CONTRIBUTED PAPERS — Tropical Pasture Species Review A REVIEW OF RESEARCH ON LEUCAENA LEUCOCEPHALA S. G. GRAY*

During the last twenty years or so there has been an increasing interest, both in Australia and in other countries, in the use of the legume Leucaena leucocephala as a forage plant for cattle in tropical and subtropical regions. The species is widely used also as a shade tree in plantation crops in some tropical countries, and for several other purposes. The present review will deal mainly with research published since about 1950 relevant to the use of the species as a forage plant.

Leucaena leucocephala is a shrub or small tree with bipinnate leaves, white flowers in globular heads, strap-shaped pods, and brown shining seeds. It belongs to the tribe Eumimoseae, of the subfamily Mimosaceae, and is native to the Central American region.

BOTANICAL NAME

The name Leucaena leucocephala (Lam.) de Wit was applied to this plant in 1961 (de Wit, 1961), replacing the name Leucaena glauca (L.) Benth. by which it had been known since 1842. When Bentham proposed the genus Leucaena in that year he included in it L. glauca (L.) Benth., based on Acacia glauca Willd., which in turn was based on Mimosa glauca L. According to de Wit, however, the plant to which Linnaeus in 1753 applied the name Mimosa glauca was in fact not the one known recently as Leucaena glauca, but the one which is now correctly known as Acacia glauca (L.) Moench. For this reason the name Leucaena glauca cannot be maintained, and so it has been replaced by Leucaena leucocephala (Lam.) de Wit, based on Mimosa leucocephala Lamarck, the earliest legitimate name which was applied to this species in 1783.

HISTORY

According to Dijkman (1950) L. leucocephala originated in Mexico, from Jalinco to Michoacan in Chiapas and Yucatan. From there it has spread and become naturalized in all the countries around the Gulf of Mexico and in the islands of the Caribbean Sea. It has not, however, been utilized to any extent as a fodder plant in these areas.

Its spread to the Pacific and Indonesia is thought by Dijkman to have been linked with the Spanish occupation of these regions which occurred during the latter half of the sixteenth century.

The leaves and seeds have been used as food by the native peoples of some of the islands in the Pacific, while in Indonesia it has been widely used for the provision of shade and soil fertility maintenance in conjunction with plantation crops. Two closely related species, namely L. pulverulenta and L. glabrata and their hybrids with L. leucocephala, have also been utilized in this way.

L. leucocephala has been naturalized for many years in several areas of tropical and subtropical Australia. It occurs sporadically in Queensland at Brisbane, Gympie, Gayndah, Rockhampton, Mackay and Innisfail, and in the Northern Territory in the vicinity of Darwin (Hutton and Gray, 1959). Collections from each of these places were grown together in nurseries at Samford, near Brisbane, and proved to be similar in type to those introduced from Hawaii and other Pacific areas. The exact manner in which the naturalized material came

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to Australia is not known, but it seems likely that seed was brought in from New Guinea, Fiji, or other parts of the Pacific (Hutton and Gray, 1959).

Systematic testing of introductions in Australia was undertaken by C.S.I.R.O. at Samford, Queensland, in 1955; by 1958 about 40 accessions from 19 countries had been tested (Gray, 1960), and up to 1967 the number tested had increased to over one hundred. Most of these have proved to be similar in type to those already naturalized in Australia. A few introductions originating in Central American countries have exhibited substantially greater vigour and taller growth.

Two cultivars were released in 1962 by the Queensland Pasture Liaison Committee. These were cv. Peru (C.P.I. 18614*) and cv. El Salvador (C.P.I. 18623), both introduced by the C.S.I.R.O. Plant Introduction Section in 1954. Peru is outstanding in vegetative vigour and yield of forage among the varieties tested in Australia. El Salvador has lower yielding ability than Peru, but is taller than any naturalized types (Hutton and Bonner, 1960).

