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Rate of Natural Outcrossing in Monoecious Cucumbers

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Abstract. The rate of natural outcrossing in cucumber (*Cucumis sativus* L.) was measured at 3 locations in North Carolina, using the dominant allele for scab resistance as the marker gene. Outcrossing was measured within and between 1.5 m plots in isolation blocks. Between-row outcrossing averaged 36% over the 3 locations, and within-row outcrossing averaged 17%. Self-pollination accounted for 47% of the pollinations. Results suggest that populations should be intercrossed either as single plants, or in family rows treated with growth regulators to control pollen flow and prevent sib- and self-pollination.

Recurrent selection schemes have been used widely in cross-pollinated crops to improve the performance of quantitative traits. Application of recurrent half-sib and full-sib family selection for improvement of yield in cucumber as recommended by Smith et al. (14) required that the best families be intercrossed once every cycle for each population being improved. Intercrossing can be carried out either by using bees in isolation blocks, or by making hand pollinations. Use of bees to intercross families requires less hand labor, but will not be useful unless the frequency of natural outcrossing is high, and unless most of the crossing occurs between

families rather than within families in the isolation block.

Lack of severe inbreeding depression in cucumber indicates that the frequency of natural self-pollination is high, or, as Allard (1) suggests, that cucurbits evolved as small populations in nature, thus having a high level of inbreeding due to intercrossing of related individuals. However, the presence of considerable heterozygosity for yield (4) suggests that inbreeding depression may occur in some populations which, in turn, may indicate a high rate of natural outcrossing in cucumbers.

Natural outcrossing of cucumbers depends on the presence of domesticated and wild bees. Honeybees (*Apis mellifera*) generally are used to intercross cucumbers in isolation blocks. Bees do not collect cucumber pollen (10) but are attracted to flowers by nectar, so they visit both staminate and pistillate flowers. Bees visit 3 times more staminate than pistillate flowers (3), however, so pollinations may not be distributed evenly in an intercross block.

The rate of natural outcrossing has been measured in a close relative of cucumber,

the muskmelon (*Cucumis melo* L.). In andromonoecious types, the rate of outcrossing generally was 0% to 20% (5, 9), although some estimates ranged as high as 68% (13). In monoecious muskmelons, outcrossing was estimated to be 73% (13). Whitaker and Bohn (15) found a high degree of variability in the rate of natural outcrossing in muskmelon, with an increase in the rate of outcrossing occurring late in the season.

Most of the cucumber populations being selected in the North Carolina State Univ. program consist of monoecious or gynodioecious families. The gynodioecious families have few staminate flowers under North Carolina conditions and thus should be nearly 100% outcrossed. However, in order to design isolation blocks for efficient natural intercrossing, it was of interest to measure the rate of natural outcrossing in monoecious plants. The objectives of this study were to measure the rate of natural outcrossing in monoecious cucumbers, and to determine the distribution of outcrossing within and between small plots in isolation blocks at several locations in North Carolina.

Experiment design. Test plots were 1.5 m long and were planted on raised, shaped beds 0.5 m wide. Rows were 1.5 m apart (center to center). Plots were overseeded and thinned to 15 plants with a 0.1 m within-row spacing to produce a typical density of 67,000 plants/ha. Treatment units consisted of 3-row plots with the outer rows planted to 'SMR 58' (a scab-resistant cultivar). That provided the pollen for any outcrossing that occurred with the center row. The center row of each 3-row plot was planted in one of 2 ways, hill or row. Hill plots had 5 seeds of 'Addis' (a scab-susceptible cultivar) planted in the middle, with the rest of the 1.5 m plot (0.75 m on each side of the hill) planted to 'SMR 58'. The hill was thinned to 1 plant before flowering began. Row plants were planted to 'Addis' for the entire 1.5 m length.

Thus, any outcrossing that occurred in the 'Addis' row plots would result in scab resistant progeny if the pollen came from 'SMR

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Table 1. The percentage of outcrossing of 'Addis' (a monoecious cucumber inbred) in 1.5 m plots and in single-plant hills at 3 locations in 1983.^a

North Carolina location	Plot type ^b	Outcrossing (%)		No. plants tested
		$\bar{x} \pm 95\% \text{ CI}$	Range	
Clayton	Hill	23 \pm 13	6-26	54
	Row	29 \pm 2	0-61	412
Clinton	Hill	77 \pm 13	0-81	35
	Row	35 \pm 2	0-72	530
Castle Hayne	Hill	59 \pm 17	41-79	85
	Row	43 \pm 2	5-87	566

^aData are means over 2 replications for each location.

