Collecting wild species of *Arachis*

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In most angiosperms, seeds, fruits and other propagules are generally quite evident at the appropriate time for collecting. *Arachis* is an exception. The cultivated groundnut (*Arachis hypogaea*) is well known for its underground fruits, which generally remain attached to the mother plants by 'pegs' as the plants are removed from the soil at harvest time. Pegs develop soon after fertilization at the base of the calyx tube of aerial flowers, which arise from axillary inflorescences hidden in the leaf axils. The peg is geotropic, reaching the ground by growth of an intercalary meristem located at the base of the ovary, proximal to the ovules (Gregory *et al.*, 1973). Maintenance of peg rigidity with tight fruit attachment after maturation is an advanced character in the genus *Arachis*, obviously selected for in the process of domestication of the cultigen. As mentioned by Gregory *et al.* (1973), cultivated groundnuts from the Guarani region, where the common method of harvest in the past was to pull up the plants by hand, have very tough pegs and the pods adhere tightly to them. In regions where pods are frequently taken individually from the plant, they may adhere much less tightly to the peg. In the wild species of *Arachis*, as a rule, a meristematic tissue also occurs between the ovules, so that an isthmus (ocasionally two or three) is formed, separating one-seeded fruit segments in a distinctly lomentiform pod (Conagin, 1959; Pattee *et al.*, 1991). The basal and intercalary portions of the peg tend to collapse at maturity. At seed maturity, the best time for germplasm collecting, seeds of wild species of *Arachis* are therefore no longer attached to the plants, or break away very easily if the soil is disturbed.

Underground fruit development in *Arachis* means that it is impossible to detect even the presence of seeds until the soil under the plants is dug up and sifted, a time-consuming procedure, let alone estimate the quantity available and their developmental stage and degree of

maturity. This is bad enough for the germplasm collector, but there is another consequence. In the absence of drastic soil disturbance, for example by animals or flooding, most seedlings will grow at only a short distance from their mother plants. The result of such relatively inefficient dispersal and limited vegetative spread by rhizomes or stolons is that populations may be dense, but are often small in extent, their perimeter remaining quite stable for many years, even decades. Apparently trivial obstacles, such as sparse groups of trees and stands of tall grass, may obscure such compact, isolated populations, perhaps the only ones for many kilometres.

The problems of wild *Arachis* collecting are not confined to those caused by the mode of fruit development, however. Locating populations at the appropriate time for seed collecting may be quite difficult because of the phenology of the species. In annual species maturation tends to be complete only after wilting of the mother plants. Experienced collectors can identify wilted stems or leaflets lying on the ground as belonging to *Arachis* species, but sometimes even such slight clues may be missing, and indirect, intuitive evidence must be used to decide whether it is worth searching for seeds in a particular area. In northern Brazil, seeds of a yet undescribed species were searched for under palm trees more because just such a location had first yielded material than because of any obvious clues to the presence of the plants. In perennial species full maturation may occur long after the peak of flowering. Therefore, showy yellow or orange flowers may help to locate plants in the field, but will not necessarily mean that mature fruits will be available. Also, some species of the Brazilian sections *Ambinervosae* and *Extranervosae* (Resslar, 1980) present at first a small number of normal, showy flowers, immediately followed by abundant cleistogamic flowers, much smaller in size and very difficult to see. The period when populations of such plants can be noticed from a distance by their flowers is extremely short.

There is a further problem. Consulting herbarium labels is common practice among plant germplasm collectors as a means of acquiring a good knowledge of target species, in particular their distributions, prior to going into the field. In the case of wild *Arachis*, this has not been a very fruitful approach. Specimens are very scarce in herbaria, since most botanists will tend not to collect plants of which they do not see fruits, and whose flowers remain turgid for only a few hours a day. Present *Arachis* collecting efforts, however, stress the need for a herbarium specimen of all accessions, even when the plants are wilted or without fruits. There is an important group of vegetative morphological characters that provide good hints for identification at the species level. Information from local people, who are usually able to associate the wild species of *Arachis* with the cultigen, mainly due to leaf and flower similarities, is extremely important when an expedition visits a new area. For example, locally used vernacular names have been found helpful in locating populations.

