SCREENING TECHNIQUES FOR HOST PLANT RESISTANCE TO COWPEA INSECT PESTS[1]

L.E.N. Jackai and S.R. Singh[2]

Abstract: As research on food crops intensifies in the tropics, the spotlight is being increasingly focused on the development of varieties with resistance to pests and diseases. Much of the time, money and patience needed to conduct research on host plant resistance (HPR) is spent on the development of methodologies that are eventually used to screen germplasm and breeding lines. The products of this screening provide the building blocks for the development of resistant varieties.

Methodologies differ from one place to another and from one crop to the other, and are usually tailored to suit local and/or individual circumstances. There is, however, a need to develop standardized methodology to make comparisons across locations easier. Any methodology is capable of improvement, but improvements come only after use and evaluation. In this paper we try to define the current status of HPR screening methodology in cowpea entomology, concentrating mainly on techniques developed and used with considerable success at IITA. In addition, the shortcomings of some of these techniques are pointed out and areas needing improvement indicated.

An important component of crop improvement programs the world over is the development of pest resistant crop varieties. The ability of a plant to resist attack by another organism is known as host plant resistance (HPR). The original definition of HPR (Painter 1951) is the "relative amount of heritable plant qualities that influence the ultimate degree of damage suffered by the plant" under a known/given insect pest population. This quality is expressed in three classical modalities:

1. Antibiosis — when the plant possesses attribute(s) that produce adverse effects on the insect's biology, behavior and/or physiology.
2. Non-preference — when the plant possesses attributes that lead to the non-use or reduced use of the plant by the insect for food, shelter, oviposition or any combination of these. This modality has been recently christened by Kogan and Ortman (1978) as "antixenosis" meaning "bad host" to reflect a characteristic of the plant, as in other modalities, rather than a response by the pest, but there is still some debate on its acceptability for common usage (Wiseman 1985).
3. Tolerance — when the plant possesses attributes which enable it to grow, repair injury and produce an acceptable yield despite supporting a pest population that would normally cause significant damage and/or kill a susceptible plant.

These terminologies have undergone various degrees of redefinition over the years (Beck 1965) but the original theme has remained, by and large, unchanged. Host plant resistance may also be rated as low, moderate or high (Horber 1980; Wiseman

[1] Paper pesented at the Regional Grain Legume Workshop, organized by IFS in Tannanarive, Madagascar, 22-28 February 1988.

[2] Grain Legume Improvement Program, International Institute of Tropical Agriculture (IITA), Oyo Road, PMB 5320, Ibadan, Nigeria.

1985), based on its manifestation in a defined test situation.

DEVELOPMENT OF RESISTANT VARIETIES

Development of resistance can be a long and expensive process, often taking over 20 years to accomplish. However, there are many examples in the literature where resistant crop varieties have been developed in a much shorter time (Gould 1983). Success in the development of resistant varieties is predicated on a number of factors. To start with, a highly diversified germplasm resource is essential to provide the genetic variability required for such work. Once this is available sources of resistance have to be identified, a process that requires the development and use of reliable screening procedures. This is probably the single most important phase of host plant resistance work, as it provides the foundation on which the development of crop resistant varieties for the future is based.

A wide range of biological information is usually required to develop appropriate screening procedures. Information on the phenological relationship between insect pest and target crop, as well as that of non-target crops or alternative hosts; plant stage(s) attacked and insect stage(s) causing damage; and developmental and behavioral profiles of the insect pest with reference to its host plant. This information is important in determining how frequently and at what levels of pest infestation screening in the field or elsewhere should be carried out. There is a continuing need to develop methods of evaluating resistance in the field, screenhouse and laboratory.

Dahms (1972) outlined 16 criteria that have been used to evaluate resistance of plants to insect damage. These include both host plant and insect responses, most of them relatively simple but others involving the use of complicated physiological and biochemical studies.

Where field screening is used it is necessary to develop appropriate sampling methods for plants or plant parts, as well as for the insect pest. Sampling is a critical component of HPR work. Poorly conceived sampling designs can result in highly misleading conclusions. Rating scales are useful for both field and greenhouse work (Davies 1985), but even the development of such scales, which are often based on degrees of damage, depends on a proper understanding of the biology of both crop and pest, as well as their interactions. Contrary to the opinion held by some workers, rating scales can be analyzed statistically (Little 1985; Little and Hills 1977).

Following the above two phases (collection and screening of germplasm), hybridization techniques are used to transfer resistance from the original sources to other genetic backgrounds, usually breeding lines or other materials with one or several desirable characteristics. This process involves close collaboration between breeders and entomologists. Because of the peculiar nature of segregating breeding material, screening procedures must be such that the transfer of resistance can be monitored either in single plants or in progeny rows at the appropriate stage of the breeding cycle.

A study of the mechanisms of resistance is the final phase in a HPR research program and may require the expertise of biochemists and physiologists. While this is important in understanding the overall resistance phenomenon, and could indeed provide clues to the development of more rapid screening procedures, it is not a prerequisite for the development of resistant varieties. The need for highly specialized personnel and sophisticated instrumentation in some cases makes its pursuit by inadequately staffed or poorly equipped programs unadvisable.

Most crop improvement programs in developing countries have a HPR component and it has received increasing emphasis in

agricultural research in Africa during the past two decades. Most national programs are involved in a lot of screening of all kinds of crops for resistance to various diseases and insect pests. A variety of methods are employed for this purpose, many of which were developed elsewhere, or are modifications of methods used on other crops. The diversity of procedures used in HPR screening is clearly evident from the publications on crop/varietal resistance that appear in scientific journals; the number of methods almost equals the number of authors! An obvious outcome of this is that comparisons of results from several locations may sometimes be quite inappropriate. Superimposed on this is the fact that the international agricultural research centers (IARCs), which have a major responsibility for the development of crop varieties for use by national agricultural research systems (NARSs), are scattered throughout the African (or other) continent in widely differing environments. Sometimes varieties or cultivars developed by an IARC, say for resistance to a particular insect pest, are sent to NARSs without complete details (including possible options) on how to evaluate the resistance. Many research stations do not have professional entomologists or well-trained technical staff, with the result that other less qualified personnel are given the responsibility of screening for insect pest resistance. This underscores the need to develop and publish screening procedures that are both practical and scientifically valid, without being complicated.

