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Heat Stress-Induced Reproductive Structure Abscission in Pepper: The Role of Hormones and Assimilates

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Heat stress-induced reproductive structure abscission in pepper:
the role of hormones and assimilates

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ABSTRACT

The loss of reproductive structures due to heat stress is a significant source of yield reduction in pepper (Capsicum annuum L.). Similarly, production of peppers in greenhouses can be reduced under the influence of low light periods during the growing season due to abscission of flowers and flower buds. The present research indicated that both heat and low light stress lead to a reduction of the assimilate levels in the reproductive structures. In the case of heat stress, acid invertase activity in the flower buds declines, leading to a reduction of reducing sugars in the reproductive structures. Concomitantly, the level of auxin in these structures slightly decreases in high temperatures. In addition, the capacity of the flower buds to translocate auxin basipetally is markedly reduced by heat stress, making the reproductive structure more susceptible to the abscission-causing influence of ethylene. Shortly before the flower and flower buds abscise, the level of ACC, the precursor of ethylene, is significantly increased by both types of stress. Ethylene evolution by these organs was more variable and less clearly linked to stress imposition. Comparison of abscission-susceptible and tolerant cultivars indicate differences in inhibition of polar auxin transport, depletion of carbohydrates in the reproductive structures during stress, and in abscission sensitivity to applied ethylene. These characteristics may be used in pepper breeding programs to develop more stress-tolerant cultivars.

OBJECTIVES OF THE RESEARCH

1. To study the mechanism of heat stress-induced abscission of pepper reproductive structures, with particular emphasis on levels of auxin, ethylene biosynthesis, and the partitioning of assimilates between young vegetative and reproductive organs.
2. To develop a general scheme of heat stress-induced reproductive structure abscission in pepper.
3. To determine the applicability of this scheme to other stresses such as low light.
4. To compare key elements in the abscission scheme in cultivars of contrasting abscission response in order to facilitate selection and breeding of abscission-resistant peppers.

EFFECT OF HEAT STRESS ON ETHYLENE BIOSYNTHESIS AND AUXIN ECONOMY IN PEPPER AND PAPRIKA

R. Goren, M. Huberman and J. Riov

I. SENSITIVITY OF PEPPER AND PAPRIKA TO HEAT STRESS ETHYLENE-INDUCED ABSCISSION

Pepper cv. 'Maor' and paprika cv. 'Lehava' were selected for testing the abscission model developed for leaves (Beyer and Morgan 1971, Morgan 1984). This model focuses mainly on the interaction between auxin and ethylene in the regulation of abscission. It is known that the above cultivars differ in their response to shade stress-induced abscission (B. Aloni, personal communication), pepper being more sensitive than paprika. In order to determine if the two cultivars respond similarly to heat stress, an experiment was designed to examine their abscission response to heat stress.

Material and Methods:

Heat stress in the present and following experiments was applied to 2- to 3 month-old pepper and paprika plants in growth chambers. The following temperature regimes were applied:

(1) 22C/17C

(2) 27C/22C

(3) 32C/27C

(4) 40C/27C

In each 24 h stress cycle, the high temperature was generally for 8 h under light, except when otherwise stated. During the experiment, the plants were kept under 70%

relative humidity and a 16 h photoperiod. Measurements and samplings were done after each 8 h stress period and/or 24 h after the initiation of each stress cycle. Ethrel was applied by spraying with 0.025% of Tween-80 as surfactant.

Results:

The effect of heat-stress on abscission of flower buds and flowers of both varieties was studied. Abscission kinetics was recorded during three stress cycles. The data confirmed that indeed the two cultivars differ in their sensitivity to heat stress (Figs. 1 and 2).

In pepper plants, abscission of flower buds in the control (22C/17C) and the two higher temperature regimes was observed during the first stress cycle (Fig. 1). Heat stress treatment resulted in increased abscission, the highest temperature being the most active in the induction of flower bud abscission (Fig. 1). Paprika flower buds showed a lower sensitivity to heat stress. In all treatments, no abscission was observed till the end of the first stress cycle (Fig. 1). Later on, abscission increased with the increase in the level of heat stress. Flowers responded similarly to heat stress (Fig. 2). From these data it is evident that paprika is less sensitive than pepper, particularly in terms of flower abscission.

Since ethylene is an important factor in the abscission process, we found it interesting to examine also the sensitivity of the two cultivars to ethylene. Plants were sprayed with ethrel and the abscission of leaves and various reproductive organs was recorded. Surprisingly, the response of the two species to ethrel was quite similar for all organs tested (Figs. 3-6). The only difference between the two species was the general slower response of paprika which was evident during the initial period of the experiment. In both species, the various organs examined differ in their abscission behavior following treatment with ethrel. Flowers and fruitlets in control plants abscised significantly during the course of the experiment (Figs.

5 and 6) whereas abscission of leaves and particularly of flower buds was quite low (Figs. 3 and 4). In treated plants, flower abscission was similar in both ethrel concentrations (Fig. 5). Flower buds and fruitlets showed a slightly higher response in the higher ethrel concentration (Figs. 4 and 6). In leaves, the increase in ethrel concentration doubled the response (Fig. 3).

