# Reproductive Efficiency in Reciprocal Crosses of *Arachis monticola* with *A. hypogaea* Subspecies[1]

H. E. Pattee[2]*, H. T. Stalker[3] and F. G. Giesbrecht[4]

**ABSTRACT**

Wild species of *Arachis* encompass a large number of species which can provide valuable genetic resources for improving *A. hypogaea* L., the domesticated peanut. *Arachis monticola* Krapov. and Rig. is the only species which is both cross compatible with *A. hypogaea* and at the same ploidy level. An evaluation of reproductive efficiency in crosses between *A. hypogaea* and *A. monticola* was conducted to better understand the potential for utilization of this germplasm. This study documents the reproductive efficiency of *A. monticola* in reciprocal crosses with *A. hypogaea* subsp. *hypogaea* var. *hypogaea* cvs. Florunner and NC 6; *A. hypogaea* subsp. *fastigiata* var. *vulgaris* cv. Argentine; and *A. hypogaea* subsp. *fastigiata* var. *fastigiata* cv. New Mexico Valencia C by using selfs as controls. A significant maternal effect was observed among selfs and hybrids for timing of fertilization. Selfs of Florunner and New Mexico Valencia C initiated fertilization by 1 d after pollination, whereas syngamy did not occur in selfs of NC 6, Argentine or *A. monticola* until after day 1. Fertilization approached 100% in *A. monticola* and *A. hypogaea* genotypes except for New Mexico Valencia C, which only had 70% of the eggs fertilized. Embryo abortion was observed in both selfs and interspecific hybrids, with the highest rates in selfs after the pegs entered the soil; but in hybrids abortion also occurred as the peg elongated. Crosses were generally more successful when *A. hypogaea* was the female parent, and developing cultivars with *A. monticola* cytoplasm will be difficult. Sixty to more than 90% of growing ovules aborted in different interspecific crosses. *Arachis monticola* selfs and hybrids most closely followed the pattern of reproductive development of *A. hypogaea* cv. Argentine, which lends support to the theory that *A. monticola* is a weedy derivative of the cultivated peanut.

Key Words: Interspecific hybridization, fertilization timing, abortion, peanut, groundnut.

Both subspecies of *Arachis hypogaea* L. are cultivated in the western hemisphere, but within the U.S., the subsp. *hypogaea* var. *hypogaea* (virginia and runner market types) predominates. Cultivars of subsp. *fastigiata* include spanish (var. *vulgaris*) and valencia (var. *fastigiata*) market types which are grown in the Southwest on a more limited land area. Although genetic resources within the domesticated species may be adequate to solve many production problems, genetic resistance to many diseases and insect pests is not readily available. Improvement of the domesticated species through interspecific hybridization is an alternative method for crop improvement, but utilization of wild *Arachis* species is severely impeded because of reproductive barriers and/or genetic incompatibility between species. Although many species within section *Arachis* have been hybridized with *A. hypogaea* (see Stalker and Moss, 1987; Stalker and Simpson, 1995), sterility due to ploidy level differences and embryo abortion still restricts efficient germplasm utilization. *Arachis monticola* Krapov. and Rig. is the only species that is both cross-compatible with *A. hypogaea* and has the same chromosome number. Two cultivars released during the early 1970s have *A. monticola* in their pedigrees (Hammons, 1970; Simpson and Smith, 1974), but little other advantage has been taken of this species in broadening the genetic diversity of *A. hypogaea*.

