Natural Crossing of Peanut in Virginia[1]

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ABSTRACT

Pedigreed natural crossing has been suggested as a technique for increasing the number of hybrids in peanut (*Arachis hypogaea* L.) breeding programs, when used in conjunction with the conventional crossing procedure. Since the success of this technique is dependent upon the natural crossing frequencies, a study was conducted from 1984 - 1986 to determine the current natural crossing frequency in Virginia peanut breeding plots. The cultivar Florigiant was used as the female parent, and the genetic line Krinkle was used as the male parent in a field plot each year. Krinkle has a dominant leaf characteristic easily identified in the hybrid progeny. Seed harvested from the Florigiant parent were planted in subsequent years, and the percentage of Krinkle plants determined for calculating the frequency of outcrossing. The frequency varied with environment from 2.8% in 1984 to 0.0% in 1986. The rate in 1984 was about four times the amount of natural crossing previously reported in Virginia. These results indicate that isolation distances between cultivars in seed fields may need to be greater than in current regulations, and cultivars need to be closely rogued for off-type plants during seed increase.

Key Words: *Arachis hypogaea,* L. groundnut, outcrossing.

Since natural crossing was first reported by van der Stok (14) in 1910 and in the U.S. in 1946 (11), almost all peanut (*Arachis Hypogaea* L.) breeders have observed natural crossing in their field plots. Heide (10) was the first to conduct tests on natural crossing and identify bees as the primary insect involved. Hammons and coworkers (6-9, 12, 13) in Georgia conducted extensive tests on natural crossing rates, and the insects involved. Results from their studies showed that at least three families and 24 species of bees are involved in natural crossing of peanut. Smaller species of bees can visit flowers several times and still obtain viable pollen. Hammons (6) found that pollen collected from bees and applied to emasculated flowers resulted in 66% of the flowers fertilized, indicating that pollen on bees is viable and capable of causing natural crosses.

Natural crossing rates of about 2% have been reported in Java and India (6), while reported rates range from 0.25% to as much as 10% in Georgia (6-8, 12-13). Previous studies in Virginia have reported rates of less than 1.0% (5,11). However, Culp *et al.* (5) felt that natural crossing should be considered when maintaining cultivar purity. The rate of natural crossing varied with the environment and genotypes used (5, 6, 8, 13).

Artificial crossing of peanuts has not changed since van der Stok first developed the procedure in 1910 (14). The amount of success varies widely and is influenced by many factors (8). The complete operation takes about ten minutes per cross and results in one to two seed. Thus, the number of hybrid seed obtained is limited compared to other crops.

Hammons (7) proposed a new technique called controlled natural crossing or more recently pedigreed natural crossing (8) as a means to supplement the number of crosses in an artificial crossing program. In a pedigreed natural cross, both the male and female parents are known. The advantages are: a) crossing is not dependent on the limited time available for a single scientist or trained assistant to perform the cross; b) identification, harvesting, and isolation of hybrids can be done by semi-skilled workers on land unsuitable for yield trials and other experiments; and c) it is more economical than the standard method of crossing. The disadvantages are: a) the male parent must have a dominant marker that is easily classified in the F_1 generation as well as the desirable characteristics needed in the breeding program; b) the pedigree is based on a parental line rather than an individual plant as in the standard method of crossing; and c) large amounts of land and/or labor may be required in identifying the hybrids.

Since previous reports (5, 11) had indicated a low natural crossing rate in Virginia, this study was initiated in 1984 to determine the current rate of natural crossing in Virginia breeding plots, and the feasibility of using the pedigreed natural crossing method in Virginia.

Materials and Methods

Natural crossing plots were established in the field at the Tidewater Agricultural Research Center in 1984 and 1986 on a Eunola loamy fine sand (fine-loamy, siliceous, thermic Aquic Hapludult) and in 1985 on a Dragston fine sandy loam (coarse-loamy, mixed, thermic Aeric Ochraquult). Plots were two 91 cm rows 18.2 m long, one 91 cm row 18.2 m long, and one 91 cm row 7.3 m long in 1984, 1985, and 1986, respectively. Plot rows were alternately planted with seed of Florigiant (2) and Krinkle (7), approximately 8.0 cm apart within the row and bordered by rows of Florigiant on either side. In 1984, plots were in a peanut field 1.0 m from a corn (*Zea mays* L.) field and 30.0 m from a drainage ditch. In 1985, plots were in a peanut field 1.0 m from a roadside ditch. In 1986, plots were in a peanut field about 20.0 m from a corn field. Rainfall and mean monthly temperatures were recorded each year (Table 1).

Florigiant, a large-seeded virginia-type peanut cultivar with a spreading growth habit and normal leaves, was used as the female parent. It has been widely grown in the Virginia-North Carolina area since 1970 and has several desirable characteristics. Krinkle, a spanish-type peanut with upright growth habit and crinkle leaves, was

Table 1. Monthly precipitation and mean temperature for 3 years (1984-86) at Suffolk, Virginia, during the growing season.

	Precipitation (cm)			Temperature (C)		
Month	1984	1985	1986	1984	1985	1986
May	12.1	5.5	2.4	19.1	19.1	19.6
Jun	3.5	15.6	10.9	23.8	22.5	24.3
Jul	21.8	10.3	16.9	23.9	24.2	26.9
Aug	10.0	9.8	21.3	24.2	23.7	23.8
Sep	7.8	25.1	1.3	19.6	21.6	21.1
Oct	2.4	13.0	7.3	18.6	18.1	17.0

[1]Cooperative investigation of U.S. Department of Agriculture, Agricultural Research Service and Virginia Polytechnic Institute and State University, Tidewater Agricultural Experiment Station, Suffolk, VA.