Even though local ecological circumstances and other factors (both socioeconomic and biological) may vary from one region, country or locality to another, there still appears to be an urgent need to put into a single document the more frequently used and time-tested techniques that are available for HPR work on cowpeas. That is the raison d'être of this paper. We cannot pretend to give an exhaustive list of all the techniques that have ever been used in cowpea resistance work (particularly as some of them have not been published) but rather present a selection of those methods that are more commonly used and have shown a good level of precision and repeatability. Most of these methods were developed and/or are currently used at the International Institute of Tropical Agriculture (IITA) in Nigeria.

IITA has a cowpea germplasm collection of over 12,000 accessions, the largest in the world, and a global mandate for the improvement of cowpea. Its Grain Legume Improvement Program, which has research and training responsibilities for cowpea, has a multidisciplinary team comprised of plant breeders, entomologists, pathologists and agronomists who work on various aspects of crop improvement, with HPR as the centerpiece.

No extensive details will be given on how to evaluate the mechanisms of resistance and/or the basis of these as they have been treated elsewhere (e.g. Maxwell and Jennings 1980; Davies 1985; Dahms 1972). In the long term it is hoped that some form of standardized procedures or techniques for screening cowpeas for resistance to insect pests can be established, with acceptable modifications to suit local conditions. Such documents are available for a number of other crops, e.g. rice (Heinrichs et al. 1985), alfalfa (USDA-ARS, ARS-NC-19, 1974) and cotton (Southern Cooperative Series Bulletin 280, 1983) (the latter two are cited by Davies 1985).

THE RANGE OF COWPEA INSECT PESTS

Insect pests damage cowpeas at all growth stages. These pests have been the subject of recent reviews (Singh and van Emden 1979; Singh and Jackai 1985; Jackai and Daoust 1986). Even though the range of pests and their relative importance may vary slightly from one location or region to another there are a number that are a regular feature of cowpea agro-ecosystems worldwide. These are generally

divided into three groups according to the growth stage of the crop.

Seedling phase: Pests attacking this stage consist of aphid (*Aphis craccivora*), beanflies (*Ophiomyia* spp.), leafhoppers (mainly *Empoasca* spp.), foliage beetles (*Ootheca* spp., *Medythia* spp. and others) and the arctiid defoliator (*Amsacta moloneyi*).

Early reproductive phase: This consists of the flower bud stage plus early flowering and is attacked mainly by the flower bud thrips (*Megalurothrips sjostedti*) and the legume pod borer (*Maruca testulalis*).

Late reproductive phase: This covers the period from late flowering through podding and is attacked primarily by the legume pod borer and the pod sucking bug complex of which two coreids (*Clavigralla* spp. and *Anoplocnemis* sp.), two alydids (*Riptortus* sp. and *Mirperus* spp.) and a number of pentatomids (e.g. *Nezara viridula* and *Aspavia armigera*) are most important. Other insects present during this phase are the cowpea seed moth (*Cydia ptychora*), the cowpea pod weevil (*Apion varius*), the cowpea curculio (*Chalcodermus aeneus*) and flower beetles (*Mylabris* spp. and *Coryna* spp.).

Other classification systems are found in the literature (e.g. Singh and van Emden 1979; Taylor 1964). Insect pests are also encountered in *storage* and only one genus, *Callosobruchus*, is known to be of economic importance. The importance attached to these pests by different workers varies, a fact that is reflected in the research emphasis that has been placed on different members of the pest complex. Aphids, leafhoppers and the storage beetle have received a great deal of attention worldwide (partly because they are easier to study) while flower thrips, pod sucking bugs and the legume pod borer have received much less attention. The latter two are among the most difficult pests to work with in cowpea HPR research.

HPR screening techniques have been tried for practically all cowpea pests, with varying degrees of success. It is clear that some of these methods have been based on a shotgun approach devoid of the necessary background knowledge of pest biology and behavior. Many such techniques have ended up in the pages of journals or in-house publications. Some of these are cited below as examples of unsatisfactory techniques, but the greater part of this paper is devoted to the description of procedures that have been used frequently and yielded satisfactory results.

SCREENING PROCEDURES

Seedling pests

Aphids

The major species encountered on cowpea is *Aphis craccivora* Koch (Homoptera: Aphididae). This is probably one of the most widely researched insects in agricultural entomology. Aphids damage young cowpea seedlings by sucking sap from the young leaves and stem tissues. Even though they also infest reproductive structures (IITA 1985) the effect is not as damaging at this stage as at the seedling stage. Screening for resistance to aphids can be conducted in field trials as well as under more controlled screenhouse tests. Most of the resistant lines developed at IITA (Table 1) have been obtained using the latter.

Field techniques. Cowpea aphid populations tend to be spotty early in the season, but if planting is delayed or made to coincide with drier periods a good infestation is generally possible. At IITA, field screening is generally conducted during the dry season (December-February), when the major pest problem on cowpea is aphids. This is, however, only possible because irrigation facilities are available. Where such facilities do not exist a late planting during the main cropping

Table 1. Cowpea varieties/cultivars with resistance to various insect pests

Insect common name (species)	Resistance source lines	Advanced breeding lines
Aphid (*Aphis craccivora* Koch)	TVu 36, TVu 408-P2, TV 410, TVu 801, TVu 2896, TVu 3000	IT81D-1020 IT82E-1-108
Leafhoppers (*Empoasca* spp.)	TVu 59, TVu 123, TV 662, TVu 1190, (VITA 3)	IT83S-742-11 1T83S-742-13 1T83S-742-14 IT83S-747-4
Beanfly (*Ophiomyia phaseoli*)	TVu 1433, TVu 3192, Farv 13	IT81D-1205-174 IT82D-644
Flower bud thrips (*Megalurothrips sjostedti*)	TVu 1509, TVu 2870	TVx 3236 IT82D-713 IT82D-716 IT84S-2246-4
Legume pod borer (*Maruca testulalis*)	TVu 946 Kamboinse local	
Pod sucking bug (*Clavigralla tomentosicollis*)	TV 1, TVu 1890	
Cowpea storage weevil (*Callosobruchus maculatus*)	TVu 625, TVu 2027, TVu 11952 TVu 11953, TVu 4200 (pod wall)	IT81D-1007 IT81D-1137 IT81D-1157 IT84S-2246-4

season will produce the same effect. Application of DDT (Don-Pedro 1980) and certain older generation synthetic pyrethroids (Matteson 1982) produces an increased aphid infestation. This is thought to result from a drastic reduction in aphid parasites and predators. The use of DDT, however, should not be encouraged. Single row plots (3-4m long) or plots consisting of four rows each four meters long can be used, depending on the amount of material to be screened. In each case resistant and/or susceptible checks are included, and tests are repeated for verification.

