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The Use of Ethephon to Regulate Sex Expression of Summer Squash for Hybrid Seed Production[1]

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Abstract. **Recommended rates of (2-chloroethyl)phosphonic acid (ethephon) application did not prevent staminate flower formation on summer squash (*Cucurbita pepo* L.) sufficient to permit hybrid seed production without defloration. The field experiments were conducted in commercial fields in which flower development occurred during warm long day conditions, which promote maleness in squash. A strongly female 'Cocozelle' inbred, a strongly male 'Straightneck' inbred and a very strongly male 'Crookneck' inbred were treated at several seedling stages with rates up to 600 ppm ethephon. Two applications of 400 to 600 ppm ethephon resulted in development of the fewest staminate flowers without significantly reducing seed yield or quality. The 'Crookneck' inbred was least responsive to ethephon.**

The ethylene-releasing chemical, ethephon, enhances the development of pistillate flowers and delays development of staminate flowers of monoecious cucurbits (5, 7). It has been suggested (4, 5, 7, 8) that ethephon could be used to prevent staminate flower development on inbred seed parents of these crops to facilitate the production of hybrid seed. The efficacy of various concentrations of ethephon applied at different stages of seedling development demonstrated its potential for hybrid seed production of cucumbers and summer squash (3, 4, 8, 9), and the chemical has been used since 1970 under the label Florel (AmChem Products Inc., Ambler, Pa.). Ethephon is widely used, especially for the commercial production of hybrid squash seed. About 113 metric tons of hybrid squash seed were produced using ethephon in 1974 (10) and even larger quantities more recently. However, the recommended rate of 88 g/ha of ethephon in 370 to 940 liters (100 to 250 ppm) applied to seedlings with 2 true leaves often does not result in the desired reduction of staminate flower development. Uneven emergence of seedlings, genetic differences in sex expression of inbreds, and variation in weather conditions contribute to variable results. Seed producers are reluctant to use multiple applications, higher rates, or applications at later stages of development due to label restrictions, uncertainty of efficacy, and possible adverse effects on yield or seed quality.

The objectives of this research were to evaluate the influence of rates and timing of ethephon applications on sex expression and seed yield and quality of three different inbred squash lines under commercial conditions.

Materials and Methods

Three monoecious inbred lines of squash differing in degree of femaleness were used as seed parents. A strongly female 'Cocozelle' type, a strongly male yellow 'Straightneck' inbred, and a very strongly male yellow 'Crookneck' were planted May 21 at Robson Seed Farms, Inc., Hall, New York, in 1975. The 'Cocozelle', and 'Straightneck' inbreds were planted June 7 and 20, respectively, in separate production fields near Hall, New York, in 1974. The soil in each case was Ontario sandy loam, a calcareous glacial till soil. Seed of the paternal parents were planted in rows 4.2 m apart; after seedling emergence 2 rows of the seed parents were planted equidistant between the paternal rows. The seedlings were thinned to 40-55 cm between plants. Fertilizer rates, cultivation and hand weeding were those normally used for production of hybrid squash seed. The pollen plants were removed after the pollination period, to prevent errors during seed harvest.

Plots in the 1974 planting consisted of four 12.2 m long rows of the seed parent, with 95 to 100 plants per plot. Plots in the 1975 planting consisted of single rows of the seed parent, 5.5 m long, with 12 to 16 plants per plot. All trials were randomized complete block designs with four replications of each treatment.

Ethephon solutions were prepared immediately before use with 0.1% Tween-20 as a wetting agent. The solutions were applied with a CO_2-pressured sprayer with 2 fine spray nozzles to give double coverage of a 25 cm band at a rate sufficient to wet the upper surfaces of the leaves to runoff. Applications were made when most plants in the plots had the desired numbers of true leaves, with the most recently expanding leaf about 2-3 cm in diameter.

Counts were made in 1974 of open pistillate flowers on the first day that significant numbers of flowers reached anthesis, which was July 9 for the 'Cocozelle' and July 25 for the 'Straightneck' inbred. Immature staminate buds were counted and removed to preclude self pollination of the seed parent. In the 1975 experiment, staminate and pistillate flowers at anthesis were recorded each day for 24 days after anthesis of the first pistillate flower. Staminate flowers were not removed since the trial was not in a production field. Beehives near the plots provided adequate bee activity for cross pollination.

In the 1974 tests the mature fruit were harvested and mechanically pulped to separate the seeds from the fruit. The total weight of wet seed and pulp residue from each plot was recorded, and a 1 liter sample of the seed and pulp slurry was saved for hand cleaning. The seed was cleaned and dried in a forced draft oven at 35°-40°C. In the 1975 trials the total number and weight of fruit was recorded, and seed was removed by hand from 3 representative fruit from each plot. Germination of the dried seed was determined by the roll towel method (1).

