EVALUATION OF BLACK JACK (*Bidens pilosa* L.) RESISTANCE TO PARAQUAT

By

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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This thesis has been submitted for examination with my approval as the University Supervisor

Date 9/4/97

Dr. J. O. Nyabundi

DEDICATION

To my beloved mother Mako Moallim, my late father Haji Mohamed Addow, my wife Fardosa Mohamed Kanyare and my beloved son Mohamed

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF APPENDICES

Abstract

Two experiments were conducted to study resistance of *Bidens. pilosa* to paraquat. A resistant and susceptible biotypes of the weed were used The first experiment looked at the effect of paraquat application rate on control of the *B. pilosa* biotypes at different stages of growth Paraquat (Gramoxone 20%) was applied at rates of 1,3,5,7, and 9 litres per hectare The growth stages comprised 2, 4, 6 and 8 weeks after emergence Assessments of the survival rate were done at three and twenty one days after each herbicide application episode Results showed that the injurious effects of the herbicide increased with increasing application rate Conversely, *B. pilosa* resistance to the herbicide increased with delayed application so that application of the herbicide in late phenological stages effected less control than early application In all application rates and growth stage, the herbicide had less effect on the resistant biotype than the susceptible one

The second experiment examined the relative growth fitness of the two biotypes in a replacement series experiment The two biotypes were grown together in different planting ratios of the 75% B1 25% B2, 50% B1 50% B2, 25% B1 75% B2 Plant height, leaf area and number of leaves were determined three weeks after emergence and at flowering In addition, number of inflorescence, fresh weight and dry weight were measured at flowering

B1 plants out-fitted B2 plants in the early stage of the growth in terms of plant height and number of leaves. At flowering stage B1 plants produced larger number of branches than B2 plants and consequently produced larger number of inflorescences.

It is suggested that plants population dynamics would favour the susceptible biotype over the resistant one if no herbicide is used. On the other hand, use of the herbicide which controls the susceptible biotype would favour dominance by the resistant one.

1. INTRODUCTION

Through the millions of years of life on earth, a continuous process of mutual evolution has taken place between plant and animal species and the various organisms that feed on them. The host plants or animals have evolved defensive mechanisms, including chemical repellents and toxins, exploiting weakness in attacking organisms. In turn the attacking organisms have evolved mechanisms that enable them to detoxify or otherwise resist the defensive chemicals of their hosts or prey. Thus, it appears that the gene pool of most of our pest species already contains genes that enable the pests to degrade enzymatically or otherwise circumvent the toxic effect of many types of chemicals that we have developed as modern pesticides. These genes may have been retained at various frequencies as part of the genetic memory of the species (Georghiou, 1986).

There are many factors affecting the development of resistance, among them, age, growth rate, morphology, physiology, biophysical process, biochemical process and genetic inheritance (Terry, 1984).

Young plants have higher proportion of meristematic tissue than older plants and are often more susceptible to herbicides at this stage. Species with a fast growth rate have higher meristematic activity and thus tends to make them more susceptible to some herbicide.

The morphology of a plant has a profound effect on the ability of certain herbicides to be retained or absorbed. The leaves, roots and growing points of weeds are areas of greatest morphological significance determining resistance.

Foliar-applied herbicides must be first retained on the leaf surface if they are to be effective Upright, narrow leaves allow less retention of herbicides than horizontal, broad leaves Retention is also reduced if leaves have surfaces that are waxy, pleated or hairy and this reduces the effectiveness of a herbicide (Terry, 1984)

The location of growing point is also important in determining the susceptibility, because it is very susceptible to herbicides Most broad-leaved have exposed growing points at the top of shoots and in leaf axil In grasses it is located at the base of the plant where it is protected from contact with post emergence herbicides

The physiology of a plant determines how much herbicide is absorbed and translocated Properties of leaf and stem surfaces can affect penetration and absorption of herbicides The rate and amount of translocation also vary widely with different plants and herbicides Constituents of plant cell may absorb some herbicides before they reach their site of action within the plant Absence of this deactivating property can make plants susceptible to herbicides Some chemical reactions, within a plant can protect it from herbicide damage or, in some cases, increase the plant susceptibility Lastly the tolerance of a plant to herbicide is largely affected by its genetically inherited characters which determine the physiological, biochemical and biophysical processes (Terry, 1984)

The idea of weeds resistant to herbicides is not new Warning about the possibility of weeds evolving resistance were issued soon after the phenoxy herbicides were introduced (Abel, 1954), however, as no confirmed cases of resistance to phenoxy herbicides occur, the warning was ignored even after the first triazine resistant weeds In Europe and the United States more than 45 weed species are resistant to triazine (Gressel,

1986) Thirteen weed species are resistant to paraquat and other herbicides All evolved from sensitive biotypes in agricultural situations (Ferrist and Vaughn, 1990)

1.1. Justifications and objectives.

Irregular weed control performances were first reported in coffee plantation in middle 1980's at Coffee Research Foundation, where paraquat had been used for over 20 years Observation made by Njoroge J M (1991), from January 1987 to January 1990 at Coffee Research Foundation reported tolerance of *B. pilosa* to paraquat

Tolerance to a herbicide could arise from variation in rate of application and could also be influenced by the phenological stage of the weed Resistance to a herbicide could be Physiological resistance which involves the resistance in the presence of the of the control agent on or in the organism or Behavariostic resistance which is resistance because of some behavariostic factor which decrease the probability of contact between the weed the control agent Therefore the true resistance " which is defined by the FAO as a decreased response of a population of animal or plant species to a pesticide or control agent as a result of their application" should be clearly distinguished from the physiological and behavariostic resistance

Herbicide treatment may fail for a variety of reasons, but genetic resistance cannot be distinguished from other causes unless comparative studies are conducted under controlled conditions (Caseley et al, 1991)

In the first experiment the responses of *B. pilosa* accessions to various rates applied in different stages will be studied with the objectives of.

1 To distinguish the true resistance which is genetic resistance from the resistance of other causes like physiological and behaviouristic resistance

2 To assess the differential tolerance of the two *B. pilosa* accessions B2 (exposed to paraquat) and B1 (Not exposed to paraquat) by varying the rate of application in different weed growth stages

Theoretical models developed to predict the rate of resistance evolution in weed population include the relative fitness of resistant and susceptible biotypes as an important parameter. In the absence of a herbicide, biotype with reduced fitness will be expected to decrease over time relative to those with greater fitness. A number of biological factors contribute to the reproductive output of a plant and hence to its fitness, such as the relative growth rate and total biomass production.

No studies related to the competition or fitness of *B. pilosa* resistant and the susceptible biotype were previously carried out, therefore the second experiment will study the growth fitness in B. pilosa biotypes under unstressed conditions with the objectives of Confirming the already detected resistance in *B. pilosa* by the 1st experiment in terms of growth fitness of the resistant and susceptible biotypes by monitoring growth parameters in a replacement series experiment without application of the herbicide.

2. LITERATURE REVIEW

2.1. *Bidens pilosa*

Bidens pilosa L. is an annual, erect herb growing to 150cm, stems and branches marked with parallel biotypes or ridges, smooth, green or with brown stripes, types found in some regions may have small, inconspicuous, white hairs on the stem, leaves opposite, petioled, pinnate, (arising on opposite sides of the midribs of the leaf) usually with three (sometimes five) ovate, acute leaflets, the upper leaflet usually large, upto 9cm long, 3cm broad, margins sharply serrate, sparsely hairy to smooth on different green colours, Inflorescence a capitulum (congested head of flowers), yellow, terminal, 7mm in diameter, on peduncles 5cm long, outer involucral bracts (rings of the bracts at the base of the flower head) oblong or more or less spoon shaped, ciliate, shorter than florets, Fruits (achene) blackish about 11mm long, narrow, ribbed, sparsely bristled to smooth, pappus a ring of awns (two to four) with recurved barbs, 3mm long (LeyRoy et al, 1977)

The bristles have played an important part in its spread It is troublesome in both field and plantation crops and is reported to a weed of 31 crops in more than 40 countries (LeyRoy et al 1977). In South Africa the early spring in growth the leaves are sometimes eaten by humans, but has low nutritive value It has pungent essential oil that may taint milk (Waterhouse, 1994)

2.1.1. Distribution and Biology

Bidens pilosa is an annual weed which originated in tropical America but is now spread throughout the warm regions of the world The weed is easily recognised by the

elongated burlike fruits which bear recurved or hooked bristles The generic name *Bidens* refers to the barbs of the fruit, suggesting "two-toothed" This weed belongs to a group which is often spoken of as the beggar ticks, stick tights, or Spanish needles (Appendix 16) The plant is in the Asteraceae (Compositae), the largest family of the flowering plants, and which include many of the weedy species widely distributed in the world The spread and e colonisation of the areas by these species can be attributed in part to its very effective pollination arrangements and to special adaptation which allow the distribution of its fruits by workers, animals, wind and water Several *Bidens* species (Appendix 17) are found far north in the temperate zone but *Bidens pilosa* prefers the warm regions It can usually be seen at all seasons in the tropics but it grows most actively in the warmer and wetter parts of the seasons The weed is found in gardens, on cultivated land, in open waste places, and along roadside It mixes easily with annuals and perennial in different types of plant communities It is reported in crops at increasing elevations in several countries but the final altitude at which it can grow, depends of course, on the climate of the regions

Bidens pilosa is easily recognised in the out-of-doors by its collection of black, barbed fruits radiating in all directions from common receptacle Very young plants have strap shaped cotyledons and purple-tinged hypocotyl Single plant have yielded 3,000 to 6,000 seeds Many of the seeds germinate readily at maturity This makes it possible to have three or four generations per year, in some areas light and good aeration are required for germination In one experiment test 35% of the weed germinated one week after harvest Whereas in another there, was 60% germination Seeds which are 3 to 5 years old may give 80% germination (Rochecouste and Vaughn, 1959)

2.1.2. Economic Importance

Bidens pilosa is a principal weed of sugar-cane in Brazil and Mexico; corn in Mexico and Mozambique, coffee in Brazil and Mexico, tea in India, cotton in Peru and Swaziland, potatoes in Colombia, Mexico and Mozambique, and citrus in Mexico and Venezuela It is a serious weed of sorghum in Hawaii, and a principal weed of vegetables in Brazil and Venezuela and of bananas and beans in Mexico In addition, it is a common weed in coffee and pyrethrum in Tanzania Latin America and East Africa have reported the most serious infestation of *B pilosa* It is a principle weed in the arable land of East Africa *Bidens pilosa* is known to be associated with the coffee for a long time It is found in all the coffee ecological zones in Kenya It is a very common weed species in East Africa and occurs in all areas as one of the most important annual weeds (Ivens, 1989) In plantation crops in which herbicide have been used to remove perennial grasses, this weed often return to become dominant (LeyRoy et al, 1977)

2.2. Paraquat:

Paraquat normally employed as its dichloride salt, (Appendix 13) is the bipyridilium herbicides finding most widespread use It is none-selective contact herbicide with rapid desiccant action (Harvey and Harper, 1982) In cropping systems it is applied before planting annual crops, during dormant stage of perennial crops, or as spray directed away from growing crops (Feurst Vaughn, 1990)

Paraquat dichloride forms colourless crystals decomposing around 300 °C with negligible vapour pressure at room temperature It is very soluble in water, slightly soluble

in short chain alcohols, insoluble in hydrocarbons This salt is stable in neutral and acid media but are oxidised under alkaline conditions Clay and organic matter rapidly and strongly adsorb paraquat, typically strong adsorption capacity vary from 20-3000 mg/Kg soil depending on the clay and organic matter content The herbicide is incompatible with anionic surface active agents

In sunlight, however, some photochemical breakdown occurs for paraquat which remains on the outside of treated plants The product of this breakdown under conditions of high light intensity is isonictonic acid and methylamine (both of which have low mammalian toxicities)

Since plants are killed rapidly in bright sunlight significant quantities of breakdown of products are formed only on the surface of dead tissue and there is no movement of the substances from dead tissue to other parts of the plants Using ^{14}C labelled paraquat for potatoes, all the ^{14}C which occurs as the residue from tubers of spraved plants can be accounted for as unchanged paraquat (Beste, 1983) Repeated use of bipyridilium herbicides in perennial crops has led to the development of tolerant weed biotypes of 13 species (LeBaron, 1991.)