Although pepper and paprika responded quite similarly to ethylene, they showed significant difference in their response to heat stress. Therefore, we felt that the two species provided a proper plant material for this project.

II. BIOSYNTHESIS OF ETHYLENE

The central role played by ethylene in abscission is reflected also in pepper and paprika reproductive structures by several lines of evidence. Abscission of flowers, buds and young fruits can be readily demonstrated with applications of ethylene generating chemicals or exposure of the plants to ethylene gas (Khademi and Khosh-Kui 1977, Beaudry and Kays 1988, Tripp and Wien 1989, and section I (Figs. 3-6). Infusion of the ethylene precursor 1-aminocyclopropane-1 carboxylic acid (ACC) into pepper bud pedicels resulted in bud abscission within 48 h (Wien et al. 1989). Elevated levels of ethylene production are common to stressed tissues (Yang and Hoffman 1984) but very little is known about the effect of heat stress on ethylene production.

Material and Methods:

Ethylene production was measured either in whole plants or in detached reproductive organs. In both cases, whole plants were exposed to a stress period of 8 h in various temperatures as indicated in the figures. Since ethylene production measurements gave inconsistent results, ethylene production was also determined immediately after the termination of the 8 h stress period. For measuring ethylene production in whole

plants, the plants were enclosed in sealed plastic bags. The air was evacuated from the bags and a known volume of air was injected into the bags. Ethylene production was measured in 1-ml samples, after a 1-h incubation period. Ethylene production in excised organs was measured by enclosing the tissue for 1 h in sealed test-tubes. Ethylene was measured by a gas chromatograph equipped with an alumina column and a flame ionizing detector.

Whole plants were subjected to the same heat stress treatments for measuring the effect of heat stress on ACC and Malonyl-ACC (MACC) content. ACC was measured every 24 h. ACC was extracted and determined according to Lizada and Yang (1979). MACC was determined in the same extracts used for ACC measurements (Riov and Yang 1982).

Results:

Measurement of the effect of heat stress on ethylene production in whole plants did not reveal any significant differences in ethylene production between stressed and non-stressed plants (Fig. 7). Measuring ethylene in whole plants is technically difficult and, therefore, we supplemented these experiments by measuring ethylene production in excised organs. As stated above, in these specific experiments, ethylene was measured immediately after the 8-h stress period and 16-h later, a period during which the plants were under non-stressed conditions, before the onset of the next stress cycle.

Generally, heat stress increased ethylene production, and this effect was more evident immediately after the stress than 16-h later (Figs. 8-13). Increased ethylene production following heat stress was observed in all organs of the two cultivars examined. However, the overall increase in ethylene production was quite low and only in a few cases a 2- to 3-fold increase was observed (Figs. 8-10). Increasing the number of stress cycles did not always increase ethylene production (cf. Fig. 8).

In addition, relatively low rates of ethylene production were sometimes observed in flowers subjected to the highest temperature regime (Figs. 9 and 12).

The above described ethylene measurements were performed in excised organs sampled from heat-stressed plants. Excision by itself may cause changes in organ physiology. Therefore, we further measured ACC content as a more accurate indicator of ethylene biosynthesis in the reproductive organs of both species.

The effect of heat stress, especially the highest temperature regime, showed a much more consistent and significant effect on the increase in ACC content (Figs. 14-16). Both cultivars responded similarly to the heat stress. The increase in ACC content following heat stress was significantly higher as compared to the control after two (Figs. 14 and 16) or three stress cycles (Fig. 15). A similar increase in ACC was found in fruitlet peduncle and sepals (data not shown).

In order to complete the data of ethylene biosynthesis, MACC was also determined in the same tissues. A representative figure (Fig. 17) shows that MACC increased significantly after one stress cycle, while a significant decrease occurred after two cycles.

III. EFFECT OF HEAT STRESS ON AUXIN TRANSPORT

Our hypothesis was that heat stress may inhibit basipetal auxin transport from the reproductive organs towards their abscission zone. The resulted reduction in endogenous IAA level in the abscission zone may be an important factor in increasing the sensitivity of the zone to ethylene action in the induction of abscission (Morgan 1984, Osborne 1989). We have previously shown that species which differ in their abscission response to ethylene also differ, among other characteristics, in their capacity to transport auxin (Riov and Goren 1989). Therefore, a series of experiments using pepper and paprika leaves and

reproductive organs was designed to study the effects of heat stress on auxin transport from the tested organs to the abscission zone. [^3H]NAA was selected for these experiments since this auxin is relatively chemically and biologically stable.

