# BREEDING OF *MACROPTILIUM ATROPURPUREUM*

E. M. HUTTON* AND L. B. BEALL*


## ABSTRACT

*Three series of* M. atropurpureum *crosses were made between* 1959 *and* 1974 *with the objective of improving dry matter yield, length of growing season and other characters in the cultivar Siratro. Series I gave no lines better than Siratro but Series II and III produced eight superior lines now being multiplied for wide scale pre-release testing. These lines have given yield increases in excess of* 25 *per cent compared to Siratro. The progress in Series II and III has resulted from incorporation within crosses of a wide range of introduced ecotypes, particularly two from dry areas unfavourable for plant growth.*

*The techniques developed have made it possible to select the most promising plants and lines with some confidence. Field evaluation for dry matter production was done in $F_2$ and $F_3$ progenies at Samford but in $F_4$'s and subsequent generations at Beerwah, Narayen and Lansdown, as well as Samford. Line-environment evaluation has been essential for selection of advanced lines with wide adaptability.*

*A number of characters with important effects on yield were given special attention in selection. These included stoloniferous development, nodulating ability, disease resistance ( virus, halo blight,* Rhizoctonia solani), *root knot nematode resistance and tolerance to low pH.*


## INTRODUCTION

Seed of Siratro (Hutton 1962) bred from two Mexican introductions of *Macroptilium atropurpureum* (syn. *Phaseolus atropurpureus*) was released in 1960 for general field evaluation and seed increase. When enough commercial seed became available it was increasingly used in eastern Australia in coastal and sub-coastal areas of summer rainfall. It also became important in the export seed trade and has been sown in a number of areas in various countries including those in South and Central America and Africa.

Before Siratro was released it was realized that it had some deficiencies and that more of these would become evident with wider usage. Therefore a new breeding program based on Siratro was commenced in 1959. The aim was to develop further populations with sufficient variability to enable the selection of improved types of this legume. At that time the gene pool of *M. atropurpureum* introductions was limited and it was not until 1964-65 that a wider range of introductions become available from Mexico and the rest of Central America. These allowed a series of wider crosses to be made with increased potential for selection of better adapted lines.

This paper describes three series of crosses made with the objective of selecting an improved type of Siratro. Also discussed are the specific aims which were involved in the program as well as the techniques used in handling and evaluating the progenies.

## BREEDING AND CHARACTERISTICS OF SIRATRO

In the breeding of Siratro (Hutton 1962) from a cross between two Mexican introductions of *M. atropurpureum* viz. CPIs 16877 and 16879, selections in the progenies were made especially for high dry matter (DM) yield, strong stoloniferous development, compatibility with an associate grass and persistence under grazing. Superior recombinants were selected in the $F_2$ generation, and the plots of $F_3$ and $F_4$

* Division of Tropical Crops and Pastures, C.S.I.R.O., Cunningham Laboratory, St. Lucia, Qld. 4067.

families were sown with Rhodes grass (*Chloris gayana*) and periodically given intensive grazing with cattle according to seasonal growth. At the $F_4$ stage the three best selections, viz. 10-12, 14-28 and 4-9, had significantly greater DM yields and stoloniferous development than either of the two parents. These selections were considered to be transgressive segregates with a marked intensification of the stoloniferous character. Further field testing of these selections proved them to be similar in most characters, so their seed was eventually combined in equal proportions as the basic Siratro seed. This was then multiplied for release and commercial use.

*M. atropurpureum* is a short-day species and raising the temperature to an optimum range of 30°/25°C is the most important factor in the induction of flowering and pod setting (Imrie 1973). The parental lines, 16877 and 16879, seed readily in southern Queensland when the photoperiod and temperatures are suitable in autumn or spring but their pods shatter easily and disperse the seed. In the progenies no progress was made towards the selection of slower shattering lines which would facilitate commercial seed harvesting. However Siratro inherited the higher seed yields of the better seeding parent, 16879, and produces high yields of hard seed which accumulate in the soil and can be mechanically recovered by a suction harvester (Hopkinson and Loch 1973; Hopkinson and Vicary 1974). The hard seed of Siratro and most *M. atropurpureum* lines favours regeneration and persistence, prevents fungi entering the seed and allows the seed surface to be cleared of fungal and other contaminants with appropriate compounds. Old crowns of Siratro may die within four years but if conditions are favourable the stand is maintained by crops of new seedlings and the development of young crowns from rooted stolons. When old areas of the legume are ploughed up even the roots will produce shoots and plants under favourable conditions.

The parental lines had root knot nematode resistance (Hutton and Beall 1957) and this character was transferred to Siratro. Its roots are not usually badly affected by *Amnemus* weevil (Braithwaite 1967) but in north Queensland they can at times be severely attacked by *Leptopius* weevils. It has proved to be relatively tolerant to a number of the legume viruses which occur in the field, but single plant populations in sparse establishments can be decimated by the disease legume 'little leaf' (Hutton and Grylls 1956), caused by a Mycoplasma (Bowyer *et al.* 1969). However 'little leaf' appears to have little effect on well established Siratro pastures (Hutton 1970a). *Rhizoctonia solani* can cause serious leaf and stem damage especially over long periods of high humidity. During the breeding of Siratro no selections were found with a useful degree of resistance to this fungus.

In southern Queensland the crowns of Siratro, *M. atropurpureum* introductions and selections from subsequent crosses all persisted under frosting. However their herbage was susceptible, so crossing did not lead to an increased supply of wintergreen leaf in the legume. A high degree of drought tolerance was maintained in the selections and has also been a feature of Siratro in most areas where it is grown. The agronomic characters of Siratro have been described by Hutton (1970b) and include quick field establishment and nodulation with the native soil rhizobia, and good growth and persistence in tropical areas with annual rainfalls of 700-1800 mm and with a well defined dry season of three to six months.

## AIMS IN IMPROVEMENT OF SIRATRO

Any improvement in Siratro should result in higher animal productivity from a pasture system based on this legume. However evaluation of new bred *M. atropurpureum* lines for their ability to increase animal production is dependent on the establishment of grazing trials when sufficient seed of the final selections is available. In the improvement program it has been necessary to assume that increased dry matter yield and other characters used as the basis for selection, will eventually result in increased animal production from this legume.