USE AND ESTABLISHMENT

The use of leucaena in plantations and as a soil improving crop is much older than its use as a source of fodder. The methods used in Indonesia for soil improvement have been described by Dijkman (1950). These are considered in two categories. The first is simply to establish the crop and then leave it undisturbed for some time so that the topsoil becomes enriched in organic material from leaf drop. Waste grasslands have been reclaimed in this way in the Philippines, in Java, and in Hawaii. The second method is to interplant with plantation crops. The leucaena acts as an aid-plant which by keeping the soil in optimum physical and biological condition ensures the best utilization of added fertilizers by the main crop. In East Java it has been estimated that a stand of 1000 leucaena plants per hectare, pruned every two months, added 35 tons of green leaves and twigs per hectare per annum.

The use of leucaena as a forage in Hawaii and elsewhere has been described by Takahashi and Ripperton (1949) and by Whyte, Nilsson-Leissner, and Trumble (1953), the methods consisting mainly of utilizing existing naturalized stands. Whyte (1944) quoted results from Hawaii in which it was found that the yield of dry matter and protein was higher when leucaena was cut four times a year than when cut three or six times.

* Commony talth Plant Introduction Number

Trials in Queensland (Anon. 1960, Humphreys, 1962) have shown that leucaena is likely to have a definite place as a browse plant in coastal and subcoastal areas in eastern and north-eastern Queensland. At Rodd's Bay near Gladstone leucaena was grown in rows 7 ft. 6 in. apart in small paddocks that could be grazed rotationally in conjunction with a larger area of native pasture with Townsville lucerne. Steers with access to leucaena gave increased weight gains in late winter and spring compared with those on pasture alone (Anon. 1966). Leucaena has also given promising results at South Johnstone in northern Queensland (unpublished data) and at Wollongbar in northern New South Wales (Aton. 1961).

AGRONOMIC CHARACTERISTICS

Dijkman (1950) has described the features that make L. leucocephala a valuable agronomic plant. The root system, consisting of a very strongly developed tap-root, with laterals growing downwards at a sharp angle to the tap-root, penetrates rapidly to considerable depths — over two metres in seedlings one year old — enabling the plant to grow in very close stands, and to break up impervious

soils. The deep root system enables leucaena to obtain nutrients from strata that would be inaccessible to many other pasture plants, and to transfer them to the topsoil through its dropped leaves. This and the good nitrogen fixing capacity that the plant possesses, provided that the appropriate *Rhizobium* is present, give leucaena considerable value as a soil-improving crop.

Experience in Indonesia and Hawaii has shown that leucaena is tolerant of a wide range of climatic and soil conditions. It thrives in naturalized stands in Hawaii from sea-level up to 1000 ft., and in Indonesia up to 1600 ft., while planted stands grow in the latter country up to nearly 5000 ft. (Takahashi and Ripperton 1949, Dijkman 1950). Areas where leucaena grows wild in Hawaii have rainfall ranging from 24 in. to 62 in. per annum, and in Indonesia from 27 to 66 in., while in Indonesia it is used in areas with rainfall up to 156 in.

Leucaena is not very specific in its soil requirements, according to Dijkman (1950); however, in many of the soils where it is grown in Hawaii and Indonesia its growth is appreciably stimulated by the application of lime and phosphate. Gantt (1953) stated that in the Philippines it is adapted to calcareous and coral soils with high pH values, although it is tolerant of acidity. Similar preferences in New Guinea and Fiji are reported by Norris (1965).

Undesirable characteristics of leucaena from the agronomic point of view are slowness of establishment, prolific seeding habit in many varieties leading to thicket production, rapid erect growth habit in some varieties, and the presence of the toxic substance mimosine. Under some circumstances leucaena may become aggressive; methods of killing the plant by the use of hormone herbicides have been studied in New Guinea (Stallwood, 1960).

