IMPROVING YIELD AND QUALITY OF LEUCAENA

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ABSTRACT

Three studies were conducted with objectives of improving yield and quality of leucaena forage.

An experiment was conducted in a split-split plot design with five harvests, four levels of irrigation and three accessions (K8, K500 and K4) as main plot, sub-plot and sub-sub-plot treatments respectively. Forage was harvested at 48 to 78 days intervals at 30-50 cm height. The initial rate of growth of K8 was the heighest and of K4 was the lowest. Forage yield was significantly high when crop was irrigated to fully compensate the evaporation losses which was the highest level irrigation treatment. Under well distributed rainfall conditions of Hawaii, soil moisture level of control treatment was well above the critical level of moisture requirement for leucaena. Therefore marginal increase in the soil moisture level through the intermediate levels of irrigation did not increase the yield. K8 and K500 yielded significantly higher than K4. There was no difference in DM yield of K8 and K500. However K8 was found to be superior to K8 for forage production due to higher protein yield, higher foliage fraction and low mimosine content. Forage yield and rate of stem elonation were high in summer and low in winter. The reverse was true for total nitrogen, mimosine and foliage fraction of the forage. Solar radiation was

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the most important factor which influenced the yield.

In a leucaena leaf meal (LLM) feeding trial on growing Japanese quail, following six deitary treatments were included to study the effects of three types of LLM varying in mimosine and tannin contents: Positive control (normal diet with corn and soymeal), negative control (15% alfalfa leaf meal), TLM (15% leucaena K8 tender leaf meal with high mimosine and low tannin), MLM (15% K8 matured leaf meal with low mimosine and high tannin), LM (15% L. diversifolia K156 leaf meal with low mimosine and low tannin) and PVP (MLM diet with 1% polyvinyl pyrrolidone). Weight gain was the lowest in TLM, highest in positive and negative controls and intermediate in MLM, LM and PVP dietary treatments. The difference was noticable by the end of the first week of the trial. Results indicated that mimosine was probably the major cause of poor growth. There was no significant variation in the weight gain due to the difference tannin content of the diets. Supplimentation of PVP was beneficial in this trial.

In another study, leucaena accessions maintained at the University of Hawaii, University of the Philippines at Los Banos and Perum Perhutani, Indonesia were screened for low mimosine and high vigor. 31 accessions from the University of Hawaii and one accession from the Perum Perhutani were selected. A forage yield trial has been laid out at the Experiment Station, Waimanalo, to test the yield potentials of these accessions.

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I. INTRODUCTION

The humid and subhumid tropics are the largest underdeveloped regions of the world. They need the technology for increased food production to meet the demands of the growing populations. In spite of the advantages of high atmospheric temperature and solar radiation, food and forage productions of these regions have been bogged down by many adverse factors such as infertile soils due to excessive cultivation, weathering and soil erosion, small land holdings, outmoded systems of cultivation and lack of suitable crops and cultivars. Under such conditions agriculture becomes uneconomical and the people dependent on agriculture can not get employment throughout the year. Due to lack of supply and buying capacity, a majority of these populations can not meet their basic nutritive needs, though their livelihood is principally dependent on agriculture and animal husbandry. It is a challenge to agricultural scientists to develop techniques for these areas which would enable the production of protein rich food and forage continuously, without a decline in crop yield and fertility, which can eradicate poverty as well as increase food resources of the world.

Mixed farming, a cultural tradition in many developing countries is another underexploited practice

which can create gainful employment in rural areas. Maintaining a few cattle, sheep, goats or poultry is not beyond the capacity of poor farmers. On the other hand it helps in recycling the available resources efficiently for increasing food production. Mixed farming is generally less affected by natural calamities such as flood, drought and typhoon, and can provide a steady income throughout the year.

Leguminous plants hold a great potential as a source of high-protein food and forage and are adapted to a wide range of adverse environmental conditions. One legume which has received much attention is leucaena (Leucaena leucocephala (Lam.) de Wit), a tropical shrub or a small tree which originated in Mexico. Initially it was grown as a shade tree in coffee plantations and as a ground cover for controlling soil erosion. During recent years, efforts are being made to promote the intensive cultivation of leucaena throughout the tropics, because of its value as a protein rich forage, fast growing tree for biomass, raw material for paper and pulp and its ability to withstand drought and alkaline soil conditions and a long productive life.

Leucaena has been referred to as the alfalfa of the tropics, though it is superior in protein and carotene contents. Moreover, alfalfa competes with food crops for good quality land, irrigation water and labor, whereas leucaena can be grown with very little care, on

unproductive land where no other crops can be grown profitably. In the present situation where animal industry in the tropics is seeking an increased supply of protein and large acreages of unproductive land is remaining fallow, leucaena may become a major forage crop of the tropics. However it is necessary to develop the ideal management system to optimize the use of available resources and to understand the toxicity problems before expanading its cultivation.

Although leucaena is a drought tolerant plant, its response to additional water during low soil moisture conditions is dramatic. No specific information is available about the requirement of water for optimum production and its variation among differnt accessions. It was therefore proposed to study the effects of different levels of irrigation on the forage yield of three accessions of leucaena.

The toxicity of leucaena forage needs intensive study, due to the presence of mimosine, tannin and other chemicals, which are absent in other forages. Mimosine toxicity problems have almost been solved in ruminants but not in non-ruminants. It is not certain whether this toxicity is exclusively due to mimosine or due to the combination of mimosine and tannin. Therefore, it was proposed to study the toxicity problems in Japanese quail and to identify the causes of toxicity by feeding leucaena containing different levels of mimosine and tannin.

Selection of low mimosine strains with good vigor can help the poultry industry with an additional source of feed at a very low cost. It was therefore proposed to screen the strains of leucaena available in Hawaii, Indonesia and the Philippines for low mimosine and high forage yield characters.

II. LITERATURE REVIEW

2.1. Origin and Distribution:

Leucaena leucocephala (Lam.) de Wit, commomly known as leucaena originated in Mexico and is also known under several common names such as, guaje, huaxin and uaxim in Mexico and Central America, lead tree, tan-tan, white popinac and hediondilla in the Caribbean islands, ipil-ipil and bayani in the Philippines, lamtoro and lanang in Indonesia, koa haole in Hawaii, vaivai in Fiji, tangantangan in Guam and subabul (formerly called as koo babul) in India. The other species in the genus Leucaena which are useful either for cultivation or breeding are L.diversifolia (Schlecht) Benth., L. esculenta (Moc. & Sesse) Benth.,L.macrophylla Benth., L.pulverulenta (Schlecht) Benth. and L.trichodes Benth. (Brewbaker and Hutton,1979).

Leucaena was first used early in 1900, by the agriculturists in Indonesia for shading and maintaining soil fertility in coffee plantations (Dijkman, 1950). During the period 1930-1935, Hawaiian ranchers recognised its forage value. Leucaena cultivation was expanded during World War II to use the forage as a substitute for concentrates (Takahashi and Ripperton, 1949). It is well established now in Australia, Fiji, Guam, Hawaii, India,

Indonesia, Malaysia, Papua New Guinea, Philippines and West⁶ and East Africa and is used for forage, fuel, paper, pulp and timber. Leucaena is also grown as a crop for soil improvement (N.A.S., 1977).

2.2.Factors affecting the forage production:

2.2.1. Environmental Factors:

Temperature, solar radiation, rainfall and soil conditions affect the adaptability and the rate of growth of leucaena, which in turn affect the forage yield.

2.2.1.1. Temperature:

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Leucaena thrives best under conditions of high temperature, once the plants are established (Savory, 1979). Variations in its performance at different altitudes and latitudes are also related to variations in temperature. It grows very well at an altitude of 1500 m along the equator in Papua New Guinea (Hill, 1971) and Indonesia (Dijkman, 1950), whereas the growth is restricted even at an elevation of 700 m in the Philippines (Farinas, 1951) and 500 m in Hawaii (N.A.S., 1977), as the cultivation shifted farther from the equator.

2.2.1.2. Solar radiation and seasonal effect:

There are no specific studies to demonstrate the effect of solar radiation independent of the effect of temperature. The rate of growth of leucaena plants is optimum under full sun (Dijkman, 1950). Shading increased

the plant height but reduced the root growth as well as forage yield (Egara and Jones, 1977). The dry matter yield was maximum under full light intensity. The yield and nitrogen content of the forage decreased when the intensity of light was 70 per cent or below, although there was a significant increase in stem elongation (Eriksen, 1978). Ferraris (1979) reported that the degree day and evaporation (radiant energy) of the growth period were positively correlated with the yield and negatively correlated with the nitrogen content of the foliage.

The seasonal effect on leucaena has been studied by several researchers. Guevarra (1976) observed that the factors causing seasonal variations were the intensity and duration of solar radiation and day and night temperatures. Kinch and Ripperton (1962), while growing Hawaiian type leucaena for forage production, recorded a weekly stem growth of 20.0 to 21.5 cm in summer and only 4.0 to 5.0 cm in winter under adequate soil moisture conditions. This affected the daily yield of forage as well as the harvesting interval. In Malawi, Savory (1979) observed the effect of seasonal variation on the branch production of Peru type leucaena.

2.2.1.3. Rainfall:

Leucanea can be grown throughout the tropics and subtropics in the annual rainfall range of 500-3000 mm (Jones, 1979). Plants grew actively even when the rainfall

during the four summer months was only 250 mm (Hutton and Gray, 1959). In dry areas of Hawaii with an average rainfall of 84 mm per month leucaena yielded enough forage to maintain one cow per 0.4 ha where other crops could not be grown satisfactorily (Henke, 1933). Leucaena is tolerant to drought but cannot yield forage under continuous drought stress. It can be grown in heavy rainfall areas as well, provided there is adequate drainage. Supplementary irrigation is beneficial while cultivating leucaena under dry conditions.

2.2.1.4. Soil requirements:

Leucaena is adapted to a wide range of soil types but the presence of lime and phosphate in the soil stimulates growth (Dijkman, 1950). Its growth could be limited by low soil pH, high levels of exchangeable aluminium or low levels of phosphorus and calcium (Tilo et al.,1981). Fox and Whitney (1981) suggested application of lime for growing leucaena in soils having problems of aluminium toxicity, manganese toxicity or calcium deficiency. Presence of Rhizobium in the soil or inoculation with an appropriate Rhizobium strain is essential to supply nitrogen to the growing plants (N.A.S.,1977). Leucaena roots do not have root hairs and hence the seedlings depend heavily on mycorrhizae for P absorption (Yost, 1980). In Hawaii, leucaena responded well to the application of N, P and Ca (Takahashi and Ripperton,

1949).

2.2.2. Management factors:

The most important factors affecting the forage yield are cultivars used, soil moisture, plant population, height of cutting and harvesting frequency.

2.2.2.1. Cultivars:

Three types of leucaena such as Hawaiian, Salvador and Peru can be grown for forage production. Cunningham is the only cultivar bred for forage production by crossing Salvador and Peru types (N.A.S.1977). Among the Salvador type, K8, K28, K29, and K67 are the most vigorous. In Peru type, there are several accessions such as K3, K4, K6, K66, K59, K62, K77, K95 and K101 (Brewbaker et al.,1972). No information is available on the forage yield of different accessions.

Forage yield of leucaena may vary from 5 to 20 t DM/ha/yr depending on the annual rainfall and temperature of the area (Jones, 1979). In Hawaii, accession K8 produced 33.5 t DM/ha/yr under adequate moisture conditions, out-yielding the Hawaiian types by 2.5 times (Brewbaker et al, 1972). In another trial, Hawaiian type K341 yielded more than K8 when the plants were harvested at 2.5 to 5.0 cm above the ground (Guevarra, 1976). However, in all other yield trials conducted in different countries, Salvador and Peru types were superior to

Hawaiian type (Hutton and Bonner, 1960; Oakes and Skov, 1967; Relwani et al., 1982; Sampet and Pattaro, 1979). Savory (1979) reported in Malawi that K8 was more vigoros than Peru, especially during the dry season. However there was no significant difference between the forage yield of K8, Peru and Cunningham. Similar results were also reported from Fiji (Partridge and Ranacou, 1973). In India, Salvador type yielded more than Peru, although the later type out-yielded the former during the winter months and Peru had higher forage fraction than that of Salvador type (Pathak et al., 1981). Shih and Hu (1981) in Taiwan recorded the highest herbage yield of 102.5 t/ha/yr from accession Sl (Santa Cruz, Mexico), followed by the other Salvador and Peru types with no significant difference, while the Hawaiian type yielded only 36 t/ha/yr. Hutton and Beattie (1976) reported Cunningham cultivar to yield 27 to 49 per cent higher than Peru, but Ferraris (1979) found In no significant difference between the above two strains, under shorter harvesting intervals and wetter conditions. In Papua New Guinea, Peru type yielded 28 t DM/ha, in nine months (Hill, 1971), whereas in Queensland it yielded 12.75 t DM/ ha followed by accession K8 (6.64 t DM/ha), Guatemalan (5.53 t DM/ha) and Hawaiian (1.52 t DM/ha) in nine months (Hutton and Bonner, 1960). In the U.S. Virgin Islands, K8 produced 20.7 t DM/ha/yr superseding other varieties (Oakes and Skov, 1967). In Fiji, K8 yielded 21.5 t DM/ha/yr (Patridge and Ranacou, 1973).

2.2.2.2. Soil Moisture:

Moisture stress affects leaf and stem production of leucaena. Under drought conditions, reductions in the size of the leaflets, number of leaflets and number of pinnules were observed. Leaves started wilting and dropping faster. Drought affected the number of stems as well as the rate of stem elongation (Takahashi and Ripperton, 1949).

In Hawaii, Kinch and Ripperton (1962) estimated a water requirement of 54 mm per t DM and a total of 1100 mm rainfall or equivalent irrigation to produce 50 t DM/ha/yr. They observed a positive correlation between the moisture supply and the forage yield in low rainfall areas. Takahashi and Ripperton (1949) produced a good crop in 4.5 months duration with only three irrigations, while Napier grass and alfalfa needed irrigation at intervals of 10 to 14 days. Their experiment consisting of three intervals of irrigations (10, 35 and 135 days), initially did not show significant difference between the former two treatments. Forage yield over a period of two years was low at 135 days irrigation interval due to lack of moisture. There was no significant difference in the yield of treatments receiving irrigations at 10 and 35 days intervals. Observations on the individual harvests indicated poor performance of leucaena under excessive moisture conditions, particularly during the cooler seasons. They observed a high

correlation between the rainfall and various growth criteria, in the treatment of 135 days interval during the dry season. Frequency of irrigation directly affected the leaf size, number of pinnules and number of leaflets. A sharp decline in stem elongation and continuous drop of lower leaves were observed during moisture stress. There was a positive correlation between stem elongation and the production of new leaves. The authors estimated that 1000 mm of well distributed annual rainfall would be adequate and, with 1250 to 1500 mm a yield of 20-23 t DM/ha/yr could be harvested. Kinch and Ripperton (1962) estimated a water requirement of 54 mm per t DM production, as 1100 mm rain was needed to produce 50 t DM/ha/yr in Hawaii.

In the U.S. Virgin Islands, Oakes and Skov (1967) observed variation in yield from 7.5 to 20.0 t DM/ ha/yr. There was a positive correlation between the rainfall and the corresponding yield, which was also confirmed by Ferraris (1979) in Australia and Hill (1971) in Papua New Guinea. Moisture stress is more severe on high yielding varieties, due to their increased productivity (Brewbaker, 1976). In Fiji, K8 strain produced 42.9 t DM/ha in two years when the annual average rainfall was 1400 mm. Yield of other Salvador and Peru strains was similar (Patridge and Ranacou, 1973). In Queensland, Australia, Peru type grows extremely well in areas recieving about 780 mm rain, of which 70 percent falls during the summer (Wilden, 1980).