Important selection criteria among progenies from the three series of crosses since Siratro was produced were yield, retention of its superior nodulating ability, ease of establishment, stoloniferous habit, persistence, and root-knot nematode resistance (Hutton *et al.* 1972). Other characters considered desirable include the regular and substantial return of hard seed to the soil so important in its persistence, and its relative unpalatability early in the season and high acceptability late in the season (Stobbs 1977).

Siratro's early and late season production is often poor with most of the growth at the height of summer. An important aim has been to increase not only the dry matter yield and competitive ability with associate grasses, but also to increase the length of the active growing period. Ability to hold leaf after frost, cold and drought would increase its nutritive value during stress periods of the year. In areas with extended moist and humid periods better resistance to leaf and stem damage from pathogens, and particularly *Rhizoctonia solani*, would be important in maintaining dry matter yield and persistence. Although halo blight (*Pseudomonas phaseolicola*) rarely affects Siratro's growth, resistance to this pathogen is needed as infected areas produce diseased seed and can be a source of infection for any bean seed crops in the vicinity (Johnson 1970, Moffett 1973). Better tolerance to the factors involved in acid soil conditions, particularly excess Al and Mn (Andrew and Hegarty 1969, Andrew, *et al.* 1973), would make Siratro better adapted to a range of more acid soils in Australia and other tropical countries. In view of the increased costs of superphosphate and other fertilizers, new bred lines with increased efficiency in the uptake of essential soil nutrients, particularly P, would also increase the adaptability of *M. atropurpureum*.

New lines need to have the potential to produce high yields of easily harvested seed so types with slower shattering pods would be an advantage. A plentiful supply of commercial seed at reasonable prices will promote a strong demand for a new cultivar. When more than one cultivar of *M. atropurpureum* is available, seed certification will be required to maintain their identity. Lines with different coloured flowers e.g. white (Hutton and Beall 1971) could be valuable for their certification.

## TECHNIQUES USED IN DEVELOPING AN IMPROVED SIRATRO

As described later there have been three series of *M. atropurpureum* crosses aimed at the improvement of Siratro. Since the development of Siratro, techniques used in the selection of superior plants and lines in *M. atropurpureum* progenies have been improved. More emphasis has been placed on evaluation of the same selected lines in the range of environments covered by CSIRO Pasture Research Stations in eastern Australia viz. Samford (27°22'S 152°53'E), Beerwah (26°51'S 153°01'E), Narayen (25°41'S 150°52'E), Lansdown (19°39'S 146°50'E). Standard pasture fertilizer applications as used in the breeding plots at the various research stations have been reduced in the last four years to bias selection towards the lines most efficient in the use of nutrients.

Hand harvested seed was usually 90-95% hard. The small amounts of $F_1$ seed were hand scarified but larger quantities of seed from $F_2$ and subsequent generations were scarified by soaking in concentrated $H_2SO_4$ for 25 minutes. Scarified seeds, just sprouted after 24-48 hours at 27°C on germination pads (dishes or trays) and moistened with sterile water containing 0.2% "Captan", were planted singly in peat pellets. Broth or peat CB756 rhizobium culture was watered around emerged seedlings which were ready for field transplanting in four to six weeks.

*M. atropurpureum* is predominantly self-pollinating and this facilitated the development of suitable field evaluation procedures for progenies of relatively large numbers of crosses. Siratro was invariably used as a control although other *M. atropurpureum* lines were also included from time to time. $F_2$ and $F_3$ and often $F_4$ populations comprised only single plants spaced 2.5 m × 2.5 m with 5 replicates. The number of crosses and the resources available determined the size of single plant progenies which were 250-500 in $F_2$, 100 in $F_3$ and 20-30 in $F_4$. Beyond $F_4$, replicated single plant populations of lines were continued and in Series II crosses this was to $F_8$ (Table 1). Replicated swards 4 m × 4 m, simulated with planted seedlings or directly seeded, were included either in the $F_4$ or $F_5$ and became the main basis for selection in the succeeding generations. Simulated swards established with seedlings 40 cm apart saved seed, but swards seeded at a rate equivalent to 6 kg $ha^{-1}$ adjusted to give uniform numbers of seedlings per unit area were preferred. At $F_9$ in Series II crosses there was enough seed of the advanced lines from long $F_8$ rows (Beerwah) to have large swards and more replication (Table 1). As a precaution against the possibility of insect induced intercrossing, basic seed of the superior advanced lines from the $F_8$ generation onwards was obtained from glasshouse grown plants.

It was essential to establish single plants and swards early in the growing season to ensure adequate growth for good differentiation between lines. With single plants, hand watering at transplanting in dry seasons obviated establishment problems. Both single plants and swards were kept separate with a regularly mown strip. All single plants and swards were kept at least two years while swards of the advanced lines in $F_7$, $F_8$, etc. were retained for about four years. An associate grass suited to the location was usually sown later in the season when the single plants and swards were well established. This enabled the various lines to express their growth potential in the first season. In the second season ability of the lines to compete with associate grasses could then be assessed.

A rating scale of 1 to 10 for estimated DM yield of each of the large number of single plants and swards was developed. After they were rated 5-10 samples representative of each rated value were cut with hand shears using a 0.5 m × 0.5 m quadrat. With the large swards, four similar hand cuts were taken in all replicates after rating. These results were used to provide a calibration line from which estimated yields of all plots were calculated. For example the ratings at the height of the growing season for the $F_3$ plants in Series II crosses (Table 1) gave a cubic relation between ratings and yield as shown in Fig. 1. It was usual to do ratings at regular intervals during the growing season and this enabled definition of growth patterns among lines and selection of the most promising plants or lines relative to Siratro. Single plants and swards were usually grazed by cattle after a rating provided the growing season was a favourable one. Swards of the advanced lines at Samford in the Series II crosses were mainly harvested and cut with a small Howard flail harvester (Shaw *et al.* 1976). There was a positive correlation between first and second year results in single plants and swards if there was a good establishment in the first year. This enabled a high proportion of the selections in the generations to $F_6$ or $F_7$ to be made in the first year and then promoted to the next generation in the following year.

