Hand-Tripped Flowers Promote Seed Production in *Arachis lignosa*, a Wild Peanut[1]

Donald J. Banks[2]

ABSTRACT

In trials conducted in the greenhouse, growth chamber, and outdoors, no pegs or pods were produced when *A. lignosa* (Chod. et Hassl.) Krap. et Greg. *nom. nud.* plants were allowed to pollinate naturally. However, hand tripping flowers, especially in the greenhouse, resulted in significant increases in pod production. The results suggest that the high degree of reproductive sterility usually noted for *A. lignosa* in culture is due to pollination failure rather than to physiological self incompatibility. The somewhat truncated shape of the stigma and its elevated position relative to the anthers probably restricts natural self-pollination without the aid of pollinating vectors such as bees.

Key Words: *Arachis hypogaea* L., *Arachis species*, groundnut, peanuts, pollination, self incompatibility, stigma morphology, wild species.

McGregor (8), Norden *et al.* (9), and Coffelt (3) reviewed the literature on natural outcrossing in cultivated peanuts (*Arachis hypogaea* L.) Previous studies have shown that the crop is highly autogamous. However, outcrossing occurs and varies, according to location, from <1 to nearly 10% with bees considered to be the primary pollen vectors.

Compared to *A. hypogaea,* information on the mode of reproduction in the wild species of *Arachis* is extremely limited (2, 6, 7, 10). In the wild species there are obvious variations in flower sizes, colors, and morphology that are suggestive of insect attraction. Consequently, cross pollination by insects probably played an important part in speciation. There is little doubt that natural outcrossing has enhanced genotypic variation considerably within the genus, especially in the case of *A. hypogaea.*

Limited seed production in some profusely flowering wild *Arachis* species grown in culture (e.g., in greenhouses, growth chambers, and field plots), where effective pollen vectors may be scarce, suggests that either self incompatibility mechanisms or pollination failures are the restrictive factors limiting seed production. Stalker *et al.* (10) indicated that more than 40% of their 290 species collections have never produced pegs or seeds in North Carolina. However, it should be noted that some wild peanut accessions succeed as pollen donors in producing interspecific hybrids although the accessions, themselves, are mostly seed-sterile. For example, Gregory and Gregory (4) successfully hybridized a seed-sterile parent, #27 (*A glabrata* Benth., coll. GK 10596, PI 276233), as a pollen donor, with 15 other parents (mostly different species). Their work suggests that pollen sterility is not the only barrier to seed production in the wild species. Bharathi and Murty (2) reported that seed failure in *A. hagenbeckii* (actually *A. glabrata* Benth. var. *hagenbeckii* [Harms ex Kuntze] F. J. Hermann) was due to endosperm malfunction. However, the exact causes of seed sterility in other wild *Arachis* species are poorly understood. According to Halward and Stalker (7), delayed pollen germination and slowed tube growth were detected in *A cardenasii* Krap. et Greg. *nom. nud.* when it was used as a female parent in hybridization attempts with interspecific hybrids involving *A hypogaea*. Consequently, self incompatibility might be suspected as a primary cause of sterility in some *Arachis* species.

A. lignosa (Chod. et Hassl.) Krap. et Greg. *nom. nud.* is a perennial species from Paraguay, South America, that produces little if any seed in the United States. According to W. C. Gregory (personal communication), following the classification system of Gregory *et al.* (5), the species belongs to the *Procumbensae* series of the *Erectoides* section. Although *A. lignosa* resembles some species of the *Arachis* section, it is not closely related to the cultivated peanut, and, in fact, will not hybridize with it.

The objective of this study was to determine the reason for the fruitless character of *A. lignosa* and to ascertain whether or not hand tripping the flowers would help promote seed production in the species.

Materials and Methods

Three flowering plants, devoid of pegs and fruits, originally derived from vegetatively propagated cuttings from a single accession of coll. GK 10598 (PI 276234) were used for the pollination treatments in three dissimilar environments. The controls consisted of three similar plants, derived as above, that were allowed to pollinate naturally.

Pollinations were made during early morning (8:00 - 9:00 a.m.) on plants of *A. lignosa* by manually tripping the flowers by pulling down on the wing petals in a flexing manner to exert the style while ejecting pollen from the keel onto the protruding stigma. The wings were then released to allow natural retraction of the style into the keel.

The environments included a fiberglass greenhouse with a range of temperatures from ca. 21 to 29 C; a growth chamber with a 12-hour, 21 C dark and a 12-hour, 29 c light cycle; and normal outdoor conditions. The outdoor site was adjacent to the greenhouse in a suburban area where no bees were observed during the summer months. The ground covers, consisting of bermudagrass, *Cynodon dactylon* (L.) Pers., gravel, and concrete (roads and walks), probably accounted for the lack of bee activity. In all trials, the plants were grown at Stillwater, Oklahoma, in sandy loam soil in 25.4 -cm clay pots and were watered and fertilized to maintain good growth.