2.2.1 Chemistry and Behaviour

2.2.2. Mode of action

Bipyridilium type herbicide (paraquat and diquat) cause wilting and rapid desiccation of the foliage to which they are applied, often within a few hours High light intensities increase the rate of development of phytotoxic symptoms, but are not essential for herbicide action Best results in the field have often been obtained by late afternoon,

rather than morning or mid day application. This appears to allow some internal transport during the night, before development of acute phytotoxicity induced by light, which could limit movement.

Translocation, following a foliar application, appears to be almost solely via the apo-plastic system (Baldwin, 1963; Slade and Bell, 1966). However after the loss of membrane integrity, induced by both herbicides, they do move into untreated leaves, presumably along with the flow of other cellular contents. They are poorly translocated from roots (Damonakis et al. 1970) because they are tightly bound to cellular components.

These herbicides are not degraded in higher plants in the usual sense. However, they are reversibly converted from the ion form to free-radical form.

This interconversion is cyclic and requires light, molecular oxygen, water, and the photosynthetic apparatus. During auto-oxidation of the paraquat free radical to the ion, four by-products are formed: (1) Hydrogen peroxide (2) Superoxide radical (3) hydroxyl radical and (4) Singlet oxygen Ashton and Crafts, 1981) (Appendix 14). Each by-products is potentially phytotoxic. However recent research suggests that the hydroxyl radical are responsible for phytotoxic symptoms.

2.2.2. Mechanism of Paraquat Resistance.

The mechanism of paraquat action involves the Photosystem one (PSI) mediated reduction of the paraquat di-cation radical (Appendix 15). This mono-cation radical reduces O_2 to O_2^-, the superoxide anion radical, resulting in the regeneration of the paraquat di-cation. Subsequently, H_2O_2 and (OH) may be produced by a variety of

reactions (Dodge, 1982 and 1983) Hydroxyl radicals are known to cause per-oxidation of unsaturated fatty acids This apparently is a cause of the observed loss of membrane integrity (Harris and Dodge, 1972, Hutchinson, 1979, Dodge, 1983)

In addition to the formation of reactive forms of O_2, the presence of paraquat causes the diversion of electrons which normally reduce NADP and the reduced state of alpha tocopherol, glutathione, and ascorbate which function in cellular protection mechanisms The action of superoxide dismutase, catalase, and peroxidase would presumably remain unaffected by this electron diversion (Feurst et al, 1985)

The superoxide radical, hydroxyl radical, hydrogen peroxide, and possibly singlet oxygen are rapidly detoxified enzymatically in the resistant biotype (Feurst and Vaughn, 1990) According to this hypothesis, enhanced activity of superoxide dismutase, catalase, peroxidase, glutathione reductase, ascorbate peroxidase, and possibly dehydroascorbate reductase detoxify the various toxic forms of oxygen and thus prevent lipid peroxidative reactions These enzymes will be referred to collectively as the "*protective enzymes*" Superoxide dismutase, ascorbate peroxidase, glutathione reductase, and dehydro-ascorbate reductase are present in chloroplast, and this detoxification pathway has been referred to as "Halliwell-Asada system" (Shaaltiel et al, 1988) Catalase and peroxidase are absent in the chloroplast Activities of superoxide dismutase, catalase and peroxidase were therefore compared in untreated leaves of 11 normal varieties and four tolerant biotypes of perennial rye grass Mean activities of superoxide dismutase (SOD), catalase, and peroxidase were respectively 56%, 32%, and 35% higher than in rye-grass of paraquat tolerant biotypes than in herbage of normal susceptible varieties Although superoxide dismutase activity in the chloroplast of tolerant plants may be adequate to convert all additional superoxide ion to

hydrogen peroxide, the elevated activity of catalase and peroxidase are not located within the organelle (Harper and Harvey, 1978) Therefore, either hydrogen peroxide diffuses out of the chloroplast to be detoxified by the enhanced extra chloroplastic activities of catalase and peroxidase, or, more probably the chloroplast have effective endogenous systems for detoxification of hydrogen peroxide, such as those involving ascorbate and glutathione (Foyer and Halliwell, 1976, Gorden and Beck, 1979)

An initial study of paraquat resistance in hairy fleabane found that the resistant biotype had a 300% increase in the enzyme superoxide dismutase (Vaughn et al, 1989) Three enzymes (superoxide dismutase, glutathione reductase, and ascorbate peroxidase) were increased in the resistant biotype chloroplast and the increase in these proteins was the reason for the resistance (Shaaltiel and Gressel, 1986)

By auto-radiography and the lack of chlorophyll fluorescence suppression in the resistant biotype, it was demonstrated that herbicidal effective level of paraquat supplied to the leaf did not even reach the chloroplast in the resistant biotype (Feurst et al, 1985) These data indicate that paraquat is sequestrated in resistant biotype at a site other than the chloroplast, such as the cell walls (Feurst et al, 1985, Shaaltiel and Gressel, 1986) The differences observed between resistant and susceptible biotype indicates that the major factor in resistance is compartmentalisation, not enzymatic protection (Vaughn et al, 1989)

Since paraquat is a divalent cation, it can be adsorbed to cellular component by ionic interaction Another possible hypothesis for the compartmentalisation is that the paraquat is actively transported into a membrane-enclosed organelle, possibly the vacuole

2.3. Bipyridilium Resistance in Weeds

Tolerance to a herbicide could arise as a result of reduced uptake by the plant However, experiments using ^{14}C-methyl labelled paraquat applied to the leaf surface or supplied to the cut ends of excised leaves have demonstrated that uptake is similar in tolerant biotypes and normal susceptible varieties (Harvey et al, 1978), hence tolerance would not appear to be due to greater absorption of the herbicide in the free space Uptake, translocation, and metabolic stability of paraquat appear similar in tolerant and normal plant varieties Thus the resistance probably arises from fundamental key differences related to the mode of action of the herbicides (Feurst and Vaughn, 1990)

Genotypes resistant to paraquat have been reported in horse-weed (*Erigeron canadensis* L.), philadelphia fleabane (*Erigeron philadelphicus* L.), ohaechinquku (*Erigeron sumatrensis* Retz) Asiatic Hawksbeard (*Youngia japonica* L. De.) from Japan, capeweed (*Arctotheta calendula* L. Levyns), wall barley (*Hordeum glaucum* Stend), hare-barley (*Hordeum leporinum* Link,) from Australia, perennial rye-grass (*Lolium perenne* L.), annual blue-grass (*Poa annua* L.) from United Kingdom, *Ceratopteris richardii* Brogn, American black night-shade (*Solanum americanum*) from U S A , hairy fleabane (*Conyza bonariensis* L. Cong) from Egypt, and horse-weed (*Conyza canadensis* L. Crong) from Hungary All the resistant cases reported, paraquat was applied several times per year for more than five years (Feurst et al , 1985)

2.3.1. Population of Genetics.

The appearance of resistance depends on characteristics of the different weeds and herbicides, which can be mathematically integrated into models If a gene or genes for resistance do not exist at some low frequency in the population, resistance will never appear in that species When resistant biotype are grown in competition with susceptible (Wild-type) biotype of the same species without herbicides, their seed yield is often about one-half that of the wild type (Rodosevich and Holt, 1982) This difference in fitness will decrease the rate of enrichment for resistance when non-persistent herbicides are used (Gressel, 1986)

Persistence of herbicide interrelates not only with fitness but also dormancy characteristics that separate weeds from crops and from other pests Weeds germinate not only throughout the season, but also over many seasons Susceptible weeds can germinate after a rapidly degraded herbicide has disappeared, they then produce more seeds before the season is over, considerably lowering the effective selection pressure Selection pressure is a result of "effective kill" which is not the same as the "knock down" after herbicide treatment Effective kill is a measure of the surviving seeds or propagules at the end of a season not after treatment (Gressel, 1986)

Every time we enrich for resistant individuals by using a herbicide the resistant seeds are diluted by a seed bank of a susceptible seeds from previous years These seeds exert a buffering effect and the appearance of resistance The interaction of selection pressure, herbicide persistence, and seed bank on the rates of enrichment for resistance can be modelled to visualise how each parameter affects the rate at which the resistance should appear (Radosevich an Holt, 1982)

2.3.2. Difference in Tolerance To Bipyridilium Herbicides.

2.3.2.1. Interspecific differences.

Although annual plants are usually highly susceptible to paraquat herbicide, differences between annual species have been recorded. The effect of resistance may have merely involved differences in spray retention as determined by leaf morphology or leaf surface characters, such as epi cuticular wax (LeBaron and Gressel, 1982).

The extent of adsorption of paraquat on the leaf surface may also be important in determining the amount of herbicide available for uptake into the cytoplasm. Brian (1967) has defined three phases of uptake of bipyridilium herbicides. An initial rapid adsorption at or near the leaf surface lasting about 30 sec. is followed by adsorption into less accessible Donnan free space, which continues up to 2 hrs. Finally there is slow accumulation, presumed to be within cell membrane. Adsorbed Bipyridilium herbicides are not readily desorbed and hence differences between species in the surface adsorption capacity can influence the amount of herbicide available for uptake into the cytoplasm.

Lignified or tannin containing tissue can strongly adsorb Bipyridilium ions, thus greater lignification may reduce the susceptibility of species to these herbicides. The total inactivation of paraquat on application to mature barks allows the herbicide to be used for weed controls in tree plantations and orchards (Gressel et al., 1982).

2.3.2.2. Intraspecific differences.

Varieties of a given species may differ in susceptibility to bipyridilium herbicides and in some species tolerance has been developed by selection. In the absence of any direct

selection pressure for development of tolerance to bipyridilium herbicides, varieties of both Italian and perennial rye-grass exhibit difference in susceptibility to paraquat Four fold differences were also found when 280 varieties of wheat were screened A tolerant line of *Poa annua* has arisen under unusually strong selective pressure as a result continued use of paraquat as the sole method of controlling annual weeds in a market garden Normal *P. annua* is killed by 0 1 to 0 2 Kg/ha of paraquat, but more than 0 8 Kg/ha is required to kill the tolerant strain Paraquat tolerance has also arisen under strong selection pressure in perennial rye-grass and has been exploited in the development for agricultural purposes of paraquat-tolerant lines of perennial rye-grass These lines exhibit tolerance at all stages of the life cycle but the degree of tolerance is dependent on growth stage, growth conditions, and method of herbicide treatment (LeBaron and Gressel, 1982)

Furthermore chloroplast isolated from paraquat-tolerant lines and normal varieties of perennial rye-grass display equal sensitivity to the herbicide, tolerant and normal genotypes did not differ in their interaction of paraquat with photo-system I (Gressel et al, 1982)

The nutritional status of the plants of the same genotype can cause variation in the susceptibility to bipyridilium herbicides Increasing the supply of nitrogen increased the susceptibility of *Agropyron repens* to paraquat This was attributed to greater spray interception by the expanded area of foliage and increased spray retention due to greater altered leaf surface characters (Lutman ad Sagar, 1975)

3. MATERIALS AND METHODS.

Two experiments were conducted in the glass-house at the University of Nairobi's Kabete field station in 1994 and 1995. The site is located on latitude 1°15 S and longitude 36°44 E and altitude of 1800m. The first experiment examined the effect of paraquat herbicide rates and plant growth stages on the resistance of *B. Pilosa*. The second experiment examined the Growth fitness of the Resistant and the Susceptible biotypes in a replacement series under unstressed condition.

Experiment I

3.1. The Effect of Paraquat Herbicide Rates on Plant Growth Stages on the Resistance of *B. pilosa*

The experiment 6 X 4 X 2 factorial structure was laid out as a split plot Design by assigning the combination of paraquat rates and growth stages into the main plot and accessions into the subplot. The treatments comprised of six levels of rates 0, 1, 3, 5, 7, 9 Nha of paraquat, four levels of *B. pilosa* growth stages 2, 4, 6, 8 weeks after emergence and two levels of accessions B1 (susceptible) and B2 (resistant). The total treatments were 48 replicated three times. The seeds of B2 were collected from coffee fields in Coffee Research Foundation (CRF) where after using paraquat for over 20 years irregular *B. pilosa* weed control of paraquat were observed (Njoroge, 1991), while B1 were collected from the areas surrounding the Coffee Research Foundation where paraquat had not extensively used.