One method of assessing the incidence of aphids is to determine the percentage of plants in each row that is infested at 10, 20 and 30 days after seedling emergence or, where larger plots are used, on the center two rows at the same time intervals. The incidence of aphids is important because they act as vectors in the transmission of cowpea aphid-borne mosaic virus. The severity of the infestation can be measured using a rating scale of 0-9 based on the number and size of colonies (Table 2). The level of infestation is assessed on 30 stands per plot (five stands per row at fixed intervals to avoid bias) and the scores averaged. It is usually not necessary to use

Table 2. Visual rating scale for cowpea resistance to aphids (in part after Listsinger et al. 1977)

Rating	Number of aphids	Appearance
0	0	no infestation
1	1 - 4	a few individual aphids
3	5 - 20	a few isolated colonies
5	21 - 100	several small colonies
7	101 - 500	large isolated colonies
9	> 500	large continuous colonies

both assessment methods, the former being adequate for most bulk screening work.

Other methods are available for determining the mechanisms of resistance involved (e.g. Davies 1985) but these are beyond the scope of this paper. Many of these use no-choice techniques to identify antibiosis and non-preference, as well as tolerance.

Screenhouse techniques. A method developed at IITA uses cowpea plants grown in wooden trays (54 x 80 x 11 cm) filled with soil to about 8 cm depth (Singh and Jackai 1985). Test materials are planted in single rows equidistant from each other. Included among these are resistant and susceptible checks (Singh 1977, 1980) where these are available. In a situation where no resistant check is available test lines are planted with a known susceptible check only. Eventually a resistant source will be identified and included in subsequent tests. When plants are about 10 days old, each plant of every test row is infested with 5 fourth instar nymphs using a camel-hair brush. Aphids for use in this test may be obtained from the field, but preferably should come from an existing aphid culture carefully maintained in a screenhouse away from parasites and/or predators. Several methods have been described for rearing aphids (Starks and Burton 1977). Infested trays are transferred to cages with fine saran mesh, small enough to allow passage of air but not insects. These cages are kept in a greenhouse. About two weeks after infestation, or as soon as the susceptible check dies, plants are rated on a 1-5 scale for damage (manifested as decreased plant vigor). The susceptible check is usually killed around 10-15 days after infestation. The rating is first done on a row basis, and later within rows. The latter is more useful when dealing with segregating breeding lines. A score of 1 indicates high resistance, 2 or 3 indicate moderate/low resistance, while 4 suggests low susceptibility and 5 high susceptibility. This process can be repeated several times for lines with scores of 1, 2 or 3, with as many replications as possible, using trays as the replications.

A similar technique is used for screening sorghum for resistance to the green bug, *Schizaphis graminium* Rondani (Starks and Burton 1977).

Leafhoppers

Most leafhoppers found on cowpea belong to the genus *Empoasca* (Homoptera: Cicadellidae). In Asia another genus, *Amrasca*, is encountered. At IITA resistance to leafhoppers has been identified mainly by using field techniques. Leafhoppers, like aphids, are a major problem during periods of low rainfall. Field screening is therefore conducted by planting test materials in late September/early October, roughly a month later than is customary (Singh and Jackai 1985). About two weeks prior to planting the test materials (which should always include a resistant and/or susceptible check) a susceptible spreader row is planted along the borders of the field. It is also customary to leave any grass around the field uncut until after the test materials are about 10 days old. These precautions help to build up the leafhopper population. Test lines are then planted in single rows, each 4 or 5m long. Checks are included once in every 10 entries. The susceptible spreader

Table 3. Visual rating scale for leafhopper damage to cowpea

Rating	Appearance
1	no visible damage
3	slight yellowing of the midrib; no cupping
5	pronounced yellowing at midrib and side veins; yellowing extends slightly between the veins; symptoms may or may not be associated with moderate cupping
7	severe yellowing of the leaves followed by drying of leaf tips and leaf margins; characteristic "hopper burn" observed
9	severe defoliation, followed by wilting, resulting in death of plants

row is cut, but left on the plot, about 10-15 days after the emergence of the test materials. This results in movement of the leafhoppers to the test materials.

Resistance rating is done on a 1-9 scale (Table 3) on a row basis around 25-30 days after seedling emergence. Resistant lines are later tested in replicated field trials. Several resistant lines have been identified using the field screening technique (Table 1).

Screenhouse tests have also been used (Raman et al. 1978) and are based on a procedure similar to that described for aphids. However, more consistent results have been obtained from field evaluation, suggesting that further refinement is required to improve the screenhouse techniques.

Beanfly

The predominant species on cowpea is *Ophiomyia phaseoli* Tryon (Diptera: Agromyzidae). This insect tunnels in the stems of cowpea seedlings causing them to swell and crack at ground level. Badly infested plants wilt off and then die. Eggs are laid in the leaves or hypocotyl by the small, shiny black adult flies. Most resistance screening is therefore targeted at the stem.

Since the insect is not presently a serious pest of cowpea in Africa little or no attention has been given to it on cowpea. However, because it is an important pest on other legumes such as beans (*Phaseolus* spp.), which are widely grown in the eastern and southern parts of Africa, as well as on soybean, we consider it a potentially important pest on cowpea and therefore useful to discuss the methods developed for beanfly resistance work elsewhere.

