Plant Physiol. (1981) 67, 120–124
0032-0889/81/67/0120/05/$00.50/0

Photosynthesis, Growth, and Ultraviolet Irradiance Absorbance of *Cucurbita pepo* L. Leaves Exposed to Ultraviolet-B Radiation (280-315 nm)[1]

Received for publication July 30, 1979 and in revised form August 11, 1980

WILLIAM B. SISSON
Jornada Experimental Range, United States Department of Agriculture, Science and Education Administration-Agricultural Research, Department of Animal and Range Science, New Mexico State University, Las Cruces, New Mexico 88001

ABSTRACT

Net photosynthesis, growth, and ultraviolet (UV) radiation absorbance were determined for the first leaf of *Cucurbita pepo* L. exposed to two levels of UV-B irradiation and a UV-B radiation-free control treatment. Absorbance by extracted flavonoid pigments and other UV-B radiation-absorbing compounds from the first leaves increased with time and level of UV-B radiation impinging on leaf surfaces. Although absorbance of UV-B radiation by extracted pigments increased substantially, UV-B radiation attenuation apparently was insufficient to protect completely the photosynthetic apparatus or leaf growth processes. Leaf expansion was repressed by daily exposure to 1365 Joules per meter per day of biologically effective UV-B radiation but not by exposure to 660 Joules per meter per day. Photosynthesis measured through ontogenesis of the first leaf was depressed by both UV-B radiation treatments. Repression of photosynthesis by UV-B radiation was especially evident during the ontogenetic period of maximum photosynthetic activity.

The lower wavelength of global irradiance is primarily a function of attenuation by ozone and, therefore, the ozone concentration in the atmosphere through which extraterrestrial UV radiation must pass prior to impinging upon the earth's surface. A reduction in atmospheric ozone concentration, as might occur through interactions with halocarbons (6, 13), would result in an ozone concentration-dependent shift from the approximate wavelength of 295 nm to shorter wavelengths within the UV-B[2] radiation waveband (280-315 nm) (11). Action spectra for this waveband are in general agreement that an exponential increase in biological effectiveness occurs from about 314 nm to wavelengths shorter than 280 nm (4, 10). The principal chromophores involved are believed to be nucleic acids and proteins (10). Thus, even a small increase in UV-B irradiance could be of significance to plant physiological processes.

The transmittance of UV-B radiation through plant leaf epidermis is generally less than 10% (14). Even with this low transmittance, several physiological processes are inhibited by plant exposure to UV-B radiation simulating various atmospheric ozone concentrations. Photosynthesis (16, 17), component reactions of photosynthesis (3), cell division rates (8), Chl concentrations (1), and plant growth (2) have been shown to be inhibited by exposure of plants to supplemental UV-B radiation. The degree of UV-B radiation-mediated inhibition appears to be species-dependent (7) and a function of the amount of UV-B radiation that penetrates plant leaves (14) and is absorbed by sensitive physiological molecules, such as proteins and nucleic acids.

Wellman (19) has shown that UV radiation induces flavonoid biosynthesis in parsley. Thus, it could be hypothesized that flavonoids and other UV-B radiation-absorbing compounds are synthesized according to the level of UV-B radiation to which plant leaves are exposed. The initial objective here was to quantify this relationship by determining the UV-B radiation absorptive properties of the flavonoids and other UV-B radiation-absorbing compounds of *Cucurbita pepo* L. leaves exposed to two levels of polychromatic UV-B radiation and a UV-B radiation-free control. The second objective was to determine if reciprocity is exhibited for photosynthesis and leaf growth of *C. pepo* exposed to UV-B radiation.