To better utilize the *Arachis* gene pool for broadening the genetic base of the domesticated peanut, a series of investigations documented the reproductive characteristics of several peanut species (Halward and Stalker, 1987a,b; Pattee and Mohapatra, 1987; Pattee and Stalker, 1991, 1992; Pattee *et al.* 1991). Peanuts differ from most plant species because flowers are produced above ground but form the fruit below the soil. Immediately following fertilization the embryo enters a growth phase until it reaches an eight- to 16-cell stage. The embryo then becomes quiescent as the peg elongates towards the soil during the following 5 to 10 d. After pegs of the species

[1] The research reported in this paper was a cooperative effort of the Agricultural Research Service, United States Department of Agriculture and the North Carolina Agric. Res. Serv., Raleigh, NC 27695-7643. Partial funding was received from the Peanut CRSP, USAID grant number DAN-4048-G-SS-2065-00. The use of trade names in this publication does not imply endorsement by the United States Department of Agriculture or the North Carolina Agric. Res. Serv. of the products named, nor criticism of similar ones not mentioned.

[2] Res. Chem., U. S. Department of Agriculture, Agricultural Research Service, Dept. of Botany, Box 7625 North Carolina State Univ., Raleigh, NC 27695-7625.

[3] Prof., Dept. of Crop Science, Box 7629, North Carolina State Univ., Raleigh, NC 27695-7629.

[4] Prof., Dept. of Statistics, North Carolina State Univ., Box 8401, Raleigh, NC 27695-8401.

*Corresponding author.

*A. hypogaea* reach the soil, they stop elongating, the apical end expands into a pod, and the embryo again enters a growth phase. Similarly, in wild *Arachis* species the peg forms a pod soon after entering the soil; however, in most of these species a secondary meristem becomes active, resulting in continued peg growth for several centimeters to a meter or more before one (or more) additional pods are produced. Significant differences between cultivated and wild species have been observed in ovary structure, onset and rate of peg growth, and presence of starch grains in the embryo sac (Pattee *et al.*, 1991); however, these studies did not include *A. monticola*. Halward and Stalker (1987a) and Pattee *et al.* (1991) reported differences in reproductive growth rates between cultivated and several diploid species, while Bharathi and Murty (1984) found no significant differences. Although information on embryo development and reproductive efficiency in hybrids between cultivated and wild taxa has appeared in the literature (Johansen and Smith, 1956; Halward and Stalker, 1987b; Ozias-Akins and Branch, 1990; Pattee and Stalker, 1992), a description of reproductive efficiency with *A. monticola* has not been published.

This study was undertaken to document reproductive efficiency using the runner market-type cv. Florunner, large seeded virginia market-type cv. NC 6, spanish market-type cv. Argentine, and valencia market-type cv. New Mexico Valencia C in reciprocal crosses with *A. monticola*. The objectives were to (a) develop a comparative basis for evaluating reproductive efficiency with crosses using different *A. hypogaea* varieties, (b) determine the greatest compatibilities between *A. hypogaea* varieties and *A. monticola*, and (c) estimate the potential for broadening germplasm diversity of *A. hypogaea*.

## Materials and Methods

The tetraploid ($2n = 4x = 40$) *A. monticola* (GKBSPSc 30062, PI 468196) was used to make reciprocal crosses to four tetraploid species *A. hypogaea* cultivars, including var. *hypogaea* (Florunner and NC 6), var. *vulgaris* (Argentine), and var. *fastigiata* (New Mexico Valencia C). *Arachis monticola* is an annual species in section *Arachis*. The accession used in this study was collected in northern Argentina during 1977. Plants were grown in a greenhouse at North Carolina State Univ., Raleigh, NC, from April through July during 1991 and 1992 in boxes filled with a growth medium of 1 part sand: 1 part Metromix™ 220 (Grace Sierra, Milpitas, CA): 1 part soil. They were fertilized regularly with a soluble nitrogen-phosphorus-potassium (20-20-20) fertilizer. Landplaster was applied as a source of calcium, which is necessary for embryo development (Cox *et al.*, 1982).