Nitrogen content of the forage was negatively correlated with the moisture availability (Ferraris, 1979).

2.2.2.3. Plant population:

Several studies were conducted on spacing management with spacing ranging from 0.6 to 3.0 m between the rows and 2.5 to 500 cm between the plants to establish a plant population between 40,000 and 400,000/ha (Takahashi and Ripperton, 1949; Kinch and Ripperton, 1962; Gonzalez, 1966, Oakes and Skov, 1967; Brewbaker et al., 1972; Guevarra, 1976). Shih and Hu (1981) observed no significant difference in the yield when the population density ranged between 50,000 and 200,000 plants/ha.

In Malawi, Savory (1979) observed a positive correlation between the yield and the plant density up to 220,000 plants/ha. Populations above 130,000 were very effective in controlling weeds. He reported a negative correlation between plant density and the number of branches per plant, as well as the forage yield per branch. These were compensated by the increased number of plants and therefore, the total yield increased with the increase in density in both Salvador and Hawaiian types (Guevarra, 1976). A plant density in the range of 80,000 to 100,000/ha might be ideal for forage production (Brewbaker, 1976). While considering the plant population, it is also important to consider the spacing between the rows. The yield was higher when the row spacing was 60 cm than at 100 cm, although the density was constant (Savory, 1979).

2.2.2.4. Height of cutting:

While deciding the height of cutting two factors will have to be taken into consideration:

A. Morphology of the cultivar or the type of leucaena

B. Systems of farming.

The Hawaiian type leucaena had a herbage yield of 50.85 t/ha/yr when cut at 5 cm above the ground compared to 43.40 and 40.28 t/ha/yr at 38 cm and 76 cm respectively (Takahashi and Ripperton, 1949). When a uniform height of 5 cm was maintained for both Hawaiian and Salvador types, the former type outyielding the later (Guevarra, 1976). Krishnamurthy and Mune Gowda (1982) reported highest herbage yield of 39.4 t/ha/yr at 150 cm cutting height followed by 33.04 t/ha/yr and 28.28 t/ha/yr at 75 cm and 15 cm respectively, in Hawaiian type. In Indonesia, cv. Cunningham yielded more when cut at 50 cm, than at 10 cm (Petheram et al., 1982). In Mauritius, cutting height between 45 cm and 90 cm was found to be ideal for Peru type (Osman, 1981a). In the Philippines, highest yield of 23.6 t DM/ha/yr was recorded when the plants were maintained at 3 m height and the leaves were plucked. The yield was further maximized by retaining 25 percent of the foliage on the plants, rather than of removing the total foliage (Mendoza et al.,1975).

In Hawaii, 15 cm height was maintained for mechanized

harvesting of Hawaiian type (Kinch and Ripperton, 1962). In India, 90 to 100 cm height was found to be the ideal for higher yields as well as minimizing the labor cost of weeding and manual harvesting (personal observations).

2.2.2.5. Harvesting interval:

Different criteria used in the past to determine the harvesting frequency were:

A. Harvesting at the onset of flowering

B. Pre determined branch length at harvesting

C. Pre determined number of harvests per year

D. Stem diameter at harvest.

Onset of flowering was used as a criterion by Anslow (1957), while studying Hawaiian type leucaena, which flowers several times a year. Hutton and Bonner (1960) considered the twig diameter at 6.4 mm as the criterion for forage harvest. Kinch and Ripperton (1962) fixed the height at cutting at 1.0 to 1.2 m, which allowed 4.6 harvests per year.

In Fiji, harvesting Peru type when the branches attain 90 cm length permitted five harvests in the first year and six harvests in the second year (Partridge and Ranacou, 1973). Branch length can be a useful criterion, provided specific heights are considered for different types. In general, the Salvador type may be allowed to grow longer than the others, followed by cv. Cunningham, Peru and Hawaiian (Personal observations).

Savory and Breen (1979) observed that the yield of Peru type was maximum when harvested four times per year instead of three or six times. Krishnamurthy and Mune Gowda (1982) reported that Hawaiian type had the greatest herbage yield when harvested at 70 day interval (39.40 t/ha/yr) followed by 60 day (35.58 t/ha/yr), 50 day (34.37 t/ha/yr) and 40 day intervals (22.78 t/ha/yr). However, the quality of the fodder was better when harvested at 50-60 day interval, as further delay caused woodiness in the stems. Osman (1981 b) observed that Peru and Hawaiian types yielded more when harvested at 90 day intervals, while Salvador type produced the same yield, when harvested at 60 days or 90 day intervals. He later reported (Osman, 1981 c) a variation in the leaf fraction of the forage ranging from 65 percent (30 day intervals) to 52 percent (150 day intervals). However, 95 percent of total herbage was edible if harvested at 60 day intervals and this decreased to 89.5 percent (Salvador type) and 93.4 percent (Hawaiian and Peru), when harvested at 120 day intervals (Osman, 1982). Partridge and Ranacou (1973) recorded a leaf to stem ratio of 65:35, when Peru type was harvested six times/yr. According to Gonzalez (1966), high yielding erect strains have a higher proportion of non-edible woody stems.

Several researchers reported that an increase in harvest interval increased the total dry matter yield but reduced the quality of the feed, because of the reduction in nitrogen content of the foliage and high proportion of

non-edible stem fraction (Sampet and Pattaro, 1979; Gonzalez, 1966; Guevarra, 1976; Takahashi and Ripperton, 1949). Harvests at monthly intervals reduced yield as well as the nutritive value (Guevarra, 1976; Ferraris, 1979). Frequent harvests at an interval of 4-6 weeks, however, reduced the woody DM yield without affecting the forage dry matter yield in Thailand (Sampet and Pattaro, 1979). In Papua-New Guinea, under adequate moisture conditions, the crop was ready for harvest in six weeks during the summer months (Hill, 1971).

While recommending eight weeks harvesting interval for warmer low land areas, Brewbaker et al. (1972) suggested that the erect strains should be harvested more frequently than the shrubby strains.

Leucaena plants have survived after continuous grazing for 16-20 years (Jones and Harrison, 1980).

2.3. Nutritive value of leucaena:

2.3.1. Nutrients:

Leucaena is a rich source of protein, carotene and minerals for cattle, sheep, goats, pigs and poultry. Several researchers have reported on the following nutritive contents of leucaena forage (Adeneye, 1979; D'Mello and Fraser, 1981; D'mello and Thomas, 1978; Joshi and Upadhyaya, 1976; Kharat et al., 1980; N.A.S., 1977; Singh and Mudgal, 1967; Stobbs and Fraser, 1971; Upadhyay et al., 1974).

Dry matter (DM): 23.7 to 36.0 percent, depending on the season, soil moisture, stage of harvest and the type of sample.

Crude protein: 20 to 30 percent, depending on the climatic conditions, growth stage and maturity and the composition of the sample material. However it can be as low as 16.5 percent (Henke et al., 1951), and as high as 47.4 percent in young shoot tips (Adeneye, 1979).

Ash: 6.0 percent in young tissues to 11 percent in mature tissues, with 0.5 to 2.8 percent Ca, 0.23 to 0.35 percent P, 1.5 to 1.75 percent K and 0.3 to 0.4 percent Mg.

Pigments: β carotene content of leucaena leaf meal (LLM) is about 536 mg/Kg DM whereas alfalfa has only 253 mg/Kg. It also contains xanthophyll (766 mg/Kg), lutein (500-550 mg/Kg) and zeaxanathin (ll0-l45 mg/Kg) as reported by D'Mello and Taplin (1978).

Metabolizable energy (ME): Classical ME is 2.67 MJ/Kg DM, N correlated ME is 2.98 MJ/Kg DM, although the gross energy is 19.60 MJ/Kg DM. It is suspected that fiber, tannin, mimosine and DHP which are present in LLM are responsible for such low ME values (D'Mello and Acamovic, 1981).

Digestibility coefficient (DC): DC of leucaena forage ranges from 64.7 to 73.25 percent (Kharat et al., 1980; Singh and Mudgal, 1967; Sobale et al., 1978). Leucaena is rich in vitamin K (180 mg/Kg leaf meal) as compared to

alfalfa (106 mg/Kg) (Chou and Ross, 1965).

2.3.2. Mimosine content:

In spite of its high nutritive value, leucaena feeding is a controversial issue, because of the presence of mimosine (B-[N-(3-hydroxy-4-oxopyridyl)]-C-aminopropionic acid), a non-protein amino acid, which can have deleterious effects on animals. Variation in mimosine concentration in different strains have been reported by several scientists (Brewbaker and Hylin, 1965; Hutton and Gray 1959; Matsumoto and Sherman, 1951; Takahashi and Ripperton, 1949). Concentrations ranging from 1.89 to 4.89 percent have been reported by Brewbaker et al, (1972) from world collections. Columbian strains had the lowest mimosine among the strains of L. leucocephala. Other species of the genus Leucaena having low mimosine are L. diversifolia (formerly known as L. buitenzorg) (2.12 %) and L. pulverulenta (1.89-2.55 %) (Brewbaker and Hylin, 1965). Carangal and Catindig (1955) reported mimosine variation in the Philippine strains ranging between 4.4 and 7.5 percent. Variation in mimosine content may be due to varietal differences, stage of maturity or type of tissue used for analysis. Takazawa and Sherman (1947) reported that young leaves contained 2-3 times more mimosine than mature leaves. Sobale et al. (1978) found that mimosine content of leaves was 15 to 20 times higher than that of stems. Mature pods had lower concentration than young pods (Kinch

and Ripperton, 1962). Mimosine in growing tips may be as high as 12 percent while the young leaves and pods may contain only 3-5 percent (Jones, 1981). Takahashi and Ripperton (1949) found no correlation between the mimosine content of the plants from dry and wet areas. Plant density and different harvesting managements also did not affect the levels of mimosine in the foliage (Guevarra et al., 1978). Mastumoto and Sherman (1948) reported that mimosine content of the forage was unaffected by drying at room temperature or dried rapidly in a force draft oven, but reduced by 40 percent when dried at high temperature. Contrary to this, Hegarty et al. (1964) observed a reduction in mimosine when air-dried at room temperature for 10 hours and the reduction was as high as 43 percent when dried at 60° C. Such reduction in mimosine in ovendried samples is partly due to its degradation to 3hydroxy-4(1H)-pyridone (DHP) through enzymic reaction (D'Mello and Fraser, 1981).

Hegarty et al. (1976) reported that ruminants can also convert mimosine to DHP, which is a potent goitrogen. In their studies with mice, Hegarty et al. (1979) showed that DHP interfered with the organic binding of iodine, rather than the iodine trapping system. Therefore the goitre was unresponsive to supplemental iodine. They confirmed that mimosine is broken down by ruminal flora to DHP, only in ruminants and opined that DHP was also involved in reducing the rate of live weight gain.

2.3.3. Presence of other Substances:

Lohan et al. (1980) reported 1.15 to 1.92 percent tannin in leucaena leaves. Jones (1979) reported 1.02 percent tannin in leucaena leaf meals (LLM) as compared to 0.013 percent in alfalfa. D'Mello and Fraser(1981) recorded 2.0 to 3.36 percent tannin. All the above researchers suggested the possibility of tannin interfering with feed quality. Kuo et al. (1982) isolated six phenolics: cis-p-coumaric, trans-p-coumaric, o-coumaric, phydroxybenzoxic, p-hydroxyphenylactic and ferulic acids as well as some unknown flavonoids from leucaena leaves. Leucaena leaf protein extracts precipitated spontaneously at room temperature due to the presence of tannin in high concentration (Telek, 1982).

Tannins adversely affect the digestibility and utilization of the nutrients present in feed (Burns and Cope, 1974). Tannins are polymeric phenolic compounds with strong protein binding properties. They occur generally in vacuoles in plant cells and are released when cell walls and membranes are broken. Tannin causes bitterness in leaves (Matches, 1973).

2.3.4. Mimosine toxicity:

2.3.4.1. Monolayer cells:

In a cell culture study, Tsai and Ling (1971) observed that mimosine and DHP inhibited cell growth and cell

division, by inhibiting DNA synthesis. These These workers also reported a decrease in the toxic effects of mimosine by the addition of Al+++ which chelated with mimosine.

2.3.4.2. Ruminants:

In Hawaii, cattle maintained partly or completely on leucaena showed an increase in growth rate and milk production, without any adverse effects (Henke, 1933; Henke et al., 1940; Henke et al., 1942; Henke and Maruyama, 1947; Takahashi and Ripperton, 1949; Kinch and Ripperton, 1962). In India, cross bred bull calves of 11-12 months age maintained exclusively on leucaena forage gained an average weight of 0.57 Kg/day compared to a group receiving 50 percent leucaena and 50 percent other roughages, which gained only 0.46 kg/day (Sobale et al., 1978). In another study, cows receiving 5 kg leucaena forage in substitution of other fodder, yielded more milk and butter fat (Damothiran and Chandrasekaran, 1982). In Mexico, cows consuming 910 Kg leucaena fodder in replacement of 75 percent rice bran or 25 percent roughage, increased their milk production (Alvarez et al., 1978a). Leucaena feeding increased the rumen mobility, flow rate of the rumen content and body weight gain, probably due to increased microbial digestion (Alvarez et al., 1978 b; Herrera et al., 1980). In Fiji, Partridge and Ranacou (1974) observed an increase in the live weight gain of steers during a period of 3.5 years when their diet contained 10-20 percent

leucaena.

Performance of steers receiving a normal diet of rice straw and concentrate (60:40) did not differ significantly from the groups which were receiving leucaena and rice straw in the ratios of 40:60 and 90:10 in the Philippines. However, the steers receiving leucaena seemed depressed (Sevilla et al., 1976).

In Mauritius <u>ad libitim</u> feeding of leucaena did not cause any adverse effect. Steers fed fresh leucaena equivalent to 2 percent of the body weight had the same growth rate as the steers receiving equivalent protein as groundnut cake (Hulman et al., 1978). Cattle in Indonesia were also not affected by mimosine toxicity (Metzner, 1976). In Western Samoa, cattle preferred leucaena over most of the common grasses (Reynolds,1978). In Queensland, Falvey (1976) reported that weaner heifers rotationally grazing leucaena up to 50 percent of the total DM gained a normal weight without showing any toxicity symptoms. Flores et al. (1979) reported an increase in milk production when the cows maintained on grass pasture were supplemented with 2 to 4 kg green leucaena forage.

There are several reports from Australia on the toxic effects of leucaena feeding. A diet composed of 80 per cent leucaena caused early parturation in heifers and the calves born had enlarged thyroids. This indicated the presence of goitrogenic compounds derived from leucaena (Hamilton et al.,1968). In other studies cattle fed leucaena had no

adverse effect on the oestrous cycle length, conception rate, gestation and milk production. However the birth weight of the calves was significantly low. The thyroids of the calves were enlarged due to hyperplasia and the levels of plasma protein bound iodine were elevated. These calves however, had normal growth rate (Donaldson et al., 1970; Hamilton et al., 1971). Steers grazing on leucaena developed a chronic toxicity, which depressed the live weight gain (Blunt and Jones, 1977). In another feeding trial of eight weeks, steers developed the hypothyroid condition rapidly. Weight gain was extremely poor and iodine supplementation was not helpful. They recovered two weeks after switching to other fodder (Jones et al., 1978). In addition to low weight and enlarged thyroid, excessive salivation and hair loss have also been noticed in Australia and Papua New Guinea (Jones et al., 1976). However in another experiment (Hutton, 1968) heifers feeding only on leucaena conceived and had normal pregnancies, which indicated the ability of the bovine rumen in some parts of Australia to convert mimosine into harmless compounds.

In Papua New Guinea, cattle grazing on leucaena and Buffel grass (50:50) gained normal weight, but continuous grazing of leucaena exclusively for 4.5 days caused probblems such as hair loss, erosion of mucosa of tongue and goitre (Holmes, 1979; 1980; 1981). Holmes et al.

