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Ethephon Treatment May Alleviate the Suppression of Female Flowers of *Cucurbita Pepo* under High Temperatures

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***Abstract*. When pumpkins are grown in elevated temperatures (32/27 °C day/night) for 1 week during flower development, fewer female flower buds are formed than at normal temperatures (20/15 °C) and only a small percentage of these reach anthesis. To determine if application of the ethylene-releasing compound ethephon can overcome the suppression of female flowers at high temperatures, 'Baby Bear' pumpkin plants were sprayed at the two-leaf stage with 100 or 300 μL·L^{-1} ethephon and then grown in hot and cool greenhouse compartments. At 20/15 °C, 17% of the first 15 main stem nodes produced female flower buds on control plants and virtually all of these developed into open flowers. The higher rate of ethephon increased female bud percentage to 37%. At 32/27 °C, only 3% of the nodes formed female flower buds and 2% flowered. Application of ethephon did not significantly increase female expression at high temperature, and none of the buds reached anthesis. Treatment with the inhibitor of ethylene action silver thiosulfate reduced female flower bud formation at the low temperature and entirely suppressed female flower buds at high temperature. In two additional experiments, these treatments were applied to two cultivars grown at a less extreme 32/20 and at 20/15 °C. Female buds and open flowers were moderately increased by ethephon in the high temperatures, suggesting that ethephon might foster female flowering in less extreme temperatures. Further work is needed to determine if ethephon treatment can overcome the heat-induced inhibition of female flowers in pumpkin under field conditions.**

The main production areas for pumpkins (*Cucurbita pepo* L.) are the central and northern regions of the United States (Peirce, 1987). This implies that pumpkins most often flower and produce fruit in moderate temperatures. When grown in warmer conditions, female flowers are suppressed (Nitsch et al., 1952; Wien et al., 2004) as a result of a reduction in female flower bud formation and a failure of these buds to come to anthesis (Wien et al., 2004).

The plant hormone ethylene appears to play several roles in plants, and some of these conflict in the cucurbit vegetables. Its central role in formation of female flowers in some cucurbits has been known since the 1960s (Rudich et al., 1969). Breeders make use of this fact by applying the ethylene-releasing compound ethephon (2-chloroethane phosphonic acid) to seedlings of *Cucumis* and *Cucurbita* to enhance female flowering and suppress male blossoms in hybrid seed production (George, 1985). Further support for ethylene's role in sex expression comes from the finding that inhibitors of ethylene action such as silver thiosulfate delay female flower production in some cucurbits (Den Nijs and Visser, 1980). However, in watermelon (*Citrullus lanatus*), even low levels of applied ethylene suppress female tendency (Christopher and Loy, 1982). In addition, ethylene functions as a stress hormone in many plants and is generated in the plant under stresses such as elevated temperature and drought (Abeles et al., 1992).

Given the contradictory roles of ethylene in cucurbits, the present experiments were conducted to see if the reduction in female flower production during high temperatures could be counteracted by treatment of the plants with ethephon. If ethylene acts as a stress hormone in pumpkin grown at high temperatures, treatment with an inhibitor of ethylene may reduce deleterious effects.

Materials and Methods

In the first two experiments, pumpkin seeds (cv. Baby Bear, Johnnyseeds.com) germinated in paper towels were planted in 15-cm diameter plastic pots filled with peat–vermiculite soilless mix and placed in two greenhouse compartments set at 20/15 (day/night) and 32/27 °C, respectively. When two true leaves had reached 2.5-cm diameter, seedlings were sprayed to runoff with aqueous solutions of the following: 1) water (control); 2) 100 μL·L^{-1} ethephon; 3) 300 μL·L^{-1} ethephon; 4) 1 mM Ag+ silver thiosulfate; and 5) 5 mM Ag+ silver thiosulfate+. Silver thiosulfate was composed of a 1:4 M ratio of silver nitrate and sodium thiosulfate. A wetting agent (0.1% liquid detergent) was added to all solutions. Within each temperature compartment, pots were arranged in five randomized blocks on the greenhouse bench with one plant per spray treatment per block spaced 45 × 100 cm apart. The experiment was conducted twice, once beginning in early June and once in mid-Sept. 2002.

A second set of experiments applied similar treatments to two cultivars of pumpkin, 'Baby Bear' and 'Schooltime' (Stokeseeds.com). Plants were again grown at 20/15 °C and at a more moderate 32/20 °C temperature. Plants were treated with aqueous sprays at the two-leaf stage with 1) water; 2) 150 μL·L^{-1} ethephon; 3) 300 μL·L^{-1} ethephon; 4) 2.5 mM Ag+ silver thiosulfate+; and 5) 5 mM Ag+ silver thiosulfate. Observations on the plants were the same as those taken in the initial experiments. There were six plants per treatment arranged in a randomized complete block design in each temperature compartment. The experiment was conducted twice starting in late March and late June 2003.

