

Growth Regulators Improve the Intercrossing Rate of Cucumber Families for Recurrent Selection

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ABSTRACT

Cucumber (*Cucumis sativus* L.) populations for recurrent selection often are intercrossed after each cycle using bees (*Apis mellifera*) in isolation blocks to save the expense of hand labor. This experiment was conducted to determine whether the rate of natural outcrossing could be increased using growth regulators, plot size management, and node of fruit position for seed harvest. The experiment was a factorial in a randomized complete block design with 2 yr (1997 and 1998), two growth regulators (treated and none), two plot sizes (single plant hills and small plots), two nodes of harvest (crown and top), and four replications (six in 1998). Plots or hills were planted to white-spined 'Sumter' and were surrounded by rows of black-spined 'Wisconsin SMR 18' pollenizer. Plots or hills of Sumter were treated with ethrel to induce gynoccy, and pollenizer rows were treated with silver nitrate to induce androecy. Untreated isolation blocks remained monoecious. Progeny of the harvested white-spined plants were planted the following year to evaluate spine color, with black-spined plants indicating an outcross. Results showed that node of harvest had no effect on outcrossing rate. However, growth regulators and plot size had a significant effect. Treated small plots, treated hills, and untreated hills had high outcrossing rates (54%) relative to untreated plots (30%). Therefore, if families are to be intercrossed in isolation blocks, they should be treated with growth regulators for maximum outcrossing.

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Abbreviations: AVG, aminoethoxyvinyl glycine; CP, composite pollenizer; GA, gibberellins.

CUCUMBER is used either fresh or processed in the United States. The total area of pickling cucumber was 45,931 ha with a production of 585,980 Mg of fruit (USDA, 2005). The total area of slicing cucumber for 1997 was 22,731 ha with a production of 437,807 Mg (USDA, 1998). The average yield of pickling cucumber in the United States has increased by 100% over the last four decades due to improved cultural practices and selection for yield and disease resistance (USDA, 1998, 2005).

Cucumber plants produce different sex phenotypes: monoecious (staminate and pistillate flowers), gynocious (pistillate flower only), and hermaphroditic (perfect flowers) (Staub et al., 2008). However, andromonoecious (perfect and staminate flowers), androecious (staminate flowers), and trimonoecious (staminate, pistillate, and perfect flowers) sex types also exist. The wild-type is monoecious with staminate flowers appearing first, followed by pistillate flowers at later nodes. Sex expression in cucumber is influenced by genotype as well as growth regulators and plant hormones (Robinson and Decker-Walters, 1997; Takahashi et al., 1983). Exogenous application of ethylene is known to promote the production of pistillate flowers in monoecious cucumber lines, resulting in increased fruit yield (Bhat et al., 2004; Cantliffe

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and Phatak, 1975; Hogue and Heeney, 1974; McMurray and Miller, 1969; Miller et al., 1970; Thappa et al., 2011; Yin and Quinn, 1995). Wang et al. (2010) reported that ethylene is involved in the arrest of stamen development in the female cucumber flower through induction of DNA damage. They further demonstrated in transgenic *Arabidopsis thaliana* (L.) Heynh. that monoecious flowers with abnormal stamens mimic female flowers. In contrast, aminoethoxyvinyl glycine (AVG), an inhibitor of ethylene biosynthesis, and AgNO₃, an inhibitor of ethylene action, as well as gibberellins (GA) suppress the development of pistillate flowers and induce staminate flowers (Atsmon and Tabbak, 1979; Elizabetha and Lush, 2010; Kwack and Fujieda, 1984; Takahashi and Jaffe, 1984; Takahashi and Suge, 1982; Tolla and Peterson, 1979). Koyama (2008) reported that staminate flowers induced with AgNO₃ at 500 mg L⁻¹ resulted in highest seed set. Yamasaki and Manabe (2011) reported that exogenous application of AgNO₃ induced both staminate and functional hermaphroditic flowers. However, functional hermaphroditic flowers are different from morphological hermaphroditic flowers because the former is induced whereas the latter is genetical. They essentially perform the same function in pollination.