MATERIALS AND METHODS

Net photosynthetic rates of single leaves were determined with an open gas circulation system incorporating a Beckman model 365[3] IR gas analyzer. Air and leaf temperatures within the gas-exchange cuvette were measured with fine-wire (copper-constantan) thermocouples. Photosynthetically active photon flux density (PAR; 400–700 nm) was continuously monitored within the cuvette at the leaf surface with a LI-COR, Inc., model LI-190SR quantum sensor. One 1,500-w Westinghouse tungsten-halogen lamp provided a photon flux density of 850 μE m^{-2} s^{-1} PAR for all photosynthetic determinations. Radiation emitted by this lamp was filtered through 3 cm water to absorb longer wavelength IR radiation and reduce the radiant heat load within the gas-exchange cuvette. Fluctuations in water vapor concentrations occurring within the cuvette were measured with Cambridge model 880 dew-point hygrometers. The flow rate of air through the cuvette was constantly monitored by a Validyne Corp. model MP45 differential pressure transducer and CD 18 carrier demodulator. A Hewlett-Packard model 3052A data acquisition system scanned each instrument several times every 10 min, and the mean values obtained were used in all calculations. Leaf areas were measured with a LI-COR, Inc., LI-3000 portable leaf-area meter.

[1] This research was supported, in part, by the Environmental Protection Agency.

[2] Biologically effective UV-B radiation (UV-B_{BE}) = $E_\lambda I_\lambda D_\lambda$, where E_λ represents the relative energy effectiveness for each wavelength (λ) as defined by the generalized plant action spectrum (5) and I_λ represents the spectral irradiance (mw m^{-2} nm^{-1}) at each wavelength.

[3] The use of trade names does not constitute an official endorsement or approval by the United States Department of Agriculture.

Photosynthetic rates are expressed on a leaf-area (one-side) basis. All photosynthetic rate determinations were made with air temperatures within the cuvette maintained at 26 C (±1 C).

Two experimental procedures were followed to assess the responses of *C. pepo* L. to UV-B radiation. In the first experiment, two UV-B radiation treatments (Fig. 1, curves A and B), and a control treatment (Fig. 1, curve C) with no UV-B radiation below 314 nm were used. Supplemental UV-B radiation was provided by two Westinghouse FS 40 "sunlamps" filtered by either 0.10-mm (treatment A; Fig. 1, curve A) or 0.38-mm (treatment B; Fig. 1, curve B) cellulose acetate. The control treatment also utilized the same lamp configuration as the UV-B radiation treatments, but the lamps were filtered by Mylar type S (0.24-mm) polyester film which absorbs all UV-B radiation emitted by the sunlamps below 314 nm. The lamp configuration used for all treatments followed the techniques described previously by Sisson and Caldwell (15). The cellulose acetate filters were replaced every 2 days to maintain the desired spectral irradiance with filters pre-exposed to the sunlamps for 8 h. The Mylar type S filters were replaced every other week. The same first leaves were used throughout the experimental period for photosynthesis and growth analysis. Plants were exposed to treatment conditions from the time the seeds were sown in the soil medium.

The spectral irradiance for each UV-B radiation treatment was weighted for biological effectiveness by the procedure outlined by Sisson and Caldwell (17). Thus, the biologically effective UV-B irradiance ($UV\text{-}B_{BE}$) represents the integrated value of the spectral irradiance of the UV-B radiation treatments (Fig. 1) weighted by the generalized plant action spectrum for UV-B damage to plants as defined by Caldwell (5). As pointed out by Caldwell (4), this action spectrum probably relates to effects where nucleic acid and protein chromophores are involved since it closely approximates the absorption spectra of nucleic acids and proteins (10). Although it may not define precisely the action spectra for plant leaf growth and photosynthesis, which is presently unknown, it does provide a useful quantification for dose-response studies involving polychromatic UV-B radiation.

