsa Craig' exhibited epinasty, reduced chlorophyll concentration, and reduced starch content. Leaves of a mutant 'Ailsa Craig', containing the *Never ripe* mutation, did not exhibit epinasty but exhibited the same amount of reduced chlorophyll concentration and starch content as normal plants. Leaves of a transgenic 'Ailsa Craig', containing an antisense gene of 1-aminocyclopropane 1-carboxylate (ACC) oxidase, were epinastic, but chlorophyll concentration and starch content were greater than in leaves of normal and mutant plants. These results suggest that transgenic plants were more tolerant of constant light than the other genotypes. Evidence from these studies indicates that ethylene, combined with constant light, has an important role in the development of constant-light injury.

Certain cultivars of potato and tomato develop a physiological injury of the foliage when grown under constant light combined with a constant temperature. The injury first becomes evident as chlorosis and necrotic spotting on the adaxial surface of young, expanding leaves (Hillman, 1956; Kristoffersen, 1963; Wheeler and Tibbitts, 1986). In potato, these symptoms are preceded by the loss of photosynthetic competence and starch content in leaf tissue of a specific developmental age, that is, in tissue undergoing a transition from heterotrophic to autotrophic function (Cushman et al., 1995). The physiological basis of this disorder is not known, but the symptoms of constant-light injury appear to be similar to other stress-induced events associated with accelerated leaf senescence. In addition, a wide range of stresses, such as exposure to ozone, heavy metals, or chilling (reviewed by Abeles et al., 1992), cause senescence-like events in leaf tissue due to the production of endogenous ethylene. As with other ethylene-mediated senescence or senescence-like events in leaves, ethylene may influence the development of symptoms associated with constant-light injury.

Many chemicals have been used in the study of ethylene and plants. The compound 2-chloroethylphosphonic acid (ethephon)

is readily absorbed by plant tissue and converted to ethylene at a steady rate over a period of several days after application (Woodrow et al., 1988). Silver thiosulfate (STS) has long been used to extend the longevity of plants and flowers of ornamental value through the silver ion's capacity to render plant tissue insensitive to ethylene, thus delaying leaf and flower senescence (Beyer, 1976; Veen, 1983). STS is a noncompetitive inhibitor of endogenous ethylene production, whereas other compounds, such as 2,5-norbornadiene (Sisler and Pian, 1973) and 1-methylcyclopropene (Serek et al., 1994), act as competitive inhibitors.

In tomato, molecular approaches have been used to study ethylene biosynthesis and perception. Transgenic plants that contained antisense constructs of ACC synthase (Oeller et al., 1991) and ACC oxidase (Hamilton et al., 1990) exhibited reduced endogenous ethylene production. Leaf discs from transgenic plants containing antisense ACC oxidase released $\approx 10\%$ of the ethylene and showed $\approx 7\%$ of the ACC oxidase activity of those from normal plants (Hamilton et al., 1990). Transgenic plants that contained a bacterial gene encoding for the enzyme ACC deaminase also exhibited a marked reduction in endogenous ethylene production (Klee et al., 1991). Fruit ripening was greatly delayed with all three of these genetic approaches.

John et al. (1995) suggested that ethylene exerted only partial control over leaf senescence in tomato. Leaf senescence was delayed 10 to 14 d in transgenic plants that contained antisense ACC oxidase compared to normal plants. Once senescence began, however, photosynthetic function and chlorophyll content declined in leaves of transgenic plants at a rate equal to that of normal plants.

Tomato mutants have also been used in ethylene research. For example, plants of the *Never ripe* (*Nr*) mutation did not exhibit leaf epinasty, the triple response of seedlings grown in the dark, flower

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petal senescence, or accelerated fruit ripening when exposed to ethylene gas (Lanahan et al., 1994).

In this report we explore the role of ethylene in the development of injury in potato and tomato plants grown under constant light. In the first study we examined the effect of an ethylene-action inhibitor, STS, or water on injury development in four potato cultivars. In the second study we examined the effect of ethephon or ethylene gas on growth of a potato cultivar normally tolerant of constant-light conditions. In the third study we examined the development of injury symptoms and the release of endogenous ethylene in 'Ailsa Craig', a tomato cultivar injured by constant light (Kristoffersen, 1963) and available with mutant and transgenic genotypes affecting ethylene perception and metabolism.