Yield

In a locality in Hawaii with 50 to 60 in. annual rainfall, Takahashi and Ripperton (1949) reported a mean annual yield of 8 to 9 tons of dry matter per acre containing 2442 to 3145 lb of protein. This was obtained by cutting plots 2 to 3 in. above ground level with a motor scythe, taking 3, 4, or 6 cuts per annum. Anslow (1957) in Mauritius, under an annual rainfall of 61.5 in., obtained 2.8 tons of dry matter per acre over twelve months containing 1696 lb of protein. These plots were cut mechanically 4 in. above ground level, 3 harvests being taken during the year.

Anslow (1959) in Mauritius described the establishment of leucaena in hedges planted between adjacent contour strips of rotational crops. These hedges were cut by hand and yielded up to 112 lb of green material per annum from ten feet of hedge. Anslow concluded that optimum yields of dry matter and protein would be obtained when hedges were trimmed five times per year.

Hutton and Bonner (1960) at Samford, Queensland, measured the yield of edible dry matter obtained by a cutting and plucking method which attempted to simulate grazing by cattle. The plants were four years old at the time of the trial. They had a stabilized framework and had been kept to a height of 4 ft. 6 in. by grazing and cutting back when necessary. After a complete grazing in September 1958, samples were taken in November, January, March, and June. Rainfall during the sampling period was 40 in. and that for the 12 months ending in September 1959 was 47 in. The yield of edible dry matter obtained over the 9 months test period for the highest yielding variety Peru was 11,236 lb per acre, containing 3217 lb of protein per acre. Hutton and Bonner concluded that the dry matter and protein production of this variety at Samford was better than that of good crops of irrigated lucerne in southern Queensland, and was comparable with the yield from high quality clover-rye grass pastures in New Zealand.

Kinch and Ripperton (1962) have reported that under continuous cropping with four to five harvests per annum, high yielding strains in Hawaii have produced eight to ten tons (dry matter) of highly palatable forage. In recent trials in the Virgin Islands Oakes and Skov (1967) reported yields of from 3 to 8 tons of dry forage per acre per annum. In these trials annual yields of protein varied from 832 to 2,550 pounds per acre. There were significant yield differences between strains.

Seed Treatment

It was pointed out by Akamine (1942, 1952) that delayed germination of leucaena seed is caused by the presence of a very thick, tough, waxy-layered seed-coat which prevents water from entering the seed. His results showed that while untreated seed germinated only to the extent of 10 to 15 per cent, mechanical scarification and acid scarification resulted in a germination of over 90 per cent, while 70 per cent germination was obtained with hot water scarification. The hot water treatment, which consisted of placing the seed in water heated to about 170°F, leaving the bag of seed in the water until the temperature dropped to about 100°F, had the additional disadvantage that the seed deteriorated rapidly under storage.

Takahashi and Ripperton (1949) recommended acid treatment consisting of immersion in commercial sulphuric acid for 15 minutes followed by thorough washing. Seed treated this way retained its viability for several months, but undesirable features of the method are risk of injury both to the seed and to the operator and the lengthy washing and drying process.

Gray (1962) found that short immersion (two minutes) in water at 80°C followed by rapid drying, was fully effective in removing germination delay, and the seed retained full viability for 15 months. The treatment resulted in the formation of a network of fine cracks in the seed-coat, evidently sufficient to allow the entry of water when the seed was sown, but not extensive enough to lower the keeping quality of the seed.

Symbiosis With Rhizobium

Cross-inoculation studies conducted in Brazil (Galli, 1958) with root-nodule bacteria of tropical legumes showed that *Leucaena leucocephala* is highly strain-specific; it became effectively nodulated when inoculated with bacteria from the same species, but failed to nodulate when cross-inoculated with any of the other legumes tested.

Trinick (1965) found that L. leucocephala failed to nodulate with slow-growing root-nodule bacteria isolated from a number of tropical legume species, but successful nodulation was obtained with strains of Rhizobium of fast-growing type.