Flowers to be selfed were tagged with numbered bands on the morning of anthesis. Flowers to be crossed were emasculated about 18 hr before anthesis and hand-pollinated between 8:00 and 9:00 A.M. the morning of anthesis. Thirteen flowers were tagged with numbered bands for each of 12 sampling stages (1, 1.5, 2, 2.5, 3, 4, 5, 6, 10, 14, 21, and 28 d after anthesis) for selfs of the *A. hypogaea* cultivars, the *A. monticola* accession, and crosses of *A. monticola* × *A. hypogaea* (in reciprocal). Samples for 1.5 and 2.5 d were omitted when *A. hypogaea* was the female parent. The 2324 harvested samples were fixed in FAA (9 parts 70% EtOH: 0.5 parts glacial acetic acid: 0.5 parts formalin) for 72 hr and then stored at 5 C in 70% EtOH until they were processed for light microscopy. A maximum of eight pegs or pods of each self and at each sampling time were dehydrated and embedded in paraffin according to Berlyn and Miksche (1976). Paraffin-embedded tissues were sectioned at 7 μm thickness and stained with safranin-fast green. Additional pollinations were made for selfs of *A. hypogaea* and *A. monticola* to better determine the percentage of pegs which reached the soil and formed pods.

Based on external morphological characteristics the pegs were designated as 'growing' or 'nongrowing' for all tissues sampled between days 1 and 7. Only growing samples were collected at day 10 and above. These were classified as 'aerial' or 'in-the-soil'. Reproductive observations were made on a maximum of four representative samples from the designated 'growing' and 'nongrowing' groups for each cross. However, in three crosses × collection-time designations there were only one or two 'nongrowing' tissues available because of early abortion and subsequent tissue decay. The standards used for rating normal *Arachis* embryo development were those described by Pattee and Mohapatra (1987) and Pattee *et al.* (1991). The zygote, proembryo, or embryo was classified as aborted if it showed cellular disorganization and/or collapse and disintegration of the cell mass. Lack of progressive development was used also as a criterion at 7 d and older. At days 7 and 10, the growth stage must have achieved D3 (Pattee and Mohapatra, 1987) or beyond to be classified as developing. To be classified as developing at day 14, the developmental stage must have reached at least D4, and at days 21 and 28 the small globular stage must have been achieved.

The curves displayed in Fig. 1a, c, e and g were obtained by using PROC NLIN in SAS (1989) to compute a weighted nonlinear regression of fraction fertile ovules on days after hand pollination. The fraction fertile ovules was estimated by the ratio of number of fertile ovules + 0.5 to number of ovules examined + 1.0. The function fitted was a quadratic curve constrained to join to a constant asymptote. Appropriate weights were computed on the basis of the assumed underlying binomial distribution.

## Results and Discussion

***Selfs***. Fertilization occurred at different rates among the *A. hypogaea* parents (Fig. 1). For NC 6, more than 90% of the eggs were fertilized between days 1 and 2 (Fig. 1a). However, about 25% of the ovules never resumed growth after the peg entered the soil even though the pod was enlarging (Fig. 1b). Of the 89 pollinations made for 10 to 28-d-old samples for NC 6, 37 resulted in a pod containing one or two growing ovules. Fertilization in Argentine followed the same time sequence as for NC 6, where fertilization occurred between days 1 and 2 (Fig. 1c). However, a significant amount of embryo abortion occurred between days 7 and 10 when the peg was elongating towards the soil, and again at the time of pod expansion. At the time of pod expansion the embryo should have resumed growth, but only 40% of the growing ovules remained viable by day 28 (Fig. 1d). In a second crossing program, 13/20 and 17/24 pollinations resulted in pods for selfs of NC 6 and Argentine, respectively. Thus, even under near ideal conditions for repro-

ductive development, a significant amount of embryo abortion occurred.