The experiment was carried out in 27.5cm diameter pots at the depth of 25cm Approximately 10 seeds of *B pilosa* were planted in each pot A week after the emergence the plants were thinned to 4 of almost uniform plants in growth per pot

Soil A mixture of forest soils, manure, gravel, coffee husk and animal blood was used as a growing medium at the ratio of 3:1.1 1.1 The mixture was sterilized in an oven at a temperature of 125°C for two hours to kill almost every seed already existing in the soil

3.1.1. Spraying and assessments

Spraying of paraquat was started as pre scheduled 2 weeks after emergence At all the four levels of the growth stages (2, 4, 6, 8 weeks after emergence), the plants were sprayed with 5 different rates 4, 12, 20, 28 36 ml of paraquat respectively which were each mixed with one litre of water These rates are equivalent to 1, 3, 5, 7, 9 l/ha respectively, when mixed with 250 l of water The recommended rate for weed control in coffee plantation is 2l/ha diluted with 250 litres of water

A small hand sprayer of 1 litre capacity was used Enough pressure was applied to produce small droplets Each plant was sprayed with just enough quantity of the herbicide to fully wet all the leaves

Visual assessments of the herbicide damage were done at 3 and 21 days after treatments A subjective score of 0 - 8 was used for the visual assessment done at 3 days after treatment

0 Completely dead

1 = all the leaves killed but the main stem and the branches are still green

2 = all leaves killed except the primordia and the most young leaves

3 = > 90% of the leaves killed

4 = 61 - 90% of the leaves killed

5 = 31 - 60% of the leaves killed

6 = < 30% of the leaves killed

7 = Sparsely burned spots spread all over the leaves

8 = Indistinguishable from the control

For the visual assessment at 21 days after emergence, a subjective score of 0-6 was used

0 = Completely dead

1 = Moribund but not all tissue dead

2 = Alive with some green tissue, but unlikely to make much further growth

3 = Very stunted but still making some growth

4 = Readily distinguishable inhibition of growth

5 = Some detectable adverse effects as compared with control, colour difference, morphology or very slight reduction in growth

6 = Indistinguishable from the control

3.1.2. Data analysis

Analysis of variance (ANOVA) was computed in respect of the visual assessment for both 3 and 21 days after herbicide applications and the mean separation were done using Least Significance Difference Test by Steel and Torrie (1985)

Experiment II

3.2. Growth fitness in *B. Pilosa* Biotypes Under Unstressed Conditions

In this experiment the growth characteristics of both biotypes B1 (susceptible) and B2 (resistant) were studied

3.2.1 Experimental Design and Treatment

The experiment was laid out in a Completely Randomised Design (CRD) The treatments comprised of four levels of planting ratio 75 25, 50 50, 25 75 of B1 and B2, respectively, and pure stands for each accessions and 2 levels of accessions (susceptible and resistant)

The B2 seeds were collected from the plants which survived the higher rates of paraquat applications in experiment I above 5 Nha B1 seeds remains the same as used in experiment I The total treatments were 5, replicated three times Both biotypes (B1 & B2) were planted according to the layout made and monitored their growth with the layout till the time of data recording reached (which is 3 weeks after transplanting)

The experiment was carried out in pots placed in glass house where only the higher temperatures above 31°C was controlled by an automatic ventilator

Seeds of both accessions were planted at high density in separately marked pots of 27 5cm in diameter and the depth of 25cm After one week a total of 30 of almost uniform plants in growth were transplanted into trays of 38 X 76 cm into their respective planting ratios The plants were spaced at 5 X 5 cm , and arranged in such a way that each plant from one accession was almost surrounded by the plant of the other accession

Soil A mixture of forest soils, manure, gravel, husk and animal blood was used as a growing medium The mixture was sterilized in an oven at a temperature of 125°C for two hours to kill almost every seed already existing in the soil

3.2.2 Measurements and observations.

The parameters measured included, Leaf area, plant height, biomass, number of branches, number of leaves and number of flowers

Three weeks after transplanting Five plants from each accession were randomly selected and tagged for data recording at three weeks after transplanting The first measurement was done at 3 weeks after transplanting. The plant height, number of leaves, and leaf area of the second leaf from top were taken 3 weeks after transplanting

At flowering stage

A week after 50% flowering date, plant height, number of leaves, number of branches, number of inflorescence and Leaf area of the second leaf from top were measured The Leaf area was measured using leaf Area Meter (Li-Cor model 3100, Li-Cor Inc ,Lincoln, Nebraska)

The 5 plants from each accession which were already labelled for the data collection, were cut from the bottom (above ground shoot), chopped and weighed for fresh weight and dried for 48 hours in the oven at 80°C, then dry weight was taken

3.2.3. Data analysis:

Analysis of variance was computed in respect of each parameter, and the mean separations were done, using Least Significance Difference Test by Steel and Torrie (1980)

4. RESULTS

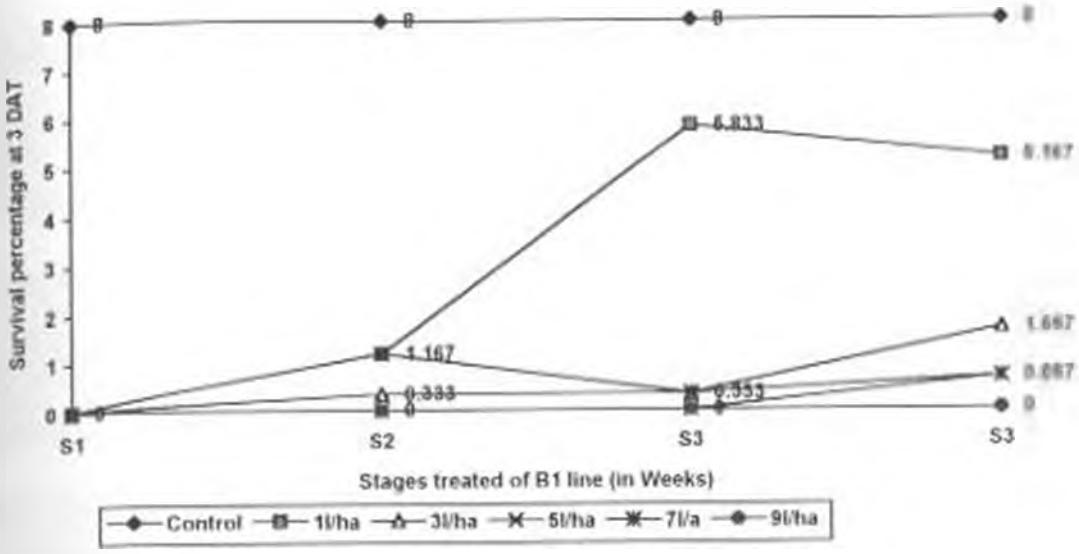
4.1. Effect Of Paraquat Herbicide Rate and Plant Growth Stages on the Resistance of *B. pilosa*

4.1.1. Assessment done 3 days after treatment

There was highly significance difference in survival score at the significance level of 1% between the control and the treatments sprayed with paraquat. B2 accession showed higher survival score than B1 plants at the significance level of 1% (Fig 1)

In B1 accession, the survival score of the plants treated with 3, 5, 7 l/ha was not significantly different from each other but there was marked difference between the plants treated with 9 l/ha in which no plants survived and plants treated with 1, 3 litres per ha. The plants treated with 3 l/ha, a rate slightly higher than the recommended rate 2 l/ha, showed significance difference from the plants treated with 1 l/ha, a rate one unit lower than the recommended rate, but showed no significance difference from the higher rates except when treated with 9 l/ha (Fig. 1a). In B2 accession, plants treated with 1 l/ha showed significantly different in the survival score than the plants treated with 3 and 5 l/ha which were not different from each other. Plants treated with 7 and 9 l/ha had shown significantly the lowest survival score and significantly from the plants treated with the rest of rates (Appendix 18). For B2 accession, the trend of the survival score followed the same as the B1 accession, which is reduction of the survival score as the rate increases, but the reduction rate was much lower than the one of B1 accession (Fig. 1b)

The survival score of the two accessions (B1 & B2) treated in four different stages had shown great difference between the stages of 2, 4 and 6, 8 weeks after emergence at the significance level of 1%, but among the groups there was no significance difference. For both accessions although B2 plants scored significantly higher points in all the stages treated, their survival scores followed the same trend, which is as the plants grow older the resistance increases. The degrees of the resistance developed were different from each other. B1 plants started to develop significantly more resistance at the sixth week of the growth than those treated at the 2nd an fourth week of growth, while B2 plants treated at the fourth week of the growth, these are the plants of one week older than the recommended stage, showed significance difference from the plants treated at the second week of growth. At the sixth week of growth plants treated showed no significance difference from those treated at the eighth week of growth (Appendix 18). The most sensitive plants observed in both accessions are those treated at the second of the growth, those are the plants of one week younger than the recommended stage (Appendix 18).



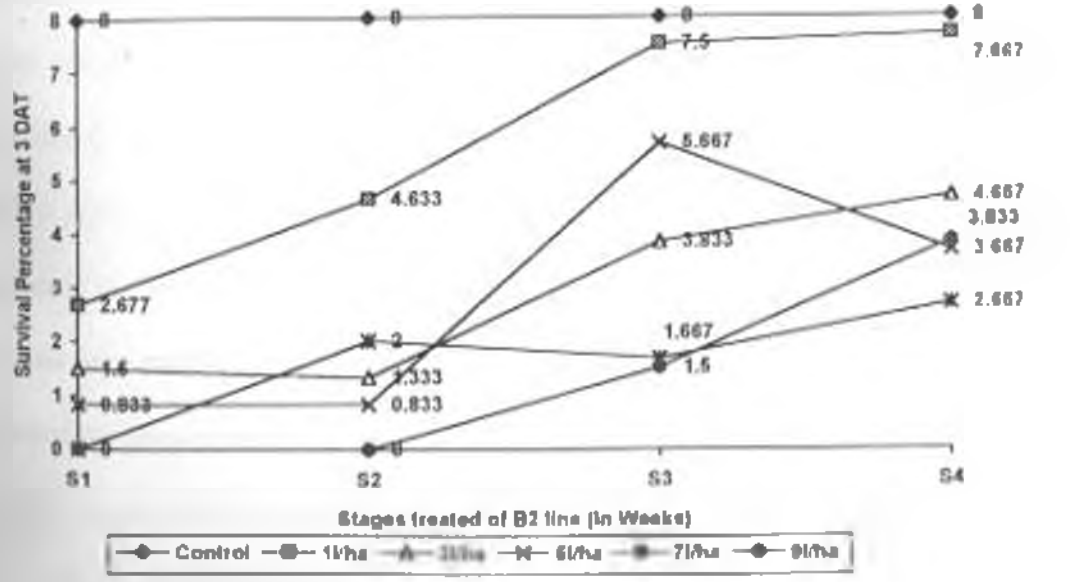


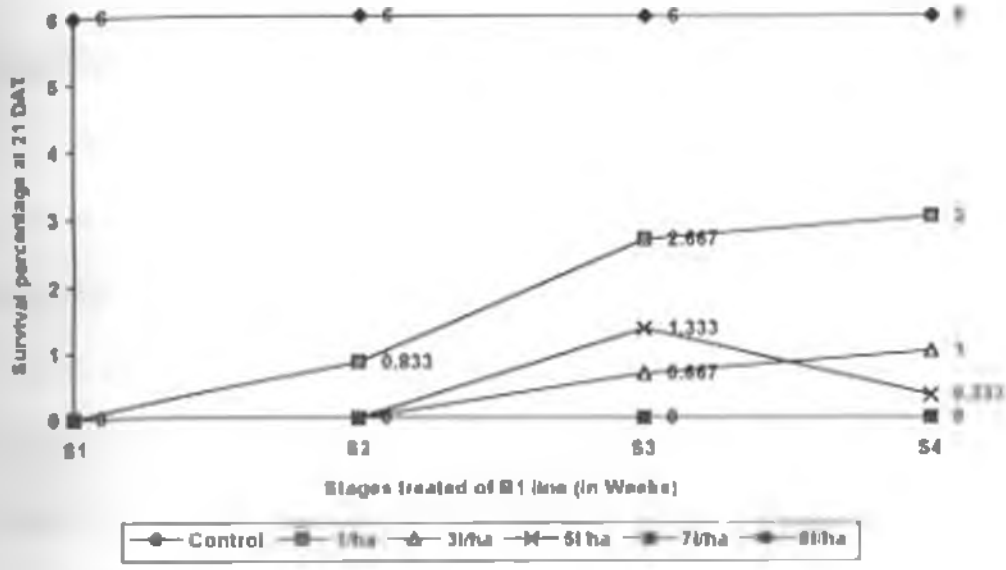
Figure 1 Survival Differences of the two accessions [B1 (a) & B2 (b)] applied with five rates of Gramoxone (1,3,5,7,9,l/ha) and the control, treated in four stages (2,4,6,8 weeks) at 3 days after treatment

4.1.2. Assessment done 21 days after treatment

There was highly significant difference in survival score at the significance level of 5% between the control and the treatments sprayed with paraquat. B2 accession showed higher survival score than B1 plants (Fig 2).