^bRow plots consisted of 1.5 m of 'Addis' in a center row with border rows of 'SMR 58' (rows 1.5 m apart center to center). Hill plots were similar except the center row consisted of 'SMR 58' with one hill of 'Addis' in the middle.

^cEstimated as the percentage of 'Addis' offspring that received the dominant allele for scab resistance by outcrossing with 'SMR 58'.

Table 2. Pollen flow in isolation blocks planted to 1.5 \times 1.5 m plots of 'Addis' (a monoecious pickling cucumber inbred).

Type of pollination	North Carolina location			Mean
	Clayton	Clinton	Castle Hayne	
Between + within row (hill plots)	23	77	59	53
Between row (row plots)	29	35	43	36
Within row ^c	0	42	16	17
Self ^d	77	23	41	47

^aCalculated as (between + within row pollination) - (between row pollination).

^bCalculated as (100) - (between + within row pollination).

58' in the 2 outer rows, and in scab susceptible progeny if pollen came from 'Addis' plants in the rest of the center row. Hill plots, on the other hand, were surrounded by 'SMR 58' (both the outer rows and the center row), so outcrossing always would result in scab resistant progeny.

The dominant allele (*Ccu*) for resistance to scab (2) caused by *Cladosporium cucumerinum* Ell. & Arth. was used as a marker to determine whether seeds were produced by self- or cross-pollination. 'SMR 58' carried the dominant alleles and 'Addis' carried the susceptible alleles. It was assumed that the frequency of scab resistant marker genes expressed in the progeny of the 'Addis' plants harvested from the center rows was equal to the frequency of cross-pollination.

The experiment was conducted at 3 North Carolina agricultural experiment stations located at Clayton, Clinton, and Castle Hayne. The 2 cultivars (Addis and SMR 58) containing the marker gene were planted at each location in 1983, and were surrounded with border rows of summer squash (*Cucurbita pepo* L. 'Zucchini Elite').

Data collection. All fruit were harvested from the marker lines at mature-seed stage, and seed from each was saved as a half-sib family (one family for each fruit harvested). All mature fruit were harvested from all plants (usually 1 to 3 per plant) and planted in a scab resistance test in 1984. The test plants, along with 'Addis' and 'SMR 58' were inoculated as described by Pierson and Walker (11). Plants were inoculated in the cotyledonary stage with 4×10^5 conidia/ml sprayed

with an artist's airbrush onto the growing point. Inoculated plants were placed in a dark incubation chamber for 48 hr at 18°C and 95% RH. Plants were rated as susceptible or resistant 8 days after inoculation. The number of plants with the normal dominant phenotype (scab resistance) out of the total was used as the measure of the rate of natural outcrossing.

Natural outcrossing. The mean rate of natural outcrossing ranged from 23% to 77% for the 3 locations tested in 1983 (Table 1). Those measurements included between- and within-plot outcrossing, since they were taken from single-plant hills. The rate of outcrossing occurring between plots generally was lower than for hill plots, ranging from 29% to 43% over locations. The percentage of outcrossing varied widely from one pollination to the next, ranging from 0% (all self-pollinated) to 87%.

Analysis of variance indicated nonsignificant ($\alpha = 0.05$) differences among locations for between- and within-plot outcrossing. This is, in part, due to the use of only 2 replications per location. It would have been difficult to use more replications and still maintain isolation, however. Means over locations indicated that the total rate of outcrossing averaged 53% (Table 2). Of that, 36% was between-row. By subtraction, within-row outcrossing was calculated to be 17%. The remainder was self-pollination, averaging 47% over locations.

Implications for crossing blocks. The intermediate amount of cross-pollination measured (53%) indicates that isolation blocks

made up of single-plant hills would not be successful in producing a well-intercrossed population.

Isolation blocks made up of families planted in plot rows would be even less intercrossed, since about 17% of the pollinations would be within family rows. The resulting population would not be random-mated, because about two-thirds of the crosses would be self- or sib-matings. Only about 36% of the pollinations would be of the type required (between-row) for proper intercrossing. Therefore, intercross blocks made up of family rows should be handled in such a way as to ensure proper pollen flow.

A possible solution would be to plant pollen rows in the isolation block such that the pollen rows consisted of a mixture of all families in the intercross block as done by Lonquist (6). In addition, the plot rows could be sprayed with ethephon [(2-chloroethyl)phosphonic acid] to produce pistillate flowers instead of staminate flowers (7) and thus prevent within-plot crossing. The composite pollinator rows could be sprayed with an ethylene inhibitor such as a silver compound (8) or gibberellic acid (12) to induce staminate flower formation uniformly throughout the different genotypes.

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