Reproductive development was observed earlier in Florunner selfs than for either NC 6 or Argentine: about 50% fertilization occurred within the first day after pollination, but continued for another 2 d by which time most (98%) of the ovules were growing (Fig. 1e). However, 50% of the growing ovules did not resume growth after pegs entered the soil (Fig. 1f). Florunner also had a slightly higher frequency of late ovule abortion as observed by the lower number of developing ovules in pods (Table 1). New Mexico Valencia C followed a similar time sequence of reproductive development as Florunner where nearly 40% of the ovules were fertilized

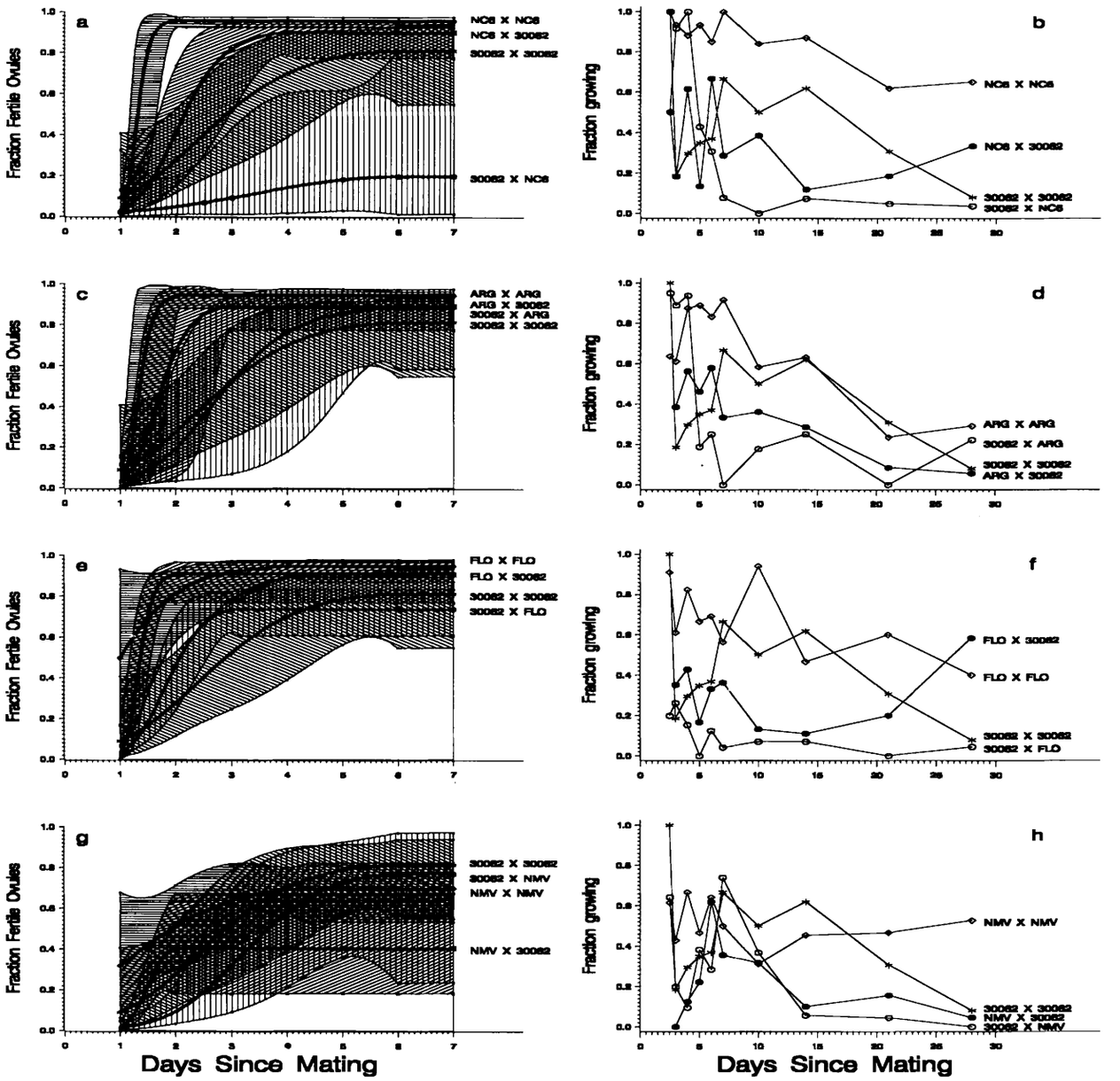


**Fig. 1. Patterns of development in *A. monticola* (30062) selfs and reciprocal crosses with *A. hypogaea* subsp. *hypogaea* var. *hypogaea* cvs. Florunner (FLO) and NC 6; *A. hypogaea* subsp. *fastigiata* var. *vulgaris* cv. Argentine (ARG); and *A. hypogaea* subsp. *fastigiata* var. *fastigiata* cv. New Mexico Valencia C (NMV). (a) Plot of the smooth curve fitted to values calculated by the equation, total number of fertilized ovules / total number of ovules observed in peanut pegs designated as growing at each collection day after pollination, in the NC 6 and *A. monticola* selfs and reciprocal crosses. The shaded band for each curve presents its 95% confidence interval. (b) Plot of values calculated by the equation, total number of pegs which commenced growth less those designated as having ceased growth / total number of crosses at each collection day after pollination, in the NC 6 and *A. monticola* selfs and reciprocal crosses. (c) Same as (a) except ARG and *A. monticola* selfs and reciprocal crosses. (d) Same as (b) except ARG and *A. monticola* selfs and reciprocal crosses. (e) Same as (a) except FLO and *A. monticola* selfs and reciprocal crosses. (f) Same as (b) except FLO and *A. monticola* selfs and reciprocal crosses. (g) Same as (a) except NMV and *A. monticola* selfs and reciprocal crosses. (h) Same as (b) except NMV and *A. monticola* selfs and reciprocal crosses.