The trial durations (from pollination commencement to harvest) were July 14 to November 12, 1976; June 26 to December 10, 1978; and August 31, 1979, to January 12, 1980, for the greenhouse, outdoor, and growth chamber experiments, respectively. The flowers that were produced on all plants were counted each day throughout the trials. Hand pollinations were performed during Monday through Friday each week. On weekends and holidays the flowers were removed from all plants during early morning (ca. 8:00 a.m.) as a guard against potential selfing. Pollinations ceased on September 7, September 21, and November 14 for the greenhouse, outdoor, and growth chamber experiments, respectively. Flowers were removed from all plants, including controls, for at least 21 days after the pollination periods ceased in order to lessen the chances of natural selfing

The plants from the outdoor trial were transferred to the greenhouse after the pollination period had ended to avoid the anticipated low temperatures during late fall and winter.

[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by USDA and does not imply its approval to the exclusion of other products that may also be suitable.

[2]Research Geneticist, USDA-ARS, Plant Science Research Laboratory, Stillwater, OK 74075.

The plants were harvested, and their firm pods (actually single-seeded segments) were counted on the final date of the duration schedule (shown above). Statistical analyses, including ANOVA and correlation coefficients procedures, were conducted using SAS.

In 1983, stigmas of *A. lignosa* and Chico (*A. hypogaea*) from fresh greenhouse flowers were mounted directly on stubs with "double-stick" adhesive tape and compared by using a scanning electron microscope to ascertain differences in morphology. Electron micrographs were made to document the findings.

Results and Discussion

Regardless of the environment in which the trials were conducted, no pegs or pods were formed on any of the naturally pollinated plants, whereas significant numbers of pods were produced on the plants with hand-tripped flowers (Table 1). The pod numbers from the greenhouse plants were significantly greater than the pod numbers of the plants from the other environments. However, the pod numbers produced in the growth chamber and the outdoor environments were not significantly different.

Table 1. Effect of pollination method on pod production in *Arachis lignosa*.

Location	Duration[a]	Flowers pollinated[b]		Pods harvested[b]	
		Natural	Tripped	Natural	Tripped
Greenhouse	56	336a	295a	0	64.0a
Growth Chamber	76	292a	134b	0	7.7b
Outdoors	88	1288a	1286a	0	17.3b

[a]No. of days from beginning to end of pollination schedule.

[b]Mean of three plants per treatment. Means followed by a different letter within rows, for flowers pollinated, or within columns, for pods harvested, are significantly different according to Duncan's New Multiple Range Test (P=0.05).

The success rate for hand tripping was highest in the greenhouse (21.7%), intermediate in the growth chamber (5.7%), and lowest outdoors (1.3%), even though more flowers were tripped on the outdoor plants. The superiority of the greenhouse environment may have been due, partially, to the high humidities that exist where a wet-pad type of cooling system is employed.

A review of the 1978 weather records for Stillwater revealed that the environment was hot and dry when the outdoor experiment was conducted. During the pollination period the average maximum temperature was 34.9 C, and there were 24 days with temperatures of 37.8 C or higher. Records of relative humidities are not available, but rainfall for the period was 86.4 cm. In view of the conditions, it seems plausible that exposure of the stigmas and pollen to hot, dry winds in the outdoor environment could have lowered the fertility of the plants growing there. It is more difficult to explain the relatively low hand-pollination success rate for the growth chamber plants, but the relative humidity may have been a factor.

Based on earlier intraspecific hybridization experiences with *A. hypogaea,* higher success rates with *A. lignosa* might have been expected than were achieved during the present study. Banks (1) reported successful pollination rates (hybrids/ pollinations) ranging from 26 to 78% in growth chamber studies. Exact reasons for the lower successes with *A. lignosa* are unknown. However, it is likely that *A. hypogaea,* with a comparatively larger stigma, is easier to pollinate than *A. Lignosa*. Also, in the case of the *A. hypogaea* hybridization trials, actual pollinations were made by physically placing pollen on the stigma with forceps, and the accuracy of each pollination was visually verified with a hand lens. Additionally, it is possible that retraction of the style into the keel of *A. lignosa* flowers could have resulted in the dislodgement of pollen from the stigma.

The flower numbers used for the natural pollination and the hand-tripped treatments were not significantly different, except for the growth chamber environment. The coefficients of variation for flowering (i.e., comparing the natural and hand-tripped treatments within environments were 33.1, 24.3, and 27.7 for the greenhouse, growth chamber, and outdoor environments, respectively. As expected, there were good correlations of flower production with pod development in all three environments. R-values, correlating flowers and pods for the hand-tripped treatments, were 0.91, 0.89, and 0.67 for the growth chamber, greenhouse, and outdoor environments, respectively.