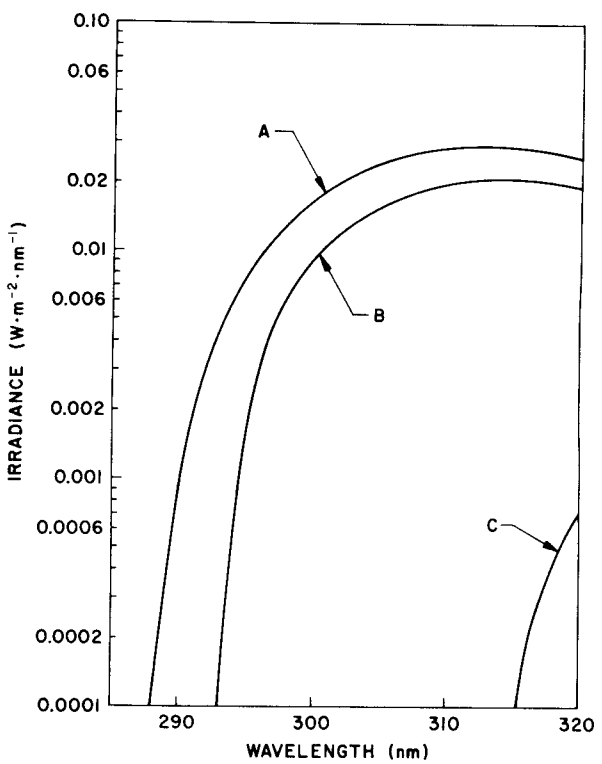

FIG. 1. Spectroradiometric measurements taken in a greenhouse during a cloudless period with the sun at an angle of 20° from the zenith for: A, two Westinghouse FS-40 sunlamps, each filtered by one layer of 0.10-mm cellulose acetate plastic film; B, two FS-4 sunlamps, each filtered with one layer of 0.38-mm cellulose acetate film; and C, two FS-40 sunlamps, each filtered with one layer of Mylar type S (0.24 mm) polyester film. Visible irradiation was 1,940 μE m^{-2} s^{-1} (400–700 nm) during the measurement period.

The $UV\text{-}B_{BE}$ for treatment A (Fig. 1, curve A) was 54.2 mw m^{-2} $UV\text{-}B_{BE}$ and that for treatment B (Fig. 1, curve B) was 26.2 mw m^{-2} $UV\text{-}B_{BE}$. The lamp/filter system was used daily for a 7-h period centered around solar noon and produced a daily biologically effective UV-B irradiance of 1,365 and 660 J m^{-2} day^{-1} for treatments A and B, respectively. These same values for the control were zero since the generalized action spectrum for plants does not include wavelengths above 313 nm.

In the second experiment, the photosynthetic responses of the first leaves of *C. pepo* were assessed after they were 13 days old and fully expanded. The lamp/filter systems used, as well as the daily period of supplemented UV-B radiation, were the same as described above. However, the levels of UV-B irradiance tested were 22.8 (575 J m^{-2} day^{-1}), 37.5 (945 J m^{-2} day^{-1}), and 53.5 (1350 J m^{-2} day^{-1}) mw m^{-2} $UV\text{-}B_{BE}$. The spectral irradiance of each treatment was similar to those illustrated in Figure 1 with the cellulose acetate-filtered lamps adjusted vertically and above the first leaf to achieve the three $UV\text{-}B_{BE}$ levels. A control treatment as described above was included for comparative analysis. Photosynthetic rates of the control plants were determined daily during this 8-day experiment for comparative purposes.

Spectral irradiance measurements were made at solar noon prior to treatment initiation and at least twice a week thereafter with an Optronics Laboratory model 721 spectroradiometer interfaced to a Hewlett-Packard model 9825A computer. Daily UV-B radiation measurements were made with an Optronics Laboratory model 25 IRL radiometer.

C. pepo seeds were germinated in 15- × 15-cm pots containing "Jiffy-7 plus" medium and maintained throughout the experimental period in a Fiberglas greenhouse. Since the first leaf was used for all analyses, plants were selected for treatment on the basis of uniformity of initiation of visible growth (experiment 1) or uniformity of size and time of initiation of first leaf (experiment 2). The first leaf was held at normal incidence to the UV-B radiation sources by white string for the duration of all experiments. Temperatures in the greenhouse fluctuated between 37 C (day) and 20 C (night). Maximum PAR during solar noon was approximately 2000 μE m^{-2} s^{-1} (400–700 nm) in the greenhouse.