Material and Methods:

Two methods were examined for measuring auxin transport capacity using either whole detached organs or petioles and peduncles: (1) Whole detached organs - Flower ovary served as a model for this method. Five ml of H_2O containing 75,000 dpm of [^3H]NAA were injected into the ovary. Flower explants were incubated individually in small vials containing 300 ml of 50 mM of ES buffer, pH 6.4. Aliquots of the receiving solution were taken for radioactivity determination. (2) Petioles and peduncles - The basal side of 10-mm sections of petioles and peduncles were placed individually on 1.5% agar discs (9 mm in diameter and 3 mm thick). The discs were put in a Petri dish on parafilm layer. The Petri dish was transferred to a humid container before starting the transport studies. Three ml of H_2O containing 200,000 dpm of [^3H]NAA were applied to each acropetal cut surface of the explants. The container was then transferred to the appropriate temperature in a controlled temperature bath. At the termination of the incubation period, each explant was deep-frozen for further analysis. Each receiver agar disc was put in a 4-ml liquid scintillation solution and [^3H]NAA was extracted overnight with constant shaking at room temperature.

Results:

Auxin transport method

Comparison of auxin transport in whole detached organs to that in petioles or peduncles showed that the latter method has several advantages. When using whole detached flowers, radioactivity in the receiving solution was 3 and 6.6%

of the total radioactivity injected after incubation periods of 3.5 and 8 h, respectively. Auxin transport capacity was much higher when employing petioles or peduncles, reaching 10 to 30% of the total radioactivity applied, depending on the organ, after a 6 h transport period. In addition, the variability between replicates was greatly reduced by using petioles and peduncles. Therefore, this method was selected for further studies.

Duration of transport

An incubation period between 2.5 to 8.5 h was examined, using sections of leaf petioles and fruitlet peduncles. Radioactivity in the receiver block increased up to 7.5 h of incubation (Fig. 18). No further increase in radioactivity was found after 7.5 h of incubation. When radioactivity was determined in the upper and lower halves of leaf petioles, it was found that there was a gradual decrease in radioactivity in the upper half of the explants up to 8.5 h while radioactivity in the lower half increased slightly between 2.5 to 7.5 h and remained unchanged thereafter (Fig. 19). Concomitantly, the radioactivity in the receiver agar block increased gradually. Similar results were obtained for sections of fruit peduncles (data not shown). Six h were selected as the standard transport period since no further increase in [^3H]NAA transport was detected in different experiments thereafter.

Temperature during transport

The effect of temperature on auxin transport capacity in leaf petiole sections and peduncle sections of various reproductive organs was examined. Auxin transport was carried out under various temperatures, ranging from 18.5 to 44°C. In all experiments with pepper, the highest auxin transport capacity was obtained at 34°C (Fig. 20 and data not presented). In the highest temperature, auxin transport capacity decreased significantly. In a more detailed experiment using pepper

fruitlet peduncle sections, the temperature during transport ranged from 30 to 47°C. Again, the peak of auxin transport capacity was obtained at 34°C and it decreased gradually as the temperature increased above 34°C (Fig. 21). Auxin transport in paprika responded similarly to temperature changes during transport (Fig. 22).

Effect of heat stress on auxin transport

Pepper plants were subjected to three stress cycles and auxin transport was determined at the end of each cycle. The data obtained clearly show that the higher temperature regimes (32°C/27°C) and (40°C/27°C) caused a significant decrease in auxin transport capacity in all organs tested (Fig. 23). In general, the inhibitory effect of heat stress on auxin transport capacity increased with time but a significant effect of heat stress was already observed after one stress cycle.

In another set of experiments the effect of high night temperatures on auxin transport in pepper leaf petioles was examined, comparing 22°C/17°C (day/night) to 27°C/40°C (day/night). The results of these experiments (Fig. 24) agree with the former data. Therefore, it can be concluded that high temperatures given either during the day or night decrease significantly auxin transport capacity.

Quite similar data were obtained when measuring the effect of heat stress on auxin transport capacity in leaves and reproductive organs of paprika (Fig. 25). However, when comparing the transport capacity data to those obtained with pepper, it is clearly evident that pepper is somewhat more sensitive to the inhibitory effect of heat stress on auxin transport capacity.

IV. CHANGES IN ENDOGENOUS IAA LEVEL UNDER HEAT STRESS

The role of auxin in the abscission model is supported by its long-known ability to inhibit abscission of explant petioles (Addicott 1982), its ability to inhibit ethylene effects both on auxin transport and abscission (Beyer 1973) and its ability to block all the specific ethylene effects on target cells in the abscission zone (Osborne 1989). The dogma is that leaves synthesize auxin, and it diffuses down the petiole to the abscission zone and inhibits the separation process. In advanced stages of abscission, auxin level in the abscission zone decreases, rendering the tissue more sensitive to ethylene action (Morgan 1984). There are only a few reports on the involvement of endogenous auxin in stress-induced reproductive organ abscission (Ofir et al. 1993 and literature cited therein). We, therefore, examined, in detail, whether a reduction in endogenous IAA level is involved in heat stress-induced abscission.

Material and Methods:

1. Extraction of IAA

Immunoassays are one of the most acceptable methods for the analysis of endogenous IAA. For the purpose of this study, a radioimmunoassay was selected. Sample purification is a crucial step in any attempt to analyze endogenous IAA level because IAA is present in trace amounts (100-200 ng/g fresh weight) in tissues which are highly pigmented. Since the pigments involved differ between species, special attention is required in order to adapt any method to the tissue in question. In the following, we summarize the different procedures which have been tested for isolation of IAA from pepper and paprika organs.