Materials and Methods

PLANT MATERIAL AND GROWTH CONDITIONS. Four cultivars of potato and three genotypes of 'Ailsa Craig' tomato were used. Tissue-cultured potato plantlets of two constant-light sensitive cultivars—Kennebec and Superior—and two constant-light tolerant cultivars—Denali and Norland—were sterile-propagated from stem cuttings in a tissue culture room under continuous lighting of $\approx 60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux (PPF) and constant 20 °C (Tibbitts et al., 1994). Tomato seedlings of 'Ailsa Craig' normal, *Nr* mutant (Lanahan et al., 1994), and antisense ACC oxidase transgenic (Hamilton et al., 1990) plants were propagated from seed in the laboratory in a tray of moist potting medium on a lab bench under $\approx 50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF.

When tissue-cultured potato plantlets were 21 d old or tomato seedlings were 10 d old, uniform plants were selected and planted into 1.8-L (15-cm-diameter) pots that contained 1 peat : 1 vermiculite (by volume) (Redi-Earth, Horticultural Products, Milpitas, Calif.) and then covered with inverted clear-plastic cups for 3 d to prevent desiccation and aid establishment. A complete nutrient solution (Hammer et al., 1978), pH 5.0 to 5.5, was delivered automatically to each pot four times daily to excess. Walk-in growth chambers were used in these studies. PPF was $370 \pm 60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from cool-white fluorescent lamps (measured at the top of the plant canopy), relative humidity (RH) was 70%, and air temperature was 18 °C for potato studies and 23 °C for tomato studies. Temperature, RH, and light-dark periods were recorded electronically every 15 min to ensure that environmental conditions were maintained. PPF was measured with a quantum sensor (LI-190; LI-COR, Lincoln, Nebr.) at the beginning of each experiment, and treatments in each experiment were blocked according to PPF level in a randomized complete-block design. Data were analyzed by using the analysis of variance procedure, and residuals were plotted using the general linear model procedure to confirm homogeneity of variances (SAS Institute, Cary, N.C.). Mean separation was determined by least significant difference (LSD).

PLANT ANALYSES. Chlorophyll was extracted from tomato leaves after determining leaflet area. Leaflets were put into test tubes to which 15 mL of chilled 100% ethanol was added. The stoppered tubes were then kept at 5 °C for 24 h. The ethanol was decanted into flasks and replaced with an equal volume of ethanol. Chilled ethanol was used the first 24 h to avoid starch breakdown in tissue that may have remained alive during the first hours of extraction. The tubes were then placed on a shaker for 24 h at room temperature, the ethanol was decanted and replaced, and the procedure was repeated once more for a total of three extractions. The extract was filtered through qualitative filter paper and brought to a final volume of 75 to 125 mL, depending on the size of the leaflets. Absorbance at 649 and 665 nm was measured with a spectropho-

tometer (DU-50; Beckman, Fullerton, Calif.). Chlorophyll concentration was determined using algorithms reported by Knudson et al. (1977). Due to large differences in the amount of injury between treatments and, thus, large variations in leaf starch content between treatments, chlorophyll concentration was expressed based on leaf area rather than mass. Chlorophyll was also extracted from potato leaflets, but only for the purpose of revealing injury in leaflets. Potato leaflets were cleared of pigments in boiling 70% ethanol for 2 min and then kept at room temperature in 70% ethanol overnight (Cushman et al., 1995).

Tomato and potato leaflets, cleared of pigments, were then stained for starch content with a solution of iodine-potassium iodide to reveal areas of reduced starch content. In tomato leaflets, relative leaflet area that exhibited reduced starch content was visually estimated using leaflets pictured in Fig. 5c as standards, representing, from left to right, 100%, 75%, 50%, 25%, and 0% of the leaflet area with reduced starch content. In potato leaflets, relative leaflet area exhibiting necrotic spotting and area devoid of starch content was visually estimated to the nearest 10%.

Ethylene release from tomato leaflets was determined as described by John et al. (1995). Each leaflet was placed in a separate test tube, 15-cm high $\times$ 1.9 cm in diameter, containing 0.5 to 1.0 mL of sterile distilled water so that the cut end of the petiole was in water. After 6 h under $\approx 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF and 25 °C, rubber stoppers were used to seal the test tubes. Ethylene was allowed to accumulate for 4 h for leaflets grown under constant light or 6 h for leaflets grown under a 16-h photoperiod before a 1-mL sample volume was withdrawn for analysis. Ethylene was quantified with a gas chromatograph (GC) (GC-9AM; Shimadzu, Columbia, Md.) equipped with a 1.8-m $\times$ 3.2-mm porapak Q column (Supelco, Bellefonte, Penn.) and a flame-ionization detector. GC settings were 40 °C in the oven, 40 mL $\cdot$ min $^{-1}$ N $_2$ carrier gas flow rate, and 390 kPa carrier gas primary pressure.