The extraction of flavonoids and other UV radiation-absorbing compounds from fresh leaf samples followed the procedures of Caldwell (4). This experiment was replicated three times, and the results of a representative analysis are presented. A Beckman model DU spectrophotometer was used to determine the *A* of the extracts between 270 and 400 nm.

RESULTS AND DISCUSSION

First-leaf areas of *C. pepo* L. exposed to two UV-B radiation treatments (treatments A and B were 26.2 and 54.2 mw m^{-2} $UV\text{-}B_{BE}$, respectively; Fig. 1) and a UV-B radiation-free control are shown in Figure 2. There were no significant differences ($P < 0.05$) between treatment B and the control leaf areas. Leaves exposed to treatment A were significantly smaller in area ($P < 0.05$) than the control leaves after 1 day treatment (Fig. 2). There were no significant differences ($P < 0.05$) between areas of leaves receiving treatments A and B until the leaves were 4 days old. When the first leaf was fully expanded after 11 days, treatment A $UV\text{-}B_{BE}$ (54.2 mw m^{-2}) resulted in an 11% reduction in total leaf area when compared to the control.

The ontogenetic course of net photosynthesis in the first leaves of *C. pepo* exposed to the two UV-B treatments and a control was

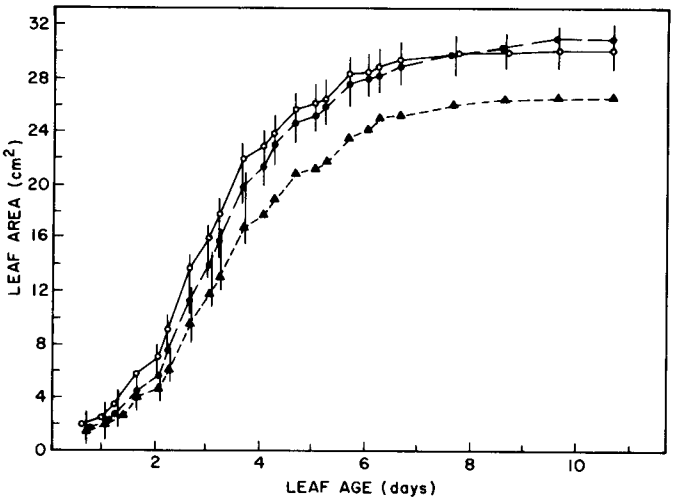

FIG. 2. Leaf area for the first leaf of *C. pepo* L. during 11 days exposure to 1,365 J m^{-2} day^{-1} UV-B_{BE} (▲-----▲), 660 J m^{-2} day^{-1} UV-B_{BE} (●---●), and a control treatment (○——○). Each point represents the mean of five or six replicates. Mean values not connected by vertical bars are significantly different ($P < 0.05$) for that day. Standard error (±) of the means ranged from 0.24 to 1.82.