Precipitation was recorded at the Robson Seed Farm, near the 1975 trial site and within 3 km of the 1974 production field trials. Temperatures were recorded at the New York State Agricultural Experiment Station at Geneva, about 13 km north of the 1975 site.

Results

Mean temperatures for the 2 seasons were similar (Table 1), except the lower temperatures and higher precipitation in late

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Table 1. Growing season weather summary, Hall, N.Y.

Month	Dates	Mean temperature (°C) 1974	Mean temperature (°C) 1975	Precipitation (mm) 1974	Precipitation (mm) 1975
May	21-31	14	21	33	10
June	1-10	19	15	15	68
	11-20	17	21	23	50
	21-30	17	21	55	0
July	1-10	23	22	0	10
	11-20	21	22	0	25
	21-31	19	22	35	20
August	1-10	20	22	35	20
	11-20	21	21	37	0
	21-31	20	18	62	108
September	1-10	16	15	40	5
	11-20	17	14	10	28
	21-30	12	13	20	128

May of 1974 delayed planting dates that year. There was a 24-day dry period during July, 1974, but the plants were relatively small at that time and were not adversely affected. During the 1975 season there was a 64-day period from late June to late August when only 85 mm of precipitation was recorded. All of the plants were noticeably wilted for several hours daily during most of the first 20 days of August, 1975.

1974 seed production field trials. Increasing rates of ethephon resulted in increased numbers of early pistillate flowers of the 'Cocozelle' inbred when applied at the first or at the first and third leaf stages (Table 2). The triple application did not show this effect, nor did treatments applied later in seedling development. All ethephon applications to the 'Straightneck' inbred at the first leaf stage resulted in greater numbers of early pistillate flowers than in the control or in treatments applied at later stages of development.

All ethephon treatments resulted in development of fewer staminate buds than on control plants, and increasing concentrations and repeated applications enhanced this effect (Table 2). The higher rates of ethephon also tended to reduce the size of staminate buds, and some treated buds aborted while still immature. The 'Straightneck' inbred was more responsive to ethephon than the 'Cocozelle' inbred, based on staminate flower production.

The ethephon treatments had no significant effect on number of fruit or seed yield. The seeds appeared normal and all had germination of 95% or more (data not shown).

1975 research plot trials. Since none of the treatments in the 1974 experiment reduced staminate flower formation on material inbred lines sufficiently to permit hybrid seed production without manual defloration, higher concentrations of ethephon were tested in 1975. Records of flowers reaching anthesis on untreated plots illustrate the differences in sex expression of the 3 inbreds (Table 3). The 'Cocozelle' inbred was the most highly female, 'Straightneck' was intermediate, and 'Crookneck' was the most highly male inbred line. Data in Table 3 are for the total 24-day flowering period but data were also summarized over four 6-day periods and are available from the authors. Applications of ethephon at the first leaf stage resulted in increased numbers of early pistillate flowers on all 3 inbreds compared to later applications and the control. Those treated at later dates tended to have more pistillate flowers during the second 6-day period following initiation of flowering than those treated at the first leaf stage. The number of pistillate flowers generally decreased during the third and fourth 6-day periods. Applications at the 2 and 4 leaf stages of the 'Crookneck' inbred resulted in more pistillate flowers during the third period than for all other treatments. Although there were differences in total numbers of pistillate flowers among treatments for all 3 inbreds, the differences were not great when compared with the effects on numbers of staminate flowers. Numbers of staminate flowers (Table 3) on all 3 inbreds were, in general, inversely proportional to the quantity of ethephon applied. No staminate flowers reached anthesis during the first 6-day period after the first pistillate flower opened. The treatments were most effective in reducing staminate flower numbers during the second 6-day period (data available from authors). Effectiveness of single and double applications in suppressing

Table 2. Effects of ethephon on flowering, fruiting and seed yield of 2 squash inbreds, 1974, Hall, N.Y.