(1981) reported that heifers maintained exclusively on leucaena for 23 months, suffered from cataracts, goitre and lingual epithelial ulceration. Their serum thyroxine (T4) levels were very high, but the ovarian activity was normal.

In the Congo, a steer maintained entirely on leucaena with minimum supplement initially consumed 21 kg green forage per day and gained weight. Later consumption increased to 32.8 kg/day and the growth rate also increased till 49 days of the experiment. From the 53rd day onwards, the animal started showing symptoms such as salivation, localized depilation, loss of apetite, reddish urine with a strong odor. On the 75th day, it became comatose with deep and slow respiration, and abundant salivation. After cessation of leucaena feeding and treating the animal, it returned to normal health in two weeks. In spite of its severe sickness, the kidney and liver were in normal condition (Compere, 1959). Stobbs and Fraser (1971) reported that cows grazing on leucaena produced tainted and off-flavored milk, though the quality was acceptable and the butter fat was high. Henke (1933) and Alvarez et al., (1978 a) also reported undesirable flavor of milk and they suggested that the cows should not be allowed to consume leucaena two hours before milking. This is a practice being followed for some other legumes as well.

Continuous feeding of 2 kg fresh leaves and pods caused hair drop of a buffalo calf in Australia. It

recovered when the feeding stopped (Letts, 1963).

Little and Hamilton (1971) observed a weight gain in ewes which were fed leucaena for 60 days prior to lambing and the lambs had normal thyroid glands. Contrary to this, ewes fed only leucaena had lambs with elevated levels of thyroxine, although the thyroid was normal size (Donaldson et al.,1970). Depilation in sheep was reported in India by Joshi and Upadhyaya (1976) who recommended leucaena feeding only to the extent of 40 per cent of the total feed. Bucks consuming 2.76 kg leucaena per 100 kg body weight per day showed alopecia symptoms and lost body weight (Upadhyay et al., 1974).

One of the earliest manifestations of leucaena toxicity in Australia was the depression in serum T4 levels (Jones, 1979). A linear relation between T4 levels and live weight gains was observed in cattle by Jones and Winter (1982). However, they opined that the lower serum T4 levels were not the only cause of low weight gain.

In Hawaii, goats maintained on leucaena had no difference in serum T4 level and thyroid weights from those on alfalfa. This was apparently due to the degradation of mimosine and DHP to nongoitrogenic compounds by cattle and goats, in Hawaii. They had very low blood levels of DHP, even after consuming large quantities of leucaena and did not suffer impaired thyroid activity (Jones, 1981). It seems that the micro-organisms capable of degrading DHP are

present in Hawaii, Indonesia, India and many other countries, but absent in Australia, Papua New Guinea and some parts of Africa (Personal communication, Dr.R.J.Jones,1982).

Hegarty et al.,(1979) suggested an approach of modifying the ruminal flora to metabolise DHP. Watson et al.,(1974) have reported the presence of an aerobic bacterium which can degrade DHP. However, it is necessary to introduce anaerobic bacteria to function in the rumen.

2.3.4.3. Non-ruminants:

Pigs consuming a diet consisting 20 per cent leucaena in Papua New Guinea, did not gain weight although they looked healthy. They gained weight when the diet had less than 20 per cent leucaena (Malyncz, 1974). However, Wyman et al., (1970) noticed a reduction in the gestation period when the gilts consumed a diet containing 12.5 per cent luecaena.

Rabbit does consuming 20 or 40 per cent leucaena in their daily diet had lower reproductive efficiency (Willett et al., 1947).

Mimosine feeding caused cessation of the oestrous cycle and complete infertility in rats (Hylin and Lichton, 1965). Joshi (1968) reported that 15 percent leucaena leaf meal in the diet reduced the food intake of rats. In addition to the reduced intake the females became either infertile or their fetuses were dead. The males had reduced libido or were infertile. However, their food intake increased when leucaena intake was restricted to 7.5 percent of the diet.

Based on a study on Swiss mice, Hegarty et al. (1976) concluded that non-ruminants do not metabolise mimosine to DHP. Mimosine inhibited the incorporation of (3H) thymides into DNA in the bone marrow system of the mouse (Hegarty et al., 1978), in accord with its acknowledged antimitotic activity. Mimosine also caused atrophy of hair follicles, whereas DHP when added to the diet produced goitre of hyper-plastic type without any other histological abnormalities. DHP inhibited the uptake of iodine by the thyroid, whereas mimosine was not goitrogenic (Hegarty et al., 1979).

Reports on the effect of leucaena on poultry are not encouraging. Leucaena supplemented ration produced the heaviest chicks (Dingayan and Fronda, 1950) and improved the egg color (Sandoval, 1954). Palafox (1948) recommended the feeding of only two to three gm fresh leaves per day, from the age of 14 days onwards. One day old chicks fed leucaena developed pasty vents. Their growth rate was poor when the ration contained as little as 5.0 - 7.5 percent LLM (D'Mello and Thomas, 1978). Springhall and Ross (1965 a) observed a delay in maturity, although there was no reduction in subsequent egg production. They attributed this poor performance to the toxicity of mimosine and low supply of energy.

Labadan et al.(1969) reported that a diet containing 10-20 percent LLM did not significantly depress weight gain and feed consumption, but affected the size of the comb and testes. They suspected mimosine as the cause of growth inhibition. Labadan (1969) further reported that the depression of growth was proportional to the level of LLM in the feed. High mortality was observed when the diet contained more than 40 percent LLM. However, the supplementation of the basal diet with 0.1 to 0.6 percent ferrous sulfate in the powder form improved the growth and feed conversion efficiency proportionately. In their feeding trial, washing of leucaena leaves, as reported earlier by Olaivar (1957) and Castillo et al. (1964), before feeding helped in reducing the mimosine concentration and improving growth.

Lopez et al. (1979) reported that layers receiving 5 percent LLM in the ration with or without the addition of ferrous sulfate were comparable to the controls. With 10 percent LLM diet, egg production decreased, but the addition of ferrous sulphate (0.2 to 0.4 %) of the feed improved egg production, egg yolk color, yolk diameter, shell thickness and weight of the egg shell. LLM had a pronounced effect on the egg yolk color, but it did not increase proportionately with the level of LLM. There was no difference in the color at 5 and 10 percent LLM levels (Springhall and Ross, 1965 b). Ross and Springhall (1963)

observed a depressed growth of chicks with 10 to 20 percent LLM in the diet. Addition of dry ferrous sulfate to the 10 percent LLM diet was effective but ineffective at 20 percent level in reducing the toxicity. Application of ferrous sulfate in solution form improved growth even at 20 percent LLM level, which might be due to the close contact of iron (Fe++) and mimosine to form an insoluble complex. Hathcock and Labadan (1975) also confirmed the detoxification effects of ferrous sulfate on mimosine. Acamovic and D'Mello (1980) initially observed no deliterious effects of feeding a diet containing 15 percent LLM on growing chicks. However, by supplementing this diet with ferrous sulfate (Fe++) or aluminium sulfate (Al+++), the mimosine content in the excreta was increased. This was an indication of chelate formation between mimosine and Fe++ or Al+++, which was undigested. Acamovic and D'Mello (1981) further noticed depressed growth of chicks of 7-21 days age consuming a diet containing 15 percent LLM, which was improved significantly by supplementing the diet with ferric sulfate powder (1.2 percent) and polyethylene glycol PEG (2.0 percent). They found that the carcasses of the birds consuming 15 percent LLM were significantly low in fat, as compared to the soybean control group (Acamovic and D'Mello, 1982 a). LLM diets (15 %) supplemented with PEG, Fe(+++) and cholesterol produced growth rates equal to those of the control without any improvement in fat content (Acamavic and D'Mello, 1982 b). They also found that

polyvinyl pyrrolidone (PVP) was less effective than PEG in improving the growth rate. Tsai and Ling (1973) found that the stability of the mimosine-Fe(+++) chelate was higher than that of Al(+++) and Fe(++).

Gonzalez Vargas and Wyllie (1982) observed an increase in the feed intake and live weight gain in pigs consuming 20 percent LLM diet. Their performance improved further, when this diet was supplemented with ferric sulfate (Fe+++) and PEG.

Thanjan (1967), in a detailed study, reported that growing Japanese quail (<u>Coturnix coturnix japonica</u>) receiving 20 percent LLM showed depressed growth, poor feeding efficiency, under-sized sex organs, delay in sexual maturity and high rate of mortality. However, histological studies did not indicate any adverse effect on the liver, spleen, intestine or thyroid. When the ration containing 20 percent LLM was supplemented with methionine (0.1%) and corn oil (6.0%), the birds showed significant improvement. Mimosine was traced in the excreta after feeding LLM, although a major portion was metabolized. Librojo and Hathcock (1974) traced mimosine, DHP and two other compounds, probably the further degraded forms of mimosine in the urine of the chicks maintained on LLM.

2.3.5. Toxicity due to other substances:

Ross et al. (1980) reported a drop in egg production from the seventh day after the quail started consuming a

diet with 20 percent LLM, while the other group receiving 1.0 percent pure mimosine (calculated to be equal to mimosine in the LLM diet) continued normal performance till 14 days. This indicated the presence of other toxic compounds in leucaena, in addition to mimosine. The presence of other toxic substances has been suspected also by Hathcock and Labadan (1975). Hathcock et al. (1975) reported that poor growth of chicks maintained on 12.9 to 30.0 percent LLM diet was due to the interaction between the dietary protein and leucaena. The toxicity of leucaena decreased as the dietary protein level increased.

Leucaena leaf protein concentrate recovered from leaf extract which precipitated spontaneously at room temperature (due to the presence of high tannin content), when used as a source of protein in the diet of rats affected the growth rate adversely (Cheeke and Telek, 1980).

A diet containing tannic acid from an external source, depressed the growth of chicks, but this was reversed when chemicals such as sodium carbonate, calcium hydroxide (0.02%), or non-ionic polymers such as PVP or Tween 80 were added to the diet. These chemicals bind tannic acid and prevent it from precipitating proteins (Rayudu et al., 1970). Ford and Hewitt (1979) reported that sorghum containing high tannin was poorly digested by the rats and chickens, and the supplementation of such diet with PEG-400

at 0.1 gm/gm protein improved the digestibility significantly.

Padgett and Ivey (1959) and Wilson et al. (1961) have indicated that the Japanese quail was suitable for pilot studies on poultry, because of their short life cycle and low feed requirements, and the physiological systems of which are similar to that of poultry.

2.4. Low mimosine cultivars:

Mimosine concentration being an inheritable character the experimental breeding of low mimosine lines has a promising future (Brewbaker and Hylin, 1965; Gonzalez et al., 1966). In a breeding trial, Gonzalez et al., (1967) were able to select plants with less than 30 percent of the normal mimosine level, from the segregating offsprings. In Australia, Hutton and Gray (1959) analyzed six strains belonging to three different types and observed wide variations in mimosine concentration both within and between the strains. They attributed the intra-strain variation to the difference in the maturity of the samples whereas the inter-strain variation could be due to the genetic differences. Their further studies suggested the existence of occasional plants with very high or very low mimosine concentration even within the strains and the possibility of selecting such seedlings for developing low mimosine lines.

Variation in mimosine was not correlated with height,

vigor, protein content or forage yield. So it is possible to breed low mimosine varieties with high vigor and high protein content (Brewbaker and Hylin, 1965; Gonzalez et al., 1967).

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III. THE EFFECTS OF DIFFERENT LEVELS OF IRRIGATION ON THE YIELD AND QUALITY OF LEUCAENA FORAGE

Leucaena has been identified as an important forage crop for the drought affected areas in the tropics. Although it is a drought tolerant crop, the level of soil moisture affects the growth and forage yield. This study was intended to compare forage yield and quality of three different accessions of leucaena, under different levels of soil moisture conditions.

3.1. MATERIALS AND METHODS

This experiment was conducted at the University of Hawaii's Waimanalo Experiment Station, which is located at coastal windward Oahu, elevation 21 m, longitude 157⁰ 43'E and latitude 21⁰, 20'.30" N. The soil of the experimental site is in the Waialua clay series, described as a Vertic Haplustoll, moderately drained with a pH of 6.0. The site is fairly level and stony. Corn was previously grown on this plot for several years.

The experimental design was a split-split plot with harvests as the main plot treatments, four levels of irrigations as the sub-plot treatments and three accessions as the sub-sub-plot treatments. There were three replications. The size of the sub-sub-plot was 8 x 2 m with two rows of 8 m long, spaced 1.0 m apart. The spacing between the plants was 8-9 cm, with a plant density of 192-

200 plants per plot or 120,000 to 125,000 plants/ha. One border plot of two rows of K8 was maintained between different irrigation treatments and also on the longitudinal sides of the end plots. Leucaena accessions used for sub-sub-plot treatment were K8 (Salvador type), K500 (cultivar Cunningham) and K4 (Peru type).

The site was prepared by tractor plowing and disc harrowing. No fertilizer was applied. The seeds scarified with sulfuric acid, were sown by hand at 1-2 cm depth on August 14, 1981. Seed germination was observed from the fifth day and about 90 percent establishment was recorded by tenth day after sowing. Resowing was done during the second week to cover the gaps.

Despite the application of a herbicide "Lasso", at the rate of 2.25 kg/ha which was sprayed two days before sowing, the field was heavily infested with weeds, the major species being the nut grass (<u>Cyperus rotundus</u>), within a week. It was effectively controlled twice, first time after two weeks and second time after six weeks of sowing, using 15 percent solution of "Roundup" (glyphosate). A hand wick applicator was used which facilitated the application without spreading the herbicide to leucaena seedlings.

In the initial stage, the crop was irrigated by a sprinkler irrigation system, generally at an interval of 7-10 days depending on the rainfall. A drip irrigation system was established at the end of the rainy season in

May 1982. Watering was regulated on the basis of rainfall and pan evaporation. The irrigation treatment 1 did not receive any irrigation except rainfall, while treatment 4 received the maximum irrigation, equivalent to or more than the moisture lost from the evaporation pan. Treatments 2 and 3 received one third and two third irrigation of treatment 4, respectively. This was done by laying out one, two and three monotubes per plant row of the treatments 2, 3 and 4 respectively. The drip system received water from a city water supply outlet and the pressure was regulated by valves and regulators to maintain a uniform discharge at all the points of the drip system. Watering was done once a week excepting in the rainy weeks. Pan evaporation reading was considered as an indicator, but on several occasions, the quantity of water in irrigation exceeded the evaporation to maintain a considerable difference in the quantity of water received by different treatments. The total soil moisture supply for different treatments was computed in mm by adding the quantity of irrigation water in mm and rainfall (excepting the runoff water) for the corresponding periods (Appendix I). The run off water was calculated, by using the following formulae (Cooley and Lane, 1982).

> 1. Q = 0, when $P \le 0.2$ S or, (P - 0.2 S)2(P + 0.8 S)

2. CN = $\frac{1000}{(10+S)}$

Where, Q = runoff volume in inches. P = storm rainfall in inches. S = retension parameter in inches CN = curve number.

Based on the soil type (Waialua silty clay), hydrologic soil group (B), general slope (<10 %) and crop cover (complete cover), CN for the experimental plot was worked out to be 49. Accordingly, runoff water was calculated only for the days when the rainfall was more than 2.08 inches. The summary of the weather data is presented in Appendix II.