In B1 accession, the survival score of the plants treated with 3, and 5 l/ha was not significantly different from each other, but significantly different from those treated with 7 and 9 l/ha which in turn were not different from each other. In B1 plants, there was a dramatic reduction of the survival score as the rate increases up to the point where no plants survived at R5 (7 l/ha) (Fig. 2a). But in B2 accession, there was no rate where all the plants are killed (Appendix 19). In B2 accession, plants treated with 1 l/ha showed significantly different in survival score than those treated with all the other rates. Plants treated with 3 and 5 l/ha, which were different from each other, showed significance difference in the survival score than those treated with 7 and 9 l/ha which in turn not different from each other (Appendix 19). For B2 accession, the trend of the survival score followed the same as the B1 accession which is a reduction of the survival score as the rate increases, but the reduction rate was much lower than the one of B1 accession (Fig. 2b).

In B1 accession plants developed significantly more resistance at 6th week of growth while in B2 accession, they started developing more resistance after the 2n week of the growth. In B2 accession, those treated at 6th week of growth developed significantly greater resistance than those treated at the 4th week of the growth which in turn were significantly different from those treated at the 2nd week of the growth. The correlation between the age and the resistance was observed significantly in the B2 accession plants (Appendix 19).



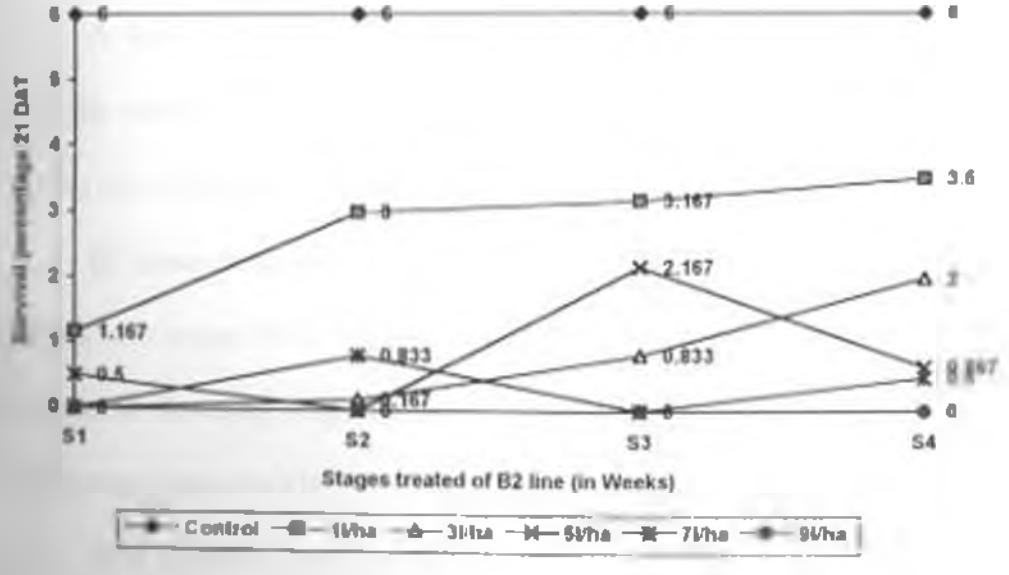


Figure 2 Survival Differences of the two accessions [B1 (a) & B2 (b)] applied with five rates of Gramoxone (1,3,5,7,9,l/ha) and the control, treated in four stages (2,4,6,8 weeks) at 21 days after treatment

4.2. Growth fitness in *Bidens pilosa* Biotypes Under Unstressed Conditions

In the early stage of the growth plants of B1 biotype grew taller plants than the B2 plants in all the planting ratio except P2 (25% B1 75% B2) which had the lowest proportion of B1 plants P4 (75% B1 25% B2) planting ratio resulted in tallest plants while P2 (25% B1 75% B2) the shortest (Fig 3 and Appendix 20) The planting ratio which had the lowest B1 proportion grew shortest plants P4 planting ratio which had the highest B1 planting proportion grew significantly the tallest plants from the rest of the planting ratios (P1, P2, P3) which showed no significance difference from each other (Appendix 20)

At flowering stage B1 plants still maintained their superiority in height, but this time in all the planting ratios (Fig 4). No significance difference was observed among the planting ratio (Appendix 21)

B1 plants developed significantly larger number of leaves than B2 plants in all the planting ratio except P2 (25% B1 75% B2) at the early stage of growth (Appendix 22), but later at flowering stage B2 plants compensated the difference and there was no significance (Appendix 23)

Leaf area showed no significance at both stages (Appendix 24 and 25)

Significantly higher number of branches were produced by B1 plants than B2 plants in all the planting ratio (Appendix 26), and in consequences number of inflorescence were found to be significantly different among the two biotypes and the planting ratio B1 plants significantly produced larger number of inflorescence than B2 plants in all the planting ratio

except P2 (25% B1 75% B2) (Fig 11) In all the planting ratio only purestand was found to be significantly different from P3 (50% B1 50% B2) and P4 (75% B1 25% B2) Thus data shows that higher number of inflorescence were produced when two biotypes (B1 & B2) are grown in competition rather than when they are grown separately B1 plants produced significantly larger number of flowers than B2 plants Surprisingly the P2 planting ratio which had the lowest B1 planting proportion produced more flowers than the purestands of both biotypes Only P3 planting ratio produced significantly more flowers than P1 and P2 but not P4 which had the highest proportion of B1 plants B1 plants in P3 planting ratio got more opportunity than all the plants in all the other planting ratios, because each B1 plant in P3 planting ratio is surrounded by four plant of B2 which are out-fit (Appendix 27)

Neither early nor late detected difference contributed to either to the fresh weight or dry weight (Appendix 28 and 29)

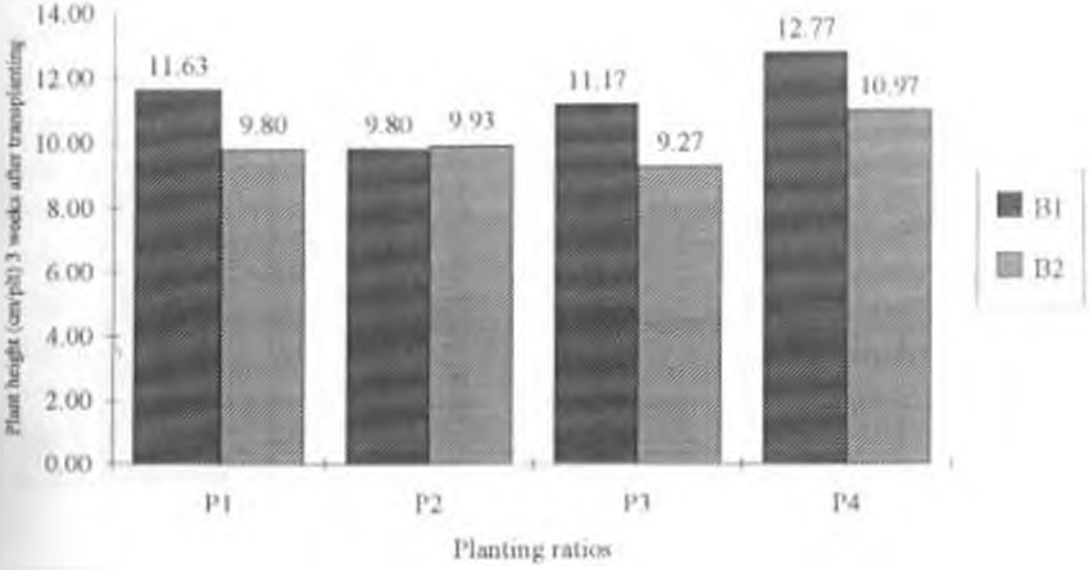


Fig 3: Plant height differences of the two biotypes (B1 & B2) planted in four different ratios P1 (purestand), P2 (25% B1 75% B2), P3 (50% B1 50% B2), and P4(75% B1 25% B2) at 3 weeks after transplanting

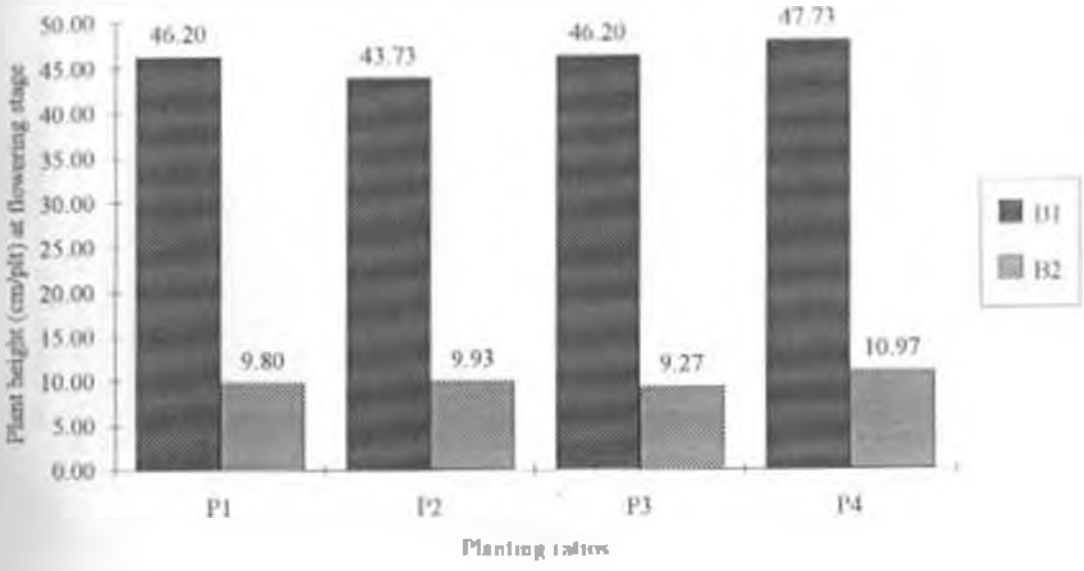


Fig 4: Plant height differences of the two biotypes (B1 & B2) planted in four different ratios P1 (purestand), P2 (25% B1 75% B2), P3 (50% B1 50% B2), and P4 75% B1 25% B2) at flowering stage