The original method that was previously developed in our laboratory (Sagee et al. 1986) was first examined. It involved extraction in 80% MeOH+0.1M NH₄-acetate containing 100µg/ml butylated hydroxytoluene (BHT) as an antioxidant. After centrifugation, the supernatant was purified and concentrated by successive column chromatography steps, using insoluble PVP, DEAE-Sephadex and Sep-Pak C₁₈ in the same order. The final solution was then methylated with diazomethane. In order to test the efficiency of the method [2-¹⁴C]IAA was used as an internal standard. It was found that about 50% of the initial [2-¹⁴C]IAA added to the extracts was recovered. However, a major problem emerged when using pepper and paprika tissues. The final methylated solution was highly pigmented in spite of the triple column chromatography steps. Further efforts were then made in order to eliminate this problem.

The elimination of pigments from the extracts was achieved by replacing the two first columns (PVP and DEAE-Sephadex) by extraction into an organic solvent. The following extraction and purification procedure was adopted: The above extraction solvent was replaced by 0.01 M phosphate buffer, pH 6.6 (according to Sundberg, 1990). The pH of the phosphate-buffer extract was raised after the extraction to pH 8.5. Phenolics and pigments were removed by diisopropyl ether. The water phase was then brought to pH 2.5 and IAA was extracted by diisopropyl ether. The diisopropyl ether phase was dried under nitrogen. The dried extract was dissolved in phosphate buffer, pH 8.0, and run through a Sep-Pak C₁₈ column, which was activated and conditioned to pH 8.0. The eluted solution was then brought to pH 2.5 with acetic acid and run again through Sep-Pak C₁₈ column preconditioned to pH 2.5. The excessive acetic acid was washed with distilled water and IAA was eluted from the column with methanol. The final methanol phase was colorless. It was brought to dryness and diazomethylated. Diethyldithiocarbamic acid was present in all the above stages. By using this extraction method, about 50% of [2-¹⁴C]IAA was recovered.

2. Radioimmunoassay

The following radioimmunoassay procedure adapted from F. Bangerth (Hohenheim University, German) was utilized for measuring IAA content in pepper and paprika. Two hundred and fifty μl of 0.1 M phosphate buffer-saline, pH 7.4, 50 μl of 0.6% BSA 50 μl of [^3H] IAA (ca. 20,000 dpm), and 50 μl of antiserum were added to the methylated extract. The samples were shaken and then incubated for 30 min at room temperature with occasional shaking. At the end of incubation, 750 μl of saturated $(\text{NH}_4)_2\text{SO}_4$ were added and the antibodies were allowed to precipitate for 30 min. The precipitate was collected by centrifugation at 1,700 g for 40 min. The supernatant was removed and 750 μl of 50% $(\text{NH}_4)_2\text{SO}_4$ were added. The samples were centrifuged as above for 30 min. The supernatant was removed and the pellet was dissolved in 200 μl of 2 $\times$ distilled water by shaking. To each sample, 2 ml of scintillation solution were added and after mixing, the radioactivity in the samples was determined.

Results:

Similar to the ethylene measurements, IAA level was determined immediately after the termination of the 8-h stress period and 16-h later (i.e. 24 h after the initiation of the stress cycle). Both pepper and paprika cultivars responded similarly to heat stress. In general, day temperature of 32°C and 40°C decreased significantly the endogenous titer of IAA (Figs. 26-29). This effect was already evident after the first 8 h heat stress cycles. When IAA was determined 16-h after the stress, no recovery was evident in all three stress cycles (up to 72 h).

IAA level in control plants was usually quite stable throughout the experimental period. Only in a few cases (Figs. 26B and 28B) some decrease in IAA level in control plants was evident, probably as a result of organ aging during the experimental period.

The data clearly show that high temperature (32°C and above) caused a quick decline in IAA levels. It seems that, at least under the present experimental conditions, the effect of heat stress was long-lasting. This conclusion is supported by the fact that the three 16 h recovery cycles did not alter the results.

V. GENERAL DISCUSSION

As noted in the above sections, it is well accepted that leaf abscission process follows the model proposed by Beyer and Morgan (1971) and Morgan (1984). In the present work, aimed to determine how heat stress induces abscission of reproductive organs of pepper and paprika, we focused on the various components of this model. Accordingly, we examined the role of ethylene biosynthesis, auxin transport and auxin level in heat stress-induced abscission. We employed mainly reproductive organs but included also leaves as a reference to the classical abscission model. Since it has been previously established that pepper and paprika differ in their sensitivity to shade stress (B. Aloni, personal communication), both plants were selected for the present project. It was hoped that if similar differences in their sensitivity exist also in relation to heat stress, it will serve to elucidate the specific role of each component of the abscission model in heat stress-induced abscission.

