Methods for the Evaluation of Forage Legumes, Grasses and Fodder Trees for Use as Livestock Feed

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1. Introduction

Forage plant evaluation, to identify legumes, grasses and fodder trees with potential for livestock feed, is carried out by many national agricultural research systems (NARS) and international research organisations throughout sub-Saharan Africa. However, to date, the approach adopted at various locations has depended on the scientists involved, the resources available and the ultimate aim of the evaluation. Although this has generated a lot of data, variation in evaluation methodology has precluded the comparison of results across sites. This manual outlines a standard evaluation methodology, which will enable researchers throughout the region to obtain meaningful results from their forage plant evaluation, whilst still allowing comparisons between sites. A companion manual, covering the evaluation of feed value (Osuji et al, 1993) has already been produced. The present document is based on experience from the forage evaluation programme at ILCA, Kaduna (Mohamed-Saleem and Otsyina, 1984; Tarawali et al, 1989; Tarawali, 1991; Peters, 1992; Peters et al, 1994a; 1994b; Tarawali, 1994a; 1994b; Tarawali et al, 1994), and fodder-tree evaluation at ILCA, Ibadan (Larbi et al, unpublished).

The experimental designs described fall into two categories. Section 2 deals with methodology for initial evaluation, including suitable methodology for small-plot evaluation trials and larger multi-locational trials such as the Regional Trials B of the RIEPT (Red Internacional de Evaluacion de Pastos Tropicales; Toledo and Schultze-Kraft, 1982). These methods can be used to generate a uniform set of information from different sites. Suggested forms for recording results are included in Appendix III. Section 3 covers methodology suitable for further evaluation of accessions identified in the initial evaluation. Appropriate experimental designs are described for estimation of the effects of grazing on plant growth, evaluation of mixtures of accessions and species and estimation of seed multiplication capacity. Researchers can then select suitable methodologies for evaluation of forages for specific farming systems.

2. Initial screening methods

Land for any trial should be cleared and the soil prepared to a fine tilth before demarcating plots and planting. A composite soil sample can be taken over the experimental area using a soil auger to collect samples to a depth of 15–20 cm. At least one sample per plot should be collected and all the cores can be bulked if the soil appears similar. If there are obvious differences, only soils of the same appearance should be bulked. The sample(s) can be analysed for nitrogen, phosphorus, soil pH, cation exchange capacity (CEC) and texture to quantify general site characteristics. Rainfall and temperature (maximum and minimum) records should be kept throughout the experiment.

Based on the site characteristics and research objectives, accessions may be pre-selected using information available in international genebank databases such as ILRI (Addis Ababa, Ethiopia), CIAT (Cali, Colombia), CSIRO (Australia), IITA (Ibadan, Nigeria) and ICRAF (Nairobi, Kenya) or by reference to literature.

Every trial should include a standard that is adapted to the locality or environment. Standards may be exotic or indigenous species. For example, in Kaduna (subhumid Nigeria) *Stylosanthes hamata* cv Verano is used as a control because this accession is currently widely used for pastures in the region.

2.1 Herbaceous legumes and grasses

The amount of seed available for research is often a limiting factor and determines the trial design. Small-plot observation trials are useful for evaluation of germplasm represented by quantities of seed too small to be sufficient for the main screening trial (less than 50 g for legumes and less than 100 g for grasses).

2.1.1 Small-plot observation trial

The small-plot observation trial can be carried out with very small amounts of seeds. For small-seeded legume species such as *Stylosanthes* or *Desmodium*, use 1 g of seeds on a 1 m^2 plot, giving a maximum of 100 to 150 plants per plot or row. Larger seeds such as *Centrosema* or *Lablab* require a larger quantity of seed to obtain an equivalent plant density per plot. Smaller amounts can be accommodated, right down to a few seeds which may also be raised in pots and transplanted to the field as spaced plants. Hard-seeded legumes require scarification before sowing (Appendix I). Grasses may need to be planted at a higher rate because the viability of grass seeds is often low. A germination/viability test may be advisable if seeds are sufficient. Seed dormancy may be a problem in grasses.

To improve establishment in areas where labour is not a limiting factor, it is possible to germinate grass seeds in petri dishes in the laboratory and raise seedling in pots for transplanting. The trial can be carried out for one or two years, during which time seeds can be collected for use in larger replicated trials.

Establishment

1. Lay out 0.5 x 2.0 m plots in a randomised complete block design with two replicates with 1 m between plots. Use three replicates if there is enough seed.

2. Plant the seeds in a single row down the centre of each plot.

3. Space small numbers of seeds along the row and mark their planting sites with small sticks.[1]

4. Do not use fertiliser and inoculants.[2]

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1 Even distribution of seeds may be aided by mixing them with sand (or fine dry soil) before sowing. Legume seeds need to be scarified before planting. Small quantities of seeds can be easily scarified by rubbing them between two sheets of sandpaper until one or two seeds break (other options for scarification are given in Appendix I). It is important to check the seed packet label and the seeds themselves to confirm whether or not they were scarified before dispatch by the genebank.

2 The aim is to identify accessions which could survive even under harsh conditions with some mismanagement. Methods of inoculation are described in Appendix II, if required.

5.   Keep the plots free of weeds to avoid competition, especially for slow-growing species. Hoe/mow or slash the vegetation between the plots to keep the area clear.

Data collection

(i)   Establishment

Seedling counts should be made four and eight weeks after planting to give an estimation of emergence.

1.   Count the total number of seedlings in the row to determine plant density per square metre.

2.   Estimate the soil cover (percentage of the plot covered by the plants).

(ii)   Disease and pest incidence

Note any obvious disease or pest problems by recording incidence and severity.[3] Score these problems at regular intervals beginning during establishment and every eight weeks thereafter.

(iii)   Flowering and seed production

1.   Monitor the plots for flowering and seed production by recording the month in which 50% of the plant canopy flowers and produces seed.

2.   Collect ripe seeds every two to three days; weigh and record the total for each month. It may be necessary to protect grass seeds from bird damage.

(iv)   Biomass production

1.   At the end of the growing season count the number of plants and cut half of each plot at 10–15 cm above the ground.

2.   Fresh weigh the plant material, take a subsample and dry in an oven to determine the dry-matter yield. This can then be converted to g dry matter/plant or kg/ha (if the number of plants varies tremendously) for comparison.

(v)   Drought tolerance

During the dry season, score the plots for drought tolerance by recording the percentage of plants in each plot that remain green or retain their leaves every six weeks.

2.1.2 Small-plot management trial

A good example for an experimental design for this type of trial is Regional Trial B of the Red Internacional de Evaluacion de Pastos Tropicales (RIEPT) as described by Toledo and Schultze-Kraft (1982). This was modified for the RABAOC (Recherche en alimentation du betail en Afrique occidentale et centrale) project (Schultze-Kraft and Toledo, unpublished) within ILCA's African Feed Resources Network (AFRNET). This type of trial may be conducted using material selected from the small-plot evaluation trial (section 2.1.1) or, if sufficient seeds are available, it may be the first evaluation trial. Unlike the small-plot observation trial, legume species in this trial are usually inoculated and fertiliser is applied to investigate its effect on biomass production.

There are three periods of evaluation in this trial. The first 12 weeks form the establishment period and data is collected on germination and soil cover. The second period of evaluation is during the period of minimum precipitation (dry season), commencing at the end of the wet season for 12 weeks. The third period is the period of maximum precipitation (wet season) which commences near the beginning of the rains (at least when the rain is well established so as to include the wettest months) and lasts for 15 weeks. Periods of maximum and minimum precipitation for Kaduna in 1991 are shown in Figure 1 as an example. The second and third periods of evaluation commence with a standardisation cut (all plots, including the sampling area and border rows are cut down to a standard height as defined below) followed by productivity measurements as detailed below. Two seasons each of maximum and minimum precipitation should be recorded.