The selection technique which was used in progenies of the crosses for improving Siratro conformed to the pedigree method (Bray and Hutton 1976). The various programs involved substantial areas of land and intense field activity in the growing season. For example, field evaluation of the Series II crossbred progenies (Table 1) used about 30 ha which was spread over four CSIRO field stations. The land was occupied at least two years by generations to $F_7$ or $F_8$ and for about four years by advanced selections under pre-release testing. It is apparent that unless considerable resources, particularly manpower, are available the field evaluation of 20 crosses is all that can be handled by a small technical field team of 3-4 people. If level areas with uniform soil and favourable but typical climatic conditions were available it is possible that those $F_2$'s with the greatest potential for agronomic improvement could be more readily selected. These $F_2$'s could then be inbred by the single seed descent method in the glasshouse or environment chambers (Imrie and Hutton 1977). This would rapidly advance the generations to $F_7$ for final field evaluation and eliminate the field work associated with the $F_3$-$F_6$ generations. Although single seed descent

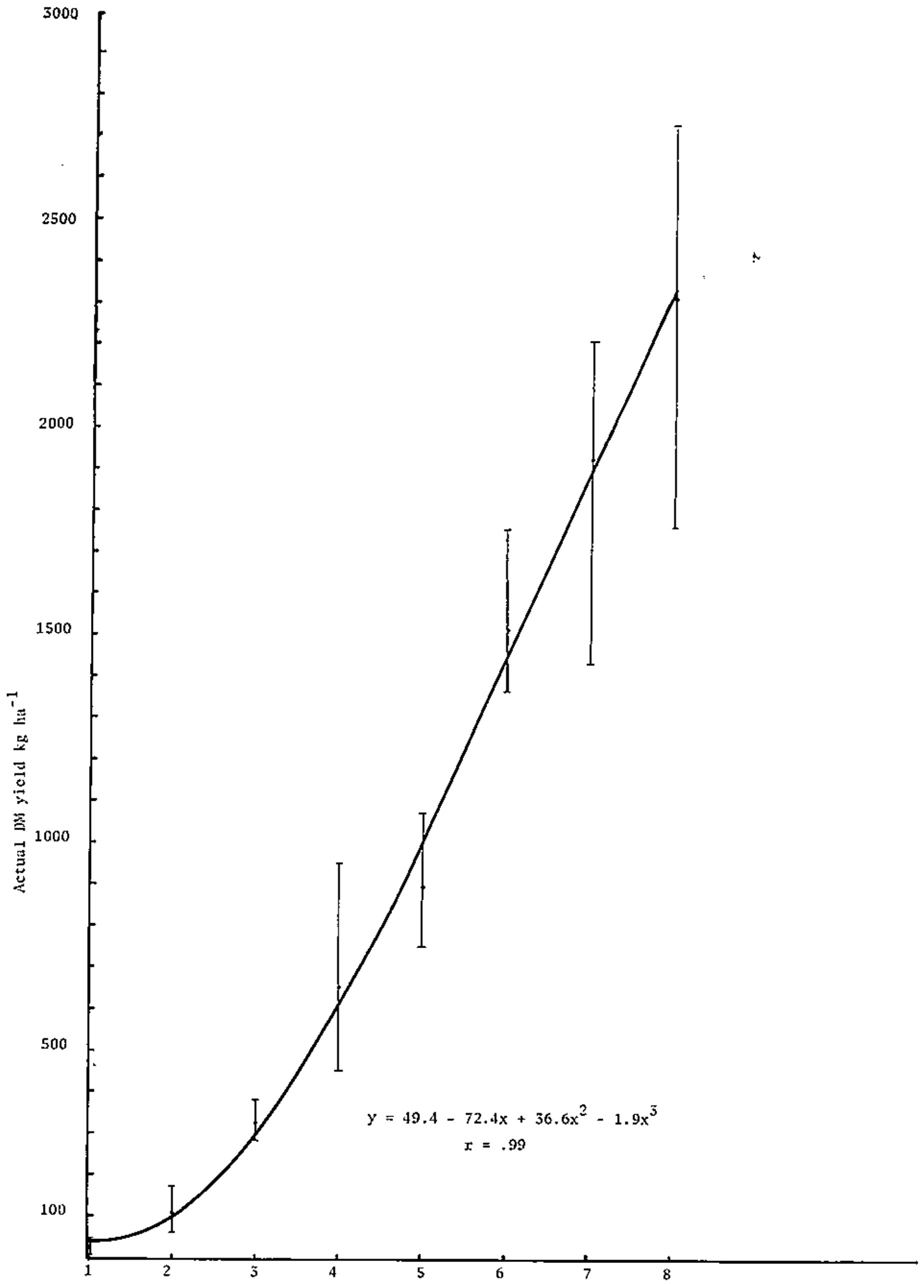


FIGURE 1
Relationship between rated and actual DM yields of all Series II $F_3$ plants. Vertical lines indicate the range of yields obtained from cuts within rated values.

saves resources the method could result in the loss of valuable recombinants which would have been selected in the field from $F_3$-$F_6$ for characters like compatibility with grasses and tolerance to grazing.

## SERIES OF CROSSES MADE SINCE SIRATRO

### *Series I*

A program to improve Siratro was developed during 1959-1963 at Samford and involved a series of 21 *M. atropurpureum* crosses. During this period only a limited number of introductions was available including several from Mexico viz. CPI's 16876 (near Durango), 16877 and 16878 (Vera Cruz), 16879 (Matlapa) and one from N.W. Argentina viz. 18556 (Guemes). These were used in crosses together with a vigorous Siratro selection (25-11) and a relatively weak pink flowered mutant derived by gamma irradiation of $F_2$ 16879 × 16877 (Hutton and Beall 1971). Among the resulting selections a high proportion was taken to the $F_4$ and $F_7$ generations and a number used in further intercrosses to increase DM production and stoloniferous development. In a range of the progenies there was a high positive correlation between DM yield and stolon root development. The progenies of crosses involving the pink flowered mutant as a parent lacked vigour and several back crosses with high yielding parents did not correct the situation.