Leaf area was measured with an area meter (LI-3100), and shoot dry mass was determined immediately after drying 2 d at 60 °C in a forced-air drying oven.

APPLYING STS TO POTATO. Spray solutions of STS were prepared from stock solutions immediately before each application. Stock solutions of sodium thiosulfate and silver nitrate were mixed in a 4:1 molar ratio to obtain a silver ion concentration of 0.5 mmol $\cdot$ L $^{-1}$ in the final spray solution. Distilled water was included as a separate treatment in all experiments. A 19-L spray chamber was located in the growth chamber where each experiment was conducted, and one plant at a time was sprayed from above through a hole in the top of the chamber. A volume of 1 mL was applied as a fine mist from an artist's air brush (model 200 GXF; Badger Air Brush, Franklin Park, Ill.) operating at 120 kPa. Plastic film was positioned around the base of each plant to prevent spray mist from contacting the growing medium. Excess mist in the spray chamber was exhausted from the growing area for 2 min before the plant was returned to its original position in the growth chamber. To prevent CO $_2$ enrichment of the growing environment, the applicator's exhalation was also vented from the growth chamber. Control plants that were unsprayed and undisturbed were included in each experiment.

To determine the effect of STS on plant growth under constant light, four separate and consecutive experiments, one for each of the four potato cultivars under investigation, were conducted with four single-plant replications of treatments and controls. Plants were grown under constant light from the time the tissue-cultured plantlets were transplanted, and distilled water or STS was applied 4, 7, 11, 14, and 18 d after transplant. The development of necrotic spotting was recorded daily. Plants were harvested 22 d after

transplant for leaf area and shoot dry mass determinations.

To determine the effects of STS on injury development in expanding leaves, two additional experiments were conducted with 'Kennebec' and 'Denali' plants established for 10 d under a 12-h light and 12-h dark photoperiod and then exposed to constant light. In the first experiment, spray applications of STS or distilled water were made on days 0, 2, and 4 of constant light, and plants were harvested on days 5, 6, or 7 of constant light. An additional spray application was made on day 6 to plants harvested on day 7. In the second experiment, spray applications of STS or distilled water were made on days 0, 2, 4, and 6 of constant light, and plants were harvested on days 7, 8, or 9 of constant light. An additional spray application was made on day 8 to plants harvested on day 9. Taken together, the two experiments provided leaf tissue over a 5-d period of injury development, from the fifth to the ninth day of constant light. Relative leaflet area that exhibited necrotic spotting or reduced starch content was determined from terminal leaflets that had been 0.5 to 1.0 mm long on the first day of constant light. Several leaflets that were not used for necrosis and starch determinations were examined for evidence of tissue toxicity to STS. Leaflets from STS- and water-sprayed plants were cleared of pigments in ethanol, placed in lactic acid, and examined under the light microscope (Cushman et al., 1995). There were four single-plant replications of treatments and unsprayed controls in both experiments.

APPLYING ETHEPHON TO POTATO. A foliar application of ethephon (Rhône-Poulenc, Research Triangle Park, N.C.) was used to encourage the development of injury in the tolerant 'Denali'. Plants were established for 8 d under a 12-h light and 12-h dark photoperiod and then exposed to constant light for the remainder of the experiment. A solution of 0.5 or 1.0 mmol·L⁻¹ ethephon or distilled water was applied on days 0, 3, 6, and 9 of constant light using the spray procedure described above. Terminal leaflets were removed from plants on day 15 of constant light, cleared of pigments to reveal necrotic spotting, and stained for starch content as described. The experiment included three single-plant replications of treatments and controls.