Treatment: Ethephon concn (ppm)	Treatment: Leaf stage(s)	Early pistillate flowers per plant[z] C	Early pistillate flowers per plant[z] S	Staminate buds per plant[y] C	Staminate buds per plant[y] S	Fruit per plant C	Fruit per plant S	Seed yield (kg/ha) C	Seed yield (kg/ha) S
125	1	0.02	0.65	6.9	5.3	2.02	1.84	660	812
250	1	0.08	0.84	6.1	3.7	2.01	2.00	616	681
375	1	0.16	0.80	4.9	3.7	2.09	2.05	840	672
125	1,3	0.05	0.61	6.5	3.4	2.12	1.82	550	778
250	1,3	0.09	0.88	5.3	1.0	2.01	2.08	590	801
375	1,3	0.11	0.80	3.1	0.2	1.90	2.04	713	812
125	1,3,5	0.05	0.82	6.4	4.1	1.96	1.95	563	806
250	1,3,5	0.05	0.65	4.3	0.5	1.96	1.98	705	711
375	1,3,5	0.02	0.80	1.5	0.1	1.92	2.02	603	700
250	2	0.04	0.08	6.3	2.8	1.94	1.81	726	650
250	3	0.03	0.11	6.3	3.4	2.13	1.91	730	717
250	2,4	0.03	0.17	3.8	1.1	2.08	1.96	896	728
375	2,4	0.01	0.23	1.6	0.9	1.96	2.17	687	879
Control	–	0.02	0.10	7.7	11.5	2.07	1.78	795	907
LSD (5%)	0.06	0.21	0.6	1.8	NS	NS	NS	NS	NS
(1%)	0.08	0.28	0.8	2.4	NS	NS	NS	NS	NS
C V		68%	18%	8%	42%	7%	9%	22%	20%

[z]At anthesis on July 9 for 'Cocozelle' (C) and July 25 for 'Straightneck' (S).

[y]Total buds removed July 11, 13, 15, and 17 for 'Cocozelle' and on July 25 for 'Straightneck'.

Table 3. Effects of ethephon on numbers of flowers per plant reaching anthesis over a 24 day period[x] on 3 squash inbreds, 1975, Hall, N.Y.

Treatment		Number of flowers					
		Cocozelle		Straightneck		Crookneck	
Ethephon concn (ppm)	Leaf stage(s)	Pistillate	Staminate	Pistillate	Staminate	Pistillate	Staminate
200	1	9.3	2.04	6.2	6.61	2.3	9.49
400	1	10.8	0.98	5.9	4.81	4.1	6.65
600	1	9.7	0.83	4.5	4.26	3.2	6.30
200	2	9.1	1.82	4.7	6.69	2.5	7.15
400	2	9.2	1.60	4.9	3.10	3.6	5.26
600	2	8.6	0.30	6.3	1.72	4.1	3.22
200	3	9.7	1.20	5.8	4.83	3.7	3.56
400	3	9.0	0.29	6.7	1.83	4.3	3.99
600	3	8.7	0.06	6.7	0.34	5.2	3.99
200	1,2	9.5	0.56	8.2	5.48	4.8	6.14
400	1,2	10.6	0.32	7.4	2.61	3.3	4.19
200	2,3	9.6	0.45	4.6	2.48	4.8	3.15
400	2,3	9.5	0.00	7.2	0.33	3.8	1.64
200	1,3	9.9	0.70	6.3	3.41	4.0	2.16
400	1,3	10.6	0.10	7.6	0.61	5.9	0.97
600	1,3	8.7	0.04	4.7	0.15	4.2	0.63
200	2,4	9.3	1.27	5.5	1.92	4.8	0.24
400	2,4	9.7	0.32	5.5	0.13	5.6	0.58
600	2,4	9.9	0.03	5.9	0.04	4.5	0.13
Control		11.2	3.20	4.6	8.78	4.2	13.33
LSD (5%)		1.3	0.65	2.0	1.77	1.6	3.33
(1%)		1.8	0.86	2.7	2.36	2.1	4.42
C V		10%	56%	24%	42%	27%	57%

[x]Flower counts started at time first female flower reached anthesis in each inbred line.

Table 4. Effects of ethephon on fruit number, fruit size, and seed yield of 3 squash inbreds, 1975, Hall, N.Y.