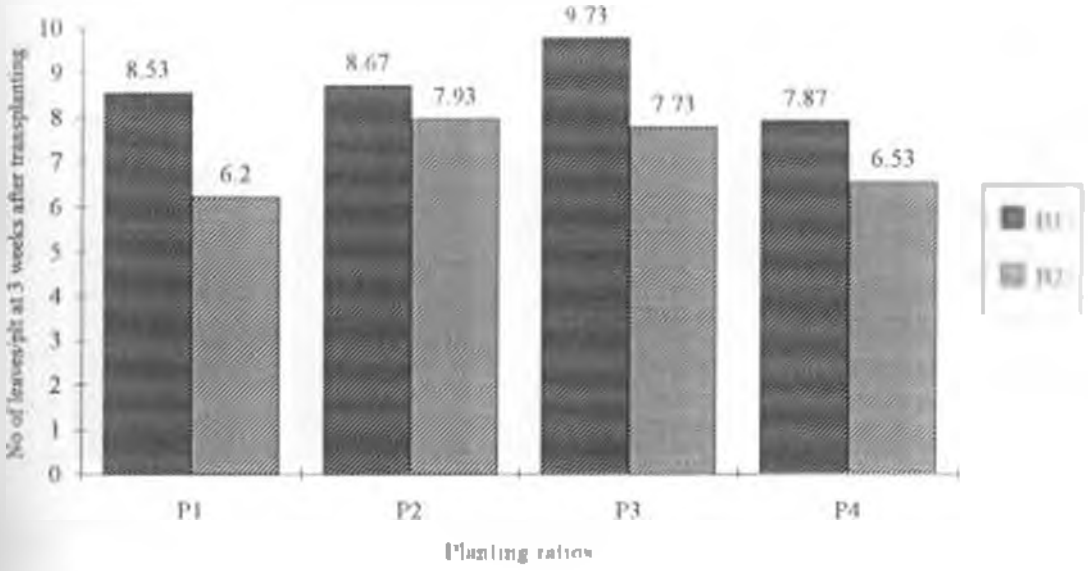


Fig 5: Differences of the number of leaves of the two biotypes (B1 & B2) planted in four different ratios P1 (purestand), P2 (25% B1 75% B2), P3 (50% B1 50% B2), and P4 (75% B1 25% B2) at 3 weeks after transplanting

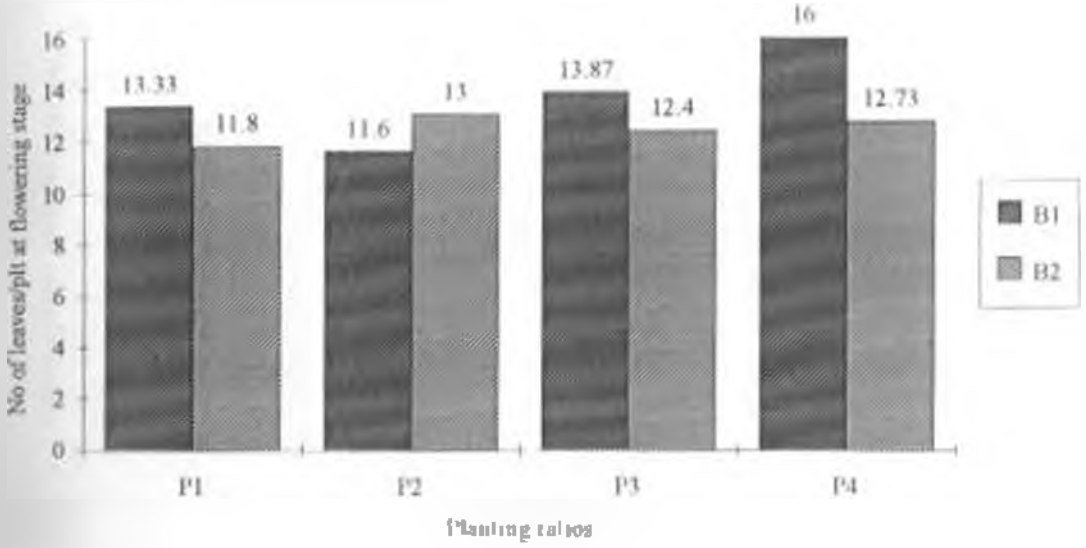


Fig 6: Differences of number of leaves of the two biotypes (B1 & B2) planted in four different ratios P1 (purestand), P2 (25% B1 75% B2), P3 (50% B1 50% B2); and P4 (75% B1 25% B2) at flowering stage

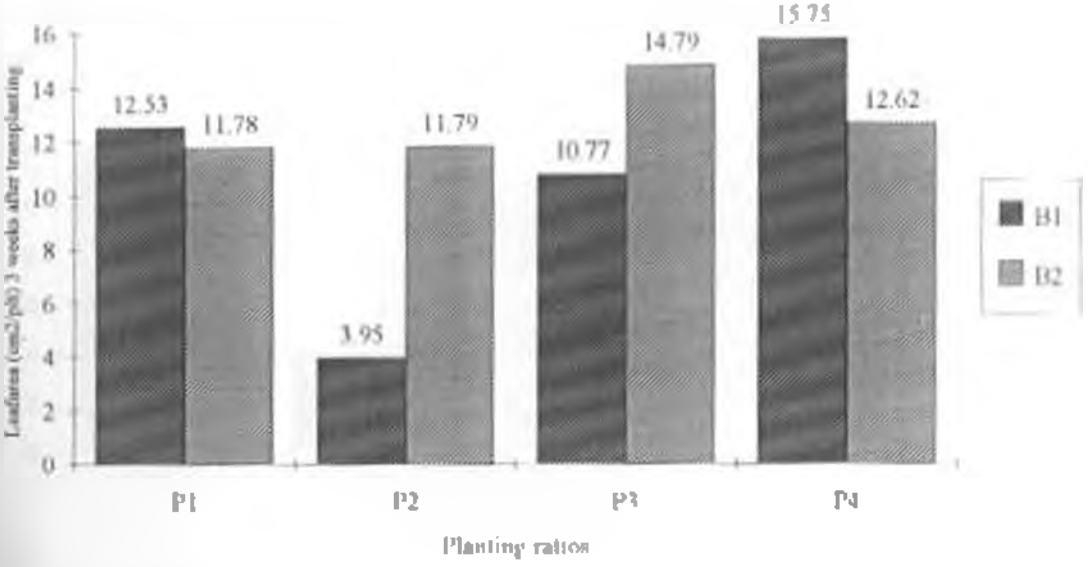


Fig 7:Leaf area differences of the two biotypes (B1& B2) planted in four different ratios P1 (purestand), P2 (25% B1 . 75% B2), P3 (50% B1 50% B2), and P4 (75% B1 25% B2) at 3 weeks after transplanting

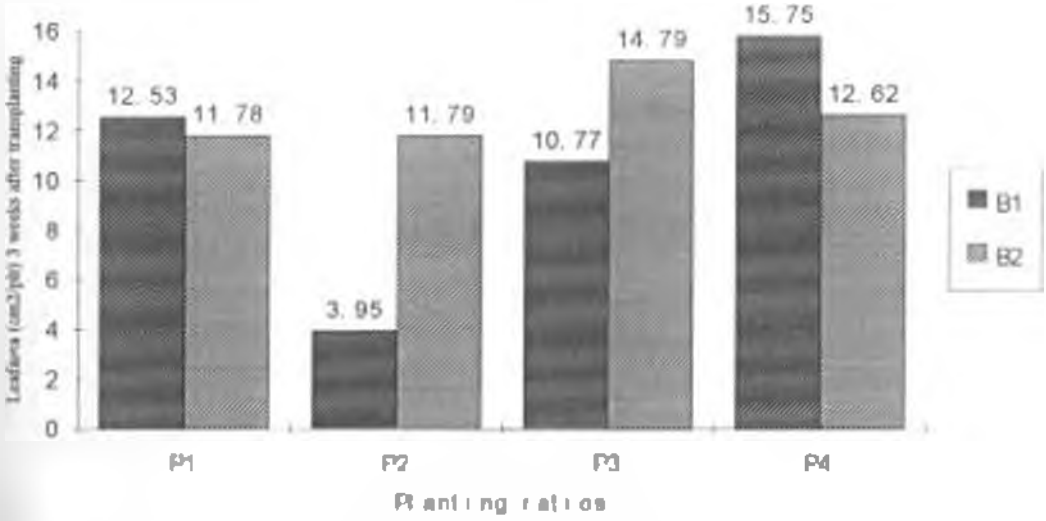


Fig: 8 Leaf area differences of the two biotypes (B1 & B2) planted in four different ratios P1 (purestand), P2 (25% B1 75% B2), P3 (50% B1 50% B2), and P4 (75% B1 25% B2) at flowering stage

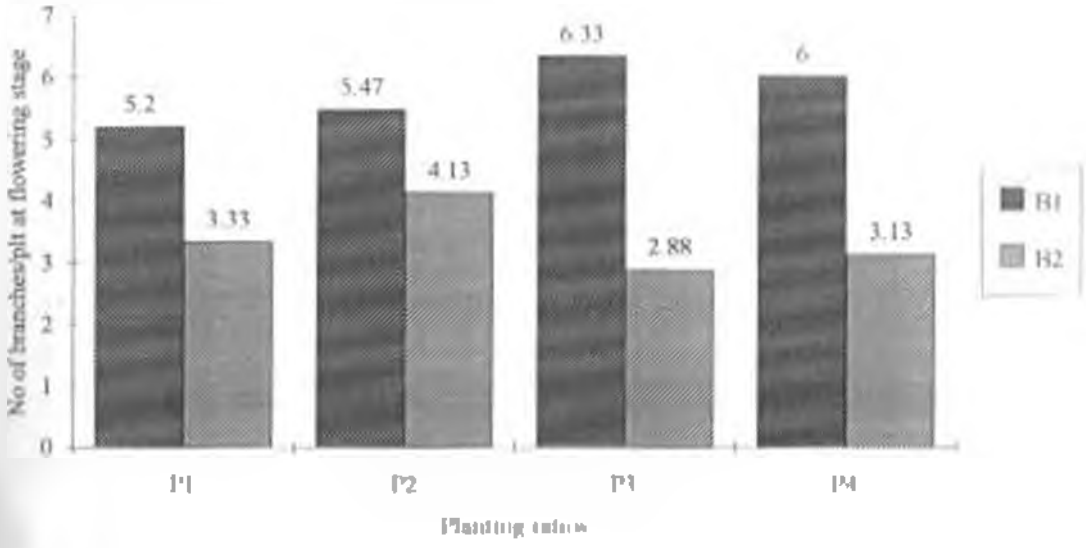


Fig 9: Differences of number of branches of the two biotypes (B1 & B2) planted in four different ratios P1 (purestand), P2 (25% B1 75% B2), P3 (50% B1 50% B2), and P4 (75% B1 25% B2) at flowering stage

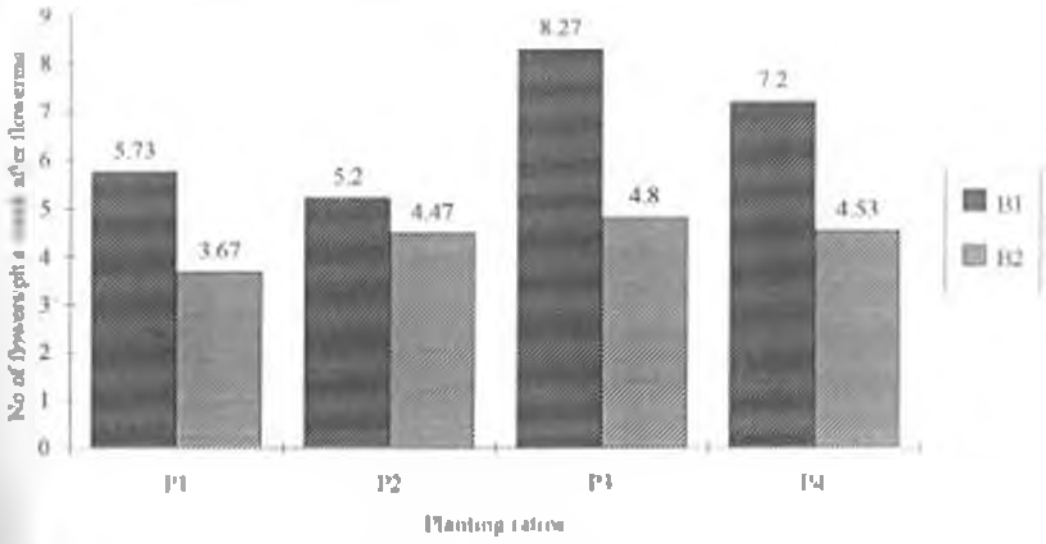


Fig: 10 Differences of number of inflorescences of the two biotypes (B1 & B2)planted in four different ratios P1 (purestand), P2 25% B1 75% B2), P3 (50% B1 50% B2), and P4 (75% B1 25% B2) at flowering stage