In all experiments, plants were observed between 35 and 50 d after sowing for formation and anthesis of female flowers and location on the main stem of these reproductive structures.

Results of both sets of experiments were statistically analyzed by calculating means and standard errors, because many zero values occurred at the higher temperature, and the temperature treatments were not replicated.

Results and Discussion

Under conditions of the first experiments, 'Baby Bear' pumpkins grown in moderate temperatures (20/15 °C) produced the first female flower buds at node 8 (Table 1). Application of ethephon in the two-leaf stage lowered location of female buds to node 6, whereas treatment with silver thiosulfate (STS), an ethylene action inhibitor, displaced the first females to nodes 10 to 13.

When the plants were grown at 32/27 °C, female primordia development was displaced to later nodes substantiating the results of previous greenhouse and field research (Wien et al., 2004). Plant reaction to ethephon application at the high temperatures was variable with some plants stimulated to produce female flower buds at lower nodes and others not so that position of the first female bud was not significantly different from the controls. The effect of STS at high temperatures was also not significantly different from control values.

When plants were grown at moderate temperatures, ethephon application increased the proportion of female flower buds and flowers reaching anthesis on the first 15 nodes (Table 1). The application of STS, especially at the higher concentration, reduced the number of female buds and flowers, confirming the important role of ethylene in pumpkin flower sex expression. At high temperatures, flower bud and flower production was

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drastically reduced in all treatments with little difference among treatments.

In the second set of experiments, both varieties of pumpkins grown at 20/15 °C reacted to ethylene and its inhibitor in a manner similar to the first experiments (Tables 2, 3, and 4). Ethephon application lowered and STS treatment slightly increased the node to first female bud. Ethephon increased both the percentage of female buds and female flowers on the first 15 nodes, whereas STS in these experiments caused a significant diminution of female tendency (Tables 3 and 4).

In contrast to the first experiments, ethephon stimulated female expression at the more moderate high temperatures used here (32/20 versus 32/27 in the first experiments). Silver thiosulfate on the other hand did not affect female flower bud and flower development differently than occurred in the high temperature controls.

The two cultivars Baby Bear and Schooltime reacted similarly to ethephon and STS treatments both at moderate and elevated temperatures, although Schooltime was reputed to have superior fruiting ability under elevated temperatures (D. Groff, personal communication). Differences in response to ethephon have been found among cultivars in *C. pepo*, but these lines have differed markedly in flowering pattern such as 'Vegetable Spaghetti' and 'Table Queen' (Edelstein et al., 1985). It remains to be seen if differences in ethephon response can be found among cultivars within the pumpkin plant and flowering type.

These experiments indicate that ethephon applications only overcome the heat-induced inhibition of female flowers in pumpkin at moderately elevated temperatures. Both cultivars showed some tendency for increased female flowering at the 32/20 °C temperatures used in the later trials, but at the 32/27 °C used in the initial experiments, the ethephon treatment effect was not evident. The experiments should be repeated under the summer field conditions of elevated temperatures as found in the southern states of the United States.

In many crops, ethylene functions as a stress hormone generated by the plant under conditions of elevated temperature, drought, anoxic conditions, and a host of other abiotic factors (Abeles et al., 1992). Such reactions have been documented for cucumber, for instance, in which exposure to a heat shock of 50 °C for 40 s resulted in a pronounced rise in ethylene 6 h later (Stermer and Hammerschmidt, 1985). It is not known if longer durations of more moderately elevated temperatures also cause cucurbit seedlings to generate ethylene. The results of the present experiments indicate that even if such treatments cause increased ethylene production by pumpkin seedlings growing at high temperatures, endogenous ethylene, and that generated by the plant when ethephon is applied did not stimulate an increase in female tendency. Furthermore, because the application of the ethylene action inhibitor STS to pumpkins grown at high temperatures did not increase female tendency, it is tempting to conclude that ethylene was not inhibiting female flowers under those conditions. Further studies are needed to determine the production and fate of stress-generated ethylene in pumpkins and other cucurbits.