Screening of cowpea for resistance to *O. phaseoli* has been conducted by IITA during the past three years in collaboration with the Asian Vegetable Research and Development Center in Taiwan using the field techniques developed by Chiang and Talekar (1980) for use on soybean. Single rows of test material are grown with known susceptible checks in the fashion described for other pests. Four weeks after planting the entries are assessed for percentage infestation. The best entries (i.e. those with the lowest infestation) are later subjected to replicated field trials. In such trials (plot sizes vary but 4 rows x 4m should be adequate) about 30 plants are sampled per plot and the number of beanfly larvae and pupae, and the extent of damage are recorded. The level of infestation per plot is measured prior to the collection of samples. Using both measurements as criteria, five cultivars were selected as resistant (IITA 1986) (Table 1).

It is conceivable that the screenhouse caging technique described for aphids could be used, with appropriate modifications, as a procedure for beanfly resistance screening. This has been attempted in the Philippines but requires further study (C. Adalla personal communication).

Other seedling pests

No systematic effort has been made to develop specific screening procedures for foliage beetles partly because of the sporadic nature of their attacks and the fact that they are not considered urgent problems. Where an attempt has been made, field plots were rated on a scale of 1-5, representing a gradation in leaf area damage and the presence/absence of adult beetles (Jackai et al. 1988).

Pests of the early reproductive phase

Two of the more important pests of cowpea, the flower bud thrips (FTh) and the legume pod borer (MPB), belong to this group. However, since the MPB is also important during the late reproductive phase, the screening procedures used for this pest are described in the next section.

Flower bud thrips

Considerable work has been done at IITA on the development of screening techniques for cowpea resistance to flower bud thrips, ***Megalurothrips sjostedti*** Tryb. (Thysanoptera: Thripidae). Screening has been highly successful in the field, but a number of screenhouse techniques have also been tried (IITA 1983).

To ensure high and uniform populations, dwarf pigeonpea (*Cajanus cajan*) is established along the borders of the field. Since this is a perennial crop, there is a special field designated for this purpose at IITA. Planting is done to ensure that the pigeonpea flowers before the cowpea. Thrips are attracted to the pigeonpea flowers where their population builds up. In the plot itself, susceptible spreader rows, usually the cowpea cultivar VITA 7, are established in a checkerboard design, 2-3 weeks prior to the planting of test lines. Thrips move from the pigeonpea to the spreader rows once the latter start to produce flower buds.

The test material is planted roughly three weeks after the spreader rows and VITA 7 is planted once in every 10 entries; 35 days later the spreader rows are uprooted and plants laid between rows of the test plants. This forces the thrips to move away from the drying plants to those of the test rows. In addition a low dosage of endosulfan (ca 200 g a.i./ha) is sprayed to reduce infestation by MPB which might otherwise interfere with the assessment of thrips damage. Visual rating is first carried out at 45 days and again at 55 days after seedling emergence, or first when racemes are about 5 mm long and then 10 days later. In the past, a damage rating scale of 1-5 was used, but this has recently been replaced with a 1-9 scale (Table 4). Rating is based on a combination of varying intensities of browning of the stipules and flower buds, non-elongation of peduncles, and flower bud abscission. These damage symptoms are used to diagnose damage caused by *M. sjostedti* but we believe other species of flower thrips cause the same or similar damage symptoms.

The distinction between susceptible cultivars and those with even low levels of resistance is very clear in field trials, so that the use of screenhouse methods is generally unnecessary. Even segregating breeding lines can be screened using this field technique. Lines with a score of 1, 2 or 3 are considered resistant. These are usually tested again in larger, replicated plots where rating is restricted to the center two rows in order to avoid any edge effect. Two germplasm lines, TVu 1509 and TVu 2870, were identified with low to moderate levels of resistance (Singh 1977). TVu 1509 has the higher level of

Table 4. Visual rating scale for flower thrips damage to cowpea

Rating	Appearance
1	no browning/drying (i.e. scaling) of stipules, leaf or flower buds; no bud abscission
3	initiation of browning of stipules, leaf or flower buds; no bud abscission
5	distinct browning/drying of stipules and leaf or flower buds; some bud abscission
7	serious bud abscission accompanied by browning/drying of stipules and buds; non-elongation of peduncles
9	very severe bud abscission, heavy browning/drying of stipules and buds; distinct non-elongation of (most or all) peduncles

Note: Scoring should be done at the stage when peduncles are about 2-3 cm long and then one week to 10 days later.

resistance and has been used in the breeding program at IITA to develop several advanced breeding lines with resistance to thrips (Table 1). A higher level of resistance than that in TVu 1509 is yet to be identified.

This technique has been criticized in the past on the erroneous assumption that test lines closest to the pigeonpea borders will receive a heavier infestation than those farther away. Carefully planned and executed studies, however, have demonstrated that this is not the case (IITA 1982). Another criticism has been that bud abscission and non-elongation of the peduncles are symptoms that can result from damage by other pests. While this is possible, the other pests that might cause similar symptoms (the legume pod borer and the pod sucking bugs) do not cause the characteristic browning associated with thrips damage to cowpea. Finally, field screening can, and has been, carried out without using permanent pigeonpea borders. The main cowpea growing season in most locations is generally suitable for field screening. The susceptible spreader rows, however, help to create a uniform insect pressure. Their use should be encouraged.

Pests of the late reproductive phase

Legume pod borer

The legume pod borer or maruca pod borer (MPB), *Maruca testulalis* Geyer (Lepidoptera: Pyralidae), is perhaps the most elusive pest in cowpea entomology. It attacks a wide range of sites including terminal shoots, young succulent stems and peduncles, flower buds, flowers and pods. Because of this multiplicity of feeding sites screening procedures have to be developed taking each site into consideration but weighting them according to their importance (Jackai 1982). Thus, as damage to some parts (e.g. flowers and pods) is more critical than that to other parts (e.g. stem) evaluation of resistance is often weighted towards the former. Field screening has been the mainstay of evaluations for resistance to MPB. Several attempts have been made to develop greenhouse techniques (Dabrowski et al. 1983) but these have generally not been repeatable by other workers.