Only two crosses in Series I gave some advanced vigorous selections with distinct promise. Among these selections 70-4, 72-43 and 72-46 were from 16879 × 16876 and 13-11 from 16877 × 16876; 13-11 was an unusual semi-erect type with small ovate leaves, dense growth and extended growing season. The parents 16879 and 16877 came from moist sub-tropical areas of southern Mexico while 16876 was a distinct ecotype from a dry stony area in the west of central Mexico. 16876 was weakly perennial, erect, with small narrow leaves, a high degree of pollen sterility and outcrossing, and substantial pod abscission. There was a high proportion of inferior plants in the hybrid progenies of these two crosses but it was apparent from the selections retained that the parent 16876 had resulted in some new and favourable genetic recombinations. However none of the selections was able to give more than a 10% DM increase over Siratro in field trials. As a result they were not released, but 70-4 and 13-11 were used as parents in the Series II crosses because of their valuable characters.

### *Series II*

The limited results from the Series I crosses based on a restricted number of introductions stimulated active field collections of a much wider range of *M. atropurpureum* ecotypes in the countries of Central America including Mexico, El Salvador, Honduras and Costa Rica. The most important collections were made in 1963-64 by Dr. H. S. McKee (Hutton 1970c) of the CSIRO Division of Plant Industry, Canberra. By 1964 some 50 new *M. atropurpureum* introductions were available.

The Series II crosses were made from 1964-66. These incorporated vigorous and diverse ecotypes from the new Central American introductions of *M. atropurpureum*, as well as Siratro selection 25-11, 13-11 and 70-4 from the Series I crosses, and a pink flowered mutant (Hutton and Beall 1971). Ten initial crosses were first made and the $F_1$'s then either intercrossed or crossed to one of the following:— another introduction, the Siratro selection, and 13-11 or 70-4. The amount of pedigree selection and numbers of single plants and swards involved in the different generations of the 20 Series II multiple crosses from 1965/66 onwards are given in Table 1.

It was apparent, even in the $F_2$, that crosses 15 and 16 had the greatest number of segregates potentially superior to Siratro. By the $F_5$ generation the selected lines from all crosses except 15 and 16 had been discarded. From $F_6$-$F_8$ pedigree selection and the associated evaluation of swards were concerned only with sorting out those

TABLE 1

*Series II* M. atropurpureum *crosses for improvement of Siratro.*

| Generation | Season | Location | No. lines | No. single plants/line (5 reps) | Swards (seeded 6 kg $ha^{-1}$) | No. crosses with selections | No. selections |
|---|---|---|---|---|---|---|---|
| $F_2$ | 1965/66 | Samford | 20 (1/cross) | 250-300 | — | 16 (15, 16, 7, 4*) | 50 |
| $F_3$ | 1966/67 | Samford | 50 | 100 | — | 14 (15, 16*) | 145 |
| $F_4$ | 1967/68 | Samford | 145 | 20-30 | — | 5 (15, 16*) | 75 |
| | | At each of Lansdown, Narayen speargrass, Narayen brigalow | 43 | 30 | — | | |
| $F_5$ | 1968/69 | Samford | 75 | 20 | Simulated[1] | 2 | 90 (Cross 16) |
| | | Lansdown | 32 | 20 | Simulated[1] | | 6 (Cross 15) |
| $F_6$ | 1969/70 | Samford | 96 | 20 | Seeded[2] | 2 | 47 (Cross 16) |
| | | Lansdown | 55 | 25 | Seeded[2] | | 8 (Cross 15) |
| $F_7$ | 1970/71 | Samford | 55 | 20 | Simulated[1] + seeded[2] | 2 | 25 (Cross 16) |
| | | Lansdown | 50 | 20 | Seeded[2] | | 1 (Cross 15) |
| $F_8$ | 1971/72 | Samford | 26 | 30 | Seeded[2] | 2 | 5 (Cross 16) |
| | | Beerwah | 20 | — | (50m rows) | | 1 (Cross 15) |
| | | Lansdown | 20 | 30 | Seeded[2] | | |
| $F_9$ | 1972/73 | Samford | 6 | — | Seeded[3] | 2 | 5 (Cross 16) |
| | | Beerwah | 6 | — | V. long seeded[4] | | 1 (Cross 15) |
| | | Lansdown | 6 | — | Long seeded[5] | | |
| | | Narayen speargrass | 5 | — | Long seeded[5] | | |
| | | Narayen brigalow | 5 | — | Long seeded[5] | | |

[1] 5 reps 4m × 4m with seedlings 40 cm apart; [2] 5 reps 4m × 4m; [3] 16 reps 5m × 5m; [4] 8 reps 125m × 1·5m; [5] 10 reps 50m × 1·5m.

*Outstanding crosses.

lines in crosses 15 and 16 markedly better than Siratro in a number of important characters. Thus efficient field evaluation of $F_2$ progenies could allow concentration on only the most promising crosses and the elimination of the rest.

Long replicated rows of closely spaced single plants from 19 $F_7$ selections and Siratro were established at Beerwah to produce enough seed for their larger scale testing in the $F_9$ generation. It was of interest that the average yield of mechanically harvested seed from six of these advanced lines was 3.7 times greater than that of Siratro; this appeared to be due to a more concentrated flowering and podding period and slower shattering pods in the new bred lines. The $F_9$ sward evaluation (Table 1) of the six lines viz. 2, 7, 13, 15 and 18 from cross 16, and 19 from cross 15, has been continued for four years over the range of environments shown. This line-environment evaluation project will be the subject of another paper. It resulted in the selection of three purple flowered lines 13, 18 and 19 which, compared with Siratro, have a longer growing season and 25% to 50% greater dry matter yield depending on the environment.