**

by day 1 (Fig. 1g). However, only 70% of the potential number of ovules in the pegs developed. A large percentage of pegs in both Florunner and New Mexico Valencia C did not reach the soil by 28 d (Fig. 1h), and fewer than 50% of pollinations resulted in pod development (Table 2).

Fertilization in *A. monticola* was delayed until after day 1 and 80% of the eggs were fertilized by day 3 (Fig. 1a). Embryo abortion was frequently observed within the first 5 d after fertilization and again after pegs entered the soil. By 28 d after pollination, fewer than 20% of the ovules were viable and only five pods were recovered from 38 pollinations (Table 2).

Although Smith (1956) reported that fertilization in peanut occurred within the first 12 hr after pollination, our study showed that fertilization did not occur for NC 6, Argentine, and *A. monticola* until after day 1. Pattee and Stalker (1992) reported similar results where syngamy occurred in *A. duranensis* Krapov. and W. C. Gregory and *A. hypogaea* at about 24 hr after pollination, and just prior to 2 d after pollination in *A. stenosperma* Krapov. and W. C. Gregory.

**Table 1. Fraction of embryos which were developing in histologically observed pods (10- through 28-d stages).**

| Female/Male | NC 6 | Florunner | N. M. Val. C | Argentine | *A. monticola* |
|---|---|---|---|---|---|
| NC 6 | 40/45 | -- | -- | -- | 14/20 |
| Florunner | -- | 28/34 | -- | -- | 24/26 |
| New Mex. Val. C | -- | -- | 30/32 | -- | 11/26 |
| Argentine | -- | -- | -- | 26/26 | 2/13 |
| *A. monticola* | 1/2 | 1/3 | 1/2 | 2/2 | 2/4 |