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3   Incidence is the percentage of the plot infected and severity is the percentage infection on those plants showing symptoms. Both should be scored on the scale 0, 5, 10, 25, 50, 75 and 100%.

Rainfall (mm)

S — Standardisation cut
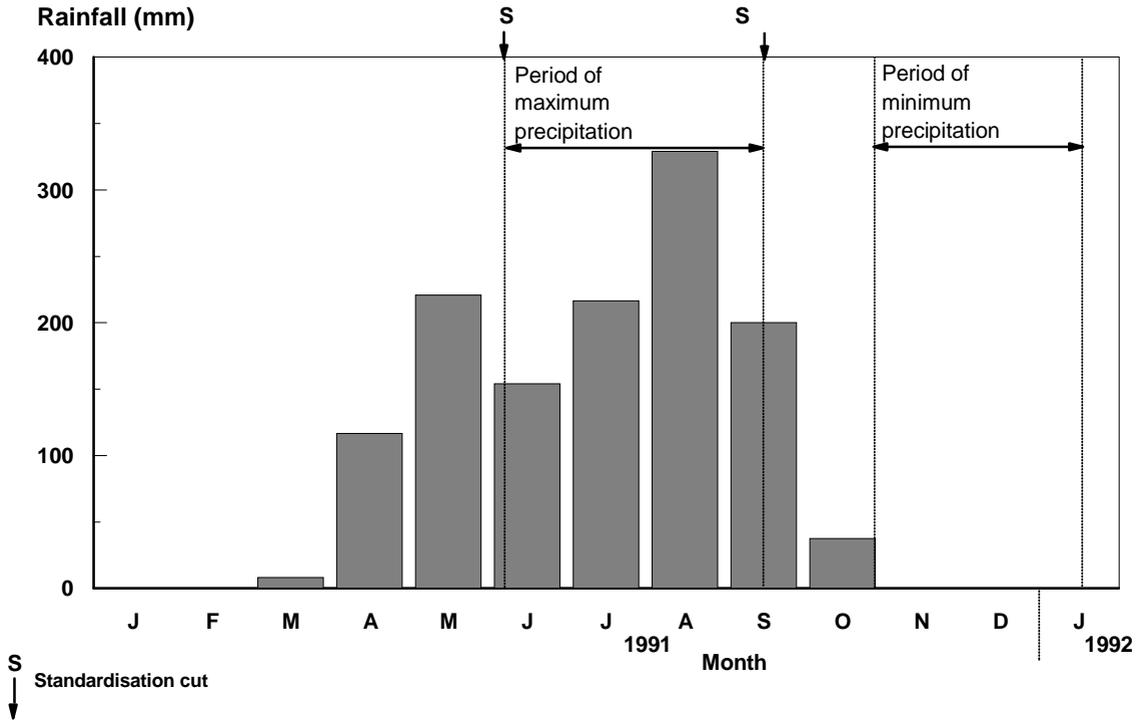
Establishment

1. Lay out 2.5 x 5 m plots with four rows of 5 m length spaced 0.5 m apart in a randomised complete block design with a split-plot arrangement (main plot = accession; subplot = harvest time) with three replicates.

2. The two central rows form the sampling area. Divide them into two or four subplots for evaluation in the dry and wet seasons, respectively (Figure 2).

3. Establish grasses and herbaceous legumes separately within the blocks and treat them as separate experiments.

4. Plant seeds evenly along the rows.[4]

5. Apply the fertiliser to both grasses and legumes at a rate of 20 kg/ha phosphorus (P) as single superphosphate (SSP) in a 20 cm band on each row at the time of planting.

6. Apply nitrogen (23 g urea in each 20 cm x 5 m band) to the grass plots eight weeks after sowing.

7. Hand weed the trial plots throughout the experiment.

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4 Inoculation can be carried out at sowing by mixing the inoculant in peat with the seeds. This method cannot be used for seeds pre-treated with chemicals and so in these instances, the inoculant in peat should be mixed with water and sprinkled on the rows once the seedlings have germinated (alternative methods of inoculation are given in Appendix II).

	Maximum precipitation	Minimum precipitation

Maximum precipitation

Minimum precipitation

1 m

Cut at:

3 and 15 weeks

6 and 15 weeks

5 m

4 m

9 and 15 weeks

12 and 15 weeks

2.5 m

Cut at:

6 and 12 weeks

12 weeks
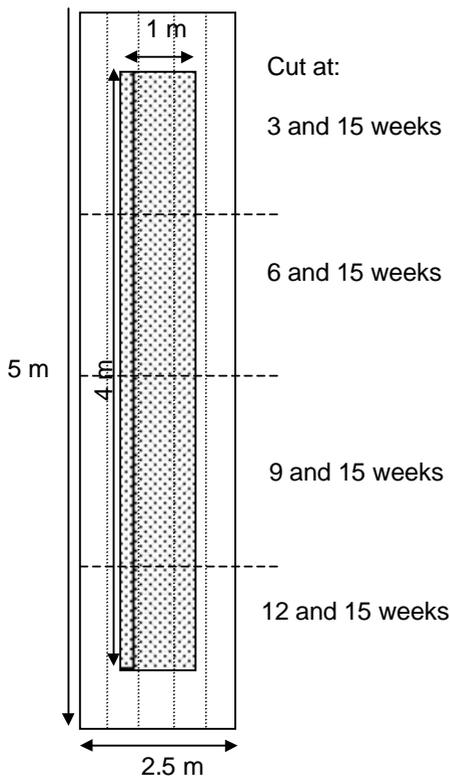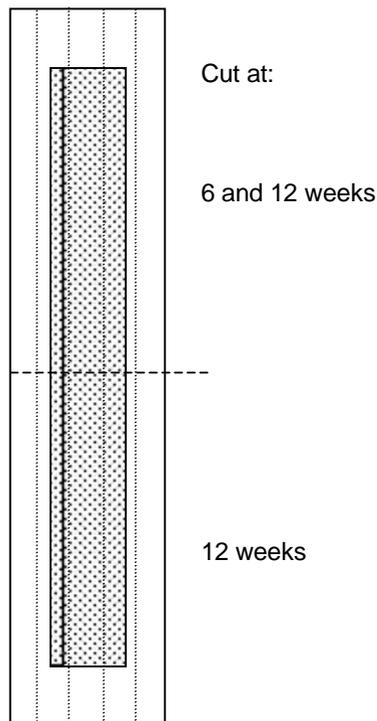
Sampling area

Cutting times are times after the standardisation cut at the beginning of each period.

Data collection

Establishment period

The evaluation should be done on an area of 1 m^2 within the two central rows (sampling area) of each plot at 4, 8 and 12 weeks after establishing the trial.

(i)   Germination and soil cover

    1.   Count the plants.

    2.   Estimate the soil cover by dividing up the 1 m^2 quadrat into 25 squares of 20 x 20 cm using string.[5]

(ii)   Disease and pest incidence

    Estimate the extent of any signs of insect attack or disease incidence as described for the small-plot observation experiment (section 2.1.1).

(iii)   Biomass production

    1.   Cut 1 m^2 within the sampling area at the end of the 12-week establishment period. [6]

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5   This makes the estimation of soil cover easier in the early stages of growth. Once the plants are larger and the observers more experienced in making cover estimates, the whole 1 m^2 quadrat can be used again.

6   In some regions this cut will coincide with the standardisation cut at the end of the wet season covering the period of minimum precipitation, whilst in other regions there may be time for an eight-week period before the rain stops and the standardisation cut is made.