Field techniques. A systematic method for screening cowpeas for resistance to MPB in field plots was developed at IITA (Jackai 1982). Over the years this method has undergone slight modifications to

Table 5. Visual rating scales for legume pod borer damage to cowpea

Pod load (PL)		Pod damage (PD)	
Rating	Degree of podding	Rating	%
1	most (>60%) peduncles bare (i.e. no pods)	1	0-10
		2	11-20
3	31-60% peduncles bare	3	21-30
		4	31-40
5	16-30% peduncles bare	5	41-50
		6	51-60
7	up to 15% peduncles bare	7	61-70
		8	71-80
9	occasional bare peduncles	9	81-100

Note: Pod evaluation index (Ipe) = PL/PD. Higher values of Ipe indicate higher resistance (with PL $\geq$ 5 and PD $\leq$ 5). Even scores (2, 4, 6, 8) are given for PL where no clear-cut (odd-numbered) score appears to fit.

increase its precision and efficiency (Jackai and Singh 1983; IITA 1986, 1987) but has, by and large, retained the basic components of the original method.

An early maturing susceptible cultivar is planted along the borders of the field, as well as perpendicular to every range of rows within the plot. This ensures that every test row has a spreader row running across each end. Alternatively, two rows of the spreader can be planted at every other range, in which case a test line has a spreader running along only one end. The latter design is preferable as it produces the same pest augmentation and distribution effect and allows adequate room for movement between plots without stepping on the crop. Two to three weeks after planting the spreader rows the test lines are planted in single 3m-long rows, with two susceptible (one early and the other medium maturing) and one resistant check included once in every 20 entries. These checks serve a dual purpose. First, they provide a means of assessing the severity and distribution pattern of MPB through larval counts in flower samples, or of assessing percentage flower infestation based on 20 flowers per row collected and examined on the spot. Second, and more commonly, they provide a basis for comparison and subsequent selection.

For effective field screening for resistance to MPB, the earlier occurring pests, flower thrips in particular, must be controlled. This is achieved by spraying the plots with monocrotophos at 250 g a.i./ha at 10-14 day intervals. It has been shown

that weekly sprays of monocrotophos (as Nuvacron® 40EC) at a dosage of 500 g a.i./ha produce a MPB larval population similar to that of unsprayed plots (Jackai 1983) and sometimes even higher (IITA 1979). This treatment also controls pod sucking bugs which would also interfere with pod evaluations for resistance to the MPB. When large amounts of germplasm or other material are being evaluated rating is limited to pod measurements, i.e. pod load (PL) and pod damage (PD). These evaluations are made using a 1-9 scale with the degrees of acceptability at opposite ends of the scale for PL and PD (Table 5). For example; a score closer to 9 is desirable for PL while one closer to 1 is desirable for PD. These evaluations are best made when the pods are mature but still green, as damage at this stage is much easier to observe than when the pods are dry. Where, unknown to the investigator beforehand, materials of greatly varying maturity are being screened, rating should be done when about 50% of the lines are mature and starting to senesce. It should be noted that for this, as in many other cases, it is preferable to screen germplasm or other genotypes in groups with similar maturity.

Materials with PL scores of 5 or better and PD scores of 5 or less are selected for a second screening in replicated 2-row plots with unprotected (i.e. with sprays of monocrotophos) and protected treatments. When the number of entries has been reduced to less than 50, more detailed measurements are taken on flower infestation in addition to PL and PD. In due course a pod evaluation index and seed damage are also computed using previously described methods (IITA 1986; Jackai 1982). This technique and its earlier versions (Singh 1980) have been used to identify resistance in TVu 946 and Kamboinse local (Table 1). More recently, a number of crosses using these two lines have shown distinct promise (IITA 1986). Breeding lines are usually scored on a progeny row basis after which single plants are selected from each of the selected rows for further testing.

Laboratory and screenhouse techniques. The use of laboratory or screenhouse procedures for evaluation of resistance to the MPB hinges on the availability of a rearing facility/insect culture which can supply adequate numbers of insects at the desired stages and times. Unfortunately, most national programs are not equipped with such a facility and are therefore unable to carry out proper laboratory and/or screenhouse resistance evaluations. Even at the international centers, where insect rearing facilities exist (Jackai and Raulston 1988), these procedures are still in the developmental phase (IITA 1985, 1986, 1987). Insects produced at IITA's rearing facility have also been used in the development of artificial infestation techniques to supplement natural field populations of MPB, but these techniques are undergoing further refinements (IITA 1985). For now, screening cowpea for resistance to the MPB by national programs will continue to depend on existing field techniques. So far these have proved satisfactory.

Pod sucking bugs

The most important members of the pod sucking bugs (PSBs) are the cowpea coreids, *Clavigralla tomentosicollis* Stäl. in west and central Africa, and *C. elongata* in east and southern Africa. These and other PSBs belong to the order Hemiptera and descriptions of their biologies may be found in the literature (Singh and Jackai 1985; Jackai and Daoust 1986). All PSBs cause damage to young developing pods by sucking sap from the seeds. This often results in aborted or badly deformed seeds and shrivelled pods. A heavy attack by PSBs during late flowering or early podding can cause extensive flower and pod abscission.

The only known research on techniques for screening cowpea for resistance to this group of pests is that at IITA. At least half a dozen different methods have been tried, both in the field and under controlled conditions in screenhouse and laboratory. Few have stood the test of time.