Indeed, both plants showed differences in their sensitivity to heat stress (Figs. 1 and 2), and these differences appeared to be based to a large extent on a differential response of auxin transport to heat stress (Figs. 23 to 25). It is surprising that heat stress induced only a small increase in ethylene production in both cultivars, and differences between them were also not large (Figs. 7 to 13). This is in contrast to the well-accepted assumption that ethylene is the most significant factor in abscission (Morgan, 1984; Osborne, 1989). Other results by our three groups also indicate that ethylene plays a major role in pepper reproductive organ abscission (this report, and Tripp and Wien, 1989; Wien and Zhang, 1991). In addition, ACC levels increased significantly in response to heat stress. It may be that the high temperatures applied, inhibited ACC oxidase as

previously reported (Kende, 1993). This may be the reason why results related to ethylene production were not as consistent as those obtained with ACC. It may also explain why sometimes ethylene production decreased rather than increasing with time under heat stress conditions (cf. Fig. 9). Temperature may have a similar control over MACC transferase activity. It is possible that the decrease in the synthesis of MACC in high temperatures (Fig. 17) is responsible, at least in part, for the significant increase in ACC content observed after two or three stress cycles.

Reduction of endogenous IAA level during abscission is well documented and this is attributed to the elevated level of ethylene during the abscission process (Riov et al. 1982 and literature cited therein). The levels of IAA in plant tissues have been shown to be affected by stress conditions. Water stress reduced both extractable and exportable (diffusible) IAA in bolls of cotton and in their abscission zone (Guinn and Brummett 1988). High and low temperatures enhanced abscission of flowers and lowered IAA levels in tomato (El Abd et al. 1986). Five hours at 38°C were sufficient to decrease auxin-like substances in flower buds and young fruits of tomato (Kuo and Tsai 1984). High night temperatures also stimulated June drop of apple fruitlets and reduced their IAA export (Bangerth 1990).

Similarly, heat stress reduced IAA level in reproductive organs of pepper and paprika (Figs. 26-29). However, the reduction of IAA level was quite low and could be observed only in some cases. Ofir et al. (1993) observed that IAA level in reproductive organs of bean subjected to heat stress was unaffected or even higher than in the control. The above observations suggest that reduction of auxin level may be, if at all, a minor factor in heat stress-induced abscission.

Inhibition of auxin transport by high temperatures seems to be the major factor leading to leaf and reproductive organs abscission in response to heat stress (Figs. 23-25). This is particularly evident in the highest temperature regime, which significantly reduced auxin transport in most organs tested. Since we measured auxin transport in excised

sections, it can be concluded that high temperature affects auxin transport directly. Ofir et al. (1993) also observed that heat stress significantly reduced the diffusion of endogenous IAA from bean reproductive organs into agar, suggesting that it was caused by a direct inhibitory effect on the transport capacity of the tissues involved. The significance of the inhibition of auxin transport capacity in pepper reproductive organ abscission was demonstrated by Wien and Zhang (1991) and Wien et al. (1992). They showed that treatment of flower pedicels with TIBA and ethylene generating chemicals at concentrations insufficient to cause complete abscission when used alone, induced an almost complete flower abscission. Whether ethylene is involved in the inhibition of auxin transport during heat stress is not yet clear. It is, however, believed that ethylene may be responsible for the inhibition of auxin transport during natural abscission (Morgan 1984).

As mentioned above, inhibition of auxin transport is a major component of the model developed for leaf abscission. The resulting reduction in endogenous IAA level in the abscission zone may lead to increased sensitivity of the zone to ethylene in the induction of abscission. Abscission can therefore occur without significant increase in ethylene production, as seems to be the case with heat stress-induced abscission.

The changes occurring during heat stress-induced abscission i.e. inhibition of auxin transport, reduction of endogenous IAA level, and stimulation of ethylene biosynthesis were observed in both cultivars. Except for the inhibition of auxin transport capacity, both cultivars did not differ much in their response to heat stress. The fact that the two cultivars differ in their sensitivity to heat stress-induced abscission as well as the reduction in auxin transport capacity points to the major role of auxin transport in heat stress-induced abscission. This does not rule out the possibility that other factors, such as changes in carbohydrate partitioning, may be involved in heat stress-induced abscission.

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1. Effect of high day temperature cycles on pepper and paprika flower abscission.

Whole plants were subjected to daily cycles of high temperature for 8 h. Flowers which abscised were collected at the end of each temperature cycle. Abscission data are presented as percentage of the total number of flowers.

2. Effect of high day temperature cycles on pepper and paprika fruitlet abscission.

Details are as in Fig. 1.

3. Effect of ethrel on pepper and paprika leaf abscission.

Whole plants were sprayed with ethrel and then grown at 27/22°C day/night temperatures. Abscission data are presented as percentage of the total number of leaves. Vertical bars indicate the $\pm$ SE of the mean.

4. Effect of ethrel on pepper and paprika flower bud abscission.

Details are as in Fig. 3.