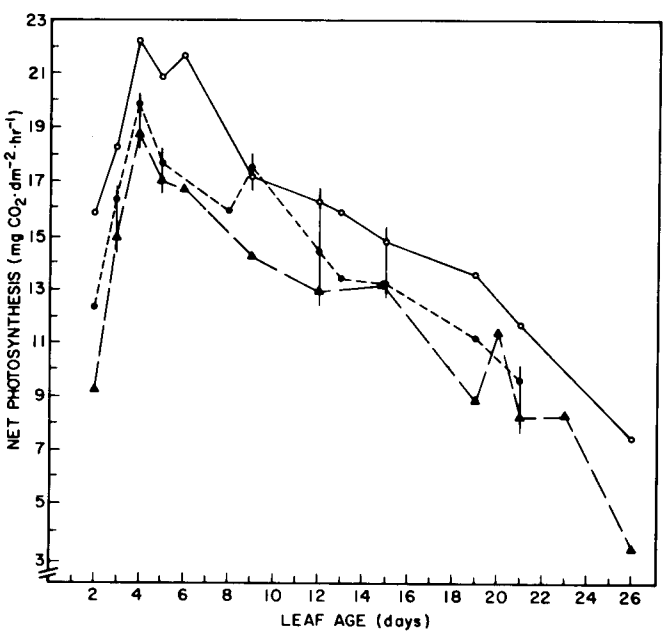

FIG. 3. Mean net photosynthesis of the first leaf of *C. pepo* L. exposed to two levels of UV-B irradiance and a UV-B radiation-free control during 26 days [(▲---▲), treatment A; (●-----●), treatment B; and (○——○), control]. See Fig. 1 and "Materials and Methods" for irradiance levels. Each point represents the mean of three to five replicates. Mean values not connected by vertical bars are significantly different ($P < 0.05$) for that day. Standard error (±) of the means ranged from 0.99 to 1.51.

determined through 26 days (Fig. 3). Maximum photosynthetic rates as related to leaf age for both treatments A and B occurred when the leaves were 4 days old and thereafter tended to decrease with time. Maximum photosynthetic rates for the control plants were evident for at least 3 days. Sisson and Caldwell (17) studied the ontogenetic course of net photosynthesis in *Rumex patientia* L. exposed to four levels of UV-B radiation (9, 8.7, 29.6, and 98.6 mw m^{-2} UV-B_{BE}) and did not find a significant decrease in the duration of maximum photosynthetic rate activity among treatments.

Except for day 9, the mean photosynthetic rates for the control treatment were higher than rates for either treatment A or B throughout the study (Fig. 3). Net photosynthetic rates for treatment B were, for the most part, higher than those for leaves exposed to treatment A. The depressed photosynthetic rates determined for the low UV-B_{BE} treatment (treatment B; 26.2 mw m^{-2} UV-B_{BE}) through 26 days exposure relative to the control rates were not reflected in the growth of the leaves (Fig. 2). Dickson and Caldwell (8) have recently shown that exposure to UV-B radiation depressed leaf growth in *R. patientia* by inhibition of cell division. If this type of inhibitory action can occur in the leaf growth processes of *C. pepo* when exposed to UV-B radiation, then the UV-B_{BE} in treatment B was apparently not sufficient to inhibit the cell division process associated with leaf growth. The reduction in photosynthetic rates in plants treated with UV-B radiation would tend to reduce the substrates available for first-leaf growth and for subsequent leaf initiation and growth. However, limited substrates for leaf expansion processes in the first leaf would not be expected since stored reserves in the cotyledons would probably provide sufficient reserves for growth processes of this leaf if translocation was not inhibited. Thus, the reduced leaf size determined in treatment A may have resulted from an inhibition of leaf expansion processes such as cell division rather than limited substrates.

Absorbances (280–400 nm) of extracts containing the flavonoid pigments and other UV-B radiation-attenuating compounds from the first leaf of *C. pepo* exposed to two levels of UV-B_{BE} and a UV-B radiation-free control treatment after 4, 6, 8, and 10 days of growth were measured (Fig. 4). The curves of all treatments for each date of analysis have similar shapes, although the magnitude of *A* increased with increasing UV-B_{BE}. This is especially evident when the control (Fig. 4A) is compared with treatment A (Fig. 4C). *A* was approximately 30% higher than the control between 280 and 315 nm for each day of analysis. Maximum UV-B radiation attenuation occurred when the leaves were 6 days old in all treatments, which coincided with maximum photosynthetic activity in the control treatment (Fig. 3), and when leaf expansion was nearly complete in all treatments (Fig. 2). Thereafter, *A* of the leaf extracts within this waveband decreased, although the relative differences remained approximately the same between treatments. Similar increases in UV-B radiation attenuation by leaf extracts have been demonstrated in the topmost leaves of *Capsicum frutescens* exposed to several levels of UV-B irradiance for more than 55 days (unpublished data). Robberecht and Caldwell (14) showed a similar increase in UV-B radiation *A* in leaf epidermal extracts at 380 nm (containing flavonoids and other related compounds)