APPLYING ETHYLENE TO POTATO. Ethylene gas was used to gain a more precise control of ethylene exposure of 'Denali' plants than could be obtained with ethephon. Plants were established for 10 d under a 16-h light and 8-h dark photoperiod and then grown under constant light for the rest of the experiment. On day 5 of constant light, nine plants were transferred to a 0.6 × 0.8 × 0.6 m (W × H × D) acrylic chamber that was positioned within an adjacent growth chamber. The plants were then exposed to air containing 0.5 to 0.8 µL·L⁻¹ ethylene. Ethylene concentrations of ≈1.0 µL·L⁻¹ have been used in other studies investigating ethylene-induced leaf senescence (Bleecker et al., 1988). Ethylene-enriched air was drawn through the chamber at a constant rate of ≈30 L·min⁻¹, and a 10-cm-diameter muffin fan circulated air within the chamber. Ethylene enrichment was achieved by exposing the incoming airstream to a 3-m section of flexible polyvinylchloride tubing (4.8 mm ID and 1.6 mm wall thickness) that was attached to a 2-L Erlenmeyer flask containing ethylene gas (99.5% ethylene, Scott Specialty Gases, Troy, Mich.). Ethylene diffused through the tubing wall and into the airstream. A 5-mL sample of the airstream was analyzed daily to ensure that desired ethylene concentrations were maintained. Plants in the exposure chamber received light, temperature, RH, and nutrient delivery as with the previous growing conditions.

After 4 d in the exposure chamber, plants were removed and returned to the original growth chamber for another 4 d. Preliminary experiments had determined that 3 to 4 d of ethylene exposure was sufficient to induce injury in 'Denali'. The postexposure

period of 4 d was provided to permit leaves to recover fully from epinasty induced while in the exposure chamber and for the leaves to become fully exposed to the light environment, ensuring that uninjured leaf tissue would contain starch. The additional 4 d also ensured that areas of reduced starch content were not induced temporarily but truly represented injured tissue. Terminal leaflets were then removed from each of the nine plants, cleared of pigments in ethanol, and stained for starch content as described. An identical experiment, but without ethylene enrichment within the exposure chamber, was conducted immediately after the conclusion of the first experiment.

EVALUATING INJURY TO TOMATO. Tomato plants of the three genotypes were grown from the time of transplant under constant light or under a 16-h light and 8-h dark photoperiod. There were six single-plant replications of each genotype. Leaflets were removed from plants of each genotype for chlorophyll and starch determinations. Counting the leaf directly above the cotyledons as one, one or two lateral leaflets were removed on days 10 or 15 of each experiment from leaf three, four, or five, depending on the size of the plant. Chlorophyll concentration and leaflet area exhibiting reduced starch content in leaflets of plants grown under constant light were expressed as a percentage of leaflets of plants grown under the noninjurious 16-h photoperiod.

To determine the rate of ethylene release from tomato leaves, seed of each genotype was planted directly into separate pots that contained moist medium, and the pots were immediately placed in a growth chamber under a 12-h light and 12-h dark photoperiod. Seedlings were thinned to one plant per pot 15 d after planting and the photoperiod was changed to constant light 22 d after planting. On day 29 of constant light, three leaflets on each of two plants of each genotype were removed from the middle portion of the plant for determination of ethylene release. The photoperiod was then changed to 12 h light and 12 h dark, and after 7 d of this noninjurious photoperiod, leaflets were again removed from plants for ethylene determinations.

Results

APPLYING STS TO POTATO. 'Kennebec' and 'Superior' plants grown under constant light produced significantly greater leaf area when treated with STS than with water (Fig. 1). Significant

Fig. 1. Effect of spray applications of an ethylene-action inhibitor, silver thiosulfate (solid bars), or distilled water (open bars) on leaf area of potato plants grown under constant light. Untreated control plants are represented by gray bars. 'Superior' and 'Kennebec' are sensitive to constant light, and 'Norland' and 'Denali' are tolerant of constant light. Means of four plants ±SE are shown. Mean separation for spray treatments was by LSD, $P \leq 0.05$. Bars without letters are not significantly different.

Fig. 2. Injury symptoms in terminal leaflets of 'Kennebec' potato plants grown under constant light. Relative leaflet area exhibiting necrotic spotting (a) and reduced starch content (b) are shown. Plants were treated with silver thiosulfate (filled circles) or distilled water (hollow circles). Means of four leaflets from separate plants $\pm$ SE are shown.

differences in shoot dry mass were identical to those of leaf area, therefore these data are not shown. In contrast, 'Denali' and 'Norland' plants grew without injury and produced similar leaf area (Fig. 1) and shoot dry mass (data not shown) regardless of treatment. In a more detailed study, leaflets from STS-treated 'Kennebec' plants exhibited far less injury, measured as reduced starch content and necrotic spotting, compared to leaflets from water-treated plants (Fig. 2a and b). STS- and water-treated 'Denali' leaflets did not exhibit reduced starch content or necrotic spotting.