2. Cut prostrate species at a height of 5–10 cm and erect or semi-erect species 10–15 cm above the soil.

3. Weigh the fresh material immediately (Tot FW).

4. Accurately weigh a sample of 150–200 g fresh material (FW ss), oven dry it at 65°C and reweigh (DW ss) to give an estimate of the dry-matter production during this period.

Dry-matter production is calculated as:

$$(\text{Tot FW} \times (\text{DW ss/FW ss})) \times 10 = \text{dry matter kg/ha}$$

where:

Tot FW = total fresh weight from 1 m^2 in g

DW ss = dry weight of the subsample in g

FW ss = fresh weight of the subsample in g

(iv) Crude-protein content

Determine the crude-protein content if facilities are available.[7] Grind the dried samples for analysis using the Kjeldahl method. Bulk replications to reduce the number of samples for analysis.

(v) Digestibility

Determine the digestibility if facilities are available. Analysis of this parameter gives an indication of the potential contribution of the plant material towards animal nutrition (Ørskov and McDonald, 1979). Use an *in vivo* estimation using the nylon-bag technique (Osuji et al, 1993) with one incubation period of 48 hours for each accession.

Period of minimum precipitation

Whole plots (including the border rows) should be cut at the end of the wet season at the recommended cutting heights. This is the standardisation cut for the period of minimum precipitation. Half of each plot should be cut after six weeks regrowth and both halves of the plots should be cut after another six weeks (Figure 2).

1. Harvest a 1 m^2 quadrat within the sampling area at each cut as described above (yield estimation) to determine dry-matter yields.

2. Cut down the remainder of the subplot.

3. Record soil cover, % green leaf and disease/pest incidence and severity at each evaluation.

4. If resources allow, analyse for crude protein and digestibility.

Period of maximum precipitation

For this period the sampling area is divided into four subplots which should be cut 3, 6, 9 and 12 weeks after the standardisation cut at the beginning of the period of maximum precipitation.

1. Weigh the material harvested from the 1 m^2 subplot (total fresh weight).

2. Take a subsample of 200–300 g fresh weight, oven dry it at 65°C and reweigh to determine dry-matter production.

3. Analyse crude protein and digestibilities for the dried samples if facilities allow.

4. Cut the border rows of the subplot after harvesting the quadrat.

5. Harvest all four subplots 15 weeks after the standardisation cut, giving another set of 3, 6, 9 and 12-week regrowth ages.

6. Record soil cover and insect/disease incidence and severity at each harvest time.

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7 Some indication of crude-protein contents is useful when designing further evaluation trials where one may need to know how much crude protein can be provided by the various accessions.

Flowering and seed production

In addition to the three replications used to monitor productivity, a fourth single-row replication should be planted to enable flowering and seed production to be observed.

1. Plant a single 5 m long row of each accession, with 10 plants spaced evenly along the row. Provide climbing accessions with trellises.

2. Monitor these plants for the onset of flowering and the month of peak flowering (50% of the row with flowers).

3. Collect ripe seeds every three days. Weigh the seeds as collected to establish the period of peak seed production.

The trial described above will enable the identification of promising material for wet and dry season use. Such material can then be incorporated into further trials as appropriate to the farming system of the area (section 4).

2.2 Fodder trees

A scheme for screening fodder trees is proposed based on the experiences from the ILCA/ICRAF/IITA collaborative multi-purpose tree evaluation research activities in southern Nigeria (Ibadan and Onne). Two stages are described within this initial evaluation section. In Stage I a large number of accessions may be screened for edaphic and climatic adaptation. In Stage II, elite genotypes from Stage I are screened for response to different management techniques and forage quality. A few selected accessions may be subjected to more detailed trials as described in section 3.

2.2.1 Initial screening trial

Establishment

1. Scarify the seeds (see Appendix I), inoculate if necessary (Appendix II) and plant in well-drained nursery bags or beds about 10 weeks before the major rains.

2. Transplant seedlings into the field when the rains have stabilised. The minimum height for transplanting should be 40–50 cm.

3. At planting apply 15–20 g of 15:15:15 N:P:K compound fertiliser around each plant. This may not be required on fertile soils.

3. Plant seedlings of each accession in 6 m long single rows, with 3 m between rows and 0.5 m between plants in a row[8] using a completely randomised design with three to four replicates depending on availability of seedlings.

4. Slash inter-row spaces to reduce weed competition.

5. Replace seedlings which die in the first eight weeks after transplanting with spare plants from the nursery.

Data collection

(i) Plant height

Measure the height of all the individual plants in each replicate with a metre stick every eight weeks and record the average for each replicate.

(ii) Seedling survival

1. Count seedlings 3 (SN_3) and 12 (SN_{12}) months after transplanting.

2. Calculate percentage seedling survival (%SS) during the establishment year as:

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8 This spacing is recommended to accommodate expected variation between species and determine yield per plant rather than yield per unit area.

$$\%SS = \frac{SN_3 - SN_{12}}{SN_3} \times 100$$

(iii) Disease and pest incidence

    1.   Score disease and pest incidence using the same system as described for herbaceous legumes and grasses (section 2.1.1), based on percentages of incidence and severity.

    2.   Record observations every four weeks during the first year of establishment.

(iv) Green-leaf retention

    1.   Determine green-leaf retention each year during the dry season from individual plants that are not pruned.

    2.   Estimate the percentage of green leaf by comparing with a plant with 100% green leaves.

(v) Biomass production

    1.   Take the first harvest when plants are 12 months old. Subsequent harvest periods should be based on phenology and climatic changes.[9]

    2.   At each harvest, cut the plants in the middle of the row to 0.5 m above the ground.

    3.   Sort materials into leaf (leaf plus fine stem up to 6 mm in diameter) and stem (greater than 6 mm diameter).

    4.   Fresh weigh this material in the field, subsample and oven dry at 60°C for 48 hours for the determination of dry matter.

(vi) Dry-season nitrogen

    If facilities allow, grind the dried biomass samples and analyse for nitrogen using the conventional Kjeldahl method.

(vii) Dry-matter degradability

    Analysis of dry-matter degradability gives an indication of the potential contribution of the plant material towards animal nutrition (Ørskov and McDonald, 1979).

    Use the nylon-bag technique (Osuji et al, 1993) with one incubation period of 48 hours for each accession.

2.2.2 Small-plot management trial

Based on the biomass- and quality-related attributes assessed in the initial screening trial, promising genotypes can be selected for further screening. At this level, dry-matter production, nitrogen content and *in vivo* digestibility can be assessed under various management regimes, such as cutting height or frequency.

Establishment

Establishment methods are similar to those described in section 2.2.1.

1.   Plant each accession in a plot of five or seven single rows, with 2 m between rows and 0.5 m between plants in a row. Each accession should be replicated three or four times (Figure 3).

2.   Impose management treatments (defoliation heights or defoliation frequencies) within the plot to give a split-plot design.

3.   Determine the agronomic data (biomass production) and nutrient content from the central rows (Figure 3). Use the outer rows for determination of digestibility and for animal feeding trials, if facilities allow.

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9   For example, in Ibadan, harvests are taken at the end of the main wet, minor wet and dry seasons.