Cross 15 was $(H5 \times 25\text{-}11)F_1 \times (H8 \times H5)F_1$ and cross 16 was $(H5 \times 25\text{-}11)F_1 \times 13\text{-}11$. They both involved Siratro selection 25-11 and the selection H5 from a dry rocky slope on the outskirts of San Salvador. In cross 15 the H8 parent was from Costa Rica and in cross 16 it appeared that 13-11 was an important factor in its success. The results from these two crosses indicate that agronomic advances in pasture legume breeding could follow from the use of parents with markedly different ecological adaptations.

*Series III*

In the extensive 1972-73 field trials with the best advanced lines from the Series II crosses (Table 1) lines 13, 15, 18 and 19 were the most promising and had significantly greater DM production than Siratro. Each line had valuable characters not present in the others; for example 13 and 15 were the best seeders, and 19 had the longest growing season. As will be described later, it was found possible to substantially upgrade the acid tolerance of 13 and the other advanced lines. In addition the halo blight resistance of all the advanced lines has been increased by single plant selection under rigorous glasshouse testing.

In 1973-74 a set of crosses was made in all possible combinations, first between several halo blight resistant (HBR) 13, 15 and 18 selections, and then between their $F_1$'s; line 19 was not included because of its low seed yields. Twenty-one $F_2$'s resulted, and an average of 400 plants of each was screened for halo blight resistance and the 326 resistant selections grown on to produce $F_3$ seed. An average of 340 seeds of each of these selections, presprouted by overnight soaking in dilute *Rhizobium* CB756 broth, was planted (20/pot) in nutrient sand culture (Andrew 1974) maintained at pH 4.2 with excess Al (10 ppm). The most vigorous acid tolerant $F_3$ seedlings were transplanted into pots to give $F_4$ seed.

A second set of six crosses was also made in 1973-74 between the HBR 13, 15 and 18 selections and two white flowered lines from several backcrosses between a white flowered Mareeba Siratro selection and HBR 2 and 13. Approximately 450 $F_2$ seedlings of each cross were first screened for a combination of vigour, acid and Al tolerance, and white flower (white flowered plants lacked stem pigmentation, which allowed early selection for this character). There were 57 $F_2$ selections and 120 plants of each were then tested for halo blight resistance and the resistant selections were then grown on to produce $F_4$ seed.

Screening the Series III $F_2$ and $F_3$ progenies eliminated 4 purple flowered crosses and resulted in $F_4$ seed of 285 $F_3$ selections with a background of acid tolerance and halo blight resistance. They also had potential for high DM production and good seed yields derived from the Series II parental lines 13, 15 and 18. Early in February 1975, $F_4$ plants of all 285 $F_3$ selections together with 22 line 13 reselections for both acid tolerance and halo blight resistance and four controls, Siratro, lines 13, 15 and 18,

TABLE 2

*Yield rating of* 285 *Series III $F_4$ lines,* 22 *Series II $F_{12}$ Line* 13 *reselections and controls—Siratro, Lines* 13, 15, 18. *Seedlings planted Beerwah February* 1975.

| | Selections | | | Lines better than Siratro 1975 ($P < 0{\cdot}01$) | | | | Lines better than Siratro 1976 ($P < 0{\cdot}01$) | | | | Final selections | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1st rating (June) | | 3rd rating (November) | | 4th rating (January) | | 6th rating (November) | | | |
| No. crosses | No. | No./ cross | Generation Planted | % | Mean Rating | % | Mean Rating | % | Mean Rating | % | Mean Rating | Cross-line | Rating |
| 6 Series III | 99 $F_3$ White | 6–30 | $F_4$ | 55 | 3·7 | 62 | 3·9 | 41 | 5·8 | 28 | 3·8 | 10–105<br>10–124 | 5·1<br>4·0 |
| 17 Series III | 186 $F_3$ Purple | 1–37 | $F_4$ | 33 | 3·5 | 63 | 4·0 | 59 | 5·8 | 28 | 3·9 | 4–49<br>24–254<br>24–255 | 4·9<br>5·4<br>5·2 |
| Line 13 Series II | 22 $F_{11}$ Reselected | | $F_{12}$ | 18 | 3·9 | 50 | 4·1 | 45 | 5·9 | 14 | 4·1 | 16–288 | 5·0 |
| *Controls* | | | | | | | | | | | | | |
| Line 13 (Series II) | | | $F_9$ | | 3·0 | | 3·6 | | 5·6 | | 3·6 | | |
| Line 15 (Series II) | | | $F_9$ | | 3·4 | | 3·8 | | 5·6 | | 3·2 | | |
| Line 18 (Series II) | | | $F_9$ | | 2·6 | | 3·2 | | 5·2 | | 2·9 | | |
| Siratro | | | | | 2·5 | | 2·5 | | 4·6 | | 2·7 | | |
| L.S.D. | $P < 0{\cdot}01$ | | | | 0·966 | | 0·951 | | 0·971 | | 0·923 | | |

were established at Beerwah in five plant rows replicated five times; the plants were one m apart in rows which were 2.5 m apart. The area planted was given the superphosphate, calcium carbonate, potash and elements needed for the Beerwah sandy podzolics (Bryan 1973) in October 1974; a year later it received 125 kg $ha^{-1}$ of superphosphate, half the usual maintenance application, with the aim of favouring those lines most efficient in nutrient uptake. The lines were rated six times for DM production at intervals from June 1975 to November 1976.

Table 2 summarises the rating results in June and November 1975 and in January and November 1976 when climatic conditions favoured most growth differentiation between lines. By the end of the first year's growth 41% white flowered and 59% purple flowered lines (Table 2) gave significantly greater DM yields than Siratro. A further season's growth reduced the proportion of both white and purple flowered lines with significantly greater yield than Siratro to 28%. From this Beerwah evaluation it was apparent that 10-105 from HBR 15 × (BC5-1 × 13H × HBR 13), 4-49 from (HBR 15 × HBR 13), 24-254 and 24-255 from (HBR 13 × HBR 15)$F_1$ × (HBR 15 × HBR 18)$F_1$ and line 13 selection 288 had the highest yield potential compared with Siratro. Another white flowered line from cross 10, viz. 10-124, was also included in this elite group because of its overall performance. It was of interest that two of the lines with the greatest promise came from cross 24 which incorporated the best lines from Series II. The selected elite lines could be considered potentially valuable elsewhere, as in the line-environment project mentioned previously, the highest yielding lines at Beerwah were also the best in other areas.