Treatment		Cocozelle			Straightneck			Crookneck		
Ethephon concn (ppm)	Leaf stage(s)	Fruit per plant	Avg fruit weight (kg)	Seed yield (kg/ha)	Fruit per plant	Avg fruit weight (kg)	Seed yield (kg/ha)	Fruit per plant	Avg fruit weight (kg)	Seed yield (kg/ha)
200	1	1.36	1.30	982	1.76	0.59	790	2.31	0.41	745
400	1	1.49	1.41	962	1.67	0.50	690	2.84	0.43	905
600	1	1.29	1.26	1010	1.69	0.44	705	2.56	0.37	740
200	2	1.38	1.39	848	1.88	0.51	850	2.37	0.37	750
400	2	1.62	1.39	1180	1.73	0.41	665	2.93	0.37	850
600	2	1.50	1.18	830	2.21	0.45	610	2.81	0.34	1060
200	3	1.42	1.38	1000	2.10	0.63	875	2.96	0.35	990
400	3	1.42	1.30	862	1.96	0.55	705	2.87	0.36	1000
600	3	1.15	1.42	813	1.96	0.50	965	2.99	0.35	818
200	1,2	1.41	1.37	1110	1.82	0.54	895	2.97	0.35	973
400	1,2	1.43	1.40	782	1.85	0.41	735	2.65	0.33	807
200	2,3	1.36	1.32	746	1.68	0.48	530	3.18	0.32	856
400	2,3	1.35	1.28	955	1.84	0.49	605	2.96	0.30	1190
200	1,3	1.42	1.44	1070	1.62	0.55	940	2.54	0.32	858
400	1,3	1.34	1.41	826	1.95	0.45	840	2.79	0.37	758
600	1,3	1.14	1.28	838	1.51	0.40	580	2.57	0.32	600
200	2,4	1.31	1.45	750	1.88	0.53	675	2.59	0.30	970
400	2,4	1.38	1.35	806	2.29	0.42	920	3.16	0.31	745
600	2,4	1.26	1.37	752	1.95	0.39	620	2.56	0.30	956
Control		1.55	1.45	1065	1.88	0.60	910	2.35	0.44	751
LSD 5%		NS	NS	NS	0.40	0.09	NS	NS	0.08	NS
1%		NS	NS	NS	NS	0.12	NS	NS	NS	NS
C V		17%	17%	30%	15%	14%	29%	17%	16%	29%

staminate flowers tended to increase with increasing age of the plants. The strongest male inbred, 'Crookneck', was the least responsive to single applications of ethephon. Applications of 400 or 600 ppm at the 1 and 3 or at the 2 and 4 leaf stages generally resulted in more than a 90% reduction in total number of staminate flowers. The 600 ppm rate applied at the 2 and 4 leaf stages was the most effective treatment for reducing staminate flower formation of the 2 inbreds with greatest male tendency.

Average fruit weight was reduced by ethephon treatments on the 'Straightneck' and 'Crookneck' inbreds (Table 4) and the reductions in fruit weight of 'Straightneck' were progressive with increasing concentration. There was no consistent effect of ethephon treatment on number of fruit produced per plant. There were no significant effects of ethephon treatments on seed yield and, although there was considerable variation in seed yield, there were no consistent trends. There were also no effects of treatment on seed size or germination (data not shown).

Discussion

Our results with repeated applications of various concentrations of ethephon higher than the label rates allowed for hybrid seed production in squash demonstrate that the label rates are too low for optimal reduction in numbers of staminate flowers. Higher rates, even when applied at two stages of seedling development, gave better control of staminate flower development and did not reduce seed yield or seed quality.

The need for higher rates than that indicated in previous research is in part due to the environmental factors influencing sex expression in cucurbits. Nitsch et al. (1952) observed that high temperature and long photoperiod promote staminate flower formation, whereas low temperature and short days favor development of pistillate flowers. Ethephon treatments are usually applied to commercial plantings of squash in the Northeastern U.S. during late June and July, when daylength and temperatures are near the maximum. In contrast, much of the early research with ethephon was done in greenhouses under short day conditions (3, 4, 5, 7). Other experiments with ethephon on squash (8, 9) were under field conditions, but treatments were applied during relatively short days. We have observed that plants are much more responsive to ethephon under greenhouse conditions than in the field at the same time of year, even though temperatures are usually higher in the greenhouse. Hillyer and Wittwer (2) reported that sex expression of 'Acorn' squash could be regulated by foliar applications of the growth regulator maleic hydrazide and they observed large differences in response to this chemical between field and greenhouse experiments. Complete suppression of male flowers was accomplished only with very high rates applied under short days and low temperatures.

Application of ethephon treatments at the first true leaf stage can be effective if all the seedlings treated are at this stage, but under field conditions results are often very erratic due to uneven emergence of seedlings. For this reason it is advisable for seedsmen to wait until the second true leaf stage (second leaf 2 to 5 cm in diameter) to apply ethephon. In addition, a single application at a given concentration of ethephon was more effective when applied at the second or third leaf stage of all 3 inbreds than when applied at the first leaf stage. To ensure effective reduction in staminate flowers it will be necessary for seedsmen to apply a second application of ethephon at the 4 leaf stage. The concentration to be used will depend on the seedsman's experience with a given inbred and the environmental conditions at the time of application. Even when ethephon treatments are effective, the maternal plants must be examined during the pollination period to remove the occasional staminate flower buds. This is necessary to ensure low percentages of selfed seed and the labor required is greatly reduced if ethephon treatment is effective.

Some of the rates of ethephon applied in this investigation were in excess of that specified on the label. In view of seedsmen's problems in obtaining effective reduction in staminate flowers with the recommended rates and our results demonstrating the need for, and safety of, higher rates, the label should be changed to permit more effective use of this growth regulator. This is especially true for strongly male inbreds which have been selected since the label rates were established.

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