**Table 2. Number of pollinations and distribution of samples collected at 21 and 28-d reproductive stages.**

| | Pollinations | Aerial pegs | Pegs In soil | Pods In soil | Total collected |
|---|---|---|---|---|---|
| | no. | no. | -- no. -- | | no. |
| **Selfs** | | | | | |
| NC 6 | 41 | 1 | 1 | 28 | 30 |
| Florunner | 30 | 2 | 9 | 13 | 24 |
| New Mex. Val. C | 34 | 4 | 12 | 14 | 30 |
| Argentine | 41 | 11 | 10 | 9 | 30 |
| *A. monticola* | 38 | 13 | 5 | 5 | 23 |
| **Crosses** | | | | | |
| *A. monticola* × NC 6 | 48 | 37 | 5 | 2 | 44 |
| *A. monticola* × Florunner | 43 | 29 | 2 | 2 | 33 |
| *A. monticola* × New Mex. Val C | 48 | 23 | 6 | 1 | 30 |
| *A. monticola* × Argentine | 43 | 35 | 3 | 4 | 42 |
| NC 6 × *A. monticola* | 54 | 31 | 3 | 11 | 45 |
| Florunner × *A. monticola* | 37 | 20 | 0 | 12 | 32 |
| New Mex. Val C × *A. monticola* | 54 | 23 | 8 | 7 | 38 |
| Argentine × *A. monticola* | 42 | 26 | 1 | 8 | 35 |

Because the timing of fertilization in the large-seeded cultivar NC 6 was nearly the same as the small-seeded cultivar Argentine, final size of reproductive tissues does not appear to be related to the pollen's ability to fertilize the egg. Further, timing of fertilization does not appear to be associated with subspecific classification because NC 6 (subsp. *hypogaea*) and Argentine (subsp. *fastigiata*) had similar sequences of development, whereas Florunner (subsp. *hypogaea*) and New Mexico Valencia C (subsp. *fastigiata*) had comparable patterns. Thus, fertilization appears to be more dependent on genotype than subspecies or variety. Three cultivars had nearly 100% fertilization without mechanical manipulation of flowers, but New Mexico Valencia C only had about 70% syngamy. The flowers were not tripped, so the reason for the low frequency in New Mexico Valencia C is unknown; but it could be due to mechanical failure of pollen shed, morphological or physiological obstructions to pollen tube growth, or to genetic causes in the form of partial self-incompatibility.

A larger percentage of embryos aborted in selfs of *A. hypogaea* and *A. monticola* than expected. Most abortion was observed after the peg entered the soil, at which time the embryo never resumed growth. Differences were then observed among the genotypes where the large-seeded type, NC 6, had about 25% abortion while 80% of the ovules aborted in *A. monticola*.

***Crosses***. Delayed fertilization was observed in NC 6 × *A. monticola* crosses as opposed to NC 6 selfs (Fig. 1a). However, syngamy occurred in nearly all ovules by 3 to 4 d and no abortion occurred during the first 7 d of reproductive development. Embryo abortion was significantly ($P \leq 0.05$) more frequent between days 7 and 15 in the NC 6 × *A. monticola* hybrids than in NC 6 selfs and, by day 28, only 40% of the hybrid embryos remained viable (Fig. 1b). In the reciprocal cross, *A. monticola* × NC 6, fertilization occurred at a significantly lower frequency than when *A. hypogaea* was the female parent (Fig. 1a). Abortion occurred most frequently as the pegs were elongating, and only a small percentage of ovules were viable by day 28 (Fig. 1b). Obtaining hybrids between NC 6 and *A. monticola* is very difficult when the wild species is the female parent.

Although fertilization was delayed when *A. monticola* was the female vs. male parent, Argentine had the most similar reproductive development to *A. monticola* for selfs and crosses (Fig. 1c,d). A large number of ovules aborted in Argentine × *A. monticola* (and reciprocal) crosses both at the time when pegs elongated and at the time of pod enlargement (Fig. 1d), but significant differences in the final percentage of fertilized ovules were not observed. Only eight pods were recovered for Argentine × *A. monticola* crosses and four for the reciprocal (Table 2). Thus, hybrids between these genotypes can be obtained, but only after relatively large numbers of pollinations are made.

Although Florunner selfs initiated syngamy before

Florunner × *A. monticola* crosses (including reciprocals), by day 3 the Florunner females in both selfs and hybrids had the same pattern of development (Fig. 1e). When *A. monticola* was the female parent in crosses, about 20% fewer eggs were fertilized than when it was used as the male parent, however, differences were statistically nonsignificant. Many ovules aborted when the peg was elongating or after the peg entered the soil, and very few viable Florunner × *A. monticola* (or reciprocal) ovules were observed at day 28 (Fig. 1f). The percentage of viable hybrid ovules was significantly less in crosses than for Florunner selfs. Fourteen pods were recovered during the study for Florunner × *A. monticola* crosses, including reciprocals, with most being obtained from Florunner as the female parent (Table 2).