Six months after sowing the plants had established well, attaining about 2 m height and had already set seeds. They were pruned for the first time on March 18, 1982, seven months after planting, at the height of 30 cm, using a motorised device with a circular brush cutting blade "bush-wacker". The weights of the biomass harvested from individual plots were recorded and the dry matter content was determined by drying the random samples at 105⁰ C for 4-5 days. The cutting height was raised by about 5 cm for subsequent harvests, with the idea of developing a good network of strong primary branches to produce more secondary branches. The thin, side branches which were not cut by the bush-wacker were trimmed by using a sickle. The first cutting was considered as the pruning to shape the

plants for forage production. Therefore the second harvest was referred to as the first forage harvest of the experiment. After pruning, the plants had negligible or no leaves on them, but after the first and the following harvests, the hedges were looking green, as they had retained some leaves within the network of the old primary and secondary branches.

The harvesting intervals varied from 48 to 78 days depending on the growth rate. It was decided to harvest the forage as late as possible, without losing the feed material in the form of woody stems, which are unfit for feeding. One of the rough indications considered for deciding the harvesting interval was the color of the bark at the base of the new shoot, when it started turning from green to pale green color. The other indications were the basal diameter of the stem (10 mm) and shoot length in the range of 120 to 150 cm. Hence no definite harvesting interval was set and the harvesting date was decided one or two weeks before the harvest, based on the above parameters.

To study the sprouting habits of new shoots, observations were made during one harvesting interval after the July harvest, on K8 accession, receiving only one third of the full irrigation (Treatment 2). These observations which were recorded after the harvest at weekly intervals, included the number of new shoots sprouted on the individual plants, length and diameter of the shoots which

sprouted during the first week after the previous harvest.

40

Five forage crops were harvested from March 19, 1982 to January 4, 1983 (293 days). Total forage yield was recorded immediately after harvesting every individual plot. Shoot samples of the forage from each plot were collected at random for separating into leaf and stem fractions and to analyze for dry matter, total nitrogen and mimosine in leaves and stems separately. However mimosine and nitrogen analysis were not done for all the replications of two harvests, as the variation in the previous harvests was found to be non-significant. While collecting the samples during the last three harvests, the longest shoot of each sample was picked to measure the stem length and diameter. It was presumed that this was one of the shoots sprouted during the first week after the previous harvest.

Average daily maximum temperature, minimum temperature and solar radiation were calculated for each crop (harvesting interval), to study the correlation and regression between the dependent and independent variables, using the Statistical Analysis System (SAS) on a computer.

Total nitrogen was estimated by the Kjeldahl method (A.O.A.C., 1965) and mimosine was estimated according to the procedure described in Appendix III (Brewbaker and Kaye, 1981; Megarrity, 1978).

Total protein yield for different treatments was calculated as below:

	yield	[(foliage fraction	protein		fractio	on	protein
 	=		 _				
yield			100)			

2. Protein % =[Total N % - (mimosine x .1414)] x 6.25

For statistical analysis of the data the splitsplit plot design was used and the effects of harvesting seasons, irrigation and cultivar and their interactions on the yield and quality of leucaena forage were calculated.

3.2. RESULTS AND DISCUSSION.

3. 2.1. Initial biomass yield :

In seven months after sowing, K8 had produced the highest biomass yield of 9.75 t DM/ha (Table 3.1), which was followed by K500 (8.67 t/ha) and K4 (4.84 t/ha). There was a significant difference in the yield of these three accessions.

All of them had attained a height of around 2.0 m and had set flowers and seeds. It was difficult to identify these accessions on the basis of growth from a distance.

3.2.2. New shoot sprouting and growth :

It was observed that each node of leucaena stem contained six dormant buds and few of these buds became active immediately (1-2 days) after harvesting the top

Table 3.1. Biomass yield of different leucaena accessions seven months after sowing.

	ہ ہے کا خذ گا ہے کر خا گا ہے ہے ہے جب ہے ہے ہی بن بن ہے ہے یہ ہے	
Accessions	Dry matter	yield
، چر دیا گا گا دار از کر دی به دو ی زیا گا گا گا گا گا با به این ک		
	kg/plot (16 sq m)	t/ha
K8	15.60 a	9.75 a
K500	13.87 b	8.67 b
K4	7.75 c	4.84 C

Means followed by the same letter are not significantly different from each other at 0.05 probability level, according to Duncan's multiple range test.

ANOV.

Sources	d.f.	Mean squares	F				
Replication	11	4.98					
Treatments	2	204.36	32.82 **				
Error	22	6.23					
Replication Treatments	11 2	4.98 204.36	32.82 **				

** Significant at 0.01 probability level.

(Table 3.2). Normally one or two buds sprouted per node although the sprouting of four shoots was not uncommon. One week after the harvest, the new buds sprouted, which accounted for 25 percent of the total new sprouts that emerged during the harvesting interval. Second week was the most active week for the sprouting of new buds, which accounted for 60 percent of the total. Sproutings decreased to 10 percent in the third week and to five percent in the fourth week. Thereafter no new buds sprouted till the next harvest.

There was a considerable variation in the growth rate of the new shoots. Only few shoots had a faster rate of growth, some others grew slowly, whereas many shoots stopped stem elongation, after they had 2-5 leaves. One of the buds which sprouted in the first week, generally grew into the tallest shoot, at the time of the harvest.

In K8, it was found that the most active period of stem elongation was during the third and fourth weeks (Figure 3.1). The average shoot length and diameter at weekly intervals are presented in Table 3.2. The data were recorded during the summer months. The rate of stem elongation varies with the season, moisture level and accessions.

3.2.3. Forage yield :

The forage yield was affected by the variation in the harvesting season, irrigation and accessions (Table 3.3,

accession, grown under irrigation for forage production, during the summer season. *						
	eeks New shoots ast sprouted **		Stem diameter			
	percent	cm				
1	25	3.0	1.7			
2	60	14.0	3.0			
3	10	45.5	4.8			
4	5	84.0	7.0			
5	-	120.0	9.0			
6	-	151.0	11.6			

Table 3.2. Weekly shoot growth measurements of leucaena K8

* Observations recorded after the harvest in July, 1982.

** Percentage of the total shoots sprouted during the harvesting interval.

4.4

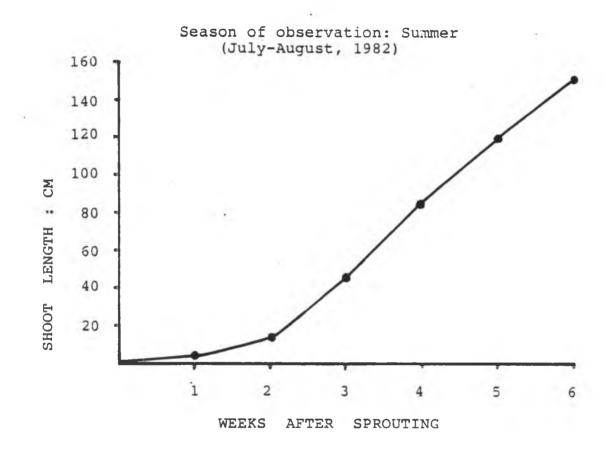


Figure 3.1. Weekly shoot growth of leucaena K8 accession maintained under irrigation, for forage production.

Treatments	Ассе	ssio	ns Me	an yield
	K8	K500	K4	
Harvests (days)	kg Di	M/16 sq m	plot/harv	est
<pre>1. May 18 (61) 2. July 13 (56) 3. Sept. 1 (50) 4. Oct. 19 (48) 5. Jan. 4 (78)</pre>	4.16 8.81 6.92 4.64 5.39	4.63 8.12 6.61 4.95 5.90	2.83 5.40 5.10 4.06 5.10	3.87 c 7.44 a 6.21 b 4.55 c 5.46 b
Total 293	29.92	30.21	22.49	
Harvest dates 1. May 18	_	_	m plot/da 0.046	
2. July 13 ·	0.156	0.145	0.097 0.102 0.084 0.065	0.133 a
Means	0.106 a	0.106 a	0.079 b	
Irrigation (mm/day)	kg Di	M/16 sq m	plot/harv	est
2. 4.438 3. 5.050	6.03 5.99	5.76 6.07	4.00 4.50 4.08 5.40	5.43 ab 5.48 ab
Means	5.99 a	6.04 a	4.49 b	

Table 3.3. Effects of harvesting season, irrigation and different accessions on leucaena forage yield.

Treatment means followed by the same letter are not significantly different from each other at 0.05 probability level, according to Duncan's multiple range test.

Table 3.3. (Continued) Effects of harvesting season, irrigation and different accessions on leucaena forage yield.

ANOV.

		*		
Sources	d.f.	Mean squares	F	
Replication Harvest Error A Irrigation Harv X Irr Error B Accessions Harv X Acc Irr X Acc Harv X Irr X Acc Error C	2 4 8 3 12 30 2 8 6 24 80	0.452 70.435 3.312 4.605 1.499 1.005 46.120 4.821 2.094 0.788 0.655	4a ± + 4a /	**
<pre>* and ** indicate probability levels</pre>		nificance at 0.05 and tively.	0.01	

3.4). Crop harvested in July yielded the highest dry matter of 7.44 kg/plot (4.65 t/ha). The per day yield was also high in July crop, which was closely followed by the September crop, without any significant difference. The crop harvested in October yielded lower, but the crops of May and January harvests yielded the lowest. The difference in the yield due to variation in the harvesting seasons was highly significant.

Due to heavy rainfall almost throughout the year excepting a few months, the difference in the levels of irrigation was small, ranging from 3.8 mm/day to 5.7 mm/day. Even then, the control treatment which grew only on rainfall had the lowest yield as compared to the treatment which received the highest level of irrigation and the difference was significant. The other two treatments receiving lower levels of irrigation were intermediate in yield as well, but the forage yield did not differ significantly from that of the lowest or the highest yielding treatments.

Among the accessions K500 yielded the highest, which was closely followed by K8, without any significant difference. K4 yielded 25 percent lower than the others. Based on the yield of five harvests (293 days) during the first year, the annual DM yields of K8, K500 and K4 accessions, under fairly well distributed rainfall conditions (1397 mm/yr) ware 21.87, 24.73 and 15.88 t/ha/yr respectively (Figure 3.2).

Table 3.4	. The	forage	yield	of	liffe	rent	accessions	of
leucaena	under	differ	ent le	evels	of	irrig	ationduring	the
	f:	irst ye	ear af	ter	estab	lish	nent.	

.....

	tments r received	Ассе К8	ssion K500	s : K4	[rrigati means	on
	mm/yr		tons DM	/ha/yr		
1.	1396.7	21.87	24.73	15.88	20.83	b
2.	1620.0	23.95	22.86	17.87	21.57	ab
3.	1843.1	23.80	24.12	16.22	21.38	ab
4.	2065.5	25.45	24.27	21.43	23.72	a
		و برده خله خله حله حله حله حله خله خله خله خله				
Mean	S	23.77 a	24.00 a	17.86 b		

Treatment means followed by the same letters are not significantly different from each other at 0.05 probability level, according to Duncan's multiple range test.

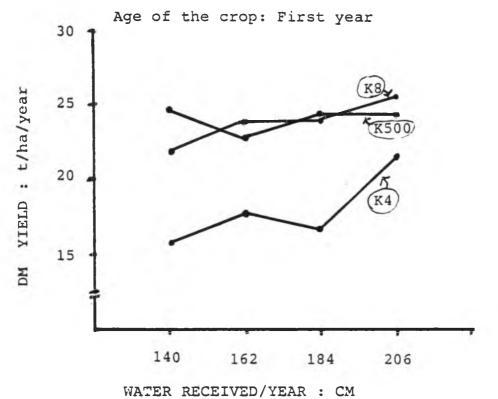


Figure 3.2. Forage yield of different leucaena accessions under different levels of irrigation.

When the crop was fully irrigated (2066 mm/yr), the DM 51 yield increased by 16 and 26 percents respectively in K8 and K4, but there was no improvement in yield of K500 (Table 3.4). The interactions between irrigation and accession as well as harvest and accession were significant.

There was a high correlation between the yield and maximum temperature, minimum temperature, solar radiation (Table 3.5). The step-wise linear regression model included only three variables such as solar radiation, irrigation and accession for formulating the following equation at 0.05 probability for predicted value of yield.

Y = 0.028 + 0.0003 S + 0.0365 I - 0.0136 A

where Y = Predicted yield

- S = Solar radiation
- I = Irrigation
- A = Accession

The above equation had a coefficient of determination (r^2) of 0.67. The r^2 had improved slightly (0.69) when a quadratic model was used, with the following equation.

Y = .0698 - .0136 A + .00000036 SSQ + .00000022 MSI

Where, A = Accession,

SSQ = Solar radiation square

MSI = Minimum temp X Solar radiation X irrigation Among the variables such as solar radiation, maximum

Table	3.5.	Correlation	matrix	of	different	variables	affecting
			leu	lcae	ena yield.		

Variables	MAX 1	MIN 2	SOL 3	IRR 4	AÇC 5	YLD 6	FOL 7	LEN 8
l.Maximum temp.							•	
2.Minimum temp.	.97**	ł						
3.Solar radiat.	.79**	.78	**					
4.Irrigation	03	.01	.06					
5.Accessions								
6.Yield	.54**	.58*1	• .73*	* .24**	31**			
7.Foliage fract.	.16*	.08	04	.08	•55**	25**		
8.Stem length	.81**	.78**	.80**	47**	27**	.65**	46**	
9.Stem diameter	.18	.21*	.20*	08	.06	.19*	22* .	27**

and minimum temperatures, which affect the seasonal variation, solar radiation seems to be the most important variable because of the highest correlation (r=0.73). In the present solar radiation range between 170.36 and 410.57 cal/sq cm/day, the relationship between the solar radiation and yield was linear. Eriksen (1978) and Ferraris (1979) also observed a linear relationship between solar radiation and leucaena dry matter yield.

Although the regression equation did not include the maximum or minimum temperature, high correlation between the yield and temperatures as well as between solar radiation and temperatures, explain the effects of maximum and minimum temperatures on the yield. Another reason why temperatures were not included in the regression equation could be their narrow fluctuation throughout the experiment, which was between 25.69 to 28.90⁰C for maximum temperature and between 20.19 to 23.34⁰C for minimum temperature. It may be useful to use the daily mean temperature to study the relationship between temperature and yield. Lower yield in the first harvest could be due to lack of nodes to produce more shoots or lack of old leaves on the stump to start carbohydrate production immediately, as observed by Mendoza et al. (1975).

The crop did respond to irrigation, but it was not dramatic, because of the unusual high rainfall, during the period of this study. Kinch and Ripperton (1962) had reported that a high yielding leucaena crop needed an

average moisture of 3.01 mm/day and in this trial the control treatment, which was not irrigated had received an average of 3.87 mm/day. However as the distribution was not uniform throughout the year, growth of the control treatment was probably affected, which was reflected in the yield.

Although many researchers had indicated a nonsignificant difference in the yield between Salvador and Peru (Partridge and Ranacou, 1973; Savory, 1979; Shih and Hu, 1981) or Cunningham and Peru (Ferraris, 1979), the difference in the yield between Peru and Salvador or Cunningham was highly significant. The results were also contradictory to the report from Australia, where Peru had out-yielded Salvador type (Hutton and Bonner, 1960). This variation might be due to the difference in the accessions of Peru type used in the trial. In the present trial under low moisture conditions Cunningham yielded more than K8 but under full irrigation the reverse was true.

3.2.4. Foliage fraction :

The ratio between the foliage and total leucaena yield, referred to as the foliage fraction, is an important character to evaluate the quality of the forage. There was a variation in the foliage fraction of different accessions. K500 and K4 had high foliage fraction compared to K8 (Table 3.6).

The growing season affected the foliage fraction of

Treatments		Ассе	s s i o	n s		
		K8	K500	K4 Me	eans	
Harvests (day	-s) -	f	oliage :	DM yield -	******	
1. May 18 (2. July 13 (3. Sept. 1 (4. Oct. 19 (5. Jan. 4 ((56) 0 (50) 0 (48) 0).58).58).62	0.62 0.63 0.65	0.62 0.62 0.66 0.66 0.67	0.60 0.61 0.62 0.64 0.64	c c b a a
Irrigation (mm/day)						
1. 3.827 2. 4.438 3. 5.050 4. 5.659	C C).59).59	0.64 0.64	0.64 0.65 0.64 0.63	0.63 0.63 0.63 0.62	a a a
Means	C).59 b	0.64 a	0.64 a		

Table 3.6. Effects of harvesting season, irrigation and different accessions on the foliage fraction of leucaena forage.