Trees for agronomic data -
split into three treatments
(T1, T2, T3) with three trees each

2.0 m

0.5 m

T1 T2 T3
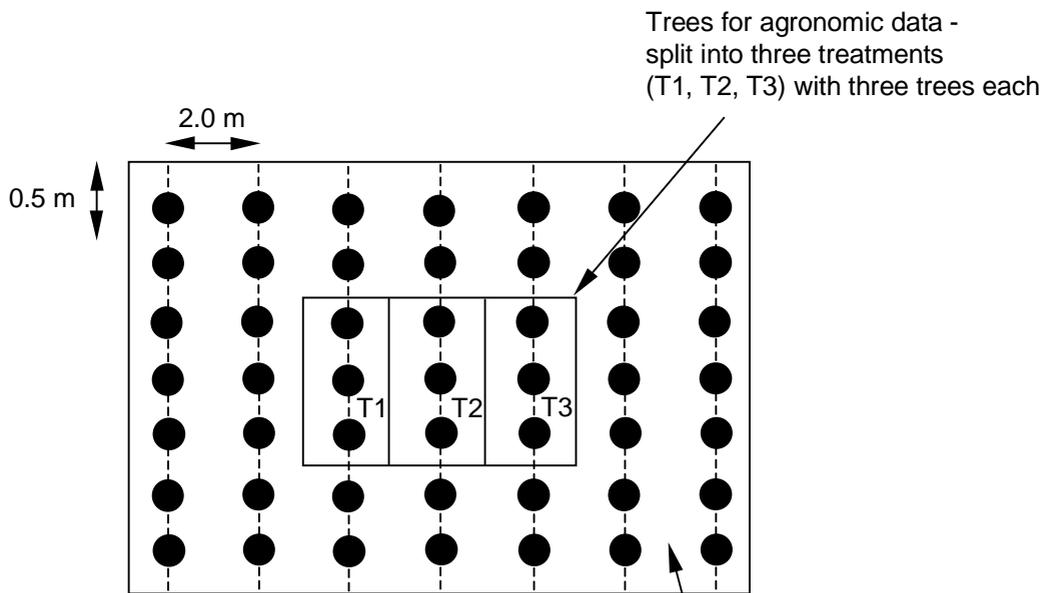
The diagram shows one plot of one accession,
each plot will be replicated three or four times
and the experiment set out in a
randomised complete block design

Border rows can be harvested
for animal trials.
Some trees may be left to monitor
dry-season performance

Data collection

(i) Biomass production

  1. At each harvest, cut plants in the central row according to the agronomic treatments imposed and
     partition into leaf (leaf plus fine stem up to 6 mm in diameter) and stem (greater than 6 mm
     diameter).

  2. Fresh weigh this material in the field, subsample and oven dry at 60°C for 48 hours for the
     determination of dry matter.

  3. Cut plants outside the sampling area to 0.5 m above the ground, bulk by accession and partition
     into leaf and stem.

  4. Use the leaf portion for assessment of forage quality (see digestibility below).

(ii) Chemical composition

  Analyse nitrogen content of the dried and ground biomass samples (leaf and stem separately) using
  the standard Kjeldahl technique (Osuji et al, 1993).

(iii) Digestibility

  1. Dry and grind samples collected from the outer rows.

  2. Test for digestibility using the nylon-bag technique with a range of incubation periods to determine
     the rate and extent of degradation (Osuji et al, 1993).

(iv) Intake and animal performance

  If facilities allow, and sufficient material is available, bulk the herbage cut from the outer rows by
  replication and use to feed animals for intake, preference and animal performance assessment.

3. Schemes for further evaluation

The initial evaluation identifies accessions with high biomass productivity and potential for use as fodder. However, these accessions still need further evaluation to assess response to defoliation under grazing or cutting, seed multiplication potential, nutritive value for use as livestock feed and performance in on-farm situations.

The methods described in this section are used to evaluate smaller numbers of selected accessions in trials related to their use and management in farming systems. The appropriate trial design should be selected according to the end use of the forage, farming system and agro-ecological zone. Only accessions identified as promising during initial screening (section 2) should be used for these trials.

3.1 Small-plot grazing trial

The aim of this trial is to give information on the performance of selected herbaceous material under grazing. It is based on a similar successful trial at Kaduna. The experiment is designed for cattle, but small ruminants could equally well be used. If facilities allow, oesophageal fistulated animals could be used to monitor diet selection and rumen-fistulated animals used for nylon-bag digestibility studies (Osuji et al, 1993).

3.1.1 Trial establishment

The recommended trial design is suitable for evaluation of six to eight selected accessions. Plots of 4 x 5 m with 2 m between plots are a suitable size for grazing.

1. Plant accessions at recommended sowing rates in four replications using a randomised complete block design.

2. Scarify legume seeds using sandpaper before planting (Appendix I).

3. Mix seeds for each plot with 300 g single superphosphate (SSP; 18% P_2O_5 = 27 kg/ha P_2O_5).

4. If grass accessions are being evaluated, apply nitrogen as urea at the rate of 400 g urea per plot (= 42 kg/ha N).

Maintenance

1. Keep the inter-plot areas slashed down low but do not weed and do not mound up the sides of the plots.

2. Do not weed the plots, but keep shrubs slashed and destroy termite mounds if necessary.

Grazing

1. If legumes are being evaluated, use four young cattle to periodically graze down the plots, starting with wet season grazing to control grasses.

2. Following establishment, observe the plots regularly. When grasses begin to dominate and are at least 10 cm higher than legume seedlings, introduce the cattle into the plots to graze down the grasses.

3. Carefully monitor grazing and remove the cattle from the plots before they damage the legumes. Cut down any remaining grass so all the plots are equal.

4. Introduce the cattle every six weeks after this initial grazing, through the remainder of the wet season and into the dry season.[10]

5. If grass accessions are being evaluated, an initial grazing is not necessary. Begin evaluation of grazing about four weeks before the end of the wet season and continue at six-weekly intervals.

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10 There should be no grazing in the dry season when regrowth is poor. In some instances, early wet-season grazing in the establishment year may not be necessary as grass infestation is less when the plots have been cleared before planting. In this case, grazing can start about four weeks before the end of the wet season and continue every six weeks after that until regrowth is too poor.

Data collection

(i) Establishment

1. Make replicated emergence counts on two quadrats of 1 m^2 per plot, four and eight weeks after establishment.

2. Estimate plant cover (sown accession/other legume/grass) using the same quadrats.

(ii) Sampling and biomass estimation

1. Cut one 1 m^2 quadrat at about 15 cm above the ground from each plot before each introduction of the animals.

2. Count the number of sown accession plants and divide the cut sample into sown accession, other grasses and other forbs.

3. Record the fresh weight of each, oven dry the samples at 65°C and reweigh.

4. Immediately after the animals have stopped grazing, cut another 1 m^2 quadrat from a different place, divide, weigh and dry as above.

(iii) Acceptability/palatability

Acceptability/palatability may be monitored in this experiment by estimating the time spent by animals on each plot. Oesophageal fistulated animals could also be used to identify species preferences (section 4.2).

(iv) Flowering

Record the date(s) when 50% of the sown accession in the plot has flowers.

(v) Disease and pest incidence

The incidence and severity of pests and diseases on the sown accession should be recorded as described earlier (section 2.1.1).

3.2 Cut-and-carry trial

Cut-and-carry trials are suitable for estimating productivity of material used for zero grazing or stall-feeding cattle or small ruminants. This is a common practice in many parts of sub-Saharan Africa including Burundi, the high plateaus of north and west Cameroon, western Kenya, Malawi and Rwanda. In these areas population density is high and the demand for crop land leaves very little for livestock grazing. Cut-and-carry feeding is popular with smallholder dairy schemes in parts of Kenya and Tanzania. The usual practice is to cultivate high yielding grass and legume species, which are then cut in rotation on a daily basis and fed to animals maintained in enclosures. If shrubs or trees are used as fodder, these may also be cut daily and used as protein supplements to low-quality feed.

3.2.1 Trial establishment

The suggested trial design is suitable for evaluating three to five selected accessions. An estimate of how much fodder will be required per animal can be obtained on the basis that an animal will consume approximately 3% of its body weight (on a dry-weight basis) per day. If legumes are to be used as supplements with grass or a basal diet, the legume should form about one-third of the feed on offer.