A study of the acid-producing characteristics of strains of *Rhizobium* from a wide range of legumes was reported by Norris (1965). He found that *Rhizobium* strains isolated from leucaena were consistently of a fast-growing acid-producing type, rather similar to strains associated with *Trifolium* spp. Norris considered this as evidence that leucaena is adapted primarily to alkaline soils. He suggested the use of lime-pelleting of seed and of lime applications to the soil for leucaena grown in Australia.

Norris (1965) tested the symbolic performance of 11 isolates of *Rhizobium* from leucaena and found wide variation in effectiveness as measured by mean plant weight.

FEEDING VALUE OF LEUCAENA

Poultry

A considerable amount of work has been done on the feeding value of leucaena when used as an ingredient of rations for poultry. Palafox (1948) reported on the use of fresh green leaves in chick rations in Hawaii. Dingayan and Fronda (1950) and Molina (1953) in the Philippines found that the addition of leucaena leaf material improved the feeding value of a basal feed for chicks. Sadoval (1955) also working in the Philippines, however, reported that in general there was no advantage in adding leucaena to a laying ration. Springhall and Ross (1965) concluded that the addition of leucaena treated with ferrous sulphate to grower rations containing sago and copra did not significantly alter egg production, egg weights, or feed conversion, although it resulted in a significantly longer time for the birds to reach sexual maturity, and significantly lighter body weight after an eight months production period. When leucaena was added to layer rations, no significant differences were found in egg production, feed conversion, or fertility in pullets. The hens fed leucaena, however, produced lighter eggs and the hens themselves gained less in weight than the control hens.

Swine

Feeding trials with swine have shown that there were no ill effects from feeding dehydrated L. leucocephala meal at 5, 10 or 15 per cent levels in the rations of growing pigs (Iwanaga, Otagaki, and Wayman, 1957). These authors concluded that this material can be regarded as a useful feed for growing and fattening swine. Deleterious effects on reproductive performance, however, were pointed out by Wayman and Iwanaga (1957) who showed that at the 15 per cent level in the ration, dried L. leucocephala feed had the effect of reducing the ability of the gilts to conceive, and also reduced the average litter size and weight.

Cattle

Although leucaena has been used quite extensively as feed for both dairy and beef cattle, especially in Hawaii (Kinch and Ripperton, 1962) very little data are available on the levels of animal production obtained. Henke, Work, and Burt (1940) reported live weight gains of up to 1.15 lb per day in steers grazing on an almost pure stand of leucaena in Hawaii. The paucity of information on this point is one of the most serious gaps in our knowledge at present concerning this legume.

TOXICITY

Non-Ruminants

Information on the toxicity found in L. leucocephala has been reviewed briefly by Owen (1958) and by Hutton and Gray (1959). Early work showed that when this species comprised a high proportion of the diet of non-ruminants, especially norses, it could cause depilation, growth reduction, and general ill-health.

Several workers (Arnold 1944, Gardner and Bennetts 1956) have ascribed the toxicity of *L. leucocephala* to its ability to accumulate selenium from soils. Yoshida (1944), however, working with rats has shown that loss of hair and other symptoms caused by diets containing material of leucaena were not due to selenium but to the amino acid mimosine which occurs in the leaves and seeds. She isolated this substance from seeds and fed it to her test animals, producing the same symptoms. Carangal and Catindig (1955) and Hegarty (1957) examined the free amino acids of leucaena chromatographically. Further investiga-

tions reported by Owen (1958) have shown conclusively that these effects in horses, pigs, and laboratory animals were caused by mimosine.

Mullenax (1963) reviewed work on leucaena with special reference to toxicity. He pointed out that although the pharmacology of mimosine is still not well understood, empirical approaches to the toxicity problem have met with some success. Mullenax, and also Seawright (1963) advocated further toxicological study.