Treatment means followed by the same letter are not significantly different from each other at 0.05 probability level, according to Duncan's multiple range test.

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Table 3.6. (Continued) Effects of harvesting season, irrigation and different accessions on the foliage fraction of leucaena forage.

ANOV.

			_
Sources	d.f.	Mean squares	F
Replication	2	0.00060	
Harvest	4	0.01217	14.37 **
Error	8	0.00085	
Irrigation	3	0.00051	0.65
Harv X Irr	12	0.00058	0.73
Error B	30	0.00080	
Accessions	2	0.04411	109.06 **
Harv X Acc	8	0.00093	2.29 *
Irr X Acc	6	0.00031	0.77
Harv X Irr X Acc	24	0.00077	1.91
Error C	80	0.00040	

* and ** indicate the significance at 0.05 and 0.01 probability levels, respectively.

the forage. Crops harvested in May and July had lower foliage fraction as compared to the crop of September, which increased further in October and January. The interaction between harvest and accessions also affected the foliage fraction significantly. There was no impact of irrigation on the foliage fraction.

The correlation between maximum temperature and the foliage fraction was low (r=0.16) and none of the other independent variables had a significant correlation (Table 3.5). It had positive correlation with yield (r=0.55) and negative correlation with stem length (r=-0.25).

The linear regression equation for the predicted value is given below.

Y = -.18 - .00017S + .0257X - .01839N + .00266 I + .0244 A

Where, S = Solar radiation

- X = Maximum temperature
- N = Minimum temperature
- I = Irrigation
- A = Accessions

The coefficient of determination was low $(r^2 = 0.50)$.

Osman (1981 c) observed a variation in foliage fraction from 52 to 65 percent depending on the harvesting intervals in Salvador type. However the foliage fraction of K8 was as high as 0.59, probably due to shorter harvesting intervals, which reduce the stem fraction. Variation due to season, although not significant, might have affected the foliage fraction due to effect on stem elongation. The effect of higher maximum temperatures seemed to be positive on the foliage fraction. It was not possible however to interpret whether the increase was due to an increase in the leaf area, leaf thickness or number of leaves. As the r^2 was very low, it was difficult to comment on the accuracy of the regression equation.

3.2.5. Foliage color :

During the initial stages of growth, leaves of K500 were dark green compared to others, but the difference was not noticable in new flushes after pruning. The basal leaves of K8 were turning more yellow than the others at the time of harvest.

It was presumed that due to narrow branching angle of K8, the basal leaves were deprived of solar radiation which resulted in early senescence.

3.2.6. Stem elongation :

Although the stem elongation rate was not uniform throughout the growing period, the data were converted into mm elongation/day for easy comparision (Table 3.7). Among the three harvests the rate of stem elongation was significantly high for the crops harvested in September (28.6 mm/day) and October (29.5 mm/day) in comparison to the crop harvested in January (18.8 mm/day). The average stem length of the the crops harvested in September and

Treatments Accessions					
	K8 K500	K4 Mean length	n		
Harvests (days)	mm at	harvest			
4. Oct. 19 (48)	1485.8 1465.8	1254.2 1431.9 a 1300.8 1416.9 a 1143.3 1200.3 b	a		
Mean length at harvest	1438.6 a 1377.8 a	a 1232.8 b			
Harvest dates	mr	m/day			
3. Sept. 1 4. Oct. 19 5. Jan. 4		25.128.627.129.517.918.8	a		
Irrigation (mm/day)					
1. 3.827 2. 4.438 3. 5.050 4. 5.659	27.7 25.0	24.1 26.6 25.6 26.1	b a		
Mean length/day	27.3 a 26.2	a 23.4 b			

Treatment means followed by the same letter are not significantly different from each other at 0.05 probability level, according to Duncan's multiple range test.

Table 3.7. (Continued) Effects of harvesting season, irrigation and different accessions on the stem elongation of leucaena forage crop.

ANOV.

		و با ۵۵ ن ن ن ن و و ب و و و ن و ۵ ن ۵ ن ۵ ن	
Sources	d.f.	Mean squares	F
Replication	2	0.0656	
Harvest	2	12.8587	114.48 **
Error A	4	0.1123	
Irrigation	3	0.1994	1.55
Harv X Irr	6	0.1397	1.08
Error B	18	0.1290	
Accessions	2	1.5152	22.84 **
Harv X Acc	4	0.1773	2.67 *
Irr X Acc	6	0.1724	2.60 *
Harv X Irr X Acc	12	0.0784	1.18
Error C	48	0.0663	

* and ** indicate the significance at 0.05 and 0.01 probability levels, respectively.

October were 143.19 cm (48 days) whereas that of January crop was only 120.03 cm, although it had the longest growing period (78 days).

The variation due to accessions was highly significant, K8 and K500 were growing longer than K4. The interactions between accessions and harvests as well as accessions and irrigation were significant. There was no significant effect of different levels of irrigation.

The rate of stem elongation had a very high positive correlation with the maximum temperature, minimum temperature, solar radiation and the forage yield, and negative correlation with irrigation, foliage fraction and stem diameter. The following regression equation $(r^2 =$ 0.73) was calculated.

Y = 15.966 + 0.230 X - 0.199 AWhere, X = Maximum temperature

A = Accession

The results are in complete agreement, with the previous observations (Kinch and Ripperton, 1962). The stem length is an important parameter useful for determining the harvesting interval. The variation in the rate of stem elongation due to season on accessions indicates that the harvesting interval should be short in summer and longer in winter. Similarly K8 and K500 accessions should be harvested more frequently than K4. Although the shading influenced the stem elongation (Egara

and Jones, 1977; Erikson, 1978), the crop harvested in January had the lowest rate of stem elongation. This was probably because the average solar radiation per day was very low (192 cal/sq cm/day, Appendix II), which might have affected the photosynthesis as well as the stem length.

3.2.7. Stem diameter:

There was a wide variation in the basal stem diameter of the tallest shoots at the time of harvesting the crop (Table 3.8). The mean stem diameter of different accessions at the time of harvest were 10.74, 9.71 mm and 9.28 mm for K8, K500 and K4 respectively. However, when the stem diameter was converted to per day basis, there were no significant differences due to any of the variables such as harvesting season, irrigation and accessions.

Stem diameter was positively correlated with stem length, yield, minimum temperature and solar radiation and negatively correlated with forage fraction (Table 3.5). However, the correlation coefficient (r) was quite low although significant at 0.05 probability level. Neither a linear nor a quadratic regression equation could be developed, as the best equation had the r^2 value of 0.046.

As the observations were taken on the longest shoot in the sample, it could be seen that all the three accessions had the same capability of producing stems of same diameter. Therefore the stem diameter alone could not give any indication about the vigor or yield of the

Table 3.8.	Effects of accessions	harvesting	season, irrigation and
different		on the stem	diameter of leucaena
		forage cr	op.

		s's i o n		
Treatments	ACCE	SSION	5	
200	K8	K500	K4	Mean dia.
Harvests (days)		- mm at ha	rvest	
3. Sept. 1 (50) 4. Oct. 19 (48) 5. Jan. 4 (78)	10.33	9.17	9.43	9.64 a
Mean diameter	10.74 a	9.71 b	9.28 b	
Harvest dates	و میں جوہ جو جو	mm/da	y	
	0.021	0.023 0.019 0.015	0.020	0.020 a
Irrigation (mm/day)				
	0.019 0.021 0.021 0.020		0.017 0.015 0.019 0.019	0.018 a
Means	0.020 a	0.019 a	0.018 a	

Treatment means followed by the same letter are not significantly different from each other at 0.05 probability level, according to Duncan's multiple range test.

Table 3.8. (Continued) Effects of harvesting season, irrigation and different accessions on the stem diameter of leucaena forage crop.

ANOV.

Sources	d.f.	Mean squares	F
ه هر به ما ما به به ما مر مر ما			
Replication	2	0.00031	
Harvest	2	0.00109	2.72 ns
Error A	4	0.00040	
Irrigation	3	0.00033	1.00
Harv X Irr	6	0.00036	1.08
Error B	18	0.00033	
Accessions	2	0.00037	0.95
Harv X Acc	4	0.00041	1.07
Irr X Acc	6	0.00046	1.20
Harv X Irr X Acc	12	0.00038	1.01
Error C	48	0.00038	

total crop. Similarly, the low correlation between the stem length and diameter (r = 0.27) indicated the relationship was weak. Stem diameter alone might not be a good critrion for determining the stage of forage harvest.

3.2.8. Total Nitrogen:

The total nitrogen content was in the range from 3.95 to 5.00 percent in the foliage and 1.12 percent to 1.28 percent in stems of leucaena (Table 3.9). The variation was due to the harvesting season and the accessions. The foliage harvested in January had higher total N than that of the July and September harvests. Among the accessions, the foliage of K8 foliage had significantly low N as compared to K500 and K4. Different levels of irrigation did not affect the foliar N content.

Total N content of the foliage had a significant positive correlation with harvest (r=0.37), accessions (r=0.32), mimosine content (r=0.35) and a negative correlation with the stem length (r=-0.47). The total N of stems varied, mainly due to the change in the harvesting seasons. All the three accessions had almost the same N content in the stem (1.19 to 1.22 percent).

Seasonal variation in the total N was in confirmation with the previous findings. Ferraris (1979) observed negative correlation between the total foliar N and temperature, as well as solar radiation. The crop Table 3.9. Effects of harvesting season, irrigation and different accessions on the total nitrogen content of leucaena forage.

1. Foliage.

Treatments	Ассе	s s i o	n s	
	K8	K500	K4	Mean N.
Harvests (days)		N in p	ercent	
2. July 13 (56) 3. Sept. 1 (50) 5. Jan. 4 (78)	4.165 3.947 4.588	4.552 4.210 4.790	4.464 4.286 4.995	4.394 b 4.148 b 4.791 a
Irrigation (mm/day)				
1. 3.827 2. 4.438 3. 5.050 4. 5.659	4.103 4.197 4.166 4.468	4.546 4.546 4.460 4.518	4.416 4.541 4.519 4.851	4.355 a 4.428 a 4.382 a 4.612 a
Means	4.233 b	4.517 a	4.582	a

Treatment means followed by the same letter are not significantly different from each other at 0.05 probability level, according to Duncan's multiple range test.

Table 3.9. (Continued) Effects of harvesting season, irrigation and different accessions on the total nitrogen content of leucaena forage.

ANOV.

Sources	d.f.	Mean squa	res	F	
Replication Narvest	2 2	- 0.134 3.797		69.40	**
Error A	4	0.055		07.40	
Irrigation	3	0.364		2.89	
larv X Irr Error B	6 18	0.087 0.126		0.69	
Accessions	2	1.236		12.68	**
larv X Acc	4	0.067		0.68	
Irr X Acc	6	0.101		1.03	
Harv X Irr X Acc Error C	12 48	0.092 4.679		0.94	
			-		
Stems.		Total N &			
2. Stems. Treatment		Total N %			
		Total N %			
Treatment Harvest dates		Total N %			
Treatment Harvest dates 2. July 13 3. Sept. 1		1.22			
Treatment Harvest dates 2. July 13		1.22			
Treatment Harvest dates 2. July 13 3. Sept. 1		1.22 1.12 1.28			
Treatment Harvest dates 2. July 13 3. Sept. 1 5. Jan. 4		1.22 1.12 1.28			
Treatment Harvest dates 2. July 13 3. Sept. 1 5. Jan. 4 Mean		1.22 1.12 1.28			
Treatment Harvest dates 2. July 13 3. Sept. 1 5. Jan. 4 Mean Accessions K8 K500		1.22 1.12 1.28 1.21 1.21			
Treatment Harvest dates 2. July 13 3. Sept. 1 5. Jan. 4 Mean Accessions K8		1.22 1.12 1.28 1.21			

harvested in January had grown under an average low temperature and low solar radiation, as compared to the other two crops. The significant correlation between total foliar N and harvest confirms this relationship. As foliar N was negatively correlated with the stem length, it could be possible that the foliar N was low in accession K8, where a portion of the leaf nitrogen might have been drawn to meet the increased rate of stem elongation. Early senescence of basal leaves of could be the other reason for low foliar nitrogen in K8. This would suggest that K8 should be harvested more frequently than the others.

3.2.9. Mimosine Content:

The mimosine content varied from 2.15 to 4.45 percent in foliage and 0.33 to 0.48 percent in stems of leucaena. This variation was due to the variation in the growing season and the difference in the mimosine content of the accessions. The mimosine content varied with the total N The foliage harvested in January had (r=0.35).significantly high mimosine content, compared to the other two crops harvested during the summer. Different levels of irrigation did not affect the mimosine content. Among the three accessions the foliar mimosine content of K500 was significantly lower than that of K8 and K4. These findings were in agreement with the earlier reports. Takahashi and Ripperton (1949) found no difference in the mimosine content of the crops of wet and dry areas. Low mimosine content of K500 was reported by Hutton and Gray (1959).

Table 3.10. Effects of harvesting season, irrigation and different accessions on mimosine content of leucaena.

Treat	tments		Ассе	ssion	n s	Mean	
			K8	K500	K4	mimosine	9
Harve	ests (da	ays)		percent m	imosine ·		
3. Se	uly 13 ept. 1 an. 4		3.00 2.85 4.45	2.60 2.15 3.49	3.26 2.65 3.91	2.95 2.55 3.95	b c a
Irriq (mm/d	gation day)						
3.	3.827 4.438 5.050 5.659	·	3.67 3.18 3.29 3.60	2.83 2.55 2.64 2.96	3.36 3.08 3.08 3.57	3.29 2.94 3.00 3.38	a a a
Mean	Mimosir	ne	3.43 a	2.75 b	3.27 a		

1. Foliage.

Treatment means followed by the same letter are not significantly different from each other at 0.05 probability level, according to Duncan's multiple range test. Table 3.10. (Continued) Effects of harvesting season, irrigation and different accessions on mimosine content of leucaena.

ANOV.

Sources	d.f.	Mean squares	F
ہ ہا کہ نہ جا چا زیا ہے جا ہے کے دو میں وہ ہے کے لیے ک	ی دور دی دو		
Replication	2	0.339	
Harvest Error A	2 4	18.692 0.102	183.04 **
Irrigation	3	1.231	1.91
Harv X Irr	6	0.405	0.63
Error B	18	0.644	
Accessions	2	4.654	11.59 **
Harv X Acc	4	0.505	1.26
Irr X Acc	б	0.047	0.12
Harv X Irr X Acc	12	0.181	0.45
Error C	48	19.266	
<pre>** indicates the</pre>	significance	at 0.01 probab	bility level.
2. Total mimosir	e in stems.		

Treatments	Total	mimosine %
Harvest dates		
2. July 13 3. Sept. 1 5. Jan. 4		0.41 0.37 0.46
Means		0.41
Accessions		
K8 K500 K4		0.48 0.33 0.43
mean		0.41

3.2.10. Protein Yield:

The protein yield in the first year after establishment ranged from 3.40 to 4.58 t/ha/yr (Table 3.11). Although the total dry matter yield of K8 and K500 did not differ significantly, K500 yielded 15 percent more protein. Among the treatments receiving different levels of irrigation, crop receiving highest level of irrigation yielded 19 percent more protein, as compared to the unirrigated crop.