Since cucumber is an allogamous (naturally outcrossing) species, recurrent selection for yield can be done by intercrossing the selected families in isolation blocks using bees to save labor. Growth regulators can be used to alter sex expression, resulting in increased intercrossing among cucumber families for population improvement. The rate (percentage) of natural outcrossing has been measured for cucumber families planted in isolation blocks and was measured as 36% (29–43%) cross-, 17% (0–42%) sib-, and 47% (23–77%) self-pollination (Wehner and Jenkins, 1985). In North Carolina, wide-base, medium-base, and elite populations of pickling and slicing cucumbers were improved by testing 352 half-sib families in two replications during the spring season. Based on fruit yield, earliness, and quality, the best 40 families were intercrossed in isolation during the summer season. Actual gain was measured after recurrent selection was practiced (Strefeler and Wehner, 1986; Wehner and Cramer, 1996) and improved populations had been released (Wehner, 1998). However, gain was found to be low and was attributed to low heritability or insufficient intercrossing.

Climatic effects have been reported on pollen flow (Gingras et al., 1999; Whitaker and Bohn, 1952). Wind velocity, temperature, and other environmental factors may influence honeybee behavior and flower set, thereby affecting outcrossing (Kalbarczyk, 2009; Robinson and Decker-Walters, 1997). Climatic conditions also influence the ratio of staminate to pistillate flowers, which also affects outcrossing.

In bee-pollinated species, a better floral display may be advantageous in attracting bees and thereby increasing

the rate of outcrossing (Augspurger, 1980; Gentry, 1974; Koptur, 1984; Schaffer and Schaffer, 1979; Udovic, 1981; Willson and Rathcke, 1974). Position of flower may play an important role in the rate of natural outcrossing. Plant breeders have suggested that there is more cross-pollination of the flowers near the crown (base) of the plant and that there is more self-pollination of the flowers near the shoot tip (top) of the plant.

This study was conducted to determine whether the rate of natural outcrossing can be improved using composite pollenizer (CP) rows and growth regulators (ethrel and AgNO₃) to encourage outcrossing with bee pollinators in isolation blocks. In addition, we were interested to determine whether harvest of early-set (crown) fruit would improve the rate of outcrossing relative to late-set (top) fruit, as is thought to be the case by some cucumber breeders. The objective of this study was to measure the rate of natural outcrossing in isolation blocks located in various environments and to determine whether the rate of natural outcrossing could be increased using growth regulators, plot size, or node of harvest.

MATERIALS AND METHODS

This study was conducted at multiple research stations in North Carolina. The experiment was a factorial in a randomized complete block design with 2 yr, two growth regulator treatments (treated and none), two harvested nodes (crown-set and top-set), and four replications. Crown-set approximated node 2, and top-set approximated node 20. Each treatment combination was planted in isolation at research stations located in Clinton, Clayton, Rocky Mount, Reidsville, Butner, Lewiston, Jackson Springs, and Kinston, NC.

Recommended horticultural practices (Schultheis, 1990) were used for all experiments. Fertilizer was incorporated before planting at a rate of 90–39–74 kg ha⁻¹ (N–P–K) (as ammonium nitrate [H₄N₂O₃]), with an additional 34 kg ha⁻¹ N (as sodium nitrate [NNaO₃]) applied at the vine tip-over stage. Curbit [ethalfuralin N-ethyl-N-(2-methyl-2-propenyl)-2, 6-dinitro-4-(trifluoromethyl)benzenamine] was applied for weed control (1.2 kg ha⁻¹ a.i. preemergence). Irrigation was applied when needed for a total (irrigation plus rainfall) of 25 to 40 mm per week.

The color of fruit spines in cucumber is controlled by a single gene, with *B* for black spines being dominant to *b* for white spines (Pierce and Wehner, 1990). All plots of Sumter, a white-spined monoecious cucumber, were surrounded by rows of Wisconsin SMR 18, a black-spined monoecious cucumber used as a CP. Therefore, the natural outcrossing rate was tracked using the *B* gene (Fig. 1). All of the black-spined progenies would be the result of outcrossing. White-spined progenies would be the result of self-pollination (hills) or self- and sib-pollination (small plots).