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used as the male parent. Krinkle-leaf is inherited as a dominant characteristic and has been used in several previous natural crossing studies (5-8). The pollen viability, pollen fertility, and flower structures of both parents are typical for peanuts (2, 7).

At harvest, Krinkle plants were hand dug and removed from the plots separately from the Florigiant plants. Pods from the Florigiant plants were harvested, dried, shelled, and planted the following year for identification of F_1 hybrids. Total plants emerged and Krinkle plants emerged were counted four weeks after planting. Identification of hybrids and non-hybrids was verified at harvest by leaf type. The percentage of Krinkle plants was used as an estimate of the percentage of natural crossing, as in previous studies. (5-8).

Results and Discussion

In 1984, 4972 total seed were harvested from the Florigiant parent plants (Table 2). Of these, 3910 emerged as plants in the field in 1985, of which 110 were Krinkle hybrids for a natural crossing rate of 2.81%. In 1985, 2245 total seed were harvested from the Florigiant parent plants. Of these, 2031 emerged as plants in the field in 1986, of which 12 were Krinkle hybrids for a natural crossing rate of 0.59%. In 1986, 583 total seed were harvested from the Florigiant parent plants. Of these, 524 seed emerged as plants in the field in 1987, of which none were Krinkle hybrids for a natural crossing rate of 0.0%. The average natural crossing rate for the three years was 1.90% and is similar to those reported previously for other locations (6-8, 12, 13). However, the 2.81% rate in 1984 is higher than previously reported for Virginia (5,11).

Table 2. Number of seed harvested, seed emerged, hybrid plants, and rates of natural crossing for 3 years (1984-86) at Suffolk, Virginia.

	Year			
Category	1984	1985	1986	Mean
Total Seed Harvested	4972	2245	583	2600
Total Plants Emerged	3910	2031	524	2155
Total Hybrid Plants	110	12	0	41
Natural Crossing Rate (%)	2.81	0.59	0.00	1.90

These results support those of other workers (5, 6, 7, 13) in that the natural crossing rate varied with season (Table 2). These rates are within the limits observed by Hammons (7, 8) when the pedigreed natural crossing method was suggested as a possible breeding procedure. Therefore, the pedigreed natural crossing method could be used as a breeding procedure in Virginia.

Support for this conclusion comes from results in three other studies in our breeding program. First, the spanish-type peanut line Chico (1) has frequent off-type plants in breeding plots in Virginia (Coffelt, unpublished data). Isolation of these off-type plants and subsequent seed increase shows that most of these plants are the result of natural crossing. Thus, Chico, if planted in a crossing block with a desired virginia-type parent, could be used as a female parent. Since work in previous studies on disease resistance has shown Chico to possess cytoplasmic factors for resistance to leaf spot and sclerotinia blight (3, 4), the use of Chico as a female parent has obvious advantages. Chico has other characteristics, such as very early maturity, which are desirable in virginia-type cultivars. The dominant characteristics of growth habit, leaf color, and plant size in Virginia-type parents are easily observed in natural crosses with Chico. Several high yielding lines have been identified in our breeding program from the off-type plants in Chico. Even though we have not utilized the pedigreed natural crossing method so that the male parent is known, these results show that the pedigreed natural crossing method could be a viable procedure.

Second, since about 1970, the predominant cultivar in Virginia has been Florigiant. However, off-type plants that could not be explained by mixture with other cultivars have been found in Florigiant fields. Two cultivars, Keel 29 and AD 1, have been developed from off-type plants in Florigiant. Florigiant is a composite of seven lines. In 1981, controlled crosses were made among the seven component lines. Subsequent evaluation and increase of seed from these crosses resulted in the isolation of breeding lines in the F_3 generation very similar to the cultivars Keel 29 and AD 1 (Coffelt and Wynne, unpublished). This provides further evidence that natural crossing does occur, and thus, supports the proposal that the pedigreed natural crossing method could be used in Virginia to supplement crosses by the conventional method.

Third, in 1985 and 1986 increase plots of seed from the Florigiant parent plots in the natural crossing block the preceding year, off-type non-krinkle plants in addition to the Krinkle hybrids were observed. These off-type plants evidently resulted from crossing among Florigiant plants or crossing with another genotype outside the natural crossing plots. The occurrence of these plants further supports the proposal that the pedigreed natural crossing method could be used in Virginia. The Krinkle hybrids and other off-type plants are being evaluated for breeding utility.

There are at least two other implications of the natural crossing rates found in this study besides assessing the feasibility of using the pedigreed natural crossing method. First, current isolation distance between cultivars of 3.0 m in Virginia may not be adequate, especially since we are now growing seed of several cultivars. Additional studies will need to be conducted to determine the minimum distance required to prevent natural crossing between or within fields. Second, the appearance of off-type plants in fields of the multi-line cultivar Florigiant indicates that in seed production of multi-line cultivars we especially need to be alert for and rogue off-type plants. Off-type plants will probably be more of a problem as component lines become more diverse in multi-line cultivars.

While field location and seasonal effects undoubtedly affected the rate, plot size also may have had some effect. The crossing rates in this study decreased with plot size to zero in a 7.3 m plot. However, the results obtained in this study do not allow the separation of the effects of environment and plot size.

Results from this study show that natural crossing can occur in Virginia at a rate at least four times greater than previously reported. The rate is high enough to utilize pedigreed natural crossing as a tool in breeding programs. However, more work needs to be done to identify optimum plot size, planting densities, and field location. Use of natural crosses could be very beneficial in supplementing crosses obtained by artificial hybridization programs.

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Accepted April 29, 1989