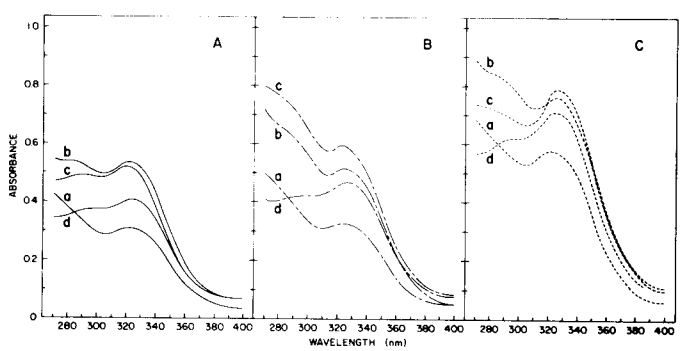

FIG. 4. Absorbance of UV radiation by extracts containing the flavonoid pigments and related compounds from the first leaf of *C. pepo* L. exposed to two levels of UV-B irradiance and a UV-B radiation-free treatment (A, control; B, treatment A; and C, treatment B) after 4(a), 6(b), 8(c), and 10(d) days. See Fig. 1 and "Materials and Methods" for irradiance levels.

from leaves exposed to UV-B radiation compared to plants grown in the absence of UV-B radiation. With one exception, the decreased transmittance was attributed to flavonoid pigments since epidermal transmittance was similar in the control and in UV-B-irradiated plants following flavonoid extraction. Even though *A* of UV-B radiation increased in plants exposed to UV-B radiation, as indicated by the *A* of extracts containing the flavonoid pigments, radiation attenuation apparently was not sufficient to provide complete protection of the photosynthetic apparatus and leaf growth processes here. Nevertheless, this capacity to increase absorption of UV-B radiation would at least partially negate the inhibitory effects of ambient UV-B radiation impinging upon leaf surfaces.

A dose-response curve for first-leaf photosynthesis of *C. pepo* exposed to three levels of UV-B_{BE} (575, 945, and 1350 J m^{-2} day^{-1}) and a UV-B radiation-free control over 8 days is presented in Figure 5. The study presented here was initiated to determine the acute effects of UV-B radiation on photosynthesis of 13-day-old leaves that were fully expanded. The data indicated that photosynthesis is depressed below the control rate after exposure to more than 3,500 J m^{-2} UV-B_{BE}. Comparable reductions in photosynthesis with similar total UV-B_{BE} doses suggested that photosynthetic inhibition is a function of accumulated dose when exposed to UV-B radiation after the leaves are fully expanded. This type of response was not consistent with that of leaves exposed to UV-B radiation through leaf ontogenesis where inhibition was greatest prior to culmination of maximum photosynthetic activity at day 9 (Fig. 3).

A reciprocal mode of action has been demonstrated in the repression of photosynthesis in *R. patientia* by UV-B radiation (17). In a recent study of the epidermal transmittance characteristics of several plants, only *R. patientia* exhibited an increase in transmittance after exposure to UV-B radiation (14). Severe epidermal tissue deterioration occurred and increased UV-B radiation penetrance 24% after 8 days treatment. Since the epidermis plays an important role in UV-B radiation attenuation, generally absorbing 90% or more of UV-B irradiance, reciprocity as demonstrated in *R. patientia* may have partially resulted through gradual deterioration of the epidermis with concomitant increases in UV-B_{BE} transmittance to the photosynthetic apparatus. The inhibition of photosynthesis that occurred during first-leaf ontogenesis does not suggest a reciprocal relationship between the repression of photosynthesis and UV-B_{BE} in *C. pepo*. The visual damage to the leaf epidermis of *R. patientia* was also not evident in *C. pepo*. Conversely, an increase in UV-B radiation *A* probably occurred in *C. pepo* with time as suggested by visually intact epidermis and substantial increases in UV-B radiation *A* by the flavonoid pigments. At the present time, it is not understood why photosynthesis of *C. pepo* was repressed in a cumulative manner in fully expanded leaves (Fig. 5) and not during leaf ontogenesis (Fig. 3).