Plots were of two types: single plant hills and small plots. Hills were planted with five seeds and thinned to two plants at emergence and one plant at tip-over stage. In single plant hills, Sumter was planted by itself, with the remainder of the row planted with the CP (Fig. 1). Small plots were 3.1 m long and 1.5 m apart (row spacing, center to center) with 1.5 m alleys

Table 1. Outcrossing rate (percentage) in cucumber isolation blocks based on treatment size and harvested node for mature fruit.[†]

Treatment Plot size	Harvested node	Percentage outcrossing over years		
		1997	1998	Mean
Growth regulators not applied				
Single-plant hill	Crown	91	38	72
	Top	90	49	64
12-plant plot	Crown	46	11	29
	Top	39	23	31
Growth regulators applied twice				
Single-plant hill	Crown	90	37	65
	Top	94	49	68
12-plant plot	Crown	73	29	55
	Top	67	31	52
Mean		74	33	53
LSD (5%)		15	15	21
F ratio				
Year	29.57*			
Growth regulator	5.55*			
Treatment size	42.72**			
Harvested node	1.24 NS [†]			

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

[†]Outcrossing rate was measured as the percentage of black-spined marker genes in the progeny of tested plants. Data are means of four replications (six in 1998) and two plants (hills) or 16 families (small plots) from isolation blocks (separated by a minimum of 2 km distance from each other). Growth regulators were ethrel and AgNO₃, with plants treated twice vs. not treated. Plot size was hill vs. small plot. Harvested node was crown vs. top for the fruit harvested for seed. Means were calculated from the raw data, not from the data shown.

[†]NS, not significant at $P \leq 0.05$.

to 4°C difference in temperature for the 2 yr. The rate of precipitation was also high in May in 1998 compared to 1997. Increased rainfall during pollination should hinder in pollinator activity and reduce the amount of cross-pollination. However, other factors such as light intensity, humidity, placement of beehives, and wind velocity also affect outcrossing and were not measured in this experiment. Large variations in weather conditions might

have lead to an altered sex ratio that resulted in significant differences in outcrossing rate for both years.

Single plant hills had higher outcrossing rates than the small plots in both treated and untreated plots (Table 1). The rate of natural outcrossing ranged from 64 to 72% in single-plant hills as compared to 29 to 55% in small plots over years and growth regulator treatment. Small plots represent families from recurrent selection, and surrounding rows of black-spined Wisconsin SMR 18 represent the composite pollenizer. Single hills of individual white-spined plants surrounded by black-spined plants permitted measurement of the outcrossing rate. Plants in small plots that were outcrosses could be pollinated by both white-spined sibs and black-spined pollenizers whereas single-plant hills that were outcrossed could be pollinated only by black-spined pollenizers. This permitted estimation of self-, sib-, and cross-pollination. Of course, use of single plants rather than families in isolation blocks would provide improved intercrossing, but we also wanted to include recurrent selection methods that make use of family testing.

Growth regulator treatment significantly increased the rate of cross-pollination (30% in untreated vs. 54% in treated plots) (Table 3). There was a 24% increase in the rate of cross-pollination when plants were treated with growth regulators. There was a significant decrease in sib-pollination. However, the rate of self-pollination was similar regardless of growth regulator treatment. Growth regulators increased the cross-pollination from 42 to 70% in 1997 and 17 to 30% in 1998 (Table 3). Therefore, it is suggested that intercrossing be improved using families treated with ethrel surrounded by CP rows treated with AgNO₃ for maximum outcrossing. Ethrel increases the production of pistillate flowers (McMurray and Miller, 1969). On the other hand, AgNO₃ is an ethylene inhibitor and increases the production of staminate flowers (Kwack and Fujieda, 1984). However, AgNO₃ is a heavy metal, so the FDA has recommended the use of alternative growth regulators such as AVG or GA.