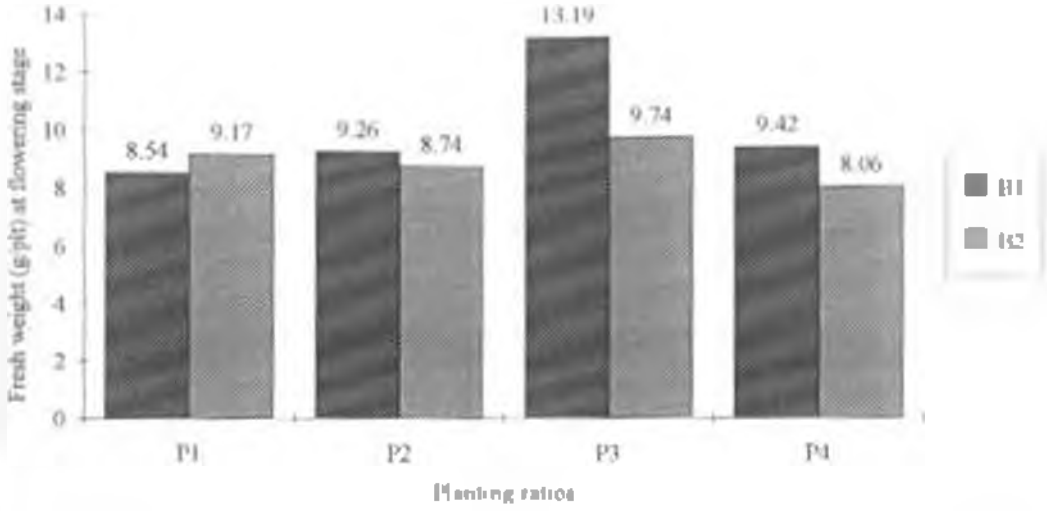


Fig: 11 Differences of fresh weight of the two biotypes (B1 & B2) planted in four different ratios P1 (purestand), P2 (25% B1 : 75% B2), P3 (50% B1 : 50% B2); and P4 (75% B1 : 25% B2) at flowering stage

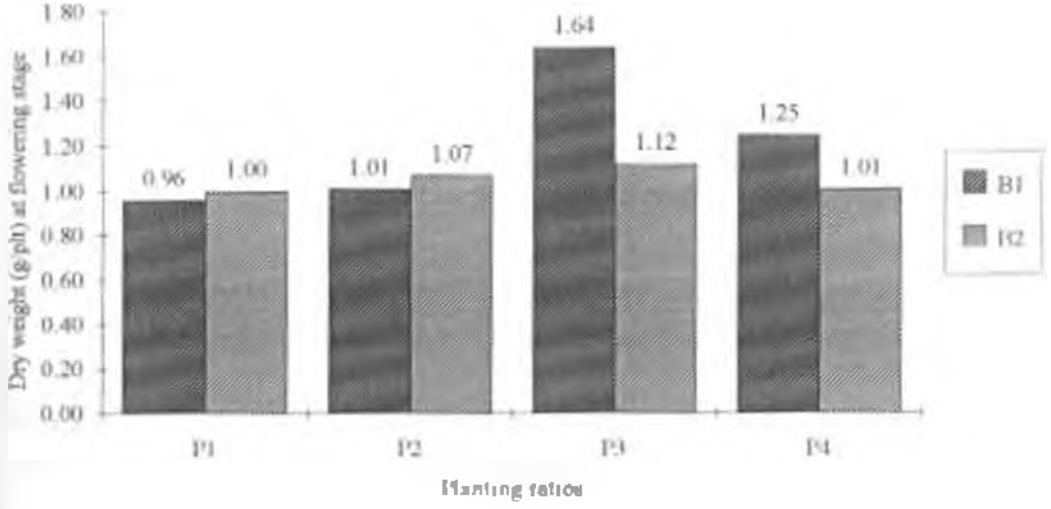


Fig: 12 Differences of dry weight of the two biotypes (B1 & B2) planted in four different ratios P1 (purestand), P2 (25% B1 75% B2), P3 (50% B1 50% B2), and P4 (75% B1 25% B2) a week after flowering date

5. DISCUSSIONS

Irregular *B pilosa* control performance were first reported in coffee plantations in middle 1980's at Coffee Research Foundation, where paraquat has been used for over 20 years (Njoroge, 1991) It is envisaged that continued application of a herbicide to populations with very low frequencies of resistant biotypes would exert greater selection pressure favouring these plants, leading rapidly to a relatively resistant population Since there is no previous database, it is very hard to say that this resistance is from an existing population of the *Bidens pilosa* weed previously resistant to the paraquat Studies of the resistance and growth fitness were carried out

Results of 1st experiment showed that B2 (Resistant) plants showed higher survival score than B1 (susceptible) plants under non-selective environment In the early stage of the growth, both accessions were less resistant to paraquat than at the later stages of the growth This shows that there is increasing resistance with the plant maturity. The increase of the resistance among the two accessions was not the same Plants of B2 accession showed marked resistance immediately after the 2nd week of growth, while B1 accession took 8 weeks to show the same degree of resistance This indicates that the degree of the resistance developed by the resistant plants grows much faster with the age of the plant than the susceptible plants This could be attributed either to the resistant plants developing some morphological structures capable of excluding the entry of the herbicide into the living part of the plant or, most probably, the resistant plants to develop increasingly protective enzymes as the plant grows older

The first plant structures that encounter herbicides are non living and include those associated with the leaf, stem and root surfaces. Movement of a herbicide across these non-living structures is complex and depends on the nature of the herbicide applied (including the formulation and ingredients), the physical properties of the cuticle (epicuticular wax, cuticular wax, cutin, pectin fibres, cell walls, and the cuticular "peg" between cell walls), the species and age of the plant, and the environment (Devine et al., 1993 a,b). Resistance developed by *Bidens pilosa* at the later stage of the growth could be attributed to the development of some morphological structures in which the movement of the herbicide is limited as observed in other case of herbicide movement in *Erigeron philadelphicus* (Tanaka et al., 1986). Marked difference of the degree of resistance was observed in the resistant biotype as the plants grows older than the susceptible biotype. In another study carried out by Ye and Gressel, 1994 concluded that the protective enzymatic activity had higher concentration rates from the age of 9 weeks of growth in paraquat resistant biotype of *Conyza bonariensis*.

Results of the 2nd experiment showed that in the early stages of the growth (3 weeks after transplanting) B1 plants grew significantly taller than B2 biotype in all the planting ratios except P2 planting ratio where B2 was grown in the proportion of 0.75 : 0.25 with B1 respectively. But in the later stage of growth B1 grew significantly taller plants than B2 plants in all the planting ratios. This could be explained that the competition between the resistant and the susceptible biotypes is fierce at the seedling stage. Since the competition level (0.75 B2 : 0.25 B1) is approaching to the purestand of the resistant (B2) biotype, it will behave almost the same as the purestand, and compensate the little suppression caused by the presence of small number of the out-fit biotype 25% in the early

stage of the growth When the wild-type (in this case susceptible) and the selected type (in this case resistant) are grown separately, there may be no significance difference between them in yield per unit area (Gressel et al , 1982)

Among the planting ratios P1 grew significantly the taller plants and P2 the shortest early in the season, but later at flowering stage no significant difference was observed

Susceptible plants developed greater number of leaves at the seedling stage than resistant plants, but measurements done at flowering stage showed that resistant plants recovered or most probably plants from the same biotype (susceptible) started competing and suppressing each other Data of another experiment carried out by Cornard and Rodesevich, 1982 indicated that the resistant biotype is more affected by the competition from the susceptible biotype of the same species than by the competition with itself

Competition ability observed in the resistant biotype of *B. pilosa* could not be attributed to the leafarea since no significance difference was detected between the resistant an the susceptible biotypes at the seedling and at flowering stage This did not support those of an earlier experiments in which the susceptible biotype of *Solanum nigrum* exhibited significantly higher growth parameter values (plant height, leafarea and number of leaves as well as root, stem and leaf dry weight) (Dominguez et al , 1994)Leaf area differences was not detected both at the seedling and at the flowering stage

Susceptible plants showed great difference of number of branches than resistant plants in only P3 and P4 planting ratios, but in P1 and P2 (Puresatand and 0 25 B1 0 75 B2 respectively). B1 maintained the highest value Accordingly susceptible plants produced higher number of inflorescence than resistant plants Radosevich and Holt, 1982 reported that the proportion of total seed output by the susceptible plants was always higher than the

proportion of total seed output by the resistant plants present at any level of competition Though seed number data was not counted, I assumed that since the number of inflorescences produced by the susceptible is significantly higher than the resistant one, the seeds of the susceptible biotype would out-number than the resistant one Data of other studies done by Domingues et al , 1994 showed that the values of seed numbers and 100 seed weight of the resistant biotype of *Solanum niger* were significantly lugher than the values of the susceptible biotype

No significance difference of fresh weight and dry weight were observed Contrary to the findings that even under non-competitive conditions, the dry matter production per plant of resistant *S. vulgaris* and *Amaranthus sp* was 25 and 40% less, respectively than that of susceptible plants (Conard and Radosevich, 1979)

It is important to note that B1 (susceptible) biotype was a better competitor than B2 (resistant) biotype in terms of plant height (at the early stage of growth), number of leaves, number of branches, number of inflorescences, but all these never contributed neither to fresh weight nor dry weight compared to the resistant biotype Review don by Holt, 1993 reported that the competition fitness between the resistant and the susceptible biotypes of different weeds behave differently Paraquat resistance of *Conyza canadensis* was less vigorous than the susceptible biotype in the absence of paraquat Field studies with resistant and susceptible biotypes of *Hordeum glaucum, Hordeum leporinum,* and *Arctotheca calendula* in Australia demonstrated a slight reduction in competitiveness of resistant biotype of *Hordeum glaucum* relative to susceptible biotype whereas resistant and susceptible biotypes of *Hordeum leporinum* were equally competitive

When the two biotypes are grown separately there was significance difference detected in all the parameters measured B1 (susceptible) was found to be having significantly higher values than B2 (resistant) in all the parameters measured This does not agree with the classical case which says " when the wild-type and the selected type are grown separately, there may be no difference between them in yield per unit area (Gressel et al, 1982)

The susceptible biotype can be more fit than the resistant biotype, as observed with other studies, at any growth stage in the life cycle because of the following factors 1) The proportion of the seeds germinating at a given time 2) The rate of germination 3) Success in establishment following self thinning 4) Any of physiological characters resulting in difference in growth rate 5) Parkinsonia plasticity 6) The seed size and yield per flower and per plant (Gressel et al , 1982)

In conclusions the results of both experiments showed that there is presence of a resistance to *B pilosa* L and that the resistant biotype is less competitive than the susceptible one attributed mainly to the average higher scores attained during the assessment of the survival score and the subsequent growth fitness observations that the susceptible out-competes than the resistant biotype under unstressed conditions As Gressel et al 1982 noted , when pressure is brought to bear on a wild population to select for a given trait, the selected individuals are less "fit" This has been described as the "cost" of selection

6. CONCLUSIONS AND RECOMMENDATIONS

The study of resistance assessment experiment has shown that there is an evidence of resistance in *Bidens pilosa* L. B2 accession has shown higher tolerance than B1 accession. Tolerance increases as *B. pilosa* matures especially after fourth week of growth.

Growth studies experiments on the biotypes of *Bidens pilosa* showed that B1 biotype out-fitted B2 line in terms of plant height, number of leaves, number of branches and number of inflorescences.

FURTHER STUDIES ON THE FOLLOWING ARE SUGGESTED

1. Study of the mechanism of the resistance should be done.
2. There is also greater need for studies on population biology in agriculture. This would give a follow-up studies to catch changing patterns of resistance as early warning system for research, extension, farmers and industry.
3. Explore viability of using higher paraquat application rates and alternate use of combination of herbicide to preclude development of economic weed resistance to any one herbicide.
4. Study on morphological characteristics of the two biotypes.

RECOMMENDATIONS

It is not easy for farmer to shift to another herbicide which sometimes is not easily available, but where it is economical it is recommendable to rotate the herbicide with different sites of action to reduce the selection pressure for the evolution of resistant biotypes. Resistance can also be avoided by understanding an analysing the interacting factors involved in changing a sensitive weed population into a resistant one.