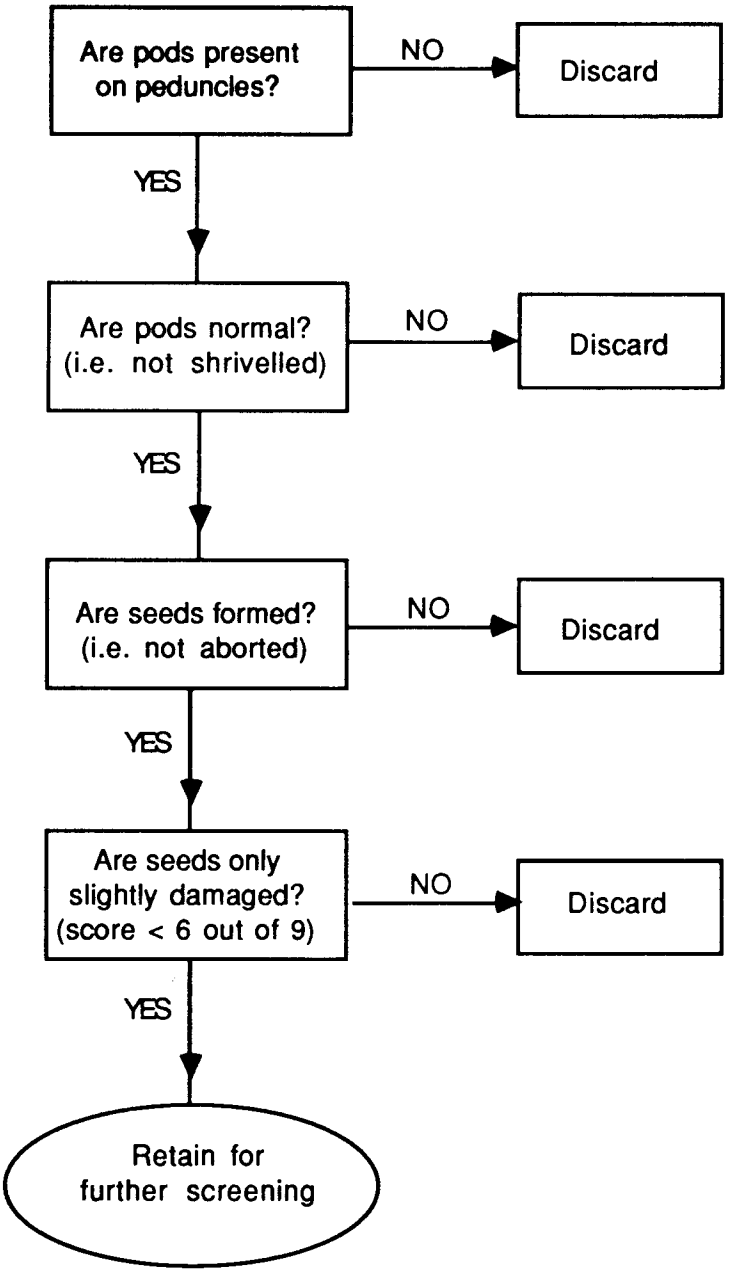

Figure 1. Schematic representation of selection procedure for mass screening of cowpeas for resistance to pod sucking bugs. (Plants should be sprayed with deltamethrin 2.5 EC at 12.5 g a.i./ha every 10 days, starting at flower bud formation, until pod borer infestation is minimal.)

Field screening. Test lines are planted in single or double row plots with a known susceptible and/or resistant check included once in every 10 or 20 entries. The planting is usually slightly delayed in order to expose the plants at the podding stage to a high PSB population pressure. The plants are sprayed with deltamethrin at 12.5 g a.i./ha every 10 days, starting at flower bud formation, to control thrips and MPB. This treatment is terminated at podding or after two sprays unless the MPB infestation continues to be high. During certain years at some locations we have had to spray up to four times, but without any discernible effect on the PSB population. At mid- to late-podding, or at full maturity if the PSB population is low, the trial is scored for damage based on a sequence of eliminative steps (Fig. 1).

Entries with a low score for seed damage are selected for further screening in replicated trials with two levels of PSB population density, obtained by spraying with deltamethrin as earlier described to maintain the status quo (a "high" density) and using half the recommended dosage of endosulfan (i.e. 250-300 g a.i./ha) + deltamethrin (i.e. 6.5 g a.i./ha) at twice the recommended interval (i.e. every 14 days) to obtain a "low" density.

Normally pod samples are taken back to the laboratory where seed damage is assessed. A resistance index (percentage seed damage in test line divided by percentage seed damage in check) is calculated and used for resistance classification and making selections for further evaluation.

Screenhouse evaluation. Test lines are planted in pots and placed in screen cages (1.20 x 1.25 x 1.30m) at the onset of flowering. Ten to twelve pairs of PSBs are introduced into each cage with five plants and allowed to feed for two weeks. Flower and pod abscission is closely monitored and any eggs are removed daily. At the end of the exposure period, counts are taken of pod production as well as an assessment of pod and seed damage, the latter usually in the laboratory. This procedure can be carried out on a choice or no-choice basis for each replication (cage). It is, however, cumbersome and time consuming, and it is not surprising that it has not been widely adopted. Modifications to it are presently being undertaken at IITA.

Other screenhouse techniques are being developed. These require relatively simple equipment — mesh-sleeve cages or plastic petri dishes and rubber bands. However, as in the above technique, a source of insects is a prerequisite. Simple rearing procedures for PSBs using dry seed have been developed recently (Jackai, in press) but conventional methods using fresh cowpea or pigeonpea pods are still useful.

Laboratory techniques consisting of a set of simple bioassays have also been developed recently (L.E.N. Jackai unpublished work; IITA 1988). These need to be adequately tested before they can be recommended for use by national research programs.

The field screening procedure described here has identified low levels of resistance in TVu 1 and TVu 1890 (Table 1).

Storage pests

Species of the genus *Callosobruchus* (Coleoptera: Bruchidae) are the most important pests attacking stored cowpeas. Its cosmopolitan nature has made it one of the most widely studied insect pests. Adult beetles lay eggs on pods (in the field) or on seeds (in storage) and larvae develop within seeds causing extensive damage.

Screening cowpea for resistance to bruchids is conventionally carried out in laboratories. The method used at IITA (Singh 1977) is an adaptation of the method used at Kansas State University (Nwanze and Horber 1975). Twenty to 40 seeds (an equal number for all test lines) of each

accession (ca 13% moisture content) are placed in small plastic boxes (5 x 5 x 2 cm). Two pairs of day-old adults are introduced into each box and allowed to oviposit for 24 hours. To ensure that there is no hidden infestation prior to carrying out this procedure the test material should be fumigated (e.g. with aluminum phosphide) for one or two days and then aerated for about the same period to get rid of the fumigant before screening. Storage of seeds at extremely low temperatures is another method of eliminating concealed infestation. In this case screening can be carried out as soon as seeds have returned to room temperature.