Underground fruit development, small populations, difficult pheno-

logy and poor representation in herbaria have led wild *Arachis* collectors to develop a strategy which often involves repeated visits to collecting sites. A good example of this and of some of the problems encountered in fieldwork is provided by accession VSGr 6416 (BRA-012726) (collectors: V = J.F.M. Valls; S = C.E. Simpson; Gr = A. Gripp), collected in Mato Grosso, Brazil. Thus far, this represents the only known population of an undescribed species in sect. *Arachis*. It has a very short life cycle and has already been included in a breeding programme as a potential source of earliness (Simpson, 1990). The species was collected for the first time in August 1981, when a very distinctive and novel fruit segment, acute and strongly nerved, was found in the soil being sifted while collecting germplasm of a plant of sect. *Extranervosae* which was very frequent at the site. After some additional searching, only two very young seedlings of the new plant were found. These were collected as live plants in pots for seed production. Some 50 seeds were later harvested from one surviving seedling and from the plant obtained by germination of the first original seed, which had about one third of each cotyledon eaten by an insect. In May 1985, another expedition visited the site. After 2 hours of intensive search by four experienced *Arachis* collectors, two of whom had made the original collection, a few additional plants were spotted and produced a small seed sample. At a better time, in October of the next year, a third collecting team reached the area, to be surprised by the sight of a continuous carpet of seedlings of the species, extending for some 500 m along both sides of the road. There were abundant seeds in the ground, this time easy to find in the burned grassland of the roadside, but they were all either germinated or rotten. Only very few plants were seen in the adjacent undisturbed grassland, obviously due to intensive grazing. By then, it was clear that the species had a very short and synchronous life cycle and that timing was critical. A fourth expedition visited the area in November 1987, hoping to collect large amounts of seed. However, it was found that the top soil had been scraped off along both sides of the road, piled up and compacted for hundreds of metres, as the base of a new paved road!

Precise notes in field books and herbarium labels are important if collecting sites are to be visited again in this way. Latitude and longitude are generally taken from maps in a somewhat imprecise way, and yet they are important references to establish the general area of search. They must be complemented by precise distances, preferably from stable geographic landmarks. For example, distances taken from city limits may result in errors of hundreds of metres in a few years. Even if a site can be accurately located again, the vagaries of the weather can also conspire against the collector. Floods can make roads impassable and submerge entire populations, for example. Annual species may not germinate and grow every year in dry climates, and even perennials may simply disappear from sight in bad years.

Once a wild *Arachis* population is located in the field, seeds are collected and herbarium specimens of the population prepared. *Rhizobium*

nodules are also collected. A description of site and plant conditions is then made in a field book. Sometimes, soil samples are taken for chemical analysis and herbarium specimens of associated species are prepared. Digging and sifting along transects across a population theoretically help to maximize variation in the samples. Digging out entire plants (which will also be useful for making herbarium specimens and collecting nodule) and sifting the soil underneath with large sieves are the most efficient way to harvest seeds (in fact, fruit segments) of wild species of *Arachis*. However, fruits recovered from the soil may be from previous years and thus have very low germination. Seed dormancy may also hinder the multiplication of poor samples. Also, as seeds of wild *Arachis* are generally loose in the soil at maturity, sampling techniques based on any '*x* seeds per mother plant' design cannot be applied. Different *Arachis* species sometimes grow in a mosaic pattern at the same site. Identification of the seeds collected may only be possible at the multiplication stage, when germinated plants show their differences. Another restriction on the use of a standard sampling procedure for all wild *Arachis* species is that very little is known of their pollination mechanisms. Although the wild species are generally considered autogamous (by analogy with the cultivated *A. hypogaea*), differences in stigma morphology and in pollination behaviour have been pointed out between annual and perennial species (Banks, 1990; Lu *et al.*, 1990). A single sampling technique will probably not be suitable for all species.

Seeds are put into cloth bags, which are then hung up to dry. Alternative or complementary collecting of entire live plants in pots, or preparation of cuttings, may be required. Collecting live plants is frequently the only way to preserve germplasm of a population *ex situ* when seeds are not produced or are not available. All collecting of live plants requires careful prior arrangements for the subsequent work of maintenance and multiplication. Rhizomatous and stoloniferous species, such as *A. glabrata* and *A. repens*, are easy to transport as cuttings wrapped in newspaper and stored in plastic bags, without additional water. They will survive for many days, especially when kept cool. They have also been transported for up to 40 days in sphagnum moss, cleaned and washed periodically. Other perennial species, which do not have rhizomes or stolons, are transplanted directly into pots, but this requires large amounts of space in the mission vehicle. A few annual species of *Arachis* do not survive for long when transplanted at the stage of fruit maturation, but may complete the maturation of a few additional fruits. *In vitro* meristem culture may be used to increase the number of individuals producing seeds (Pittman *et al.*, 1983). Complementary *in situ* conservation has been suggested for some species and situations (Valls, 1985). This may be very important in future in buying time until special techniques for the conservation of important germplasm of difficult species are developed (Simpson, 1988).