The *A. monticola* × New Mexico Valencia C crosses had the same pattern of early embryo development as selfs of either species, but fertilization was significantly lower for New Mexico Valencia C females when crossed with *A. monticola* (Fig. 1g). Abortion occurred earlier in these crosses than for other cultivars, and the data indicated that a progressive amount of abortion occurred from a few days after fertilization until 28 d (Fig. 1h). Ovules also aborted after pods developed in New Mexico Valencia C × *A. monticola* crosses (Table 1). More than 90% abortion occurred by day 28, and the percentage of viable ovules was significantly less in crosses than for New Mexico Valencia C selfs. Only one viable embryo was observed by 28 d after pollination when *A. monticola* was the female parent.

When developing pods were divided into 'slow growth' and 'normal growth' classes, most abortion occurred in the 'slow growth' pods. For example, 13 of 70 ovules (18.6%) in the 'slow growth' group aborted, whereas only 15 of 244 ovules (6.1%) in the 'normally growing' group aborted. Of the nine ovules observed in pegs or pods below the soil from interspecific hybrids with *A. monticola* as the female parent, only five were viable. Although viable ovules were observed when *A. monticola* was either the female or male parent at 28 d after pollination, few pods were observed when the wild species was used as the female (Tables 1 and 2). The data indicate that *A. monticola* is cross-compatible with *A. hypogaea*, but hybrids are difficult to produce for individual cross combinations. Fertilization is less of a problem than abortion in interspecific hybrids, but significant maternal effects were observed both in fertilization percentage and abortion where *A. monticola* was generally, but not always, a better male than female parent. When using *A. monticola* as a female, one must also be aware that this species has fragile pegs which are easily broken prior to harvest, thus making hybrid recovery difficult. These factors do not eliminate the usefulness of *A. monticola* for altering the cytoplasmic genome or introgressing nuclear genes, but for some hybrid combinations, great efforts will be required to obtain mature hybrid plants.

Krapovickas and Rigoni (1957) described *A. monticola* as a distinct species primarily based on morphological characteristics. In large part because *A. monticola* is found in the northern Argentina to southern Bolivia region of South America, the domesticated species is believed to have originated in this area. The origin of *A. monticola* has been questioned, however, and whether it is a progenitor species of *A. hypogaea* or a weedy derivative has been debated (Stalker and Simpson, 1995). The evidence for the progenitor theory comes from *A. monticola* being the only other tetraploid species in section *Arachis*. However, Stalker and Dalmacio (1986) concluded that it is karyotypically more similar to *A. hypogaea* var. *vulgaris*, which is evolutionarily the most advanced of the peanut varieties (Stalker and Simpson, 1995). This reproductive study indicates that among the four cultivars observed, *A. monticola* is most similar to Argentine, and thus adds some support to the weedy-derivative theory. Further, progeny from a subsp. *hypogaea* × subsp. *fastigiata* type once gave rise to plant types and pods indistinguishable from *A. monticola* (Wynne and Stalker, unpub.). Although hybridizing *A. hypogaea* and *A. monticola* may be difficult, they are not so isolated as to be classified as incompatible, but rather 'difficult to hybridize', and the *A. monticola* gene pool is more accessible for crop improvement than other *Arachis* species. Based on karyotypes of *A. monticola* and *A. hypogaea* (Stalker and Dalmacio, 1986), meiotic pairing in hybrids (Raman, 1959) and molecular marker analyses (Halward *et al.*, 1992), the two taxa are very similar and genetically should be considered as the same biological species. A review of the taxonomy of the domesticated species and its relationship to *A. monticola* is thus believed to be warranted.