Table 1. Effect of ethephon and the ethylene action inhibitor silver thiosulfate (STS) on location of the first female bud, the percent of female nodes, and the percent of female flowers on the first 15 nodes of the main stem grown in two temperature regimes in two greenhouse experiments with 'Baby Bear' pumpkin.[z]

	Node of first female flower bud		Female flower buds (%)		Female flowers (%)	
Treatment	20/15	32/27	20/15	32/27	20/15	32/27
Water	8.5 ± 0.5[y]	19.8 ± 4.3	16.7 ± 3.5	3.3 ± 5.6	16.0 ± 3.4	2.7 ± 4.6
Ethephon, 100 μL·L^{-1}	6.5 ± 0.7	14.4 ± 7.3	20.0 ± 4.7	4.0 ± 4.7	15.3 ± 6.3	2.7 ± 4.6
Ethephon, 300 μL·L^{-1}	6.0 ± 0.5	15.8 ± 8.2	36.7 ± 10.5	4.0 ± 4.7	28.7 ± 5.5	0
STS, 1 mM Ag+	10.0 ± 2.5	20.5 ± 2.7	15.3 ± 6.3	0.7 ± 2.1	13.3 ± 6.3	0
STS, 5 mM Ag+	13.0 ± 0.9	21.5 ± 1.8	10.0 ± 3.5	0	10.0 ± 3.5	0

[z]Data are averages of the results of two experiments.
[y]Mean ± SE for five replications.

Table 2. Node of first female flower bud of two pumpkin cultivars, as influenced by the application of foliar sprays of ethephon, and the inhibitor of ethylene action STS and by subsequent growth under 32/20 or 20/15 °C day/night temperatures.[z]

	Baby Bear		Schooltime	
Treatment	20/15	32/20	20/15	32/20
Water	10.4 ± 2.3[y]	15.8 ± 2.6	10.6 ± 2.5	16.5 ± 1.2
Ethephon, 150 μL·L^{-1}	7.4 ± 1.4	9.8 ± 4.1	7.7 ± 1.1	8.3 ± 3.4
Ethephon, 300 μL·L^{-1}	6.8 ± 1.2	8.8 ± 3.3	7.0 ± 1.0	6.9 ± 1.1
STS, 2.5 mM Ag+	11.8 ± 2.8	17.0 ± 1.9	14.4 ± 2.2	17.4 ± 2.4
STS, 5 mM Ag+	13.3 ± 2.6	17.6 ± 1.7	13.9 ± 2.7	18.7 ± 1.4

[z]Data are averages of the results of two experiments.
[y]Mean ± SE for six replications.

Table 3. Percent female buds in the first 15 nodes of two pumpkin cultivars, as influenced by the application of foliar sprays of ethephon and the inhibitor of ethylene action STS, and by subsequent growth under 32/20 or 20/15 °C day/night temperatures.[z]

	Baby Bear		Schooltime	
Treatment	20/15	32/20	20/15	32/20
Water	12.2 ± 4.8[y]	2.8 ± 4.4	11.1 ± 4.3	1.1 ± 2.6
Ethephon, 150 μL·L^{-1}	18.3 ± 7.6	11.1 ± 8.2	20.0 ± 4.0	15.0 ± 8.6
Ethephon, 300 μL·L^{-1}	25.6 ± 6.8	13.3 ± 8.0	30.0 ± 3.4	23.3 ± 8.3
STS, 2.5 mM Ag+	10.6 ± 5.3	0.6 ± 1.9	5.0 ± 4.1	1.1 ± 2.6
STS, 5 mM Ag+	6.7 ± 5.7	0.6 ± 1.9	5.0 ± 4.1	0

[z]Data are averages of the results of two experiments.
[y]Mean ± SE of six replications.

Table 4. Percent female flowers on the first 15 nodes of two pumpkin cultivars, as influenced by the application of foliar sprays of ethephon and the inhibitor of ethylene action STS, and by subsequent growth under 32/20 or 20/15 °C day/night temperatures.[z]

	Baby Bear		Schooltime	
Treatment	20/15	32/20	20/15	32/20
Water	11.6 ± 5.0[y]	2.8 ± 4.4	9.4 ± 5.3	0.6 ± 1.9
Ethephon, 150 μL·L^{-1}	12.2 ± 7.4	5.6 ± 6.9	17.8 ± 5.2	14.4 ± 8.9
Ethephon, 300 μL·L^{-1}	18.9 ± 10.2	5.6 ± 8.0	27.8 ± 5.5	18.3 ± 12.4
STS, 2.5 mM Ag+	10.0 ± 5.3	0	2.8 ± 3.4	1.1 ± 2.6
STS, 5 mM Ag+	5.6 ± 5.6	0	4.4 ± 4.3	0

[z]Data are averages of the results of two experiments.
[y]Mean ± SE for six replications.

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