The scanning electron microscope study revealed that there are distinct differences in the morphology of the stigmas of *A. lignosa* and *A. hypogaea* (Fig. 1 and 2). The stigma of *A. hypogaea* is enlarged and distinctly globose, and provides a good receptacle for pollen, whereas the stigma of *A. lignosa* is smaller and somewhat truncated which makes pollen positioning upon it difficult. Difficulties in applying pollen to the stigmas of *A. lignosa* had been noted in earlier hybridization trials (Banks, unpublished). Additionally, it was found that the stigma of *A. lignosa* is positioned higher, relative to the anthers, than in *A. hypogaea* (Fig. 3). This floral arrangement would make successful self pollinations, even by tripping, somewhat difficult. The above findings should help explain why *A. lignosa* did not respond as well to the pollination treatments.

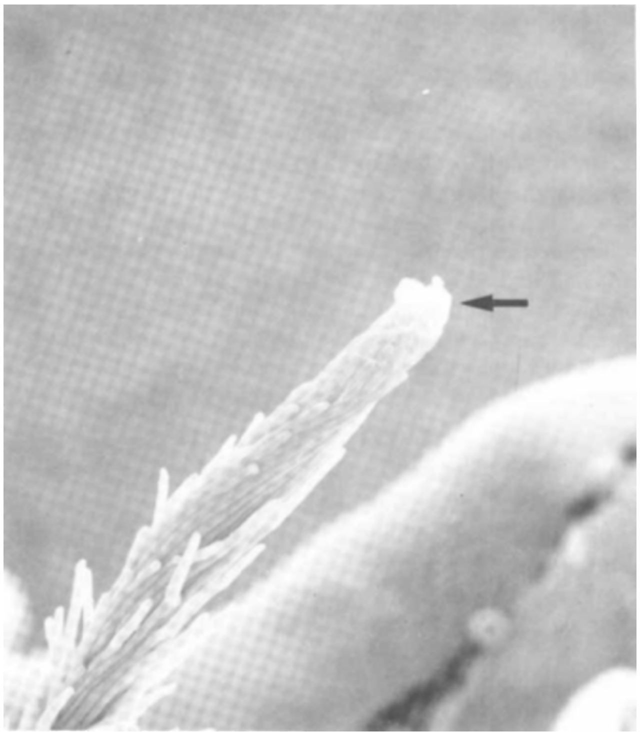

Fig. 1. Style and stigma (arrow) of *A. lignosa* showing the truncated end. (320x)

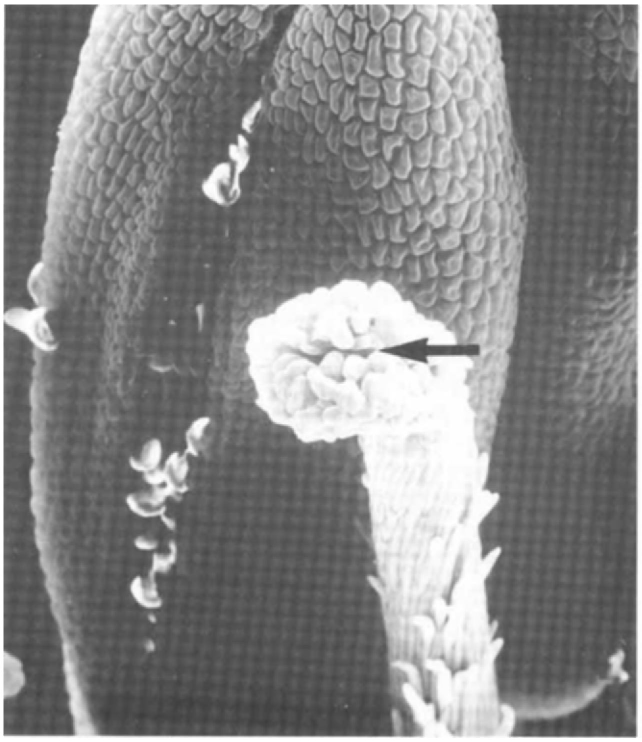

Fig. 2. Style and stigma (arrow) of A. hypogaea (cv. Chico) showing the globose end. Note the anther (background) overtopping the stigma. (320x)

Fig. 3. Stamens and pistil of A. lignosa showing the elevated stigma. (27x)

Conclusions

The present experiment, involving three different environments (i.e., greenhouse, growth chamber, and outdoors) demonstrated that seed production is possible in *A. lignosa* when effective pollination techniques are employed. Consequently, physiological self incompatibility can be discounted as the primary mechanism restricting fertility in this species. It appears probable that natural selfing in *A. lignosa* is ineffective in promoting seed production mainly because pollen is not properly positioned on the stigma to promote pollen germination, and therefore, fertilizations do not occur. Thus, the morphology of the flower, especially stigma shape and position, limits natural selfing in this species. There are likely some other species of *Arachis*, like *A. lignosa,* that fail to produce seed chiefly because of pollination failure. Similar studies with other species of *Arachis* are warranted. This study provides a procedure for producing seeds of A. lignosa which will insure its survival under cultivated conditions.

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