## DEVELOPMENT AND MULTIPLICATION OF ELITE LINES FROM *M. ATROPURPUREUM* CROSSES

Progenies of the best 45 Series III $F_4$ selections (Table 2), derivatives of Series II viz. 4 reselected line 13's and the ordinary lines 13, 15, 18 and 19, together with Siratro, were screened for HBR plants; these selections were then grown on to produce $F_6$, $F_{14}$ and $F_{10}$ seed respectively. Progenies from these HBR selections were grown in nutrient sand culture maintained at pH 4.2 and 10 ppm Al; the most vigorous plants after 6½ weeks growth were transplanted to produce $F_7$ seed of the Series III lines and seed of reselected 13(288), 18(18-3) and 19(19-3). This seed was used in a large final screening of the progenies for acid and Al tolerant plants; the vigorous tolerant seedlings were transplanted to produce $F_8$ seed of the five best Series III lines (Table 2) and $F_{16}$ seed of 13(288) and $F_{12}$ seed of 18-3 and 19-3.

The selection procedures outlined with Series II and III progenies gave eight promising advanced lines more vigorous than Siratro. Compared with Siratro, they also have halo blight resistance and acid tolerance, as well as higher yielding ability at a range of pHs up to 8.2. These elite advanced lines, viz. 13(288), 18-3 and 19-3 from Series II (Table 1) and 4-49, 10-105, 10-124, 24-254 and 24-255 from Series III (Table 2), are to be multiplied during the 1977 dry season at the Kimberley Research Station near Kununurra, N.W. Western Australia. Towards the end of 1977 there should be sufficient seed of the new lines available for wide scale prerelease testing in the 1977-78 summer season.

## SELECTION FOR CHARACTERS OTHER THAN YIELD IN *M. ATROPURPUREUM* CROSSBRED PROGENIES

### *Stoloniferous development*

Stoloniferous development in the Series II $F_9$ lines and Siratro established at four contrasting sites (Table 1) was compared after three seasons growth. At the end of the 1975 dry season random samples measuring 40 cm × 40 cm of surface soil to a depth of 10-15 cm were examined at Samford, Beerwah, Narayen and Lansdown. In each sample trailing stems with roots (stolons) and without roots, and also the stolon roots, were measured and counted. For ease of comparison the observed results were calculated on the basis of stem length in metres per square metre (m $m^{-2}$, Table 3).

TABLE 3

*Trailing stems with roots (stolons) and without roots after three seasons in advanced lines derived from Series II* M. atropurpureum *crosses.*

| Line | SAMFORD[1] | | | BEERWAH[2] | | | NARAYEN[3] | | | LANSDOWN[4] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Length (m m$^{-2}$) | | | Length (m m$^{-2}$) | | | Length (m m$^{-2}$) | | | Length (m m$^{-2}$) | | |
| | Stems without roots | Stolons | No. stolon roots m$^{-2}$ | Stems without roots | Stolons | No. stolon roots m$^{-2}$ | Stems without roots | Stolons | No. stolon roots m$^{-2}$ | Stems without roots | Stolons | No. stolon roots m$^{-2}$ |
| 2 | 2·92 | 3·92 | 52·3 | 11·32 | 4·88 | 58·1 | 2·16 | 1·16 | 7·5 | 2·00 | 4·6 | 65·8 |
| 13 | 3·92 | 6·44 | 101·7 | 17·40 | 6·00 | 77·9 | 2·16 | 1·00 | 6·9 | 4·32 | 10·52 | 120·8 |
| 15 | 3·56 | 1·84 | 25·9 | 13·16 | 2·96 | 29·8 | 0·52 | 0·32 | 3·5 | 3·00 | 2·68 | 40·4 |
| 18 | 3·08 | 3·32 | 54·2 | 15·16 | 6·04 | 69·4 | 1·16 | 0·16 | 1·7 | 4·84 | 5·32 | 55·8 |
| 19 | 5·04 | 5·12 | 88·0 | 17·04 | 6·68 | 84·4 | 1·60 | 1·76 | 17·5 | 3·32 | 7·52 | 100·4 |
| Siratro | 2·92 | 4·60 | 63·2 | 7·36 | 6·04 | 59·6 | 2·60 | 2·60 | 20·6 | 2·16 | 8·68 | 103·3 |
| L.S.D. $P < 0{\cdot}05$ | 1·21 | 1·18 | 22·2 | 3·05 | 1·05 | 18·1 | 1·42 | 1·01 | 10·3 | 2·07 | 3·54 | 43·3 |

[1] 4 samples in each of 12 reps; [2] 8 samples in each of 6 reps; [3] 5 samples in each of 6 reps; [4] 5 samples in each of 3 reps.

There were marked differences in stoloniferous development between lines as well as sites (Table 3). The lines had rooted stolons in varying degrees but Siratro was the most consistent in root production and maintained high stolon root numbers at all sites. Of the bred lines, 13 and 19 compared favourably with Siratro and were superior to Siratro at two sites. It was of interest that for all lines and Siratro at all sites, except Narayen, a mean of 88% of stolon roots were 1 mm diam., 10% 2 mm diam., and 2% 3 mm diam. At Narayen only a few stolon roots developed, most being 1 mm diam. It is apparent from Table 3 that it is possible to select lines with stoloniferous development comparable to Siratro at most sites from crosses involving this cultivar.

*Nodulating ability*

The best selections from both Series II and Series III have been grown in several nutrient sand culture experiments under a range of pH and nutrient conditions; inoculation of seed at planting with CB 756 was a standard procedure. Examination of root material from these has shown no significant differences in degree of nodulation between the advanced lines and Siratro. The results of one of the experiments grown for 18 weeks in 1972 is given in Table 4.