The protein yield seems to be a realistic parameter, to compare different treatments, than the DM yield alone, if the crop is valued on the basis of its nutritive value. It is particularly important for leucaena, where the dry matter yield increase with the delay in the harvesting interval, but this increase might not increase the protein yield proportionately, because of the reduction in the leaf fraction of the forage due to a prolonged harvesting interval.

3.3. SUMMARY.

A forage yield trial of leucaena was conducted at the Agricultural Experiment station of the University of Hawaii at Waimanalo, Oahu, to study the effect of different levels of irrgation on the forage yield and quality of three leucaena cultivars. The experiment was laid out in a split-split plot experimental design, using harvests as the Table 3.11. Effects of irrigation and different accessions on the total protein yield of leucaena forage during first year afterthe establishment.

Treatment	Pro	tein	Leaf:St	emratio	Forage	Protein
	Leaf	Stem	Leaf	Stem	yield	yield
Irrigation	- perc	cent -			t/ha	a/yr
mm/day						
1. 3.827	24.31	7.18	.626	.374	20.83	3.729
2. 4.438	25.08	7.18	.626	.374	21.57	3.765
3. 5.050	24.74	7.18	.625	.375	21.38	3.890
4. 5.659	25.84	7.18	.619	.381	23.72	4.443
Accessions						
K8	23.42	7.14	.593	.407	23.77	3.992
K500	25.80	7.33	.636	.364	24.00	4.578
K4	25.75	7.06	.641	.359	17.86	3.401

main plot treatment, irrigation as the sub plot treatment and the cultivars as the sub-sub plot treatment. There were four irrigation treatments with three levels of irrigation and a control. The highest level of irrigation treatment was a full irrigation at weekly interval, to compensate for the evapotranspiration loss moisture, whereas the other two treatments received one third and two third of the full irrigation and the control treatment was maintained only by rainfall. The cultivars used were accessions K8, K500 and K4. There were five harvests and three replications.

The trial was established by direct sowing of the scarified leucaena seeds on August 14, 1981. All the plots received uniform irrigation for seven months, till their establishment. The plants were pruned at 30 cm height for the first time after seven months. A drip irrigation system was established to provide different levels of irrigation. The cutting height was raised by 5 cm during the subsequent harvests. The harvesting interval fluctuated from 48 to 78 days, depending on the growth. The criteria used for deciding stage of harvest were, length of new shoots (120-150 cm), stem diameter (10 cm) and the color of the stem at the base of the new shoots (turning from green to pale green). After the pruning in March, 1982 till January, 1983, there were five forage harvests in 287 days. During the initial stage of establishment, the rate of growth was highest in K8,

followed by K500 and K4, which was reflected on the biomass yield, at pruning. K8 yielded twice as much as K4, but the difference was not wide between K8 and K500,

The new shoots started sprouting within 1-2 days after the harvest and in two weeks about 85 percent of the sproutings were completed. The rate of stem elongation was maximum during the third and the fourth weeks. These observations were made only on K8 during the summer season.

The forage yield varied significantly due to the harvesting season, levels of irrigation and the accessions. The growth rate was highest during the summer and hence the harvesting interval was shorter. The yield was higher in summer in spite of the shorter harvesting interval. Solar radiation, maximum and minimum temperatures which caused the seasonal variation, had a significant correlation with the yield. In Hawaii, where the temperature variation between the night and day as well as between the seasons was very narrow, the solar radiation had the greatest influence on the forage yield. The treatment which did not receive irrigation, had the lowest yield compared to the treatment which received the highest level of irrigation. The yield of other irrigation treatments did not differ significantly, although the yield was higher than that of control. This was probably because of the heavy rainy season which provided adequate moisture to the control treatment as well and hence the difference in the level of irrigation was marginal. Among the cultivars, accession K8

and K500 yielded significantly higher than K4. The protein yield of K500 was more than K8 by 15 percent. K8 was low in foliage fraction and foliar nitrogen as compared to K500 and K4. Low foliar N content might be due to their faster rates of stem elongation and early senescence of lower The mimosine content was low in K500 as compared leaves. to K8 and K4. There was a significant correlation between the nitrogen and mimosine content, and their variation in the foliage was also seasonal. Foliage harvested in winter was high in both nitrogen and mimosine, as compared to that of summer. The levels of irrigation did not affect either the foliage fraction or the nitrogen and mimosine content. It was concluded that K500 (Cunningham) was superior for forage production as compared to K8 (Hawaiian gaint) and K4 (Peru).

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IV. EFFECT OF LEUCAENA LEAF MEAL FEEDING ON GROWING JAPANESE QUAIL.

The objective of this feeding trial was to study the toxicity problems of leucaena in poultry, so that suitable corrective measures can be taken while using leucaena leaf meal (LLM) in poultry ration. As Japanese quail (<u>Coturnix</u> <u>coturnix japonica</u>) have a similar physiological system to the chicken, but a small body size with short life cycle, Japanese quail was used for conducting this trial.

4.1. MATERIALS AND METHODS.

The feeding trial was conducted at the University of Hawaii, Upper Manoa campus facilities for four weeks, using 10-day-old Japanese quail. The quail were obtained from the University of Hawaii, Livestock Research Station, Waialee, Oahu, where they were hatched and transported in cartons to Manoa three days before the trial was started. A mortality of 35 percent was observed over the next three days, probably due to exhaustion and suffocation during transportation. Most of the dead birds were small in size compared to the survivors which weighed in the range of 10 to 26 gm at 10 days age. They were divided into 18 units of approximately equal weight, with seven birds per unit. A battery brooder with 18 independent compartments was used to house the birds. Each compartment had a small heating section with the temperature controlled around 37 ⁰ C and a fluorescent light source provided light throughout the trial. The birds had free access to feed and water.

The experiment was set up as a randomized complete block design with three replications and six treatments. Three different types of leucaena leaf meals (LLM) were used in the trial. These were leucaena K8 tender leaf meal (TLM), leucaena K8 matured leaf meal (MLM) and leucaena K156 (Leucanena diversifolia) leaf meal (LM). TLM which had high mimosine and low tannin was prepared by picking the shoot tips and tender leaves of K8 accession and MLM which had low mimosine and high tannin was produced from the mature leaves of K8. LM which was low in both mimosine and tannin was produced from the harvested forage, without any specific selection of leaves. These leaf meals were produced by air-drying the green foliage and grinding it to 2 mm or less. The nutritive values of these leaf meals is presented in Table 4.1. The composition of the experimental diets is presented in Table 4.2. The above three LLMs constituted 15 percent of the three dietary treatments. Another diet was composed of MLM diet and polyvinyl pyrrolidone (PVP) in the ratio of 99:1. Two other diets were introduced without LLM, as positive and negative controls. The positive control was a standard diet whereas the negative control had 15 percent alfalfa leaf meal (ALM), which is roughly comparable to LLM in composition but without mimosine and tannin. The proximate

Table 4.1. The proximate analysis of different leaf meals
used in the experimental diets.Type of leaf meal Dry matter Protein Mimosine Tannin------ percent ------
percent (TLM)K8 tender leaf
meal (TLM)K8 mature leaf
meal (MLM)92.8
(LM)K156 leaf meal
(LM)93.2
(LM)Alfalfa leaf
meal (ALM)90.4
20.4520.45
-
0.63

Protein = (Total Nitrogen X 6.25) - mimosine.

Protein, mimosine and tannin were calculated on dry matter basis.

Ingredients Positive Negative TLM * MLM * LM * PVP * control control ----- percent -----Choline0.1600.1600.1600.1600.1600.1600.1600.160CaCO33.0003.0003.0003.0003.0003.0002.970Def. Phos.3.5003.5003.5003.5003.5003.5003.600Methionine0.1000.1000.1000.1000.1000.099Meat & Bone Meal5.0005.0005.0005.0005.0004.950Tuna Meal5.0005.0005.0005.0005.0004.950Min. mix (80080)0.3000.3000.3000.3000.3000.3000.297Vit. mix0.0380.0380.0380.0380.0380.0380.038Corn39.5031.1032.8032.2031.9031.88Soy Meal37.4033.8032.1032.7033.0032.37Tallow0.6993.0002.7602.8402.9702.812Cellulose2.310-----0.2400.1600.0300.158Alfalfa Meal3.00015.00----------15.0015.0014.85Polvvinvl----------15.0015.0015.0014.85 Leucaena Meal Polyvinyl ----- 1.000 ----Pyrrolidone (PVP) Proximate analysis ----- percent on dry matter basis ------Crude protein
Ether extract28.83
3.7829.27
6.3029.63
5.9029.26
6.7829.37
6.5629.41
6.55Ash12.3013.0812.2012.8313.0112.70Mimosine----1.100.440.300.43Tannin0.0190.0950.2250.6450.1730.639Dry matter89.0689.3089.4389.9389.7489.80 ----- Cal/kg Energy (cal/kg) 2600 2600 2600 2600 2600 2574 Total energy was calculated by using the values suggested by Tanaka (1982). * TLM = Leucaena K8 tender leaf meal MLM = Leucaena K8 matured leaf meal LM = Leucaena K156 leaf meal PVP = MLM diet + PVP (99:1)

Table 4.2. Composition of the experimental diets.

analysis of the feeds was carried out according to A.O.A.C.(1965) methods. Total nitrogen of the feeds and excreta was estimated by Kjeldahl method and protein was calculated as below:

Protein = (Total N X 6.25) - Mimosine

Mimosine was analyzed by colorimetric method as described in Appendix III (Brewbaker and Kaye, 1981; Megarrity, 1978), which records the total quantity of mimosine and DHP. The electrophoresis technique was used to estimate the quantity of mimosine and DHP in the droppings. Tannin analysis of the leaf meal was carried out by the vanillin-HCl method (Burns, 1963), and expressed in percent catechin equivalent.

The quail of each unit were weighed collectively at the end of each week. Feed consumption, water intake and weight of the droppings were also recorded weekly. Feed spilled in the dropping trays were accounted for as wastage. The final weights of the quail were obtained by weighing the birds individually by sex.

Two birds from different treatments died during the course of the trial. The weights of the dead birds were included for calculating feed conversion (FC), but deleted for calculating the average cumulative weight gain per bird. The following formulae were used to calculate FC and digestibility coefficient of dry matter (DM DC).

The quantity of mimosine excreted in relation to the total quantity consumed was calculated as below.

Mimosine (1 - DM DC) X Mimosine in dry excreta (%) excreted = ----- X 100 (percent) Mimosine in dry feed (%)

4. 2. RESULTS AND DISCUSSION.

4.2.1. Body weight gain:

There was a distinct difference in the growth of different dietary groups at the end of the first week(Table

The TLM group had the lowest weight gain and the 4.3). positive and negative controls had the highest gain. The other LLM treatments such as MLM, LM and PVP showed intermediate weight gains. Although the difference in the weight gain among the above three treatments was not statistically significant, the quail of LM treatment had a higher weight gain than the other LLM treatments (Table The same trend was observed throughout the 4.4). trial (Figure 4.1). However the overall weight gain of the male quail on the positive control and the females on the negative control did not differ significantly from the MLM, LM and PVP dietary treatments. Inclusion of 1.00 percent PVP in the MLM diet did not improve the weight gain.

The quail on the TLM diet were very small, less active, with dry and loose skin, compared to the birds on other diets. None of the birds lost their feathers.

These results were in agreement with the results of other researchers where the feeding of LLM beyond 10 percent of the diet depressed growth rate. Among the LLM dietary groups, the growth rate was most seriously affected by TLM diet, which had the highest level of mimosine (1.10 percent) and low tannin. Among the low mimosine diets, tannin content was very low (0.1725 percent) in LM diet and high in MLM and PVP diets (0.64 percent), but the difference in the weight gain was not significant. Therefore, the major cause of poor growth due to leucaena feeding in quail is presumed to be due to mimosine. This

Dietary		Mean body weight						
treatments		Weeks	after ex	periment				
	0	1	2	3	4			
	·		gm/bird -					
Positive control	18.4	46.7	73.6	96.8	114.26			
Negative control	18.2	44.9	71.5	97.7	117.20			
TL M	17.9	36.1	49.6	66.2	81.19			
MLM	17.9	41.5	58.8	81.1	96.53			
LM	18.1	42.5	62.7	87.6	102.77			
PVP	17.9	41.6	58.0	79.9	97.15			

Table 4.3. Effects of different experimental diets on the cumulative weight of growing Japanese quail.

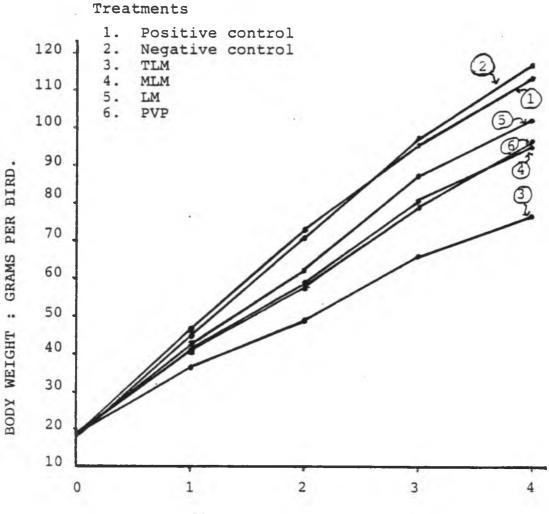
Treatments	Weight		gain i	gain in 4 weeks			Average	Water consumed
							FC	per feed
			gm/bi	rd		-	feed/gain	ml/gm
Positive control		a	86.5 (11)	b	95.86	a	3.54 a	2.18
Negative control			97.7 (13)	a	99.00	a	3 . 63 a	2.28
TLM	60.3 (12)	С	66.6 (9)	с	63.29	С	3.93 b	2.66
MLM	81.6 (9)	b	77.3 (11)	b	78.63	b	4.04 b	2.39
LM	83 . 2 (12)		87.2 (9)	b	84.67	b	4.30 c	2.36
PVP	83.5 (9)	b	77.7 (11)	b	79.25	b	4.41 c	2.66
LSD .05	19.7		10.2		9.92		0.25	ns

1. Figures in parentheses indicate the number of birds.

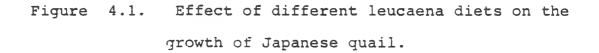
2. Treatment means in the same column followed by the same letter are not significantly different from each other at 0.05 probability level according to Duncan's multiple range test.

Table 4.4. Effect of different experimental diets on growing Japanese quail.

Average weekly body weight







was also observed by other researchers (Castillo et al., 1964; Labadan, 1969; Lopez et al., 1979).

There was no significant adverse effects of tannin on the growth of young quail, although, Acamovic and D'Mello (1981, 1982 a; 1982 b) observed a reduction in the growth rate only when the mimosine toxicity of LLM was overcome by the addition of ferrous sulfate. The probable reason could be that the problem of tannin was insignificant in the presence of mimosine which severely supressed the growth. Therefore the effect of PVP in improving the poor weight gain could not be seen in this trial. In the earlier trials (Acamovic and D'Mello, 1981; 1982 a and b; Ford and Hewitt, 1979; Rayudu et al., 1970), non-ionic polymers such as PEG or PVP were effective in overcoming the adverse effects of tannin only when mimosine was absent or detoxifed by adding iron ions.

The non-significant difference between the weight gains of the males of positive control as well as the females of negative control and their corresponding sex groups in MLM, LM and PVP groups is probably due to the small number of observations representing these groups.

4.2.2. Feed conversion (FC):

The overall FC was highest for the positive control diet followed by the negetive control diet, intermediate for TLM and MLM diets and lowest for LM and PVP diets (Table 4.4), which differed significantly. FC was high in the first week and reduced progressively as the body weight

increased(Table 4.5).