5. Effect of ethrel on pepper and paprika flower abscission.
 Details are as in Fig. 3.

6. Effect of ethrel on pepper and paprika fruitlet abscission.
 Details are as in Fig. 3.

7. Effect of high day temperature cycles on ethylene production by whole pepper and paprika plants.

Whole pepper were subjected to daily cycles of high temperature for 8 h. At the end of of each 8-h high temperature period, plants were enclosed individually in a polyethylene bag. Air was removed from the bags by a vacuum pump and replaced by a constant volume of 500 ml air. At the end of the incubation period, air samples of 1 ml were taken for ethylene measurements. Vertical bars indicate the $\pm$ SE of mean.

8. Effect of high day temperature cycles on ethylene production by pepper leaves.

Whole plants were subjected to daily cycles of high temperature for 8 h. Ethylene was measured either after each 8-h of high temperature period (A) or at the end of each temperature cycle (B). For ethylene measurement, leaves were enclosed individually in a 29-ml test-tube. At the end of a 1-h incubation period, air samples of 1 ml were taken for ethylene measurements. Vertical bars indicate the $\pm$ SE of the mean.

9. Effect of high day temperature cycles on ethylene production by pepper flowers.

For ethylene measurement, two 2 flowers were enclosed in a 14-ml test-tube. Details are as in Fig. 8.

10. Effect of high day temperature cycles on ethylene production by pepper flower buds.

For ethylene measurement, each four flower buds were enclosed in a 9-ml test-tube. Details are as in Fig. 8.

11. Effect of high day temperature cycles on ethylene production by paprika leaves.

For ethylene measurement, leaves were enclosed individually in a 29-ml test-tube. Details are as in Fig. 8.

12. Effect of high day temperature cycles on ethylene production by paprika flowers.

For ethylene measurement, two flowers were enclosed in a 14-ml test-tube. Details are as in Fig. 8.

13. Effect of high day temperature cycles on ethylene production by paprika flower buds.

For ethylene measurement, each four flower buds were enclosed in a 9-ml test-tube. Details are as in Fig. 8.

14. Effect of high day temperature cycles on ACC content in flower buds of pepper (A) and paprika (B).

Whole plants were subjected to daily cycles of high temperature for 8 h. ACC content in flower buds was measured at the end of the temperature cycles. Vertical bars indicated the $\pm$ SE of the mean.

15. Effect of high day temperature cycles on ACC content in ovaries and flower peduncle of pepper (A) and paprika (B). Details are as in Fig. 14.

16. Effect of high day temperature cycles on ACC content in flower petals of pepper (A) and paprika (B). Details are as in Fig. 14.

17. Effect of high day temperature cycles (40/27°C day/night) on MACC content in petals and ovaries of pepper.
Details are as in Fig. 14.

18. Transport capacity of [^3H]NAA in leaf petiole and fruitlet and fruit peduncle sections of pepper during various transport periods. Different letters indicate significant differences ($p=0.05$).

19. Distribution of radioactivity in the upper and lower halves of leaf petiole and fruitlet peduncle sections of pepper following various periods of [^3H]NAA transport. Different letters indicate significant differences ($p=0.05$).

20. Effect of temperature during transport on [³H]NAA transport capacity in pepper fruitlet peduncle sections.

Fruitlets were tested 12 and 16 days after petal abscission.

[³H]NAA transport duration was 6 h. Different letters indicate significant differences (p=0.05).

21. Effect of temperature during transport on [³H]NAA transport capacity in pepper fruitlet peduncle sections.

Details are as in Fig. 20.

22. Effect of temperature during transport on [³H]NAA transport capacity in paprika fruitlet and fruit peduncle sections.

Organs were tested 12 and 24 days, respectively, after petal abscission. Vertical bars indicate the $\pm$ SE of the mean. Details are as in Fig. 20.

23. Effect of high day temperature cycles on $[^3\text{H}]\text{NAA}$ transport capacity in pepper leaf petiole and flower and fruitlet peduncle sections.

Whole plants were subjected to daily cycles of high temperature for 8 h. The sections were sampled daily after each 8-h high temperature period. Fruitlet peduncle section were sampled 12 days after petal drop. Vertical bars indicate the $\pm$ SE of the mean.

24. Effect of high night temperatures cycles on [³H]NAA transport capacity in pepper leaf petiole and flower and fruitlet peduncle sections.

In each group of data, different letters indicate significant differences (p=0.05). Details are as in Fig. 23.

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25. Effect of high day temperature cycles on $[^3\text{H}]\text{NAA}$ transport capacity in paprika leaf petiole and flower and fruitlet peduncle sections.

Details are as in Fig. 23.

26. Effect of high day temperature cycles on IAA content in pepper flower peduncle.

Whole plants were subjected to daily cycles of high temperature for 8 h. IAA content was measured after each 8-h high temperature period (A) and at the end of each temperature cycle (B). Vertical bars indicate the SE of the mean.

27. Effect of high day temperature cycles on IAA content in pepper ovaries.

Details are as in Fig. 26.