Boxes with infested seeds are generally left at 28°C and 70-80% relative humidity, but where rooms with temperature and humidity control are not available an ordinary laboratory room will suffice. Five days after infestation the number of eggs laid per seed lot is counted. By this time the eggs are easily observed and larvae may have already hatched and moved into the seed. Starting from 25 days after infestation the adults that emerge each day are counted and removed. This is done for up to four weeks, or until no further emergence occurs on the susceptible control. At the end of this period the percentage adult amergence is determined using the number of eggs laid as an estimate of the expected number of adults. A suitability index (= growth index, GI) is calculated using the formula GI = log S/T, where S = percentage adult emergence and T = mean development time (Howe 1971). On the basis of this, as well as the level of oviposition, an estimate of the resistance status of the test materials is made.

Pod-wall resistance has been less well studied. To screen for this factor, different containers (e.g. paper bags) are used to hold 6-12 pods which are infested with 3-6 pairs of adult bruchids for 24 hours. After this the insects are removed and the same data collected as for seeds.

In screening for resistance to storage pests, Ife brown (a susceptible line) is often used as a check but other checks can be used. TVu 2027 was identified as having moderate levels of seed resistance (Singh 1977) from over 10,000 accessions that were screened. Since then this line has been used as the resistant check at IITA. Several breeding lines have now been developed with almost identical levels of resistance as TVu 2027 (Singh et al. 1985) (Table 1). These are available upon request. The maintenance of a continuous culture of *C. maculatus* (or other bruchid species) is important for a successful screening program for resistance to this insect. Appropriate methods have been described (Singh and Jackai 1985).

Techniques used for other cowpea pests that are considered of minor importance can be found in the review by Singh and Jackai (1985). It is hoped that in due course specific screening procedures will be developed for pests with restricted/localized importance such as *Apion varius*, meloid beetles, the seed moth (Ofuya and Akingbohungbe 1986) and defoliators such as *Amsacta moloneyi* (N'Doye 1978). What we have attempted to do in this paper is to summarize the various methods available for use in screening cowpeas for resistance to insect pests. When field tests are used for screening it is important that the prevailing insect population be monitored (by sampling) and reported along with the results of the screening tests. This is important in comparing the performance of resistant varieties under different ecological conditions or in different locations.

Sampling procedures used for most cowpea pests are identical to those described for closely related pests on soybean (Kogan and Herzog 1980). Those for flower thrips have been reported recently by Salifu and Singh (1987) and for MPB by Jackai (1982) and Jackai and Lawson (1987), while methods used for sampling PSBs appear in various IITA Annual Reports (e.g. IITA 1983, 1984, 1985, 1987) as well as

in Todd and Herzog (1980) and Suh et al. (1986). The method for sampling Bruchidae described by Alzouma (1988) can be used to determine field infestation levels of both *C. maculatus* and *Bruchidius atrolineatus*. These methods are also useful for screening insecticides. Even though most methods used for sampling cowpea pests require further research to improve their precision and reduce cost, as has been recently done for FTh (Salifu and Singh 1987; Salifu and Hodgson 1987), they are quite adequate for use in resistance screening and pest monitoring.

No research method is beyond improvement. Consequently, the various methods described here are constantly under review. The authors would be glad to receive suggestions for improvements or new procedures for host plant resistance screening.

REFERENCES

Alzouma, I. 1988. Cycle biologique, reproduction et developpement de *Bruchidius atrolineatus* Pis (Coleoptere Bruchidae) en zone Sahelienne. Importance de leur connaisance pour un controle efficace de ce ravageur dues graines de *Vigna unguiculata* L. Walp. Paper presented at the Regional Grain Legume Workshop organized by IFS, 22-27 February 1988, Tannanarive, Madagascar.

Beck, S.D. 1965. Resistance of plants to insects. Annual Review of Entomology 10: 207-232.

Chiang, H.S. and N.S. Talekar. 1980. Identification of sources of resistance to beanfly and two other agromyzid flies in soybean and mungbean. Journal of Economic Entomology 12: 260-265.

Dabrowski, Z.T., D.O.M. Bungu and R.S. Ochieng. 1983. Studies on the legume pod borer, *Maruca testulalis* (Geyer). 3. Methods used in cowpea screening for resistance. Insect Science and its Application 4: 141-145.

Dahms, R.G. 1972. Techniques in the evaluation and development of host plant resistance. Journal of Environmental Quality 1: 254-259.

Davies, F.M. 1985. Entomological techniques and methodologies used in research programs on plant resistance to insects. Insect Science and its Application 6: 391-400.

Don-Pedro, K.N. 1980. A population explosion of *Aphis craccivora* Koch following DDT application in a cowpea plot (*Vigna unguiculata*) cultivar (Prima) in Nigeria. Journal of Natural History 14: 117-119.

Gould, F. 1983. Genetics of plant-herbivore systems: interactions between applied and basic study. Pages 599-653 *in* Variable plants and herbivores in natural and managed systems, edited by R.F. Denno and M.S. McClure. Academic Press, N.Y.

Heinrichs, E.A., F.G. Medrano and H.R. Rapusas. 1985. Genetic evaluation for insect resistance in rice. International Rice Research Institute, Los Banos, Philipines.

Horber, E. 1980. Types and classification of resistance. Pages 15-21 *in* Breeding plants resistant to insects, edited by F.G. Maxwell and P.R. Jennings. John Wiley and Sons, N.Y.

Howe, R.W. 1973. A parameter for expressing suitability of an environment for insect development. Journal of Stored Product Research 7: 63-64.

IITA (International Institute of Tropical Agriculture). 1979. Annual report for 1978. Ibadan, Nigeria.

IITA (International Institute of Tropical Agriculture). 1982. Annual report for 1981. Ibadan, Nigeria.

IITA (International Institute of Tropical Agriculture). 1983. Annual report for 1982. Ibadan, Nigeria.

IITA (International Institute of Tropical Agriculture). 1984. Annual report for 1983. Ibadan, Nigeria. 218 pp.

IITA (International Institute of Tropical Agriculture). 1985. Annual report for 1984. Ibadan, Nigeria.

IITA (International Institute of Tropical Agriculture). 1986. Grain Legume Improvement Program annual report for 1985. Ibadan, Nigeria. 303 pp.

IITA (International Institute of Tropical Agriculture). 1987. Annual report and research highlights for 1986. Ibadan, Nigeria.