TABLE 4

*Yield, nodulation, nitrogen and phosphorus content of leaves and nodules of nine Series II $F_7$ lines grown in sand culture*, 1972.

| Line No. | Mean Total DM g/pot* | Mean % nodule DM in roots | Mean % N | | Mean % P | |
|---|---|---|---|---|---|---|
| | | | Leaf | Nodule | Leaf | Nodule |
| 1 | 27·3 | 11·0 | 3·30 | 6·05 | 0·21 | 0·40 |
| 2 | 33·2 | 9·1 | 3·18 | 4·54 | 0·20 | 0·35 |
| 3 | 28·0 | 8·3 | 3·22 | 5·39 | 0·18 | 0·36 |
| 4 | 29·6 | 7·2 | 2·87 | 5·48 | 0·20 | 0·36 |
| 5 | 29·5 | 10·0 | 2·75 | 5·69 | 0·20 | 0·31 |
| 6 | 32·9 | 9·9 | 2·54 | 5·18 | 0·20 | 0·38 |
| 7 | 26·7 | 9·9 | 3·65 | 5·90 | 0·19 | 0·37 |
| 8 | 29·3 | 8·5 | 3·30 | 5·89 | 0·22 | 0·41 |
| 9 | 28·7 | 9·5 | 3·13 | 5·90 | 0·23 | 0·41 |
| Siratro | 25·1 | 8·1 | 3·22 | 4·98 | 0·20 | 0·38 |
| L.S.D. $P < 0{\cdot}05$ | 5·4 | NS | NS | NS | NS | NS |

*Four replicates, each replicate one pot of four plants.

Some of the lines gave significantly higher DM yields than Siratro (Table 4). There were no significant differences between the lines and Siratro in the percentage of nodular tissue in the root system. In all lines and Siratro the N and P levels were normal in the leaves (Andrew and Robins 1969a, b) and as expected these were higher in the nodules.

*Disease resistance*

*Legume 'little leaf'*

The disease legume 'little leaf' (Hutton and Grylls 1956) mentioned earlier has similar effects on Siratro and the bred *M. atropurpureum* lines. As it is usually a problem only in spaced single plants, and not in swards, breeding for resistance to this disease has not been attempted.

*Passionfruit woodiness virus*

Early in the season, a number of plants of the *M. atropurpureum* lines in the field at Samford often had a transient mottling in the young leaves. This was found by Queensland Department of Primary Industries plant pathologists to be due to passionfruit woodiness virus (Teakle and Wildermuth 1967). Infection became more evident when plants were transferred from the field to pots in the glasshouse. In a nutrient sand culture experiment the virus was transferred to seedlings by rubbing their leaves with inoculum made by grinding up mottled leaves of infected plants in a suitable buffer. Infection with passionfruit woodiness virus had no significant effect on growth and N and P concentrations of the bred lines and Siratro. Up to the present, the virus problems which have occurred in Siratro in northern Australia have not warranted any resistance breeding programs.

*Halo blight*

In 1973 450 plants of each Series II $F_9$ line and Siratro were inoculated with halo blight in a controlled replicated experiment in the glasshouse. The halo blight culture which included a range of biotypes was supplied by Miss Melda Moffett of the Plant Pathology Laboratory, Queensland Department of Primary Industries. Every seedling was initially inoculated at the first trifoliate stage and then again 10 days later, the inoculum being sprayed on both sides of the leaf. The plants were grown in a chamber maintained at approximately 80% relative humidity and a temperature of 17° to 27°C and symptoms were observed 14 and 25 days after the second inoculation. All plants showed symptoms which were rated according to their severity; spotting/mottling restricted to inoculated leaves was rated 1 but when systemic it was rated 2-5 as associated stunting and leaf drop increased. Only plants rated 1 were regarded as halo blight resistant, and the numbers of these in the different lines for the two ratings are given in Table 5.

TABLE 5

*Levels of halo blight resistance and* Rhizoctonia solani *tolerance in advanced lines from Series II* M. atropurpureum *crosses.*

| | Lines | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 7 | 13 | 15 | 18 | 19 | Siratro |
| HBR Selections/450 plants† | (3)1 | (4)1 | (30)10 | (40)7 | (80)21 | (26)10 | (1)0 |
| Estimated Mean % plant defoliated *R. solani*‡ | 43 | 39 | 38 | 44 | 43 | 40 | 59 |

†Numbers of selections—in bracket after 1st rating, outside bracket after 2nd rating.
‡LSD between any two means *$P < 0{\cdot}05$ 5·5, **$P < 0{\cdot}01$ 7·4.

Siratro had a dearth of HBR plants (Table 5) whereas there were promising numbers in the advanced lines, particularly 13, 15, 18 and 19. With halo blight, Johnson (1970) found a low level of resistance in the parents of Siratro but a high level in CPI 16876, an important basic parent in the cross 16 lines, viz. 2, 7, 13, 15 and 18. Further halo blight resistance screening of the progenies from these selections (Table 5) showed a marked progressive increase in the proportion of resistant plants, e.g. in progenies of the HBR 13 selections there was up to a 12-fold increase. It is apparent that even as late as the $F_9$ stage it is possible to select plants for characters like halo blight resistance.

*Rhizoctonia solani*

Controlled glasshouse screening of introductions and bred lines of *M. atropurpureum* infected with *R. solani* has not resulted in selection of resistant types. However degrees of tolerance among lines to natural infection by the fungus have been observed in the field, particularly at Beerwah and Lansdown. Long periods of warm humid weather with frequent rain are necessary to define tolerance differences between lines and these conditions usually occur at Beerwah in summer. The advanced lines in the replicated yield trial at Beerwah were rated in March 1976 for the proportion of dead tissue present in the swards (Table 5).

All the advanced lines and particularly 7, 13 and 19 were apparently more tolerant to *R. solani* than Siratro (Table 5). Although the better tolerance of the bred lines represents an advance, *M. atropurpureum* cultivars will still need to be grown for best results in areas with definite dry periods from time to time throughout the year.