When examined under a light microscope for visible evidence of STS toxicity, a few necrotic epidermal cells were evident on the adaxial surface of STS-treated 'Kennebec' and 'Denali' leaflets. These necrotic cells were most often located at the base of hair-like trichomes and may have developed due to an accumulation of spray droplets on the structure and a high concentration of STS at the base. In a survey of water-treated leaflets, necrotic epidermal cells were not observed at any time in either cultivar.

APPLYING ETHEPHON TO POTATO. Applications of ethephon at $0.5 \text{ mmol} \cdot \text{L}^{-1}$ induced mild chlorosis and reduced starch content in leaflets of the tolerant 'Denali' (Fig. 3a). Applications of ethephon at $1.0 \text{ mmol} \cdot \text{L}^{-1}$ also induced epinasty, necrotic spotting, and severely stunted growth (data not shown). Epinasty was not induced on any of the leaves or leaflets to which $0.5 \text{ mmol} \cdot \text{L}^{-1}$ ethephon was applied. Injury symptoms developed in a pattern somewhat typical of constant-light injury, with injury occurring toward the base of older leaflets and the apex of younger leaflets, but the pattern was not as well defined as reported for leaflets of 'Kennebec' plants grown under constant light (Cushman et al., 1995). Regardless of concentration, ethephon treatment caused young leaves of the apical region of all 'Denali' plants to darken, apparently by increasing anthocyanin pigmentation of the leaf

tissue. Ethephon also caused axillary shoots to elongate beyond that of control plants. None of these symptoms developed in leaflets of water-treated plants, and starch loss did not occur in these leaflets (Fig. 3b).

APPLYING ETHYLENE TO POTATO. Injury symptoms were induced in the leaflets of 'Denali' plants exposed to 0.5 to $0.8 \text{ } \mu\text{L} \cdot \text{L}^{-1}$ ethylene gas. Necrosis (Fig. 4a) and reduced starch content (Fig. 4b) developed to a greater extent than in plants to which ethephon was applied, and leaves became epinastic during the latter 2 d of the 4-d ethylene exposure. Control plants, grown under identical conditions but without ethylene, exhibited no epinasty or necrotic spotting, and two out of nine plants exhibited a minimum amount of reduced starch content (Fig. 4c).

EVALUATING INJURY TO TOMATO. Young leaves on normal and transgenic 'Ailsa Craig' tomato plants exhibited leaf and petiole epinasty when grown under constant light (Fig. 5a). Leaves often twisted into a corkscrew configuration but then almost completely recovered from epinasty as they expanded fully. Leaves on mutant plants seldom became epinastic under constant-light conditions. Epinasty did not develop on any of the three genotypes when grown under a noninjurious 16-h photoperiod.

All three 'Ailsa Craig' genotypes exhibited interveinal chlorosis and reduced starch content when grown under constant light. Leaves on mutant and transgenic plants appeared to exhibit less chlorosis than leaves on normal plants (Fig. 5b), but reductions in

Fig. 3. Injury symptoms in 'Denali' potato plants grown under constant light. Shown are terminal leaflets removed from three adjacent leaves and stained for starch content. Ethephon (a) or distilled water (b) was sprayed on plants during growth. Light-colored areas indicate greatly reduced starch content. 'Denali' plants normally are not injured by constant light.

Discussion

Our results with STS, a well-known inhibitor of ethylene action, provide evidence that ethylene has an important causal role in the development of constant-light injury in potato. Large reductions in injury were achieved by reducing leaf-tissue sensitivity to ethylene. Ethylene, therefore, appears to be necessary for the development of injury symptoms in constant-light sensitive potato cultivars such as 'Kennebec' and 'Superior'. STS did not adversely affect growth and leaf expansion of the constant-light tolerant 'Denali' and 'Norland' potato, indicating that the concentration of STS used in this study was adequate to control ethylene sensitivity, but not excessive to cause altered plant growth.

Fig. 4. Injury symptoms in 'Denali' potato plants grown under constant light. Shown are terminal leaflets removed from five adjacent leaves. Necrosis (a) and reduced starch content (b) were induced in leaflets after a 4-d exposure to 0.5 to 0.8 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene. (c) Leaflets from a control plant are shown. This plant exhibited more injury than any other control plant. Light-colored areas in b and c indicate greatly reduced starch content. Areas of reduced starch content at the base of leaflets in c are due to shading from overlapping lateral leaflets.

chlorophyll concentration (Fig. 6a) were statistically significant only for transgenic plants. Reductions in starch content (Fig. 6b) were also statistically significant only for transgenic plants. Patterns of interveinal chlorosis were often similar to patterns of reduced starch content (Fig. 5 b and c). A few plants developed necrotic spotting, but when it occurred, it developed only on leaves of normal plants. Chlorosis, reduced starch content, and necrotic spotting did not develop in any of the plants grown under a noninjurious 16-h photoperiod.