The use of iron salts to decrease toxicity has been mentioned by Yoshida (1944), while the effectiveness of ferrous sulphate for this purpose was described in 1951 by Matsumoto and his co-workers (Matsumoto and Sherman, 1951; Matsumoto, Smith and Sherman, 1951). Ross and Springhall (1963), working with diets for poultry, reported that the addition of ferrous sulphate in solution to leucaena material prior to mixing with the balance of the ration was effective in reducing toxicity. Additional improvement was achieved when the iron-treated leucaena was allowed to stand for at least a week before being mixed with the other ingredients.

Hylin and Lichton (1965) reported that feeding mimosine isolated from leucaena seed to fertile, female rats caused cessation of the oestrous cycle and complete infertility. As little as 0.5 per cent in the diet on prolonged feeding caused irregular and atypical oestrous cycling. These authors pointed out that the mechanism of the action of mimosine is not known, but their results were suggestive of interference with gonadotrophin production or release. Fertility was restored when the rats were returned to a mimosine-free diet. Montagna and Yun (1963) fed leucaena seeds to mice resulting in gross damage and degeneration to the hair follicles; they concluded that the toxic principle leucaenine (mimosine) seems to be a mitotic inhibitor. This finding has been supported by Pritchard and Court (1968), who found that clumped metaphases followed treatment with mimosine of root tips of Vicia faba, Leucaena leucocephala, and other plants, and concluded that mimosine may be classified as a c-mitotic agent.

Further studies by Bindon and Lamond (1966) using mice showed that mimosine extracted from leucaena caused the death of embryos when fed to pregnant females. Leaf material at levels up to 30 per cent of the diet had only slight effects on pregnancy. Lactation, as measured by weaning weights of litters, was reduced in mice fed on mimosine, but was unaffected by diets containing leucaena. Response by the mouse to gonadotrophin was reduced following a 3-day feeding period on leucaena.

Ruminants

It was formerly believed that ruminants did not suffer ill effects from leucaena, although Damseaux (1956) had reported symptoms in Suffolk sheep fed on this plant. Whyte, Nilsson-Leissner and Trumble (1953) reported that leucaena was suspected of causing sterility in cows, although other work at the University Experiment Station in Hawaii (Anon., 1948) had shown no loss in reproductive efficiency of cows fed on this species.

Compère (1959) reported toxic symptoms in both ruminants and non-ruminants when fed large quantities of leucaena forage. He considered that the rumen of cattle was capable of destroying the toxic agent, and suggested the excessive intake of digestible protein as a possible cause of the toxic effect. Letts (1963) described a buffalo calf that suffered loss of hair following feeding with a diet containing leucaena, but which recovered when this was removed from the diet.

Hegarty, Schinckel, and Court (1964) found that sheep fed on a diet consisting entirely of leucaena shed their fleece, the depilatory agent being mimosine.

The depilatory effect of leucaena was influenced by the level and method of feeding. It was found that in the rumen of the sheep extensive degradation of mimosine to 3,4-dihydroxypyridine (DHP) takes place, but that sheep cannot detoxify mimosine after absorption. The experimental results suggested that sheep can be conditioned to consume a diet consisting entirely of leucaena without untoward effects, and that the absence of toxic symptoms in conditioned sheep appeared to be due to increased detoxification in the rumen.

Variation in Mimosine Content

Differences in mimosine content between varieties of L. leucocephala have been reported by Matsumoto and Sherman (1948), Carangal and Catindig (1955), Hutton and Gray (1959), Brewbaker and Hylin (1965), and Gonzalez, Brewbaker and Hammill (1967). These findings were all based on the analytical method of the first-named authors, using dried leaf material for analysis.

Hegarty, Court, and Thorne (1964) described a method for the determination of mimosine and DHP in leaf extracts and seeds; they found that appreciable destruction of mimosine, with the formation of DHP, occurs during the drying of fresh leaves, even under mild conditions. This finding leaves the results obtained using the method of Matsumoto and Sherman with leaf material open to criticism, so that up to the present there is no satisfactory evidence of genetic variation in mimosine content in this species.