### *Root knot nematode resistance*

Retention of high root knot nematode resistance in crossbred progenies involving Siratro and other *M. atropurpureum* lines was an important objective in the breeding program. An investigation of the reactions of a wide range of *M. atropurpureum* introductions and some bred lines to four root knot nematode species (Hutton *et al.* 1972) clearly indicated a high level of resistance in most *M. atropurpureum* material. Thus it has been possible in the breeding work outlined with the Series II and III crosses to concentrate selection on characters other than root knot nematode resistance in the knowledge that this will be at a high level in any new line selected.

### *Tolerance to low pH*

Studies of the reactions in the advanced Series II lines to low pH and manganese excess in nutrient sand culture have shown tolerance differences between them and the results obtained will be presented in another paper. The emphasis in this paper has been on selection for tolerance to low pH and excess Al, especially in the Series III crossbred progenies. This has been successful in the selection of Series III lines with a high degree of tolerance to these stresses under controlled conditions; the selected lines also grow well at pHs up to 8.2.

Concurrent research has been in progress to upgrade the acid tolerance of the $F_9$ Series II lines (Table 1), and particular attention has been given to the promising line 13 in which lack of acid tolerance was a serious deficiency. In a population of line 13 plants grown in nutrient sand culture at pH 4.2 and Al excess, most plants become yellow and stunted; a few were green and relatively vigorous and were transplanted to produce seed. The seed of the most vigorous acid tolerant 13, viz. line 288, was used in a comparative experiment which also included ordinary line 13 and lines 15, 18, 19 and Siratro. One hundred plants of each line were established in November 1975 in nutrient sand culture maintained at pH 4.2 and 10 ppm Al. After 6½ weeks growth all plants were assessed for DM production; those designated *a* were yellow and averaged 3-5 cm high, *b* were light green and 5-9 cm high, and *c* were deep green and 9-13 cm high.

TABLE 6

*Differences between Series II $F_9$ lines in tolerance to pH* 4·2 *and* 10 *ppm Al*.

| Lines | % plants in growth rating classes *a* | *b* | *c* | % Plants Nodulated | Mean no. of nods/ nodulated plant |
|---|---|---|---|---|---|
| 288(13) | 22 | 53 | 25 | 72 | 4·1 |
| 13 | 66 | 33 | 1 | 32 | 2·9 |
| 15 | 56 | 43 | 1 | 51 | 2·4 |
| 18 | 48 | 40 | 12 | 64 | 2·8 |
| 19 | 55 | 42 | 3 | 49 | 2·6 |
| Siratro | 81 | 19 | 0 | 63 | 2·1 |
| L.S.D. $P < 0·05$ | 32 | 31 | 19 | 31 | 1·3 |

*a* yellow mean height 3–5 cm.
*b* light green mean height 5–9 cm
*c* deep green mean height 9–13 cm.

The new line 288 was significantly more acid tolerant than Siratro and the other lines, including 13 from which it was selected (Table 6). It had not only the highest proportion of tolerant plants, but also the most nodulated plants and nodules per nodulated plant. Line 18 was the second most tolerant line while Siratro was the least tolerant, a majority of its plants being yellowed and stunted by the treatment. This was in spite of Siratro having a relatively high proportion of nodulated plants. It was evident that selection for acid tolerance, like halo blight resistance, could be achieved in an $F_9$ generation. Thus with regard to unselected characters like these, advanced populations retain a considerable level of heterogeneity.

### *Efficiency in P uptake*

Andrew *et al.* (1973) and Andrew and Vanden Berg (1973) have shown in the more sensitive legumes that soluble Al in the nutrient substrate restricts uptake of P and Ca and reduces efficiency of translocation of P from roots to the tops. Thus it is possible that selection of *M. atropurpureum* lines for tolerance to low pH and Al excess, may increase their efficiency of P and Ca uptake and P translocation. In a preliminary nutrient sand culture experiment using a very low concentration of P, some Series II lines showed a better ability to take up P than Siratro and a number of other lines. In another glasshouse experiment using the infertile Beerwah soil with rock phosphate and superphosphate treatments, two of the four Series II lines grown were more efficient in P uptake from rock phosphate (Andrew, personal communication). There is a distinct need to understand the factors involved in P uptake and translocation in *M. atropurpureum* lines. This would increase the possibility of breeding cultivars of this legume able to maintain good growth in pastures on poorer soils or fertilized with relatively low quantities of superphosphate.

## CONCLUSIONS

Our breeding investigations with *M. atropurpureum* have defined some important factors requiring consideration in any future improvement work with this legume. These have relevance to breeding of other herbaceous trailing legumes. They include the following:

(1) It appeared that the crosses between the common *M. atropurpureum* ecotypes from moister Central American areas gave little improvement over Siratro. Most improvement came from the parents CPI 16876 and San Salvador selection H5 from dry areas unfavourable for plant growth. In pasture legume breeding, crosses involving diverse ecotypes from radically different environments could be a valuable source of agronomic advances.

(2) If $F_2$ progenies were grown under uniform conditions it should be possible to select with some degree of accuracy only those crosses with most potential on the basis of overall performance. This would eliminate the relatively large number of less promising crosses, save resources and allow concentration on those most likely to give significant improvement.

(3) Screening of progenies for important physiological characters like acid tolerance and halo blight resistance can be done under controlled glasshouse conditions and the selections rapidly advanced through several subsequent generations. It is apparent that variability for such characters is still present in advanced generations. Further field evaluation will be necessary to demonstrate the agronomic superiority of these glasshouse selected lines.

(4) A range of contrasting environments for field evaluation of selections from the $F_4$ generation onwards is essential for selecting those with wide adaptability. These are the types needed at present in Northern Australia and other tropical areas where there is extensive pasture development.

(5) Tolerance to drought and cold do not appear to have been increased in this program. These characteristics would enhance the dry season value of cultivars, so there is a need to determine whether it is possible to integrate these characters into new lines.

(6) With increasing costs of superphosphate and other fertilizers there is a need for lines of *M. atropurpureum* and other legumes more efficient in the use of nutrients, particularly P. Consideration should therefore be given to selection of progenies using minimal applications of superphosphate.

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