Alternative methods of weed control should be encouraged, eg Animal grazing in perennial crops cultural weed management which includes (Hand weeding, mechanical, tillage, burning, flooding, mulching (non-living material, crop rotation and etc.), introducing natural enemies of Bidens pilosa as a biological control B Pilosa is known to be a particularly promising target for biological control

7. REFERENCES

Abel, A L 1954 The rotation of weed killing In *Proc Br Weed Cont*, Cliftonville, Margate, England

Alembi, Daniel 1993 paraquat and atrazine resistance in *Bidens pilosa* L in Kenya M sc Thesis, University of Bath

Ashton, F M and A S Crafts 1981 "Bipyridiums" In Ashton, F M and A S Crafts, eds. *Mode Of Action Of Herbicides*, Wiley, New York pp 164-179

Baldwin, B C 1963 Translocation of diquat in plants *Nature*, **198** 872

Baur, J B , R W Bovey, P S Baur and Z El-Seify 1969 Effect of paraquat on the ultrastructure of mesquite mesophyll cells *Weed Res* , **9** 81

Beste, C E 1983 *Herbicide Handbook for the Weed Science Society of America* 5th ed WSSA Illinois pp 362-366

Brian, R C 1967 The uptake and adsorption of diquat and paraquat by tomato, sugar beet and cocksfoot *Ann. Appl. Biol* , **59**. 91

Caseley, J C , Cussain and R K Atkin 1991 *Herbicide resistance in weeds and crops* Butterworth Heinmann, Oxford

Cornard, S G and S R Radosevich 1979 Ecological fitness of *Senecio vulgaris* and *Amaranthus retroflexus* biotypes susceptible and resistant to atrazine *J. Appl. col* **16** 171

Damonakis, M , D S H Drennan, J D Freyer and K Holly 1970 The adsorption and mobility of paraquat on different soils an soil constituent *Weed Res* **10:** 264

Devine, M D , Duke, S O , and Fedtke, C 1993a *Physiology of Herbicide Action* Prentice Hall, Englewood Cliffs, NJ

Devine, M D Hall, J C , Romano, M L , Marles, M A S , Thomson, L W , and Shimabukuro, H R H 1993b Diclofop and fenoxaprop resistance in wild oats is associated with an altered effect on the plasma membrane electrogenic potential *Pestic. Biochem Physiol* **45**, 167-177

Dodge, A D 1982 The role of light and oxygen in the action of photosynthetic inhibition herbicides In DE moreland, JB St John, FD Hess, eds. *Biochemical responses induced by herbicides* ACS symposium series No **181** pp 57-77

Dodge, A D 1983 Toxic oxygen species and herbicide action In J Myamaoto, PI Kearney, eds, *IUPAC pesticide chemistry* Human Welfare and the environment Pergamon Press, New York, pp 59-66

Donunguez, C M Tena and R De Prado 1994 Photosynthetic activity, growth and productivity in a s-triazine resistant biotype of *Solanum nigrum*. *Plant Physiology and Biochemistry* Paris **32** 627-632

Feurst, E P Vaughn, K C 1990 Mechanism of paraquat resistance *Weed Technology* **4** 150-156

Feurst, E P , H Y Nakatam, A D Dodge, D penner, and C J Arntzen 1985 paraquat resistance in *Conyza Plant Physiol* 77 984-989

Foyer, C H and B Halliwell 1976 The presence of glutathione and glutathione reductase in chloroplast a proposed role in ascorbic acid metabolism *Planta*, **133** 21

Georghion, G P 1986 The magnitude of the resistant problem In *Pesticide resistant Strategies and Tactics for management* National Academic Press, Washighton D C

Gorden, D and E Beck 1979 H_2O_2 destruction by ascorbate dependent system from chloroplast *Biochem. Biophys. Acta* **546** 426

Gressel, J 1985 Herbicide tolerance and resistance Alteration of site of activity *Weed physiology*. 2 159-189

Gressel, J 1986 Modes and Genetics of Herbicide Resistance in Plants. In *Pesticide resistance strategies and tactics for management* National Academic Press, Washington D C

Gressel, J and G Ben-Sinai In Press Low intra-specific competitive fitness in a triazine resistant, nearly nuclear isogenic biotype of *Brassica napus*. *Plant Sci Lett* 38

Gressel, J H O Ammon, H Fogelfors, J Gasquez Qonkey, H Kees 1982 Discovery and distribution of herbicide resistant weeds outside North America In H M LeBaron and Gressel, J eds, *Herbicide Resistance in plants* John Wiley and Sons, NewYork, pp 31-56

Harper, D B and B M R Harvey 1978 Mechanism of paraquat tolerance in perennial rye-grass II Role of superoxide dismutase, catalase and peroxidase *Plant Cell Environ*, 1 211

Harris, N and A D Dodge 1972 The effect of paraquat on Flax cotyledons leaves Physiological and biochemical changes. *Planta*, 104 210

Harvey, B M R , J Muldoon and D B Harper 1978 Mechanism of paraquat tolerance in perennial ryegrass I Uptake, metabolism and translocation of paraquat *Plant Cell Environ* , 1 203

Harvey, B M R , D B Harper 1982 Tolerance to Bipyridilium herbicides In H M LeBaron and J Gressel, eds *Herbicide resistance in plants* John Wiley and Sons, New York pp 215-233

Holt, J S 1993 Mechanism and Agronomic Aspects of Herbicide Resistance *Annu Rev Plant Physiol. And Plant Mol. Biol* **44**: 203-229

Hutchinson, J M 1979 Hydrogen peroxide production and lipid peroxidation induced by paraquat in isolated cells and chloroplast of spinach (*Spinacea oleracea* L) Ph D thesis University of California

Ivens, G W 1989 In *Weeds in East Africa* Oxford, Nairobi p 124

LeBaron, H M and J Gressel 1982. *Herbicide resistance in plants* John Wiley and Sons, New York

LeBaron H M 1991 Distribution and seriousness of herbicide-resistant weed infestations world wide In J C Caseley, G W Cussan and R K Atkin Eds *Herbicide Resistance in Weeds and Crops* Butterworth-Heinemann, Oxford pp 27–44

Lev Roy G Holm, Donald L Plucknell, Juan V Pancho and James P Harberger 1977 *The World's worst weeds distribution and biology* East West Centre Book University of Hawaii, Honolulu pp 184-195

Luitman P J W and G R Sagar 1975 The influence of the nitrogen status of the Oat plants (*Avena sativa L.*) on the interception and the retention of the foliar sprays *Weed Res* 15:217

Njoroge, J M 1991 Tolerance of *Bidens pilosa* L and *Parthenium hysterophorus* L to paraquat (Gromoxone) in Kenya coffee *Kenya coffee* **vol. 56** No 651, 999-1002

Powels, S B , Howart, P D 1990, Herbicide resistance weeds in Australia *Weed Technology* **4** 178-185

Radosevich, S R and J S Holt 1982 Physiological response and fitness of susceptible and resistant biotypes to triazine herbicide In H M LeBaron and J Gressel eds *Herbicide Resistant in Plants*, Newyork John Wiley and Sons pp 163-184

Rochecouste, E and R Vaughn 1959 Weeds of Mauritius *Bidens pilosa* L leaflet series I Mauritius Sugar Industry Institute

Shaalfiel, Y and J Gressel 1987 Kinetic analysis of resistance to paraquat in *Conyza Plant Physiol* **85** 869-871

Shaaltiel, Y , and J Gressel, 1986 Multi enzymatic oxygen radical detoxification system correlated with Paraquat resistance in *Conyza bonariensis*. *Pestic Biochem Physiol* **26** 22-28

Shaaltiel, Y , A Glazier, P I Bocion and J Gressel 1988 Cross tolerance to herbicide and environmental oxidants of plant biotype tolerant to paraquat, sulfur dioxide and ozone *Pest Biochem. Physiol* **31** 12-23

Slade, P , and E G Bell 1966 The movement of paraquat in plants *Weed Res* **6**: 267

Slade, P 1966 The fate of paraquat applied to the plants *Weed Res* **6** 158

Tanaka, Y , H Chusaka and H Saka 1986 Movement of paraquat in resistant and susceptible biotype of *Erigeron philadelphicus* and *E. canadensis* *Physiol. Planta* **66** 605-608

Terry, P J and Michieka, R W 1987 Common weeds of East Africa FAO, Rome p 30

Tolbert, N E 1971 Microbodies - peroxisomes and glyoxysomes, Ann Rev *Plant Physiol* **22** 45

Terry, P.J., G.A. Mathews, J.G. Boonman 1984 *A guide to Weed Control in East African Crops* Kenya Literature Bureau

Vaughn, K.C. and E.P. Fuerst. 1985 Structural and physiological studies of paraquat-resistant *Conyza Pestic. Biochem. Physiol* **24** 86-94

Vaughn, K.C., M.A. Vaughn and Patrick Camilleri 1989 Lack of cross-resistance of paraquat resistant Hairy fleabane (*Conyza bonariensis*) to other oxygen generators indicates enzymatic protection is not the resistance mechanism *Weed Sci* **37** 5-11

Waterhouse, D.F. 1994 *Biological control of weeds* Southeast Asian Prospects Brown Prior Anderson Pty Ltd pp 26-33

Wilcut, J.W. and Charles W. Swann 1990 Timing of paraquat applications for weed control in Virginia-type peanuts *Weed Sci* **38** 558-562

Worthing, Charles R., Raymond J. Hance 1991 *The pesticide manual* A world compendium, 9th ed. Unwin Brothers Limited Old, Surrey PP 646-647

Ye, B. and J. Gressel, 1994 Constitutive Variation of Ascorbate Peroxidase Activity During Development Parallels that of Superoxide Dismutase and Glutathione Reductase in Paraquat-resistant *Conyza bonariensis Plant Science Limerick* **102** 147-151

Youngman, R J and A D Dodge. 1981 On the mechanism of paraquat resistance to *Conyza* sp In Akoyunoglou, ed Proc Int Congr Photosynth **Vol. 6** Balaban Press, Tel Aviv, Israel pp 537-544

Youngman, R J and A D Dodge 1981 On the mechanism of paraquat resistance in *Conyza* sp In Proc 5th int congr photosynth G Akoyunoglou (Ed) Balaban International Science Services, 2242 Mt Carmel Ave , Glenside, Pa 19038

8. APPENDICES

Appendix 1 ANOVA table for the survival score of the assessment done 3 days after treatment

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
2	Rate	5	970.754	194.151	208.5163 ***
4	Stage	3	115.287	38.429	41.2782 ***
6	Rate x Stage	15	85.862	5.724	6.1486 ***
-7	Error	48	44.687	0.931	
8	Biotype	1	100.668	100.668	153.9524 ***
10	Rate x Biotype	5	28.460	5.692	8.7048 ***
12	Stage x Biotype	3	16.359	5.453	8.3393 ***
14	RateXSatgeXBiot	15	32.957	2.197	3.3601 ***
-15	Error	48	31.387		
Total		143	1126.420		

C.V. 28.37%

Appendix 2 ANOVA table for the survival score of the assessment done 21 days after treatment

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
2	Rate	5	626.089	125.202	186.8295 ***
4	Stage	3	16.977	5.659	8.4447 ***
6	Rate x Stage	15	29.512	1.967	2.9359 **
-7	Error	48	32.167	0.670	
8	Biotype	1	5.252	5.252	5.8000 *
10	Rate x Stage	5	1.259	0.852	0.8920 ns
12	Stage x Biotype	3	0.519	0.173	0.1812 ns
14	RateXStageXBiotype	15	1.762	0.317	0.3325 ns
-15	Error	48	45.833	0.955	
	Total	143	765.290		

C.V. 61.05%

* = Significant at 5% significance level
** = Significant at 1% significance level
*** = Significant at 0.5% significance level
ns = not significant

Appendix 3 ANOVA table for the plant height of the two biotypes (B1 & B2) at 3 weeks after transplanting

Source	SS	df	MS	F
Main Effects				
biotype	12 042	1	12 042	11 496 **
population	15 710	3	5 237	4 999 *
Interaction				
biotype x population	4 848	3	1 616	1 543 ns
Error	16 760	16	1 048	
Total	49 360	23		