An understanding of the genetic variation encompassed by each accession and by the collection as a whole is only attained with

subsequent characterization. This will not only affect decisions about use in breeding, but also allows the identification of geographic areas of high diversity, where additional collecting work may need to be carried out. Intensive efforts to characterize and evaluate wild *Arachis* germplasm are under way in many countries and institutions, involving taxonomy, cytogenetics, breeding behaviour, genome analysis, crossing behaviour, pest and disease resistance, potential for forage use and so on (Singh and Moss, 1982; Pompeu, 1983; Grof, 1985; Simpson *et al.*, 1985; Subramaniam *et al.*, 1985; Kretschmer and Wilson, 1988; Moss *et al.*, 1989; Nelson *et al.*, 1989; Cook *et al.*, 1990; Lu *et al.*, 1990; Stalker, 1990, 1991; Kochert *et al.*, 1991; Singh *et al.*, 1991; Stalker *et al.*, 1991). Many scientists directly involved in *Arachis* characterization and evaluation research have had an opportunity to participate in collecting mission(s) (Table 35.1). A good link thus exists between the collectors and users of the germplasm. Decisions on priority areas and priority species for collecting have been made by specialists who have had access to up-to-date characterization and evaluation information (Valls *et al.*, 1985; Simpson, 1990).

Wild species of *Arachis* are native to five countries, some 60 species occurring in Brazil, 15 in Bolivia, 12 in Paraguay, seven in Argentina and two in Uruguay. Many more countries grow the cultigen and are interested in its improvement. It is thus not surprising that, though expeditions have always included local scientists, international cooperation has been essential in the continuing build-up of a comprehensive collection of wild *Arachis*. This has required much careful planning. Each country has different legal requirements for collecting work and the possibilities for local support also vary widely. The planning of expeditions has taken months and sometimes even years (Simpson, 1984). Field activities have been supported by the International Board for Plant Genetic Resources (IBPGR), the US Department of Agriculture (USDA), EMBRAPA and other institutions. Subsamples of each accession are always deposited in the host country. They are usually also shared with other national institutions which did not participate in the mission but which have a clear commitment to the conservation of *Arachis* germplasm (Simpson, 1980, 1984, 1991; Williams, 1989). An international cooperative approach was also used for the development of a list of descriptors for wild *Arachis* germplasm (IBPGR and ICRISAT, 1992).

Special care has been taken to involve promising young scientists and even undergraduate students in collecting (Simpson, 1990). For example, from 1981 to 1992, a period of intensive collecting, especially in Brazil, 28 scientists or highly trained technicians participated in field missions, representing 14 institutions in six countries. They collected accessions from all eight taxonomic sections of the genus (Krapovickas, 1990) (Table 35.1). Despite all this effort, the general feeling among *Arachis* workers is that much fieldwork remains to be done. A reservoir of expertise is being built up: the solutions to the unique problems of wild *Arachis* collecting which have been developed over scores of

Table 35.1 Expeditions for collecting of wild *Arachis* germplasm in South America, 1981–92. Number of accessions collected in each country and sections of species collected in each expedition.

		Countries					Sections							
Year	Collectors	ARG	BOL	BRA	PRY	URY	AM	AR	CA	ER	EX	PR	RH	TR
1981	VW			6					+		+		+	
	VVeSv			7			+				+			+
	VSGr		1	40				+			+	+	+	
1982	VKRSv			38			+	+			+			
	ScVn	10					+							
	VSW			17			+		+		+			+
1983	VKVeSv			27			+	+			+			+
	KSSc	5	2				+							
	VSMGeSv			18				+					+	
1984	VRGeSv			76				+		+		+	+	
	VSStGdW			25				+			+		+	
1985	Mt					6		+						
	VVeSv			45			+	+			+			
	VKSSv			26				+			+	+	+	
	VPoBi			27				+		+	+	+	+	
1986	VSW			14	1			+		+	+	+	+	
	VPoJSv			11				+		+		+		
1987	VRSv			35			+				+			
	VeSv			5						+			+	
1988	VFdSv			11						+			+	
	Wi		4					+						
1990	Wi	5	4					+						
	VGaRoSv			9				+			+			
1991	VPmSv			22			+	+			+			
	VFaPzSv			13			+		+					+
1992	VPzVaW			29			+		+		+			
	VSPmWiSvVePzRs			17			+	+	+		+		+	

Key: Collectors: Bi = Bianchetti; Fa = Faraco; Fd = França-Dantas; Ga = Galgaro; Gd = Godoy; Ge = Gerin; Gr = Gripp; J = Jank; K = Krapovickas; M = Moss; Mt = Millot; Pm = Pittman; Po = Pott; Pz = Pizarro; Q = Quarin; R = Rao; Ro = Rocha; Rs = Santos; S = Simpson; Sc = Schinini; St = Stalker; Sv = Silva; V = Valls; Va = Valente; Ve = Veiga; Vn = Vanni; W = Werneck; Wi = Williams. Countries: ARG = Argentina; BOL = Bolivia; BRA = Brazil; PRY = Paraguay; URY = Uruguay. Sections: AM = *Ambinervosae*; AR = *Arachis*; CA = *Caulorhizae*; ER = *Erectoides*; EX = *Extranervosae*; PR = *Procumbensae*; RH = *Rhizomatosae*; TR = *Triseminalae*.

missions will need to be further refined and applied for many years to come.

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