## Acknowledgments

We thank Mrs. Cynthia Hammond for her excellent technical assistance.

## Literature Cited

Berlyn, G. P., and J. P. Miksche. 1976. Botanical Microtechnique and Cytochemistry. The Iowa State Univ. Press, Ames.

Bharathi, M., and U. R. Murty. 1984. Comparative embryology of wild and cultivated species of *Arachis*. Phytomorphology 34:48-56.

Cox, F. R., F. Adams, and B. B. Tucker. 1982. Liming, fertilization, and mineral nutrition, pp. 139-163. *In* H. E. Pattee and C. T. Young (eds) Peanut Science and Technology. Amer. Peanut Res. Educ. Soc., Stillwater, OK.

Halward, T. M., and H. T. Stalker. 1987a. Comparison of embryo development in wild and cultivated *Arachis* species. Ann. Bot. 59:9-14.

Halward, T. M., and H. T. Stalker. 1987b. Incompatibility mechanisms in interspecific peanut hybrids. Crop Sci. 27:456-460.

Halward, T., T. Stalker, E. LaRue, and G. Kochert. 1992. Use of single-primer DNA amplification in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol. Biol. 18:315-325.

Hammons, R. O. 1970. Registration of Spancross peanuts. Crop Sci. 10:459.

Johansen, E. L., and B. W. Smith 1956. *Arachis hypogaea* × *Arachis diogoi*. Embryo and seed failure. Amer. J. Bot. 43:250-258.

Krapovickas, A., and V. A. Rigoni. 1957. Nuevas especies de *Arachis* vinculadas al problem del origen del mani. Darwiniana 11:431-455.

Ozias-Akins, P., and W. D. Branch. 1990. Incompatibility during late embryogeny in some crosses of *Arachis hypogaea* × *A. stenosperma* and the utility of three tissue culture methods for hybrid rescue. Proc. Amer. Peanut Res. Educ. Soc. 22:51 (abstr.).

Pattee H. E., and S. C. Mohapatra. 1987. Anatomical changes during ontogeny of the peanut (*Arachis hypogaea* L.) fruit: Mature megagametophyte through heart-shaped embryo. Bot. Gaz. 148:156-164.

Pattee, H. E., and H. T. Stalker. 1991. Comparative embryo sac

morphology at anthesis of cultivated and wild species of *Arachis*. Ann. Bot. 68:511-517.

Pattee, H. E., and H. T. Stalker, 1992. Reproductive efficiency in reciprocal crosses of *Arachis duranensis* and *A. stenosperma* with *A. hypogaea* cv. NC 6. Peanut Sci. 19:46-51.

Pattee, H. E., H. T. Stalker, and F. G. Giesbrecht. 1991. Comparative peg, ovary and ovule ontogeny of selected cultivated and wild-type *Arachis* species. Bot. Gaz. 152:64-71.

Raman, V. S. 1959. Studies in the genus *Arachis*. VII. A natural interspecific hybrid. Indian Oilseeds J. 3:226-228.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Vers. 6, 4th Ed., Vol 2. Cary, NC.

Simpson, C. E., and O. D. Smith. 1974. Tamnut 74. Texas Agric. Exp. Leaflet L-1348.

Smith, B. W. 1956. Normal megasporogenesis and syngamy with occasional single fertilization. Amer. J. Bot. 43:81-89.

Stalker, H. T., and R. D. Dalmacio. 1986. Karyotype analysis and relationships among varieties of *Arachis hypogaea* L. Cytologia 51:617-629.

Stalker, H.T., and J. P. Moss. 1987. Speciation, cytogenetics and utilization of *Arachis* species. Adv. Agron. 41:1-40.

Stalker, H. T., and C. E. Simpson. 1995. Genetic resources in *Arachis*, pp. 14-53. *In* H. E. Pattee and H. T. Stalker (eds.) Advances in Peanut Science. Amer. Peanut Res. Educ. Soc., Stillwater, OK.

Accepted 22 Nov. 1997