Table 2. Temperature (°C) and precipitation (mm) data for different research stations of North Carolina in 1997 and 1998.

Location	May		June		July		August									
	1997		1998		1997		1998									
	Temp [†]	PPT [‡]	Temp	PPT	Temp	PPT	Temp	PPT								
Clinton	18.6	37.6	21.4	145.8	23.0	40.1	26.2	46.7	27.0	276.6	27.7	61.5	24.7	81.0	26.0	184.1
Clayton	19.1	43.9	21.4	72.6	22.8	NA [§]	26.1	39.8	26.5	NA	26.8	153.9	25.2	NA	25.7	109.2
Reidsville	16.6	44.9	19.9	NA	21.1	93.9	24.1	83.8	25.2	100.3	25.9	71.6	23.9	18.3	24.1	148.8
Jackson springs	17.9	25.9	21.1	123.9	21.9	48.0	25.8	24.9	25.9	260.3	26.9	70.4	24.3	31.2	25.3	100.0
Rocky Mount	18.9	35.8	21.2	74.4	22.8	72.1	25.6	69.8	26.0	125.2	26.4	105.1	24.7	41.4	25.8	110.7
Lewiston	18.8	37.8	20.7	89.9	22.7	76.4	25.2	78.5	26.2	129.5	26.4	116.6	24.8	38.1	26.1	111.5
Butner	19.3	28.4	NA	85.8	23.1	90.1	24.4	47.0	27.6	106.9	25.6	123.0	25.4	7.1	24.2	104.9
Kinston	18.2	63.7	20.9	115.0	22.5	45.9	25.5	110.7	26.3	223.7	26.1	155.7	26.3	128.5	24.4	195.6
Mean	18.3	39.8	20.9	101.0	22.2	66.6	25.6	62.7	26.2	174.7	26.6	107.2	24.8	49.4	25.3	133.1

[†]Temp, temperature (°C).

[‡]PPT, precipitation (mm).

[§]NA, not available.

Table 3. Calculated pollen flow in cucumber isolation blocks based on growth regulator application.[†]

Pollination type	Growth regulators	Percentage outcrossing over years		
		1997	1998	Mean
Self-pollination	Not applied	10	56	31
	Applied twice	8	57	33
Cross-pollination (between and within)	Not applied	90	44	69
	Applied twice	92	43	67
Cross-pollination (between plot)	Not applied	42	17	30
	Applied twice	70	30	54
Sib-pollination (within plot)	Not applied	48	27	39
	Applied twice	22	13	13

[†] Data are means of four replications (six in 1998) and two plants (hills) or 16 families (small plots) for both years. Growth regulators were ethrel and AgNO₃, with plants treated twice vs. not treated. Plot size was hill vs. small plot.

Crown-set fruits are set early whereas top-set fruits are set late in the season. Crown-set fruits were expected to have more outcrossing than top-set fruits. However, node of fruit set did not affect the outcrossing rate in cucumber. This suggests that honeybee activity is enough or uniform throughout the flowering period to ensure maximum cross-pollination irrespective of node of flower set. In addition, application of growth regulators for 2 wk would keep the sex expression changed for optimum intercrossing for 2 wk, followed by reversion. If reversion were a problem, then outcrossing rate would change from crown-set to top-set fruits; however, the outcrossing rate was not affected by node of harvest for the fruit.

CONCLUSIONS

Cucumber is an allogamous (naturally cross-pollinated) crop that is suited to breeding methods requiring intercrossing of selected families each cycle of recurrent selection. For those breeding methods, a high rate of outcrossing is needed.

The rate of natural outcrossing varies over years and locations, depending on the environment as well as other factors such as the activity of honey bees. A significant increase in outcrossing rate was observed when growth regulators (AgNO₃ and ethrel) were applied to plants designated as male and female parents. Outcrossing can be increased using isolation blocks made up of family plots treated with ethrel and composite pollenizer rows treated with AgNO₃.

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