1. Prepare the land as described earlier (section 2 under initial screening methods). For cattle, 0.25 to 0.5 ha plots are necessary to meet the feed requirements of one animal for two to three months. Smaller plots of 0.10 to 0.25 ha per animal should be sufficient for small ruminants.

2. Leave a 2-m pathway around the plots to allow easy access for cutting.

3. Scarify legume seeds (Appendix I) before sowing.

4. To stimulate early development of legumes apply 150 kg/ha single superphosphate at planting. Apply farmyard manure to encourage the growth of grasses.

Maintenance

Weeding during the initial establishment phase suppresses competition from weeds, but once the sown material is established this should no longer be necessary. Subsequent elimination of shrubs or very persistent/vigorous weeds may continue to be necessary.

Animals selected for the trial should undergo routine veterinary treatment such as deworming and vaccination before the trial.

Cutting and feeding forage

For feeding trials involving several accessions, use at least five cows or six to eight small ruminants per treatment. If the forage has a moisture content over 80%, the material may be cut the day before feeding because wilting of such material can improve dry-matter intake. Material with a lower moisture content should be cut and fed on the same day. Chopping forage before offering it to the animals improves feed intake and limits selectivity.

1. Feed the animals 50% of their daily requirement in the morning and 50% in the evening.[11]

2. Record the weight of forage offered and residue remaining at each feeding time.[12]

3. Monitor the dry-matter content of the forage offered and the residue to accurately estimate dry-matter intake.[13]

4. Weigh animals weekly or fortnightly. If lactation is also being monitored, record daily milk production and growth of suckling animals.

Accessions that are suitable for use in cut-and-carry farming systems are those which show good regrowth in response to cutting, have a high biomass and produce good liveweight gains from the experimental animals.

3.3 Seed multiplication trial

Seed-multiplication potential of promising accessions should be monitored because planting material is essential for widespread use as a forage. Seeds are also required for further trials and on-farm testing. Accessions with poor seed-production capacity are not easily disseminated or accepted. The following experimental design may be used to assess the seed-production capacity of promising accessions. Seed production plots can also be established to produce large amounts of seeds for further trials and release to farmers (Kachelriess and Tarawali, 1994).

3.3.1 Trial establishment

Isolation is necessary when growing accessions of outcrossing species in the same area to ensure genetic purity. An isolation distance of 100 m is required when pollination is by insects and one of 200 m when pollination is by wind. Separate apomicts and self-pollinating species by about 5 m between accessions to avoid mixing. Isolation may also be necessary to prevent the spread of insect pests and diseases between accessions. In this case a distance of 100 m is recommended.

1. Lay out 4 x 5 m (or larger) plots in a randomised complete block design with four replicates of each accession with 2 m between plots for estimation of seed-production capacity.

2. Scarify legume seeds using sandpaper before planting (Appendix I).

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11 Feeding twice a day improves intake. The first two weeks of the feeding regime should be regarded as the adaptation period during which the microbial population of the rumen and the whole digestive tract of the animals should adjust. Usually, at the beginning of the trial, animals tend to eat small quantities of the feed but this increases as they become more familiar with the material. After about 10 days it is possible to predict the daily feed intake of the animals.

12 An animal is satiated from eating a forage if a part of the daily ration (not the inedible fraction) remains. To minimise wastage, it is advisable to provide the estimated daily feed requirement plus 15% to ensure the animal is satiated.

13 Subsequent chemical analysis of the forage offered and the residue should provide a good estimate of nutrients consumed.

3. Mix seeds for each plot with 300 g single superphosphate (SSP; 18% P_2O_5 = 27 kg/ha P_2O_5). If grass accessions are being evaluated, apply nitrogen as urea at the rate of 400 g urea per plot (= 42 kg/ha N).

Plots established for other trials can also be maintained and allowed to grow without further cutting or grazing to estimate seed-production potential.

Plant the seeds in rows to allow easier management at weeding, rogueing and harvest. Closer planting distances between rows ensures a good stand, reduces weeding and increases seed yield per hectare. As a general guide, sowing rates for seed-production evaluation are usually double those used for estimates of forage production. Legume accessions with creeping growth habits usually produce more seeds when grown on trellises. Wider spacing is required if seeds are produced on trellises.

Maintenance

Keep the plots well weeded to reduce both competition and the quantity of weed seeds in the final seed harvest. Spraying for insects and diseases may also be necessary to produce high quality clean seeds.

Data collection

Plants should be monitored for the onset of flowering, 50% flowering (when 50% of the plot is flowering) and the month of peak flowering.

There are two common methods for estimation of seed-production capacity.

Method I

1. Harvest ripe seeds every two or three days.

2. Determine the total seed-production capacity by measuring the weight of seeds produced per plot on a monthly basis.

3. Multiply the results of (2) to seed yield per hectare per year.

Method II[14]

1. Estimate the period of peak seed maturity and harvest the whole plot by cutting back.

2. Leave the foliage to dry then thresh it.

3. Calculate the seed yield per plot per unit time.

3.4 Evaluation of mixtures of accessions

In many cases smallholder farmers grow forages as mixtures and not as pure stands. It is therefore important to test the combining ability and yields of promising accessions in mixtures. Three types of mixtures can be considered for evaluation, namely grass–legume, legume–legume and fodder tree–herbaceous forage. A large amount of research has been done on the assessment of productivity of mixtures of grasses and legumes and on alley cropping of fodder trees with herbaceous materials. However, evaluation of legume–legume mixtures is a relatively new research area.

3.4.1 Grass–legume

Further evaluation of performance in mixtures should only be done using accessions which have been identified as promising from initial trials. Evaluation is more straightforward if combinations of one grass and one legume are compared. The methodology described for the small-plot grazing trial (3.1.1) or the cut-and-carry trial (3.1.2) should be used.

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14 This method is less labour intensive and therefore more economical, although a lower seed yield is obtained. In a species such as *Arachis pintoi* which produces subterranean seeds, this is the only possible method of harvest because the plants have to be cut back to collect the seeds by sieving from the soil.

1. To establish plots, plant the grass in rows 0.5 m apart then broadcast the legume over the whole plot area.

2. Include controls of each grass accession alone and each legume accession alone.

3. If facilities are available, analyse for crude protein and digestibility of the dried samples to give an idea of the feed value of the mixtures.

3.4.2 Legume–legume

Accessions of legumes identified through earlier evaluation can also be evaluated in combination in a similar way. In this case combinations of two or three accessions may be evaluated. A combination of a legume that is good in the establishment period with one that does well in subsequent wet seasons and one that produces well in the dry season would be appropriate to give the potential for sustainable, year-round production. It is also recommended that legume–legume combinations are evaluated using the small-plot grazing trial (3.1.1) or cut-and-carry trial (3.1.2) methodologies already described.

3.4.3 Fodder trees–herbaceous mixtures

IITA and ICRAF have developed screening methodologies to assess the agronomic potential of multi-purpose trees for alley farming or intensive feed gardens. Although these methodologies were developed to estimate productivity of herbaceous crop species with multi-purpose trees, they can be easily adapted for herbaceous forage species and fodder tree combinations. Accessions identified as promising from initial evaluation trials described in section 2.2 should be used. Appropriate methodologies are described in Tripathi and Psychas (1992).

At this stage an assessment of the value of promising accessions as livestock feed requires nutritional studies such as nylon-bag digestibilities, if these were not done during small-plot management trials. Details of these methodologies are presented in the feed evaluation manual (Osuji et al, 1993).

3.5 On-farm trials

The forages identified through initial evaluation and on-station research will ultimately be used on-farm. Conditions on local farms may be very different from those on-station. For forages to be accepted, the promising accessions must also perform well under these conditions. On-farm research is necessary to test and validate technologies developed on-station and to adapt these technologies to farmer conditions. It is also useful for demonstration and transfer of technology. On-farm research should consider the biological performance of the forage and the socio-economic aspects of its adoption and role in the farming system.