28. Effect of high day temperature cycles on IAA content in paprika flower peduncles.
Details are as in Fig. 26.

29. Effect of high day temperature cycles on IAA content in paprika ovaries.
Details are as in Fig. 26.

DESCRIPTION OF COOPERATION

Cooperation in the project began during the planning of the submission of the research proposal to BARD. The investigators met at scientific meetings in the US, and Aloni and Wien wrote the final version of the research proposal in Ithaca together.

During the duration of the grant period, the Hebrew University and Volcani Center groups were in frequent contact and worked out the methodology of growing satisfactory plant material together. Both groups used the same cultivars and environmental conditions for much of the research.

Communication between Israeli and US investigators has also been good. Wien spent a week in Israel in early December, 1991, discussing the research accomplishments and future plans with both groups. He also presented a seminar at the Volcani Center on a fruit malformation of tomato induced by low temperatures. Meetings of the investigators also occurred at scientific meetings in Europe and North America every year during the grant period.

A paper summarizing the research results of the project was written jointly and presented by Wien at a Crop Stress conference in Taiwan in 1992.

EVALUATION OF RESEARCH ACHIEVEMENTS

Research on reproductive structure abscission of pepper (Capsicum annuum) represented an opportunity to combine the study of the role of hormones in abscission with a consideration of assimilate supply to flowers and flower buds. Previous work on reproductive structure abscission has generally focussed on one aspect or the other, and rarely considered them together.

In the following paragraphs, we will discuss the research accomplishments under the objectives outlined in the research proposal. More detailed consideration of the results can be found in the discussion sections of the individual experiments. In this section, the term "pepper" refers to both bell pepper and paprika types.

1. MECHANISM OF HEAT STRESS-INDUCED ABSCISSION OF PEPPER

The model for the abscission of leaves developed by Beyer and Morgan (1971), indicating central roles for the hormones auxin and ethylene, appears to be applicable to reproductive structure abscission in pepper as well. Our research has demonstrated that auxin production by pepper reproductive structures is reduced in heat stress, and the capacity of the pepper flower and fruit pedicel to transport auxin is markedly inhibited.

Evidence for the involvement of ethylene in pepper abscission comes from several sources. Heat treatment of pepper plants led to increases in the precursor of ethylene, ACC, in all plant parts measured. In addition, application of the ethylene action inhibitor silver thiosulfate significantly reduced reproductive structure abscission in explants as well as in field experiments carried out in Israel and New York. Finally, treatment of pepper plants with ethephon resulted in severe reproductive structure abscission.

It has been more difficult to demonstrate that ethylene production is increased by heat treatment of pepper plants. This is partly due to the variability inherent in ethylene measurements, especially on intact plants, which may differ in size, leaf area and other factors. In addition, ethylene production by detached plant parts may be an artifact of the wounding of the plant part by detachment. Thirdly, imposition of heat stress may inhibit the activity of ACC oxidase (Kende, 1993), so that ACC, but not ethylene levels, rise in response to heat stress.

The role of ethylene in controlling abscission may not be related only to its absolute concentration but also to the ratio of ethylene to auxin in the tissue. It could be postulated that even relatively low ethylene concentrations would suffice to induce abscission in cases where tissue auxin levels had decreased to a lower level. In such a case the tissue sensitivity to ethylene would be increased sufficiently to allow the initiation by ethylene of the separation process.

The production of ethylene by reproductive structures that are about to abscise may be preceded by a reduction of assimilates in these organs. The Volcani group as part of this research demonstrated that heat stress reduced the export of sucrose from photosynthesizing leaves and decreased the relative amounts of assimilates imported into reproductive structures, particularly flower buds. The decreased sink activity as a result of heat stress may have been influenced by the reduced activity of acid invertase in the reproductive structures. Acid invertase activity in adjacent young leaves was not inhibited by high temperatures, allowing these structures to serve as alternate sinks for metabolites.

2. SCHEME FOR HEAT STRESS-INDUCED REPRODUCTIVE STRUCTURE ABSCISSION IN PEPPER

It is now possible to determine the effect of heat stress on pepper reproductive structure abscission by constructing an overall abscission scheme from the results of the individual experiments conducted here. This scheme must however still be verified by measurement of the time course of all the key events on plants in one experiment.

The imposition of one high temperature cycle was sufficient to significantly reduce both reproductive structure auxin production and the polar transport of auxin through the pedicel. Acid invertase activity in the flower buds began to decrease by two cycles, leading to a shortage of assimilates in these structures. Levels of the ethylene precursor ACC stayed similar to control values for two high temperature cycles, but rose on day 3. This sequence of events may indicate a link between the reduction in bud auxin levels and reduced acid invertase activity. Morris and Arthur (1986) have demonstrated such a connection in the stimulation of acid invertase activity after application of auxin to expanding bean stem internodes. Russell and Morris (1982) showed that reduced acid invertase activity in aborting tomato inflorescences was linked to lower activity of growth-promoting compounds (gibberellins and cytokinins) in these reproductive structures.