Although feed spilled into the dropping trays was accounted for while calculating the feed consumption, a small portion of the wastage in the form of dust particles could not be separated from the excreta of the LLM dietary treatments. Such wastage was high in the LM treatment. Although the same grinder was used for grinding all of the leaf meals, LM appeared to be ground finer, probably due to small size of the leaflets. The birds on the PVP diet were often found with a thick coating of feed set hard on their In some cases, they were unable to get rid of such beaks. coats and these had to be removed by hand. The reduction in the feed conversion of LLM diets was earlier reported by several workers. Among the LLM dietary treatments, TLM and MLM had intermediate FC. There was no significant difference among the two groups although the mimosine content of the TLM diet was higher than that of MLM diet. It is suspected that the better FC of small sized birds of TLM group might have compensated for the poor FC due to high mimosine content. Lower FC of LM and PVP diets, even though the mimosine content did not differ from that of MLM diet, might be due to the wastage of feed in the form of fine dust and formation of hard feed coats, respectively. The sticking of feed to the beaks was observed only in PVP group and hence the presence of PVP was suspected to be the It might be beneficial to make pellets of the diets cause. after mixing the ration. Wastage of feed can be reduced by

ا ها ها بنا که ها ها چا جا ها ها بنا ها ب				
ietary		Weekly	feed/gain	
treatments	1	2	3	4
Positive control	2.18	2.88	4.10	6.01
Negative control	2.61	2.75	3.73	6.05
TLM	2.61	3.98	4.55	5.90
ML M	2.40	3.74	4.33	6.02
LM	2.51	4.09	4.62	7.00
PVP	2.68	3.87	4.24	6.25
			• • • • • • • • • • • • • • • • • •	

Table 4.5. Effect of different experimental diets on weekiy feed conversion values of growing Japanese quail.

grinding the leaf meal more coarsely.

4.2.3. Water consumption:

The quail on TLM and PVP diets consumed more water per unit feed consumed than did the other groups, but the difference was non-significant (Table 4.4).

4.2.4. DM Digestibility coefficient (DM DC):

The positive control diet had the highest DM DC which was significantly different from the other treatments. The negative control diet had the lowest DM DC although it did not differ from MLM, TLM and LM dietary groups (Table 4.6). Lower digestibility of the leaf meal diets might be due to the presence of high fiber content. However, low DM DC of the feed did not affect the performance of the birds.

4.2.5. Analysis of the excreta samples:

The droppings of the positive and negative controls were solid and dry compared to the LLM dietary treatments. The droppings of TLM and PVP treatments were semisolid and dark in color.

The apparent protein digestibility of the diets ranged from 46 to 58 percent, but no definite pattern was observed (Table 4.6).

Mimosine content in the droppings of the TLM group was the highest and the LM group was the lowest. The other two LLM dietary groups had intermediate levels of mimosine and the difference was significant. Only 55 to 70 percent of Table 4. 6. Dry matter digestibility, apparent protein digestibility of the experimental diets and mimosine excretion of growing Japanese quail fed different experimental diets.

Treatments	DM DC	Apparent protein digestibility *	Mimosine excreted		
		percent			
Positive control	0 .63 a	54.34			
Negative control	0.56 c	54.51			
TLM diet	0.57 bc	58.46	54.55		
MLM diet	0.57 bc	54.79	70.45		
LM diet	0.57 bc	46.35	56.67		
PVP diet	0.60 b	55 .94	65.12		
ے کے اگر کہ کا کے لیے نہن نہا جب کے لیے ک		*******			

LSD 0.05 0.03

Treatment means in the same column followed by the same letter are not significantly different from each other at 0.05 probability level according to Duncan's multiple range test.

DM DC = Dry matter digestibility coefficient of the feed.

* Apparent protein digestibility -- feces and urine not separated.

the consumed mimosine was found in the droppings (Table 4.6). The electrophoresis analysis indicated that about 75 percent of the total mimosine content was in the form of mimosine and the rest in the form of DHP. Presence of mimosine and DHP in the excreta was earlier reported by Thanjan (1967) and Librojo and Hathcock (1974). Although there was no clear evidence as to whether the remaining mimosine was degraded or absorbed in the system, it was probable that part of it was absorbed by the system and thus supressed growth.

4.3. SUMMARY:

A feeding trial was conducted for four weeks to study the effect of leucaena leaf meals (LLM) on 10-day-old Japanese quail. Three different types of LLM (K8 tender leaf meal (TLM) with high mimosine and low tannin, K8 mature leaf meal (MLM) with low mimosine and high tannin and K156 (Leucaena diversifolia) leaf meal (LM) with low mimosine and low tannin), were used as 15 percent of the diet and compared with a positive control diet, a negative control diet with 15 percent alfalfa leaf meal and the MLM diet with 1.00 percent PVP.

The diet with the highest mimosine content (1.1%) severely restricted growth rate. This was significantly visible one week after the commencement of the feeding trial. The diets with low mimosine (0.30 to 0.44%) also

depressed weight gain as compared to positive and negative²² control diets. The quail maintained on the diet with medium mimosine-high tannin had a lower weight gain as compared to that of the low mimosine-low tannin diet, although the difference was non-significant. The tannin content in the diets did not appear to adversely affect quail growth possibly because the weight gain may have been already suppressed due to mimosine toxicity. The addition of PVP to the MLM diet had no effect on growth, but depressed feed efficiency and increased the water intake. The feed conversions of all the LLM diets were lower than those of positive and negative control diets. Usage of fine leaf meal powder may have futher reduced the FC of LM diet.

The dry matter digestibility coefficients of LLM diets as well as alfalfa leaf meal diet were lower than the positive control but it did not apper to affect the body weight gain or FC.

The apparent protein digestibility did not follow any particular pattern with the composition of the different diets. Only about 55-70 percent of the total mimosine consumed through the diet was excreted through the droppings. The electrophoresis analysis indicated that of the total mimosine excreted, about 75 percent was in the form of mimosine and the rest in the form of DHP.

V. SELECTION OF LOW MIMOSINE STRAINS OF LEUCAENA FOR FORAGE PRODUCTION.

The objective of this study was to screen different accessions of leucaena for strains having a combination of low mimosine and high vigor for fodder production.

5.1. MATERIALS AND METHODS.

5.1.1. Sources of different strains of leucaena:

The University of Hawaii has a world collection of leucaena. From this collection, a plantation of more than 400 accessions are maintained at the University of Hawaii Experiment Station, Waimanalo, Oahu. These accessions were planted during 1981-82, in a single replication of 10 plants each. Accessions Kl to Kl00 were not included in the present study, as these were already studied earlier (Brewbaker et al., 1972). In addition, the accessions maintained at the Institute of Plant Breeding, University of the Philippines at Los Banos, Philippines and the Forest Development Corporation (Perum Perhutani), Jakarta, Indonesia were also used for screening.

5.1.2. Criteria for selection.

The height and trunk diameter of trees of each accession, aged around two years, were measured. Other characters such as leaf size, branching habit and branch angle could not be taken into consideration, as it was difficult to measure them, in a closely spaced plantation.

Each accession was given a rank of one to five on the basis of height and trunk diameter. The highest rank was one and the lowest rank was five. Mimosine content of all the accessions, excepting a few which were extremely poor in vigor, was analyzed using the method given in Appendix III (Brewbaker and Kaye, 1981; Megarrity, 1978).

All of the accessions containing less than 2.0 percent mimosine, irrespective of their vigor, and accessions ranked 2 or below in vigor, irrespective of their mimosine content, were selected for inclusion in the forage yield trial. 252 accessions screened at the Waimanalo Experiment Station have been listed in Appendix IV with the other details such as the country of origin, United States Department of Agriculture Plant Introduction number (USDA PI No.), growth habit, mimosine content in the leaves and vigor of the plant. Some of the accessions are yet to receive the PI Number.

Growth habit of the plants indicated the type of leucaena accession. Hawaiian or "common" types were shrubby (S), the Salvador types were erect (E) and the Peru types were semi-erect (SE).

More than 100 accessions maintained at the Institute of Plant Breeding, UPLB, Philippines were screened and 22 accessions were tentetively selected on the basis of an unpulished report on their low mimosine content(Appendix V). Leaf samples of these accessions were collected and brought to the University of Hawaii for mimosine analysis. Only one out of eight accessions observed was selected from Indonesia because of high vigor.

There was wide variation in the mimosine content, which was presumed due to not only genetic variation, but also to variation in the season when the samples were taken, and the age of the leaf samples. The values given in Appendix IV were therefore used only for primary screening.

5.1.3. Forage yield:

Among the accessions listed in Appendix IV, 31 were selected for the forage yield trial. In . addition, six accessions which are in common use for forage production were included as controls. Acid scarified seeds were dibbled in dibble tubes, using peat-vermiculite media, on September 4, 1982, to start the nursery. As seeds of some of the accessions were in short supply, the trial was conducted using an augmented block There were 26 accessions with four replications, design. one with three replications, eight with two replications and two with a single replication. The size of a plot was 3.6 x 0.75 m with 24 plants in a single row of 3.6 m. Therefore the spacing was 0.75 x 0.15 m with a plant population of 88,900 plants/ha. The site was prepared for

planting by plowing and disc harrowing. For controlling weeds, "Lasso" was sprayed at 2.25 kg/ha two days before planting. Seedlings were removed from dibble tubes and transplanted on November 8, 1982. A basal dose of 16:16:16 fertilizer was applied in the furrows at the rate of 2 gm/plant (175 kg/ha).

Growth of the seedlings was slow in the initial stage, probably due to low temperature. Growth started to income in mid January,1983.

Leaf samples from these seedlings were collected on February 1, 1983 for analyzing the mimosine content. The plants were not ready for the observations on growth.

5.2. RESULTS AND DISCUSSION.

Mimosine content of the accessions included in the forage trial varied from 1.84 to 3.79 percent (Table 5.1). The foliage of cv. Cunningham (K500) had 2.68 percent mimosine. There were 11 accessions which contained less than 2.5 percent mimosine, three of them below 2.0 percent.

Mimosine analysis of the foliage samples of the accessions selected in the Philippines showed that all except accession 55 (K22 accession of the University of Hawaii), more than 3.0 percent mimosine (Appendix V). Therefore none of the accessions of the IPB, UPLB, Philippines were selected for the forage trial.

The only strain from Indonesia selected on the basis

Table 5.1. Mimosine content of leucaena accessions selected for forage yield trial.

Acc. no.	USDA PI number	Origin	Growth habit	Vigor	Mimosine %	No. of reps.
K4	284758	Australia	S-E	Control	2.85	4
K6	284758	New Guinea		Control		4
K8	263695	Mexico	Е	Control		4
K62	286295	Ivory Coas	t S-E	Control	2.29	4
K102	313957	Bolivia	E	2.5	2.83	2
K140	324393	Mexico	Е	2.0	2.53	2 2 2
K152	324402	Mexico	S E	2.5	2.61	2
K156*	324356	Mexico		Control	1.84	4
K217	324310	Salvador	E	1.0	1.89	4
K318	-	Thailand	E	2.5	2.55	4
K358	-	Mexico	Ε	1.0V	2.40	4
K360	-	Mexico	E	2.5V	2.76	2 2
K365	-	Mexico	Έ	2.5	2.52	2
K378	-	Mexico	E	2.5	2.80	4
K382	-	Mexico	E	2.5	2.85	2 2 2
K397	-	Mexico	Ε	2.0	3.28	2
K418	443481	Salvador	E	2.0V	3.79	
K448	443573	Mexico	E	2.0V	3.22	4
K455	443576	Mexico	E	3.0	2.79	4
K493	442833	Philippine	es E	1.5	2.52	4
K499		Mexico	E	1.0V	2.95	4
K500	-	Australia	S-E	Control		4
K513	443587	Mexico	E	3.0V	2.84	4
K517	443591	Mexico	E	1.5	3.07	4
K538 -	443610	Mexico	E	2.0	1.85	4
K592*		Mexico	Е	1.5	2.47	4
K601	443657	Mexico	E	2.5V	2.11	4
K604	443660	Mexico	Е	1.0	3.18	4
K614	443670	Mexico	Е	1.0V	2.47	4
K617	443673	Mexico	E	2.0	2.76	1
K633	443688	Mexico	E	2.0V	3.00	4
K634	443689	Mexico	E	2.0	2.65	3
K635	443690	Mexico	Е	1.0V	2.90	l
K636	443740	Mexico	Е	<1.0	2.47	4
K638	443692	Mexico	E	1.5	2.67	4
K654	443700	Mexico	E	3.5	2.64	4
K655	443701	Mexico	Е	2.5	2.01	4

* Species other than Leucaena leucocephala K156: Leucaena leucocephala, K592: Leucaena esculenta.

Leaf samples for mimosine analysis were taken on February 1, 1983 from seedlings planted in the forage trial.

Growth habit: S = shrub, E = erect and S-E = semi-erect. Vigor rating : Most vigorous = 1; least vigorous = 5.

of high vigor had 2.86 percent mimosine. Therefore this strain was selected for inclusion in the forage trial.

Mimosine analysis of leaf samples collected from the seedlings established in the forage yield trial showed higher mimosine content in several accessions compared to the earlier analysis (Appendix IV). This variation was suspected to be because leaves were sampled in different seasons of the year. It is therefore suggested to conduct mimosine analysis during different seasons, before the final selection is made.

Variation in the results of mimosine analysis of the accessions of the Philippines conducted at the UPLB, Philippines and the University of Hawaii was presumed to be due to variation in the sampling season and difference in the method of mimosine analysis.

No final selection for high yielding low mimosine accessions could be made, as the data on growth rate and forage yield were not available.

5.3. SUMMARY:

This study was conducted to select low mimosine strains of leucaena with high forage yield from the accessions collected at the University of Hawaii,

Institute of Plant Breeding University of the Philippines at Los Banos, and Perum Perhutani, Indonesia. These accessions were ranked from 1 to 5 in vigor depending on the height and trunk diameter with 1 representing highest vigor and 5 lowest vigor. The leaves of these accessions were analyzed for mimosine content. The accessions having mimosine content below 2 percent and accessions having a vigor below 2 were selected for the forage field trial. 31 accessions of the University of Hawaii and one accession from the Perum Perhutani, Indonesia qualified for the entry in the forage yield trial on the basis of these characteristics. Seedlings of these accessions were planted at the University of Hawaii Experiment Station, at Waimanalo in November 1982.

Mimosine content of the foliage of seedlings of these accessions was analyzed in February, 1983. Mimosine content was higher compared to the results of the earliar analysis, probably due to different sampling season. It is recommended to analyze the foliage for mimosine content, during different seasons. Final selection of high yielding, low mimosine accessions could not be done as sufficient data on Vigor and forage yield will not be available, till May, 1984.

Harvests	Irr	igation	treatmen	lts
No. Date	1	2	3	4
		mm/c	lay	
l. May 18, 1982	2.53	2.53	2.53	2.53
2. July 13, 1982	3.39	4.44	5.48	6.51
3. Sept. 1, 1982	4.34	5.29	6.24	7.19
4. Oct. 19, 1982	0.91	1.93	2.95	3.96
5. Jan. 4, 1983	6.32	6.59	6.68	7.13
Mean	3.827	4.438	5.050	5.659

Appendix I. The quantity of water received by different irrigation treatments during different harvests in the leucaena forage yield trial.

Appendix	II. Average	daily ma	aximum and	minimum	temperatures
and solar	radiation	during	different	harvests	in the
	leuca	ena fora	ge yield [.]	trial.	

Harvests	Maximum Minimum temperature temperature		Solar	
No. Date	remberarare	cemperature		
0.0	degre	e C	Cal/sq.cm	
1. May 18, 1982	25.69	20.19	170.36	
2. July 13, 1982	27.55	21.71	410.57	
3. Sept. 1, 1982	28.80	23.34	348.05	
4. Oct. 19, 1982	28.90	22.62	334.78	
5. Jan. 4, 1983	26.31	20.19	191.60	

Appendix III. Mimosine analysis procedure.