On-farm research may take several different forms. It may be completely controlled by the researcher who lays out a replicated trial design on the farmer's field and controls management and collection of data. Farmer involvement can be increased so that the farmer and researcher take joint responsibility for the trial, with frequent visits and backup being provided by the researcher. At the other extreme the farmer may be completely responsible for the trial and therefore make decisions on management and use of the forage produced, although the researcher will make regular observations, collect data and provide advice to the farmer.

The trial design for on-farm experiments must vary to fit the requirements of the farming system and individual farmer's needs and land availability. Researcher-controlled trials should use standard designs such as the grazing trial (section 3.1) or cut-and-carry trial (section 3.2). Simpler trial designs should be used as the amount of farmer participation increases. In experiments where farmers are used as replicates the number of accessions, plot size and management should be uniform across farmers to allow statistical analysis of results.

4. Planning an evaluation strategy

4.1 Selecting appropriate trials for farming systems and agro-ecological zones

The initial screening methods are suitable for all sites and should produce a uniform set of data which may be compared between sites. Whether legumes, grasses and shrubs are all evaluated at a site will depend on the aim of the evaluation, the farming systems, cultural practices and opportunity for intervention, along with the soil type and agro-ecological zone (AEZ). The type of material required by the farmer for his livestock will therefore influence the selection of species and evaluation strategy. The researcher can then select the appropriate initial evaluation trial design from those suggested in this manual.

Subsequent evaluation needs to take into account not only the type of material required but how it will be used. For grazing *in situ*, evaluation using the small-plot grazing trial (section 3.1) would be most appropriate, whether for pure legumes or grasses, or combinations (section 3.4). If zero grazing is feasible, the cut-and-carry trial (section 3.2) could be used, again with pure or mixed introductions (section 3.4). For shrubs, alley farming would be appropriate where crop–livestock integration is important, such as in areas of the humid zone. Intensive feed gardens could be useful in the humid zone and in drier areas (e.g. subhumid zone where no hardpan exists or semi-arid zone).

4.2 Introduction of animals

Since the ultimate aim of forage plant evaluation is to improve livestock productivity, it is important to include the animals as soon as possible in the evaluation procedure. However, this has to be balanced against the resources required to involve animals at an early stage of evaluation when many accessions are being tested. One valuable assessment of the potential of forage plants for animals can be obtained using the nylon-bag technique to assess *in vivo* rumen digestibility (Osuji et al, 1993). This method requires only a few animals with rumen fistulae and could be introduced as early as the RABAOC trial (section 2.1.2) or the small-plot management trial (section 2.2.2).

Oesophageal fistulated animals may be used to monitor diet selection in the trials designed for further evaluation (section 3). Animal preference for different forages can be assessed using either the difference or the occupancy-rate methods (Cook, 1978; Owen-Smith and Cooper, 1987; Koreth and Suth, 1991). For the difference method, the forage on offer is sampled and weighed immediately before and after grazing to estimate the amount consumed (as described for the small-plot grazing trial, section 3.1.1). The occupancy-rate method uses a preference index to compare the amount of time spent on each species by recording which is being grazed at five-minute intervals throughout the grazing period.

Further details of methods of assessing feed value using animals are given in the feed evaluation manual (Osuji et al, 1993).

Most of the research using animals should concentrate on the effect of the animal on the plants and the potential value of the material to the animals (crude-protein content, digestibility, presence of toxins etc). Large-scale grazing trials in which animal productivity *per se* is monitored are very expensive. Such trials are usually not necessary in forage-evaluation programmes since most of the material being tested is already recognised as beneficial to livestock. If a forage legume has a good crude-protein content, is readily digestible, has no toxins and is readily accepted then it should have a positive effect on animal productivity.

5. Experimental design and analysis

5.1 Selection of appropriate experimental design

Evaluation involves screening large numbers of accessions to select the more promising ones. Although a graphical screening method exists, a method based on the analysis of variance is recommended. For initial screening of large numbers of accessions a series of trials which include a common control treatment (standard or check) is used. Separated analysis of variance and Dunnett's test will identify superior accessions from each trial. Dunnett's one-tailed test will test if any accession is significantly better (or worse) than the control. Promising accessions of interest can then be selected and evaluated at a second stage, using a randomised complete block design because of its simplicity and precision. The numbers of accessions to be tested should not be more than 15 in one trial.

5.2 Analysis of data

Analysis of the data will depend on the experimental design selected and the sampling method. Two distinct cases are shown as examples:

Case 1: Subplots are randomly allocated to each harvest time with one subplot being harvested at each time.

A correct analysis for this type of sampling is to consider time of harvest as a subplot factor and analyse the data as a split-plot design with accessions as a main plot factor.

In general, a format for an analysis of variance is as follows:

Source	df
Main plot comparisons	
Accessions (T)	$t–1$
Blocks (B)	$b–1$
Main plot error (T x B)	$(t–1) (b–1)$
Subplot comparisons	
Time of harvest (D)	$d–1$
Time of harvest x Accessions (D x T)	$(d–1) (t–1)$
Time of harvest x Blocks (D x B)	$(d–1) (b–1)$
Time of harvest x Accessions x Blocks (D x T x B)	$(d–1) (t–1)$

Often the D x B and D x T x B sums of squares are pooled to give the subplot error. Before this pooling, the size of D x B mean square and D x T x B mean square are compared. If the D x B mean square is less than the D x T x B mean square, then pooling is necessary. Correct standard errors for different comparisons should be derived accordingly.

Case 2: A single plot is repeatedly harvested.

The methods described in this manual require accessions to be repeatedly measured for a series of preselected characters at regular intervals on the same plots. It is incorrect in this case to regard time as a split unit factor. The reason for such data recording is to examine how effects of treatment vary with time (accessions x time of harvest interaction).

Three approaches for analysing repeated measurement data are available:

1.   Straightforward analysis

2.   Split-plot analysis (Greenhouse and Geisser's approach)

3.   Multivariate analysis

The straightforward approach is recommended because of its simplicity and validity. The other approaches will therefore not be considered in this text.

With the straightforward approach, the scientist should specify relevant contrasts for predetermined tests. Time–trend analysis using linear, quadratic or higher order polynomial contrasts can be performed. A linear contrast tests whether there is a linear trend over time of harvest. T-test or simple analysis of variance are used for performing various tests.

5.3 Comparisons between sites

The main objective of comparison between sites is to define genotype–environment interaction. Accessions can only be compared between sites if the following requirements are met:

- use of same experimental design
- common treatments (accessions) through sites
- same plot size
- same number of replications if possible
- randomisation of treatments is not standardised.

Use a randomised complete block design with four replicates. Replicates are used within sites to give an adequate estimate of experimental error from each site. They may be reduced if the number of sites is increased. For multi-locational analysis, only common treatments should be considered. Selection of sites should consider environmental characteristics whose interaction with treatments are of interest.

Steps for combined analysis:

Step 1: Perform individual analyses of variance of trials.

Step 2: Select trials with same residual error (same precision) by applying a test for homogeneity of variances (Bartlett's test, Hartley's test etc).

Step 3: Perform combined analysis of trials with same variances. In the resulting analysis of variance table, first look into the interaction treatments x sites before drawing any conclusion.

The corresponding analysis of variance table is as follows:

Source of variation	df
Sites (S)	$s-1$
Blocks within sites	$s(b-1)$
Treatments (T)	$t-1$
Sites x treatments (S x T)	$(s-1)(t-1)$
Error	$s(b-1)(t-1)$
Total	$sbt-1$

In this table, sites and treatment effects are tested against mean square of the sites x treatments interaction term. The remaining terms are tested using the error mean square term. Sites are not considered as a random variable.