IITA (International Institute of Tropical Agriculture). 1988. Annual report and research highlights 1987/88. Ibadan, Nigeria. 161 pp.

Jackai, L.E.N. 1982. A field screening technique for resistance of cowpea (*Vigna unguiculata*) to the pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). Bulletin of Entomological Research 72: 145-156.

Jackai L.E.N. 1983. Efficacy of insecticide application at different times of day against the legume pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae), on cowpea in Nigeria. Protection Ecology 5: 245-251.

Jackai, L.E.N. Laboratory screening of cowpeas for resistance to the cowpea coreid, *Clavigralla tomentosicollis* Stäl. (Hemiptera: Coreidae). Journal of Economic Entomology (in press).

Jackai, L.E.N. and S.R. Singh. 1983. Varietal resistance in integrated pest management of cowpea (*Vigna unguiculata*) pests. Insect Science and its Application 4: 199-204.

Jackai, L.E.N. and R.A. Daoust. 1986. Insect pests of cowpeas. Annual Review of Entomology 31: 95-119.

Jackai, L.E.N. and T.L. Lawson. 1987. Insect pest surveys on cowpea (*Vigna unguiculata*, Walp.) and the possible effects of some climatic factors on the population trends of the legume pod borer and pod sucking bugs. Pages 245-256 *in* Proceedings, Agrometeorology and Crop Protection in the Lowland Humid and Sub-humid Tropics. WMO, Geneva.

Jackai, L.E.N. and J.R. Raulston. 1988. Rearing the legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) on artificial diet. Tropical Pest Management 34: 168-172.

Jackai, L.E.N., J.M.F. Roberts and S.R. Singh. 1988. Cowpea seed treatment with carbosulfan: potential for control of seedling pests. Crop Protection 7: 384-390.

Kogan, M. and D.C. Herzog. 1980. Sampling methods in soybean entomology. Springer-Verlag, New York. 487 pp.

Kogan, M. and E.E. Ortman. 1978. Antixenosis — a new term proposed to replace Painter's "non-preference" modality of resistance. Entomological Society of America Bulletin 24.

Litsinger, J.A., C.B. Quirino, M.D. Lumaban and J.P. Bandong. 1977. Grain legume pest complex of three Philippine rice-based cropping systems. Cropping Systems Program, International Rice Research Institute, Los Banos, Philippines. 39 pp.

Little, T.M. 1985. Analysis of percentage and rating scale data. Hortscience 20: 642-644.

Little, T.M. and F.J. Hills. 1977. Agricultural experimentation — design and analysis. John Wiley and Sons, N.Y. 350 pp.

Matterson, P.C. 1982. The effects of intercropping with cereal and minimal permethrin application on insect pests of cowpeas and their natural enemies in Nigeria. Tropical Pest Management 28: 373:380.

Maxwell, F.G. and P.R. Jennings. 1980. Breeding plants resistant to insects. John Wiley and Sons, N.Y. 683 pp.

N'Doye, M. 1978. Donnés nouvelles sur la biologie et l'ecologie au Senegal de la chinelle poilue du niebe, *Amsacta moloneyi* Drc. (Lepidoptera: Arctiidae). Cahier ORSTOM, Série Biologie 13: 321-331.

Nwanze, K.F. and E. Horber. 1975. Laboratory techniques for screening cowpea for resistance to *Callosobruchus maculatus* F. Environmental Entomology 4: 415-419.

Ofuya, T.I. and A.E. Akingbohungbe. 1986. Aspects of varietal resistance in cowpea to the black cowpea moth, *Cydia ptychora* (Meyrick) (Lepidoptera: Totricidae). Insect Science and its Application 7: 777-780.

Painter, R.H. 1951. Insect resistance in crop plants. McMillan, N.Y.

Raman, K.V., S.R. Singh and H.F. van Emden. 1978. Yield losses in cowpea flowering leafhopper damage. Journal of Economic Entomology 71: 936-938.

Salifu, A.B. and S.R. Singh. 1987. Evaluation of sampling methods for *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) on cowpea. Bulletin of Entomological Research 77: 451-456.

Salifu, A.B. and C.J. Hogson. 1987. Dispersion patterns and sequential sampling plans for *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) in cowpeas. Bulletin of Entomological Research 77: 441-449.

Singh, B.B., S.R. Singh and O. Ajadi. 1985. Bruchid resistance in cowpea. Crop Science 25: 736-739.

Singh, S.R. 1977. Cowpea cultivars resistant to insect pests in world germplasm collection. Tropical Grain Legume Bulletin 9: 1-7.

Singh, S.R. 1980. Biology of cowpea pests and potential for host plant resistance. Pages 398-421 *in* Biology and breeding for resistance to arthropods and pathogens in agricultural plants, edited by M.K. Harris. Bulletin MP-1451, Texas A & M Univ.

Singh S.R. and L.E.N. Jackai. 1985. Insect pests of cowpeas in Africa: their life cycle, economic importance and potential for control. Pages 217-231 *in* Cowpea research production and utilization, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, N.Y.

Singh, S.R. and H.F. van Emden. 1979. Insect pests of grain legumes. Annual Review of Entomology 24: 255-278.

Suh, J.B., L.E.N. Jackai and W.N.O. Hammond. 1986. Observations on pod sucking bug populations on cowpea at Mokwa, Nigeria. Tropical Grain Legume Bulletin 33: 17-19.

Starks, K.J. and R.L. Burton. 1977. Greenbugs: determining biotypes, culturing and screening for plant resistance with notes on rearing parasitoids. USDA-ARS Technical Bulletin 1556.

Taylor, T.A. 1964. The field pest problems on cowpeas, *Vigna sinensis* L. in southern Nigeria. Nigeria Grower and Producer 3: 1-4.

Todd, J.W. and D.C. Herzog. 1980. Sampling phytophagous pentatomidae. Pages 438-478 *in* Sampling methods in soybean entomology, edited by M. Kogan and D.C. Herzog. Springer-Verlag, N.Y. 487 pp.

Wiseman, B.R. 1985. Types and mechanisms of host plant resistance to insect attack. Insect Science and its Application 6: 239-242.