- 1. Collect first and second fully opened leaves on healthy branches. Sample size should be about 10 grams.
- 2. Dry at 65° C in a forced air dryer until no further loss of weight occurs.
- 3. Weigh out 1.0 gm dried leaflets in volumetric flask and fill to 100 ml with Regent A (0.1 N HCl), and macerate in homogenizer at 700 RPM for one minute.
- 4. Place 10 ml aliquot of the macerate in a boiling bath tube. Add 15 ml Regent B (0.1 N HCl with charcoal), and boil for 15 mim.
- 5. Filter through #2 Whatman paper.
- Take 2 ml filtrate and add 5 ml Regent C plus 1 ml Regent D. Allow the samples for 15 min. in dark for color development.
- 7. Read optical density at 535 nm, correlating against a blank achived by diluting a duplicate 2 ml sample of filtrate with 5 ml Regent C plus 1 ml water.

Regent solutions:

- A. 0.1 N HC1
- B. 1 liter 0.1 N HCl with 1.5 gm activated carbon, keep in suspension during use with magnetic stirrer.
- C. Diluent solution of 1 gm (Na2 EDTA.2H2O) in 4 liters water.
- D. 60% FeCl3 solution, obtained by dissolving 4 gm FeCl3.6 H2O on 500 ml 0.1 N HCl.

Mimosine determination:

1. Prepare a calibration curve using solutions containing between 0.0025 and 0.025 percent mimosine (in 0.1 N HCl), treating 2 ml aliquats as in steps 6 and 7 above.

2. Determine OD of Sample (corrected against blank), apply to curve from step 1. Mimosine percent = OD X 250.

References: Brewbaker and Kaye (1981) and Megarrity (1978).

Accessi	on Country		Growth	Mimosine	Dlant
Number	of origin	PINO.		percent	
Hamber		FINO.	nabic	percent	vigor
101	Virgin Is.	312118	S-E		2.5
102	Bolivia	313957	Е	2.23	2.5
108	Cameroon	317918	S	-	3.5
109	Puerto Rico	-	S	-	3.5 3.0
110	Costa Rica	237147	S		3.0
115	Tanzania	319842	S	-	3.5
116	Venezuela	319843	S	-	4.0
117	Venezuela	319844	S-E	-	4.0
118	Venezuela	319845	S	-	3.0
119	Venezuela	319846	S	-	3.5
120	Argentina	-	S	-	3.5
121	U.S.Virgin Is.	-	S	-	3.0
122	Puerto Rico		S-E	3.07	2.0
131	Mexico	324375	S	-	3.0
132	Mexico	324391	E	2.47	2.5
140	Mexico	324393	Ε	2.75	2.0
141	Mexico	324394	E	3.64	2.0
144	Mexico	324397	E	-	4.0
152	Mexico	324402	S	2.50	2.5
161	Mexico	324404	ន ន ន ន	-	3.5
170	Mexico	324405	S	-	4.0
189 198	Colombia	324426	5	-	4.0
198	Colombia Colombia	324435	S	- E	3.0
200	Colombia	324434 324437	S	3.00	3.0 2.5
203	Colombia	324437	S	3.00	3.0
205	Colombia	324443	5		4.0
207	Colombia	324444	S S		4.0
208	Colombia	324445	G		4.0
209	Colombia	324446	S S	2.75	2.0
217	Salvador	324310	Ē	3.18	1.0
218	Salvador	324311	ŝ	-	3.0 V
271	Argentina	321077	S	-	3.0 V
273	Brazil	337088	S	-	4.0
275	Australia	331797	S S	-	4.0 5.0 3.0 2.5
276	Australia	331798	S-E	-	5.0
304	Benin	330481	S	-	3.0
306	Costa Rica	338606	E	-	2.5
307	Costa Rica	338607	2	2.11	
313	Thailand	-	S	-	3.0
314	Thailand	-	S	2.01	4.0
315	Thailand	-	នល ស ស ស ស	-	4.0
316	Thailand	-	S	-	4.0

Accessi	on Country	USDA	Growth	Mimosine	Plant
Number	of origin	PINO.	habit	percent	vigor
317	Thailand		S		3.5
318	Thailand	_	E	3.29	2.5
319	Thailand	=	S	_	3.5
320	Thailand	-	S	-	3.5
321	Thailand	-	S	-	3.0
322	Thailand	-	S	_	4.0
323	Thailand	-	S	- 00	4.0
324 325	Thailand Thailand	-	S S	2.88 3.90	3.0 3.0
325	Thailand	-	S	3.43	2.0
329	Honduras	_	S	2.76	3.0
336	Mexico	342957	S	2.99	3.5
337	Mexico	342956	Ē	2.73	2.0
338	Mexico	342958	Е	2.40	1.5
358	Mexico	-	Е	2.55	1.0 V
359	Mexico	-	E	3.17	2.5
360	Mexico	-	E	2.45	2.5 V
361	Mexico	-	S	2.64	4.0
362	Mexico	-	E	1.88	4.0
363	Mexico	=	E E	3.20	2.5
364 365	Mexico Mexico	-	Ē	2.67	4.0 V
365	Mexico	_	E	2.07	2.5 3.5
367	Mexico	-	E	1.82	4.5
368	Mexico		S	-	5.0
369	Mexico	-	E	2.50	1.5
370	Mexico	_	Ē	_	3.0
371	Mexico	-	E	-	3.5
372	Mexico	-	S	4.25	1.5 V
373	Mexico	-	E	-	3.0
374	Belize	-	S	-	4.5
375	Salvador	-	E	-	4.0
378 382	Mexico Mexico	_	E	2.67	2.0
386	Mexico	-	E E	3.50 1.78	2.5 3.0
387	Mexico	-	Ē	2.75	3.0
388	Mexico	_	S	3.84	4.0
389	Mexico	_	S	-	4.0
390	Mexico	_	Ē	-	3.0
392	Mexico	-	Ē	2.73	2.0
394	Mexico	-	Е	3.00	2.5
395	Mexico	-	E	3.09	2.5

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Appendix IV. (Continued) Details of leucaena accessions screened at the University of Hawaii Experiment Station, Waimanalo.

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Accessi	on Country	USDA	Growth	Mimosine	Plant
Number	of origin	PINO.	habit	percent	vigor
397	Mexico		E	2.64	2.0
400	Cameroons	_	S	-	5.0
403	Indonesia	-	S	2.90	4.5
404	Indonesia	-	S	-	4.5
415	Salvador	443478	E	2.37	4.0
416	Salvador	443479	S	3.43	5.0
417	Salvador	443480	E	2.23	5.0
418	Salvador	443481	E	2.07	2.0 V
419	Salvador	443482	E	1.77	2.5
432	Mexico	443561	E	2.33	3.5
433	Mexico	443562	S	3.46	4.0
434	Mexico	443563	S	1.71	3.0 V
435	Mexico	443564	S	2.49	3.5
436	Mexico	443565	E	2.01	3.0
443	Mexico	443571	S	1.86	3.5
446	Mexico	443572	E	2.93	3.0 V
448	Mexico	443573	E	2.20	2.0 V
449	Mexico	443574	E		2.5
452 455	Mexico	443575	E	2.10	2.5
460	Mexico Mexico	443576	E	2.50	3.0
485	Nicaragua	443578	E	-	2.5
486	Nicaragua	443473	S-E	2.45	4.0
488	Colombia	443474 44347 0	S-E	1.88	3.5
493	Philippines	442833	S	2.18	4.5
494	Philippines	442832	E	2.40	1.5
497	Hawaii	442830	S S	2.93	3.0
498	Bolivia	442827	S E	2.96	5.0
499	Mexico		E	2.06 2.20	4.0
500	Australia		S-E	2.43	1.0 V 2.0
507	Mexico	443581	Ē	1.77	3.0
508	Mexico	443582	Ē	3.34	
50 9	Mexico	443583	Ē	2.37	4.0 2.5
510	Mexico	443584	Ē	2.25	
511	Mexico	443585	Ē	1.55	4.0 3.5
512	Mexico	443586		2.09	2.0 V
513	Mexico	443587		2.76	3.0
514	Mexico	443588		1.55	3.5
515	Mexico	443589		2.67	2.5 V
516	Mexico	443590		1.67	3.5
517	Mexico	443591		2.70	1.5
518	Mexico	443592	E	1.71	4.0

Accessi	on Country	USDA	Growth	Mimosine	Plant
Number	of origin	PINO.	habit	percent	vigor
519	Mexico	443593	S	4.04	4.5
520	Mexico	443594	S	2.37	4.0
521	Mexico	443595	S	1.78	4.0
522	Mexico	443596	E	2.15	2.5
523	Mexico	443597	E	2.79	3.5
524	Mexico	443598	E	3.50	3.0
525	Mexico	443599	S	2.45	3.5
526	Mexico	443600	S	3.16	4.0
527	Mexico	443601	E	2.02	4.0
528	Mexico	443602	E	3.11	3.5
529	Mexico	443603	E	2.61	3.0
530	Mexico	443604	S	1.96	4.0
531	Mexico	443605	S	2.41	4.5
532	Mexico	443606	S	2.83	3.5
533	Mexico	443607	E	2.62	3.0
536	Mexico	443608		2.18	2.5
537	Mexico	443609		2.15 1.39	2.5
538	Mexico	443610	E	1.39	2.0
539	Mexico	443612	Ε	2.50	3.5
540	Mexico	443613	E	3.09	3.0
541	Mexico	443614	E E	1.48	3.0
542	Mexico	443615	E	2.03	3.5
543	Mexico	443616	E	1.67	3.0
544	Mexico	443617	E	1.52	4.0
548	Mexico	443619	E	2.04	4.0
549 550	Mexico Mexico	443620 443621	E E	1.15	2.0 V
551	Mexico	443621	Ē	2.59 4.43	3.0
552	Mexico	443623	E	3.15	4.0 3.0
553	Mexico	443624	E	2.13	2.5
554	Mexico	443625	Ē	1.93	3.0
558	Mexico	443627	Ē	1.98	3.0
559	Mexico	443628	E	1.80	2.0
562	Mexico	443629	Ē	1.80	2.0
564	Mexico	443630	S-E	3.46	3.0
565	Mexico	443631	E	3.41	1.5
568	Mexico	443632	E	2.86	3.0
569	Mexico	443633	Ē	2.29	2.5
570	Mexico	443634	E	3.24	2.5
571	Mexico	443635	E	2.56	2.0
572	Mexico	443636	Ε	1.36	3.5
573	Mexico	443637	E	1.93	4.0
574	Mexico	443638	E	2.19	4.0

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Accessi	on Country	USDA	Growth	Mimosine	Plant
Number	of origin	PINO.	habit	percent	vigor
575	Mexico	443639	Е	3.03	4.5
576	Mexico	443640	E	2.35	4.0
578	Mexico	443641	S	1.90	4.0
582	Mexico	443642	E	3.63	4.0
584	Mexico	443643	E	-	2.5
586	Mexico	443644	S	1.84	4.0
587	Mexico	443527	E	2.25	4.0
588	Mexico	443645	E	2.14	3.0 V
589	Mexico	443646	E	1.96	4.0
590	Mexico	443647	E	3.16	3.0
591	Mexico	443648	Е	4.28	2.5
592	Mexico	443537	E	2.34	1.5
593	Mexico	443649	E	-	4.0
594	Mexico	443650	E	1.40	3.0
595	Mexico	443651	E	1.92	2.0
596	Mexico	443653	S	3.90	4.0
597	Mexico	443654	E	1.93	3.0
598 599	Mexico	443655	E	1.64	2.0
	Mexico	443656	Е	2.86	2.5
601	Mexico	443657		2.31	2.5
603 604	Mexico	443659	E	1.44	2.0
605	Mexico	443660	E	2.08	1.0
606	Mexico	443661	E	1.48	1.5
607	Mexico Mexico	443662	S	1.51	4.0
609	Mexico	443663	E	1.48	1.0
610	Mexico	443664 443666	E	2.89	2.5 V
611	Mexico	443667	E E	2.76 2.17	2.5
612	Mexico	443668	E	2.17	3.0 3.0
613	Mexico	443669	E	2.07	3.0
614	Mexico	443670	E	2.36	1.0 V
615	Mexico	443671	Ē	2.75	1.0 1.5
616	Mexico	443672	Ē	1.94	1.5
617	Mexico	443673	Ē	2.42	2.0
618	Mexico	443674	Ē	3.57	3.0
619	Mexico	443675	Ē	3.97	1.5
620	Mexico	443676	Ē	3.08	2.0
621	Mexico	443677	Ē	2.27	2.5
622*	Mexico	443538	Ē	1.15	1.5
623	Mexico	443678	Ē	5.21	3.0
624	Mexico	443679	E	2.36	3.0

Accessi	on Country	USDA	Growth	Mimosine	Plant
Number	of origin	PINO.	habit	percent	vigor
625	Mexico	443680	E	2.34	3.0
627	Mexico	443682	E	1.64	3.0
628	Mexico	444683	E	2.63	2.0 V
629	Mexico	443684	E	4.34	2.5
630	Mexico	443685	Ε	2.11	3.0
631	Mexico	443686	Ε	1.99	2.5
632	Mexico	443687	Ε	2.36	4.0
633	Mexico	443688	E	1.58	2.0 V
634	Mexico	443689	E	2.20	2.0
635	Mexico	443690	E	2.15	1.0 V
636	Mexico	443740	E	2.41	<1.0
637	Mexico	443691	E	4.43	3.5
638	Mexico	443692	E	1.53	1.5
639	Mexico	443693	E	4.19	3.0
640	Mexico	443694	E	2.66	2.5 V
641	Mexico	443695	E	2.80	2.5
644*	Mexico	443541	E	2.59	4.0
647	Mexico	443696	E	1.96	3.5
648	Mexico	443697	E	1.14	2.0 2.5
652	Mexico	443698	E	2.85	2.5
653 654	Mexico	443699	E	1.49	2.5
655	Mexico	443700	E	2.20	3.5
656	Mexico Mexico	443701	E	2.80	2.0
658	Mexico	443702	E	2.44	2.5
659	Mexico	443704	E	2.44	2.5
661	Mexico	443705	E	1.99	2.0
662	Mexico	443707		2.08	2.0 V
663	Mexico	443708	E	2.50	2.5 V
664	Mexico	443709	E	3.08	2.0
665	Mexico	443710	E	2.12	2.5
666	Mexico	443711	E	1.84	2.0
667	Mexico	443712 443713	E	2.39	3.5
669	Mexico	443713	E	2.81	2.5 V
670	Mexico	443715	S-E	-	4.0
671	Mexico	443717	E	1.84	3.0
672	Mexico	443718	E	1.38	3.5
673	Mexico	443718	E	2.44	4.0
674	Mexico	443719	e e	2.86	3.0
675	Mexico	443721		2.63	3.5
	HENTON	443/21	E	1.78	3.0

Accessi	on Country	USDA	Growth	Mimosine	Plant
Number	of origin	PINO.	habit	percent	vigor
<b>6</b> 76	Malaysia	-	S	2.63	5.0
678	Thailand	-	E	2.27	1.5 V

* Accessions other than Leucaena leucocephala.

K622, K644 belong to the species Leucaena esculenta.

Vigor rank followed by V indicated the variation between the plants of the accession.

Growth habit: S = shrub type, E = erect type, S-E = semi-erect.

Vigor was rated visually by observation of plant height and stem diameter. A rating of 1 was most vigorous and 5 least vigorous. Appendix V. Leucaena accessions screened at the Institute

of Plant Breeding, University of the Philippines,

## Los Banos.

*Source: Unpublished data collected personally from IPB, UPLB, Philippines.

** Mimosine content reported by IPB based on the analysis conducted according to Matsumoto and Sherman (1951).

Vigor rating : Highest vigor = 1, lowest vigor = 5.

Growth habit : All the above accessions are of shrub type.

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