Further analysis (stability analysis) of this interaction can be performed.

5.4 Grouping of similar accessions

Cluster analysis is a technique for grouping objects into clusters, so that objects with similar characteristics are in the same cluster. Cluster analysis is more useful as a classification tool than a screening tool. Principal component analysis can also help examine relationships among several quantitative variables.

5.5 Managing data collected from trials

The most convenient way to handle the data for all the trial designs is to enter results on DBase files which may then be used for SAS analysis.

Suggested format for DBase file:

SPECIES - species

ACCN NO - accession number; use number only, no letters

REP - replication

GERM_4 - plant count (1 m^2), 4 weeks after sowing

GERM_8 - plant count, (1 m^2) 8 weeks after sowing

COVER_4 - soil cover (1 m^2) 4 weeks after sowing

COVER_8 - soil cover (1 m^2) 8 weeks after sowing

FLOW_50 - month (numeric value, e.g. July = 7) of 50% flowering

NO_PLTS - number of plants harvested

TOT_DW - total dry weight of harvested plants

DM_G_PLT - dry matter g/plant (use the REPLACE command and the previous two fields)

SDS_7 - total seed weight for July

SDS_8 - total seed weight for August

SDS_ etc......- total seed weights for each month

DGHT_12 - drought tolerance score December

DGHT_2 - drought tolerance score February

DGHT_4 - drought tolerance score April

DISMAXI - maximum disease incidence recorded (%)

DISMAXS - maximum disease severity recorded (%)

A DBase file in this form is convenient to use in a SAS analysis such as Ward's pattern analysis. If the number of accessions is small, they can also be divided up by simply sorting the DBase file on the characters of interest.

5.6 Analysis of trial designs in this manual

The experiments described in this manual are all simple, agronomic trials requiring randomised complete block or split-plot designs. Analysis is therefore straightforward and involves the use of means, analysis of variance and least significant difference (LSD) tests. Pattern or cluster analysis may be useful in the early evaluation stages if many accessions are used.

The following analyses are recommended:

RABAOC-style trial — Analysis of variance and LSD are sufficient. Harvest times can be considered as splits and the trial analysed as a split-plot design.

Initial evaluation—shrubs — Analysis of variance, LSD.

Small-plot management—shrubs — Analysis of variance, LSD.

Grazing trial	For each grazing/sampling time, analysis of variance for each of the components—sown accession, grass, forbs— and an LSD for each group.
Cut-and-carry trial	Analysis of variance, LSD.
Seed multiplication	Statistical analysis not always necessary.
Evaluation of mixtures	Analysis of variance, LSD.

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Acronyms and abbreviations

AEZ	Agro-ecological zone
AFRNET	Animal Feed Resources Network
CIAT	Centro Internacional de Agricultura Tropical, Cali, Colombia
CSIRO	Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia
IARC	International Agricultural Research Centre
ICRAF	International Centre for Research in Agroforestry, Nairobi, Kenya
IITA	International Institute of Tropical Agriculture, Ibadan, Nigeria
ILCA	International Livestock Centre for Africa, Addis Ababa, Ethiopia
ILRI	International Livestock Research Institute, Nairobi, Kenya
MPT	Multi-purpose tree
NARS	National Agricultural Research Systems
RABAOC	Recherche en alimentation du bétail en Afrique occidentale et centrale
RIEPT	Red Internacional de Evaluacion de Pastos Tropicales

Appendix I

Methods for scarification

Legumes (herbaceous and shrub)

Many forage legumes have seeds which are dormant because of their hard impermeable seed coats. Dormancy resulting from hardseededness is indicated by lack of imbibition. An imbibition of at least 60% (for irregular rainfall areas) and at most 90% (according to the seed trade) is expected. Hardseededness can be broken by thinning or breaking the seed coat. Three common scarification techniques are used to soften or break the seed coat. These are mechanical, chemical and heat scarification. Mechanical scarification is simple, cheap and safe and is recommended for the majority of legume seeds.

Hardseededness varies between seedlots as well as within and between species. It is affected considerably by the pre- and post-harvest conditions. No one treatment can therefore be considered as a precise prescription and it is best to test small quantities of seeds first, if possible.

Mechanical scarification

1. Scarify small quantities of seeds by hand using sandpaper.
2. Rub the seeds with sandpaper until one or two seeds break and damage to the seed coats is visible.
3. For larger quantities of seed, use a box lined with sandpaper. Ensure that only one layer of seed is present so that some seeds are not missed.
4. Rub the seeds in the box with a block of wood covered with sandpaper.

Manual cutting or "nipping" of large seeds is possible but time consuming. Care should be taken to damage the seed coat at the end furthest away from the embryo to avoid killing the seed.

Larger quantities of seeds are usually more conveniently scarified using scarifiers or other machines. A speed of 500–1000 rpm is sufficient for abrasion. This can be provided by driven cylinders lined with carborundum paper or consisting of woven wire with different apertures against which nylon or wire brushes and even metal beaters revolve. The clearance is adjusted according to the size of the seeds being scarified. Commercial rice polishers can be used to abrade the seed coat, but need some experimentation to determine optimum operating conditions. Hammer mills with speeds of 2450 rpm using a screen of 4.75 mm have been used for *Medicago* species. Very large amounts of seeds may be scarified by mixing with building stone and running in a cement mixer for 2–3 minutes.

Chemical treatment

The seed coat may also be damaged by immersing the seeds in concentrated sulphuric acid for 7–20 minutes (depending on the species and thickness of the seed coat) followed by washing and drying. However, this treatment can damage the seeds and different species have different degrees of resistance. Generally this is an expensive and dangerous method and therefore considered unsuitable for field use.

Heat treatment

Some seeds, especially those with waxy or oily seed coats, can be effectively scarified using hot, but not boiling, water. Extreme temperatures can have a rapid effect (seconds to minutes) but have been found to accelerate ageing in seeds, so should only be used just before sowing. Heated revolving drums have been used in Australia but are an expensive alternative to hot water. Half a 200-litre drum can accommodate one or two sacks of seed.

Seeds can be immersed in hot water of varying temperatures to ensure more uniform and precitable germination rates. A good practical way of doing this is to soak the seeds in very hot water for a few seconds, followed by overnight drying. It is important to ensure that the seeds are all fully immersed and in contact with the water.

Grasses

Grass treatments are usually carried out to facilitate seed to soil contact rather than to break specific dormancies. Deawning/debearding can be done using a cone thresher or hammer mill. A speed of 2450 rpm with a 4.75 mm mesh screen has given satisfactory results for *Cenchrus ciliaris*, *Panicum* species, *Urocloa* species and *Chloris gayana*. Some *Brachiaria* seeds may also require scarification which is usually done using concentrated sulphuric acid for 10–15 minutes. Scarified grass seeds are more prone to attack by harvester ants and therefore require treatment with an insecticide such as Lindane before sowing.

Appendix II

Methods for inoculation of legumes

Rhizobium are bacteria which are able to form a symbiotic relationship with legumes. The legume plant develops root nodules where the rhizobia fix nitrogen which then becomes available to the plant. There are different strains of *Rhizobium*. Some are very specific to species or even genotypes whilst others are non-selective. Most soils already contain native rhizobia but application of the appropriate specific *Rhizobium* can give substantial increases in plant growth and yield.

The appropriate *Rhizobium* culture is usually supplied mixed with an inert material, such as dry peat or charcoal. This is called the inoculant. It is important to keep the inoculant cool and away from direct sunlight. The inoculant needs to be applied to the legume seeds before sowing or to the soil after sowing as a suspension. Applying the inoculant to the soil is less effective but necessary if the seeds have been treated with chemicals (fungicides, pesticides etc) which would kill the bacteria. Recommended amounts are 6–8 g of inoculant per kg of seed.