GENETICS AND BREEDING

Varieties of L. leucocephala have been grouped (Hutton and Gray 1959, Gray 1960) into three types based on growth habit. Those designated Type 1 are bushy, relatively short, early flowering and low yielding. This type, as well as occurring in Central America, is widely naturalized in the Pacific region and in northern Australia. Type 2 varieties are tall and erect, sparsely branched at the base, late flowering and high yielding. Several varieties introduced into Australia from El Salvador and Guatemala are of this type. Type 3 is tall, late maturing and high yielding, and strongly branched at the base. The variety Peru is of this type.

The flowering and seed-setting process has been described in detail (Hutton and Gray 1959; Gray 1960). Anthesis occurs early in the morning. The clumped pollen falls directly on to the stigmas and pollen grains become lodged in the stigmatic cups, where they germinate immediately. This process results in a high degree of self-pollination although cross-pollination can readily occur. Emasculation was effected by shaking the flower heads for three minutes in a 0.1 per cent solution of Gardinol K, a sulphonated lauryl alcohol, soon after anther dehiscence between 8 a.m. and 9 a.m. This process removes or renders inviable the pollen grains in the stigmatic cups. After the heads have dried, pollen from the male parent may be applied to the stigmas. Crosses between varieties were made successfully both in the field and with potted plants in the glasshouse.

The somatic chromosome number 2n=104 for L. leucocephala was reported by Tijo (1948). This has been confirmed by Frahm-Leliveld (1960) and by Pritchard and Gould (1964).

Gonzalez, Brewbaker and Hamill (1967) found that L. leucocephala hybridized readily with several other species in the genus, and reported wide variation in mimosine content in the segregating generations of crosses between strains of L. leucocephala.

The mode of inheritance of characters related to growth habit and size in L. leucocephala has been investigated by Gray (1967a). Erect habit was found

to be dominant over bushy habit, and absence of strong basal branching was dominant over its presence. In each case the F_2 segregation conformed to a 3:1 ratio, and two major gene pairs have been postulated. In these crosses, quantitative characters stem length and stem number appeared to be controlled by multiple genes affecting vegetative vigour, and these genes appeared to be transmitted independently from those controlling branching habit.

A diallel series of intervarietal crosses was used (Gray 1967b) to obtain estimates of general and specific combining ability in quantitative characters. These estimates were used to evaluate parents and crosses for breeding. Partition of variation in stem length, using Mather's method (Gray 1967c), showed the presence of a strong additive component, but not of any non-additive component.

A breeding programme to develop types adapted to grazing under Australian conditions was commenced in 1956 at Samford, Queensland (Hutton and Gray, 1959, Gray 1967a). The major objective is to combine high density of branching with high forage yield. Since plant vigour was found to be under the control of additively acting genes independent of those controlling branching habit, this should be possible. Promising lines derived from crosses between the shrubby Hawaiian type and the taller more vigorous introductions from Central America are currently being tested at Samford and Townsville, Queensland.

CONCLUSION

The work on L. leucocephala reviewed in this paper has approached the species from several different aspects — agronomic, chemical, and genetic. All these are relevant to the problem of adapting the species as a forage plant for use in tropical and subtropical regions.

This plant could make a substantial contribution to the protein requirements of cattle over a very large area in northern Australia, and it is essential that further work should be done to bring this about. There are several obvious gaps in our present knowledge of this species, one of the most serious being lack of information on the effect of this legume on production in cattle. The basic work that has been done on laboratory animals and other non-ruminants needs to be supplemented by further work with ruminants, especially in relation to reproduction and lactation. Extensive field testing is required to determine its geographical limitations more precisely, and methods of establishment and management under Australian conditions need to be investigated more intensively.

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