Leaflets from normal and mutant plants released more ethylene when grown under constant-light conditions than when grown under noninjurious conditions (Fig. 7). In contrast, transgenic plants released relatively little ethylene under either light environment.

Fig. 5. Injury symptoms in three genotypes of 'Ailsa Craig' tomato grown under constant light. (a) Young leaves of normal plants that exhibited epinasty. (b) Pairs of lateral leaflets from transgenic (left), normal (center), or mutant (right) plants that showed differences in interveinal chlorosis. (c) Relative leaflet area that exhibited reduced starch content in lateral leaflets of normal plants ranging from $\approx 100\%$ (left) to $\approx 0\%$ (right). Light-colored areas indicate greatly reduced starch content.

Fig. 6. Injury in three genotypes of 'Ailsa Craig' tomato after 20 d of growth under constant light. Chlorophyll concentration (a) and relative leaflet area that exhibited reduced starch content (b) are expressed as a percentage of control plants of each of the three genotypes grown under a noninjurious 16-h light and 8-h dark photoperiod. Means of six leaflets from separate plants $\pm$ SE are shown. Mean separation was by LSD, $P \leq 0.05$.

Our results also show that ethylene, applied either as a gas or an ethylene-releasing compound, induced injury symptoms in a potato cultivar normally tolerant of constant-light conditions. Ethylene, combined with constant light, was sufficient to induce necrotic spotting and areas of reduced starch content in 'Denali' leaflets in a pattern typical of injury in sensitive cultivars. These results are consistent with ethylene having a causal role in the development of this disorder.

Although transgenic tomato plants exhibited a similar degree of epinasty as normal plants, transgenic plants were less injured by constant light than normal or mutant plants. Transgenic plants may have been less injured as a result of greatly reduced endogenous

Fig. 7. Ethylene release from leaflets of three genotypes of 'Ailsa Craig' tomato. Plants were grown under constant-light conditions (gray bars) or a noninjurious 12-h light and 12-h dark photoperiod (black bars). Means of six leaflets $\pm$ SE are shown.

ethylene production under constant-light conditions. It has been suggested, however, that the quantity of ethylene produced by transgenic plants is still sufficient to be physiologically significant (Picton et al., 1993); therefore, this reduced quantity of ethylene may have been sufficient to induce some injury. Ethylene produced by plants of the other genotypes may have also contributed to injury in transgenic plants because all three genotypes were grown together in the same chamber. John et al. (1995) reported that differences in the initiation of leaf senescence were not as great when transgenic and normal plants were grown in the same growth chamber as when grown in separate chambers.

Plants of the *Nr* mutant exhibited some of the symptoms associated with constant-light injury, such as chlorosis and reduced starch content, but not other symptoms, such as leaf and petiole epinasty. The *Nr* mutant has been reported to be insensitive to many ethylene-mediated responses, and leaves of the *Nr* mutant do not become epinastic in the presence of ethylene. Instead, leaves become epinastic upon application of 2,4-D, a compound with auxin activity (Lanahan et al., 1994). Therefore, the lack of epinasty in mutant plants and the development of epinasty in normal and transgenic plants suggest that ethylene, and not auxin, induces the epinasty of tomato leaves observed under constant-light conditions. The fact that all other injury symptoms were almost as severe in the mutant as in normal plants, however, confounds the role of ethylene in the development of injury in the mutant. It is possible that our results with the mutant genotype indicate that epinasty is induced by the same causal factors as other symptoms of constant-light injury but is controlled by a different mechanism, a mechanism affected by this particular mutation.

In conclusion, results from these studies appear to indicate that ethylene is necessary and, combined with constant light, sufficient to cause the senescence-like symptoms associated with constant-light injury. Applying ethylene, through liquid sprays and gas exposure, and STS, an ethylene-action inhibitor, provides the most convincing evidence to support this conclusion. Our comparisons of tomato genotypes that have varied ethylene metabolism and perception tend also to support the role of ethylene in the development of this injury.

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