Inoculation of seed

Although this method is not as effective as other seed inoculation methods, it does have the advantage of being very simple. Inoculant may be applied dry or as a slurry. Using a slurry is a more effective method.

A.   Dry application

Mix the dry inoculant with the seed immediately before planting.

B.   Slurry application

1.   Mix the inoculant with water to make just enough slurry to wet the seeds and coat them thoroughly without making them too wet.[15]

2.   Dry the seeds in a shaded area before planting; plant within 24 hours.

It is possible to inoculate seeds in the evening and dry them overnight before planting early the next morning.

If the soil is particularly acid, lime pellets may be used together with the inoculant. Inoculate the seeds using the slurry method as described above then roll them in finely ground limestone or powdered rock phosphate.

Soil inoculation

The inoculant can be added directly to the soil by placing alongside the seed. This may be difficult because the quantity of inoculant is usually small. The inoculum can also be mixed with water to form a suspension then sprayed beside or beneath the seed in the early stages of germination.

Further details and hints on inoculation can be found in FAO (1984).

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15   The water may contain an adhesive such as gum arabic or household sugar (10% solution) to form a suspension (approximately 2 ml water per gram of inoculant).

Appendix III

Formats for results

Format sheets are very useful for recording data in an organised way and ensuring that all data are collected. The following are sample format sheets that can be used to collect minimum data from initial screening trials. At some sites, additional data may be collected and formats for this can easily be prepared.

Format 1a General site and climatic data

Format 1b Climate

Format 2a Small-plot observation trial – Establishment period

Format 2b Small-plot observation trial – Biomass production

Format 2c Small-plot observation trial – Dry-season data

Format 2d Small-plot observation trial – Flowering and seed production

Format 3a RABAOC-style trial – Establishment period

Format 3b RABAOC-style trial – Biomass production

Format 3c RABAOC-style trial – Flowering and seed production

Format 4a Initial evaluation, shrubs – Height and survival

Format 4b Initial evaluation, shrubs – Biomass production

Format 5a Small-plot management trial, shrubs – Biomass production

Format 5b Small-plot management trial, shrubs – Quality assessment

General site and climatic data

General

Country:_____ Latitude:____° ____'N/S

Scientist(s):_____Longitude:__ °____'E/W

Institute:_____ Altitude: _____m asl

Site:_____

Original ecosystem:_____

Ecological zone:_____

Climate – Summary

Year:_____

Minimum temperature:_____°C

Maximum temperature:_____°C Month:_____

Total rainfall:_____mm   Month:_____

No. of rainy days:_____

Wet season:_____(month) to _____ (month)

Wettest month(s):_____

Soil

Classification (name,system):_____

Depth of sample:_____to___cm

Clay:____%   N:___.___%

(<0.002 mm)   C:___.___%

Silt:_____%   P:___.___ppm

(0.02-0.002 mm)   (method:____)

Sand:_____%   CEC:___meq/100g

(2.00-0.02 mm)   pH (H$_2$O):_____

Gravel:_____%

(>2.00 mm)

Format 1b

Climate

Year:_____

	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sep			Oct			Nov			Dec		
	Tmax	Tmin	mm	Tmax	Tmin	mm	Tmax	Tmin	mm	Tmax	Tmin	mm	Tmax	Tmin	mm	Tmax	Tmin	mm	Tmax	Tmin	mm	Tmax	Tmin	mm	Tmax	Tmin	mm	Tmax	Tmin	Mm	Tmax	Tmin	Mm	Tmax	Tmin	mm
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Mean																																				

Format 2a

Small-plot observation trial
Establishment period

Site:_____   **Trial:**_____   **Date planted:**_____

Species	Accession no.	Repli-cate No.	4 weeks				8 weeks			
			No. of plants	%cover	Disease/pest		No. of plants	% cover	Disease/pest	
					Incidenece/severity	Name			Incidence/severity	Name

Small-pot observation trial
Biomass production

Site:_____ **Trial:**_____ **Date planted:**_____ **Date harvested:**_____

Species	Accession No.	Repli-cate No.	No. of plants	Total fresh weight (g)	Subsample Fresh weight (g)	Subsample dry weight (g)	Dry-matter yield (kg/ha)	Disease/pest Incidence/severity	Name

Format 2c

Small-plot observation trial
Dry-season data

Site:_____   Trial:_____   Date planted:_____   Date harvested:_____

Species	Accession no.	Repli-cate no.	Date:_____ % green	Disease/pest Incidence/severity	Name	Date:_____ % green	Disease/pest Incidence/severity	Name	Date:____ % green	Disease/pest Incidence/severity	Name

Format 2d

Small-plot observation trial
Flowering and seed production

Site:_____   **Trial:**_____   **Date planted:**_____   **Date harvested:**_____

Species	Accession no.	Repli-cate no.	50% flowering month	Seed production					
				Month:____ (g)	Month:____ (g)	Month:_____ (g)	Month:_____ (g)	Month:____ (g)	Month:___ (g)

Format 3a

RABAOC-style trial
Establishment period

Site:_____   Trial:_____   Date planted:_____

Species	Accession no.	Repli-cate no.	Age (weeks)	No. of plants (per m^2)	Soil cover (%)	Disease/pest			
						Incidence/severity	Name	Incidence/severity	Name

Format 3b

RABAOC-style trial
Biomass production

Production period (Max/min):__

Site:_____   **Trial:_____**   **Date planted: _____**   **Date harvested:_____**

Species	Accession no.	Repli-cate no.	Age (weeks)	Cover (%)	(%) green	Total fresh weight (g)	Subsample fresh weight (g)	Subsample dry weight (g)	Dry-matter yield (kg/ha)	Disease/pest Incidence/severity	Name

Format 3c

RABAOC-style trial
Flowering and seed production

Site:_____   Trial:_____   Date planted:_____

Species	Accession no.	Repli-cate no.	50% flowering month	Maximum flowering Month	Seed production				
					Month:_____ (g)	Month:_____ (g)	Month:_____ (g)	Month:_____ (g)	Month:_____ (g)

Format 4a

Initial evaluation, shrubs
Height and survival

Site:_____   Trial:_____   Date planted:_____   Date measured:_____

Species	Accession no.	Repli-cate no.	Age (weeks)	Average height (cm)	No. of plants	% seedling survival	Disease/pest			
							Incidence/severity	Name	Incidence/severity	Name

Format 4b

Initial evaluation, shrubs
Biomass production

Site:_____   Trial:_____   Date planted: _____   Date harvested:_____

Species	Accession no.	Repli-cate no.	Age (weeks)	(%) green	No. of trees	Total fresh weight (g)		Subsample fresh weight (g)		Subsample dry weight (g)		Dry-matter yield (kg/ha)		Disease/pest	
						Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Incidence/severity	Name

Format 5a

Small-plot management trial, shrubs
Biomass production

Site:_____   Trial:_____   Date planted: _____   Date harvested:_____

Species	Accession no.	Repli-cate no.	Tree man-age-ment	Age (weeks)	No. of trees	Total fresh weight (g)		Subsample fresh weight (g)		Subsample dry weight (g)		Dry-matter yield (kg/ha)		Disease/pest	
						Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Incidence/severity	Name

Format 5b

Small-plot management trial, shrubs
Quality assessment

Site:_____   Trial:_____   Date planted:_____   Date harvested:_____

Species	Accession no.	Repli-cate No.	Tree man-age-ment	Age (weeks)	Nitrogen content (%)	Digestibility			
						(Hours:____)	(Hours:____)	(Hours:____)	(Hours:____)