How lower auxin or assimilate levels might lead to a stimulation of ethylene production is still unclear. One possibility is that low carbohydrate levels in reproductive structures may bring about the release of cell wall fragments, that may elicit ethylene production, as has been postulated in climacteric fruit (Brady and Speirs, 1991). Others conjecture that high tissue carbohydrate levels may inhibit activity of ethylene-synthesizing enzymes (Mayak and Borochev, 1984). To determine which of these mechanisms may be operating in heat stress-induced abscission will require more research.

3. A COMPARISON OF ABSCISSION CAUSED BY HEAT AND SHADE

The mechanism of shade-induced abscission has not been investigated as comprehensively as that caused by heat stress, but our understanding of heat stress-induced abscission can nevertheless serve as a useful point of comparison. Reducing ambient light by 80% results in a rapid decline in the reproductive structure assimilate levels, followed by increase in ACC and ethylene levels. As with heat stress, the reproductive structure abscission resulting from exposure to low light could be prevented by the application of the ethylene action inhibitor STS. Unlike heat stress, in which young leaves appear to form the principal competing sink, with shade stress, the mature leaves appear to retain assimilates rather than translocating them to the rapidly developing reproductive structures as expressed by longer retention of carbohydrates in the leaves. Although we have not compared acid invertase activity in flower buds of shade-stressed and normal plants, analysis of carbohydrates in these structures indicates that acid invertase is unlikely to have been negatively affected by the stress. The proportion of reducing sugars to sucrose showed no consistent trend before and after the imposition of shade stress.

The effect of shade stress on reproductive structure auxin levels, and on capacity of pedicels for polar auxin transport still needs to be determined. Given the production of ethylene during shade stress, one would expect to find a diminution in auxin content and transport capacity (Morgan, 1984).

4. SCREENING PEPPER CULTIVARS FOR SUSCEPTIBILITY TO HEAT OR SHADE STRESS

With the improved understanding of the mechanism of stress-induced reproductive structure abscission has come the development of screening techniques that avoid the need to expose the plants to the stress. The central role of ethylene-auxin balance in the

abscission process caused by both stresses, and the finding that pepper lines may differ in sensitivity to ethylene allows the identification of less susceptible individuals by exposing them to low levels of ethephon (50-100mg/l for whole plants, 0.5-1 mg/l for explants) (Tripp and Wien, 1989, and present research). The inconsistency in the results of ethephon screening between the Rehovot group on the one hand, and the Volcani and Cornell groups on the other, will require a repetition of these tests in greenhouse and field. Such experiments are planned at Cornell for late 1994 and 1995.

Pepper cultivars that differ in susceptibility to heat-induced abscission also show altered degree of inhibition of polar auxin transport. It may be possible to test the auxin transport capacity of fruitlet pedicels of a large number of lines, to identify those with improved stress resistance.

Pepper flowers accumulate sugars, as do sink leaves, during the day period and utilize them for maintenance and growth during the night period. Flowers of a cultivar with low sensitivity to heat-induced abscission (paprika cv. 'Lehava') accumulate more starch during the day period, particularly in their ovules, than a sensitive cultivar (Bell pepper cv. 'Maor'). Shading causes reduction in starch accumulation in both cultivars, but in the less sensitive the reduction is much less pronounced. Utilization of starch during the night period in both cultivars seems not to be limiting. The differences in starch accumulation in flowers of different cultivars was not observed in sink leaves, indicating that it was flower specific. Similar differences in starch accumulation was observed in new greenhouse - grown bell pepper cultivars with different susceptibility to flower abscission. Reducing the source size (by removing source leaves on the main stem and on the side shoot, adjacent to the tested flower) rapidly decreased starch accumulation shortly following the treatment and enhanced flower abscission in the long run. Ethephon treatment reduced and NAA treatment enhanced starch accumulation in the flowers and respectively increased and decreased flower abscission. These results suggest that carbohydrate reserves which are being accumulated during the day period are crucial for the maintenance of the flower during the night period. High night

temperatures may affect flower abscission by enhancing metabolization of these reserves, presumably through respiration. It is also suggested that cultivars with higher capacity for starch accumulation in their flowers during the day period are the less sensitive to night time, high temperature-induced flower abscission.

5. BENEFITS TO AGRICULTURE

The research has resulted in several findings that will have a positive influence on the production of peppers. A major benefit has been the development of screening techniques which will permit the easier identification of lines that will resist the loss of reproductive structures under high temperature conditions. The techniques will also help in the development of pepper cultivars that will fruit successfully under the low light conditions prevalent in greenhouses during the winter season in temperate climates. The finding that pepper cultivars will retain flowers and flower buds in one week of total darkness, as long as temperatures do not exceed 15 C points to a practical method of abscission prevention of use to greenhouse growers with good temperature control.

The ethylene action inhibitor silver thiosulfate was shown to be an effective preventive agent of reproductive structure abscission under high temperature as well as low light conditions. Fruits retained by the plants were in many cases misshapen and not marketable, with a significant increase in seedlessness. In addition, the presence of the heavy metal silver in the compound will prevent its use in food crops. However, application of STS to pepper crops to mitigate the effects of stress on fruitset may have practical value for seed production purposes.

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