The daily dose of UV-B_{BE} utilized in the two UV-B radiation treatments of the present study was relatively low. The UV-B_{BE} would be approximately equivalent to 40 (treatment A) and 10% (treatment B) reductions in atmospheric ozone during cloudless days in mid-April at 32° N latitude (11). However, Dütsch (9) has shown that year-to-year average atmospheric ozone concentrations may fluctuate by 10%. Thus, the low UV-B_{BE} treatment (treatment B; 660 J m^{-2} day^{-1}) would approximate ambient UV-B radiation levels in mid-April when minimal atmospheric ozone concentrations prevail. During mid-June, when solar zenith angles are maximum and atmospheric ozone concentrations tend to be minimal (12), the higher UV-B_{BE} treatment (treatment A; 1365 J m^{-2} day^{-1}) would approximate ambient daily UV-B_{BE} (1390 J m^{-2} day^{-1}) (11) during cloudless days (32° N latitude). Thus, results here might be equated to ambient levels of UV-B_{BE} during mid-April (treatment B) and mid-June (treatment A) in a field situation. The repression of photosynthesis (Fig. 3) and leaf growth (Fig. 2) that occurred support the hypothesis that existing ambient UV-B radiation levels inhibit certain physiological processes as previously suggested by Sisson and Caldwell (17) and Teramura *et al.* (18). The UV-B_{BE} impinging upon the leaf surface of the plants used in this experiment would probably be higher than that impinging on leaves in a field situation for equivalent ambient UV-B_{BE} since the first leaves were held at normal incidence to the UV-B radiation sources and were not shaded by later leaves. This difference may not be particularly large since a major portion of ambient solar UV-B radiation is received as diffuse, rather than direct beam radiation.

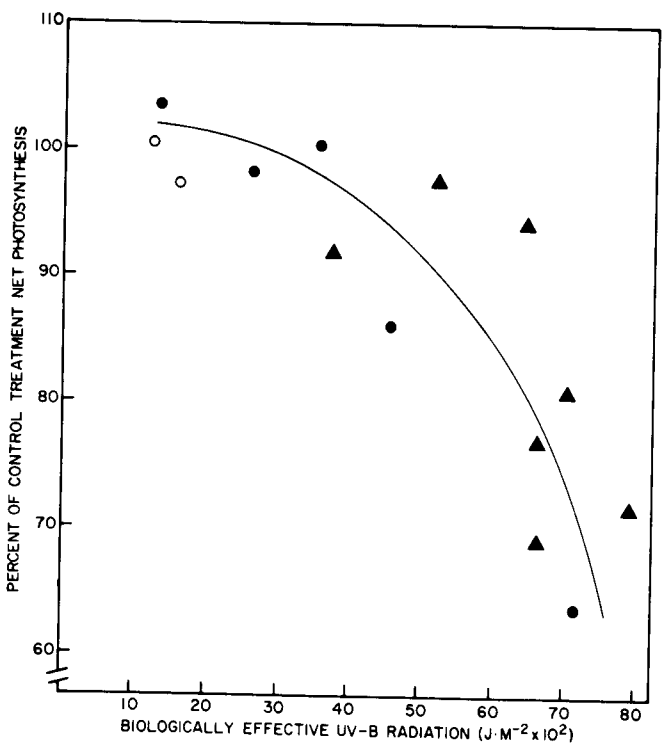

FIG. 5. Percent of control treatment (no UV-B radiation below 314 nm) net photosynthesis for the first leaf of *C. pepo* L. exposed to 575 (○), 945 (●), and 1,350 (▲) J m^{-2} day^{-1} UV-B_{BE} during 8 days treatment. Each point represents a single photosynthetic rate determination. The leaves were 13 days old when treatment was initiated.

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