C.V 9.57%

Appendix 4 ANOVA table for the number of leaves of the two biotypes (B1 & B2) at 3 weeks after transplanting

Source	SS	df	MS	F
Main Effects				
biotype	11 760	1	11 760	7 007 *
population	6 893	3	2 298	1 369 ns
Interaction				
biotype x population	3 107	3	1 036	0.617 ns
Error	26 853	16	1 678	
Total	48 613	23		

C.V 16.26%

Appendix 5 ANOVA table for the leaf area of the two biotypes (B1 & B2) at 3 weeks after transplanting

Source	SS	df	MS	F
Main Effects				
biotype	1 480	1	1 480	0 217 ns
population	13 025	3	4 342	0 636 ns
Interaction				
biotype x population	45 077	3	15 026	2 201 ns
Error	109 245	16	6 828	
Total	168 827	23		

C V 20 11%

* = Significant at 5% significance level
** = Significant at 1% significance level
ns = Not significant

Appendix 6 ANOVA table for the plant height of the two biotypes (B1 & B2) at flowering stage

Source	SS	df	MS	F
Main Effects				
biotype	159.135	1	159 135	6 749 *
population	10 778	3	3 593	0 152 ns
Interaction				
biotype x population	15 592	3	5.197	0 220 ns
Error	377.253	16	23 578	
Total	562 758	23		

C V 11 19%

Appendix 7 ANOVA table for the number of leaves of the two biotypes (B1 & B2) at flowering stage

Source	SS	df	MS	F
Main Effects				
biotype	8 882	1	8 882	1 063 ns
population	15 178	3	5 059	1 745 ns
Interaction				
biotype x population	16 818	3	5 606	1 933 ns
Error	46 400	16	2 900	
Total	87 278	23		

C V 13 01%

Appendix 8 ANOVA table for the number of branches of the two biotypes (B1 & B2) at flowering stage

Source	SS	df	MS	F
Main Effects				
biotype	34 082	1	34 082	15 215 **
population	0 872	3	0 291	0 130 ns
Interaction				
biotype x population	4 165	3	1 388	0 620 ns
Error	35 840	16	2 240	
Total	74 958	23		

C V 32 83%

* – significant at 5% of significance level

** Significant at 1% of significance level

ns = not significant

Appendix 9 ANOVA table for the leaf area of the two biotypes (B1 & B2) at flowering stage

Source	SS	df	MS	F
Main Effects				
biotype	94 685	1	94 685	0 525 ns
population	262 458	3	87 486	0 485 ns
Interaction				
biotype x population	603 313	3	201 104	1 115 ns
Error	2885 198	16	180 325	
Total	3845 653	23		

C V 34 38%

Appendix 10 ANOVA table for the number of inflorescence of the two biotypes (B1 & B2) at flowering stage

Source	SS	df	MS	F
Main Effects				
biotype	29 927	1	29 927	19 841 ***
population	13 713	3	4 571	3 031 ns
Interaction				
biotype x population	5 980	3	1 993	1 322 ns
Error	24 133	16	1 508	
Total	73 753	23		

C.V 22 39%

Appendix 11 ANOVA table for the fiesh weight of the two biotypes (B1 & B2) at flowering stage

Source	SS	df	MS	F
Main Effects				
biotype	8 319	1	8 319	0 870 ns
population	30 590	3	10.197	1 066 ns
Interaction				
biotype x population	13 324	3	4 441	0 464 ns
Error	153 009	16	9 563	
Total	205 242	23		

C V 32 5%

*** Significant at 0 5% of significance level
ns = Not significant

Appendix 12 ANOVA table for the dry weight of the two biotypes (B1 & B2) at flowering stage

Source	SS	df	MS	F
Main Effects				
biotype	0 167	1	0 167	1 226 ns
population	0 566	3	0 189	1 388 ns
Interaction				
biotype x population	0 338	3	0 113	0 829 ns
Error	2 176	16	0 136	
Total	3 247	23		

C V 32 49%

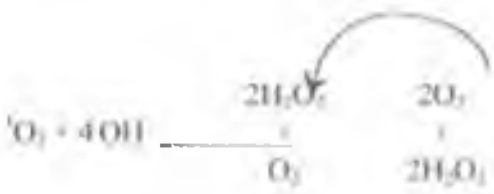
ns = Not significant

Appendix 13 Paraquat chemical formula (LeBaron and Gressel, 1982)

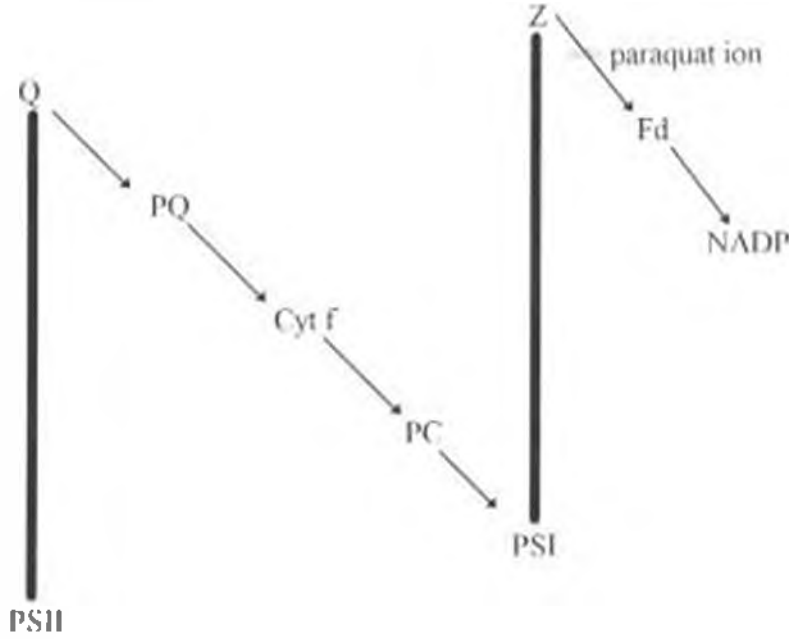
Appendix 14 Free radical formation from paraquat ion and auto-oxidation of free radical yielding H_2O_2 (Hydrogen peroxide) and O_2^- (Superoxide radical), and subsequently, OH (Hydroxyl radical) and $^1O_2$ (Singlet Oxygen) (Ashton and Crafts, 1981)

Paraquat ion [2X]

Paraquat [X]
Free radical



Appendix 15 Reduction of paraquat cation in photosynthetic electro transport Q, primary electron acceptor of PSII, PQ, plasto-quinone, cyt, cytochrome, PC, plastocyanin, Z, Primary electron acceptor of photosystem I, Fd, ferredoxin (LeBaron and Gressel, 1982)



Appendix 16 Common names of *Bidens pilosa* (Alembi 1993)

English	Hairy beggar ticks
	Black jack
	Spanush needle
	Black fellows
	Cobblers peg
	Farmers friend
Portuguese	Picao-preto
	Picao do campo
	Pico picho
French	Sornet
Polynesian names	Fisiuli
	Kofe Tonge - Niue
Philipine names	Pisau pisau, Nguad, Puriket (Bon)
Australia	Black fellows
South Africa	Gewone knapsekerel
Zibabwe	Nyamaradzo

East Africa
Black jack (E), Ida (Atesa), Ekanoganogia (Ekagusii), Enyabarashana (Runyankore, Rukiga), Kichoma nguu (Kiswahili), Labika (Acholi), Muceege (Kikuyu), Murashe (Runyankore), Nyabaraasana (Lutoro, Lunyoro), Nyanyiek-mom (Luo), Onorot (Lango), Ssere (Luganda), Olukuye (Luhya)

Ethiopia	Chigogot, chibu, yesitana mertle, Abare, Zagogo
Sierra Leone	Dada
Liberia	Niani (Kru-Guere)
Upper volta	Nanguadian-Mauding
Ivory Coast	Solole (Dan)
Ghana	Asedura (Asante)
Nigeria	Abere (Yoruba)

Appendix 17 Plant species relative of *B. pilosa* (LeyRoy et al , 1977)

Kerneria dubia Cass
Kerneria tetragona Moench
Bidens sundaicus Brume
Bidens subalternans DC
Bidens quadrangularis DC
Bidens elgonensis (Sherff)
Bidens mototonensis (Sherff)
Bidens biternata (Lour) Mer
Bidens uncumbens (Sherff)
Bidens schimperi Sch Bip
Bidens linearis (Sherff)
Bidens superba (Sherff)
Bidens ruepellii (Sch Bip) Sherff
Bidens ugandensis Sherff
Bidens convacea (O Hoffm) Sherff
Bidens kilimandscharica (O Hoffm) Sherff
Bidens grantii (Oliv) Sherff
Bidens grantii (Oliv) Sherff
Bidens tripartita L
Bidens biternata L.
Bidens steppia Sherff

Appendix 18 Means of the survival score of the accessions (B1 & B2) applied with six different rates (0, 1, 3, 5, 7, 9 l/ha) at four different stages (2, 4, 6, 8 weeks after emergence) at 3 days after treatment

	B1					B2				
R/S	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
R1	8.000	8.000	8.000	8.000	8.000^{a}	8.000	8.000	8.000	8.000	8.000^{a}
R2	0.000	1.167	5.833	5.167	3.042^{b}	2.667	4.633	7.500	7.667	5.617^{b}
R3	0.000	0.333	0.333	1.667	0.583^{c}	1.500	1.333	3.833	4.667	2.833^{c}
R4	0.000	1.167	0.333	0.667	0.292^{c}	0.833	0.833	5.667	3.667	2.750^{c}
R5	0.000	0.000	0.000	0.667	0.167^{c}	0.000	2.000	1.667	2.667	1.583^{d}
R6	0.000	0.000	0.000	0.000	0.000^{c}	0.000	0.000	1.500	3.833	1.333^{d}
Mean	1.333,	1.611,	2.417,	2.694,	2.014	2.167,	2.800,	4.694,	5.083,	3.686

$LSD_{0.05}$ = 0.792

SE for rate = 0.1970

SE for stage = 0.1608

SE for biotype = 0.0953

Within the column means followed by the same suprscript, and within the rows means followed by the same subscript are not significant different at 5% level of significance according to the Least Significance Difference Test

Appendix 19 Means of the survival score of two accessions (B1 & B2) applied with six different rates (0, 1, 3, 5, 7, 9 Nha) at four different stages (2, 4, 6, 8 weeks after emergence) at 21 days after treatment

	B1					B2				
R S	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
R1	6.000	6.000	6.000	6.000	6.000[a]	6.000	6.000	6.000	6.000	6.000[a]
R2	0.000	0.833	2.667	3.000	1.625[b]	1.167	3.000	3.167	3.500	2.708[b]
R3	0.000	0.000	0.667	1.000	0.417[c]	0.000	0.167	0.833	2.000	0.750[c]
R4	0.000	0.000	1.333	0.333	0.417[c]	0.500	0.000	2.167	0.667	0.833[c]
R5	0.000	0.000	0.000	0.000	0.000[d]	0.000	0.833	0.000	0.500	0.333[d]
R6	0.000	0.000	0.000	0.000	0.000[d]	0.000	0.000	0.000	0.000	0.125[d]
Mean	1.000,	1.139,	1.778,	1.722,	1.410	1.278,	1.667,	2.028,	2.194,	1.792

$LSD_{0.05}$ = 0.673

SE for rate = 0.167

SE for stage = 0.1364

SE for biotype = 0.1152

Within the column, means followed by the same suprscript, are not significantly different at 5% level of significance, and within the rows means followed by the same subscript are not significant different at 5% level of significance according to the Least Significance Difference Test

Appendix 20: *Bidens pilosa* plant height differences of the two biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand), P_2 (25% B_1 75% B_2), P_3 (50% B_1 50% B_2), and P_4 (75% B_1 25% B_2) at 3 weeks after transplanting

	Biotype			
Population	B1	B2	Total	Mean
P1	11 63	9 80	21 43	10 715a
P2	9 80	9 93	19 73	9 865a
P3	11 17	9 27	20 44	10 22a
P4	12 77	10 97	23 74	11 87b
Mean	11 343x	9 993y		10 668

Biotype $LSD_{0.05}$ = 0 512; $LSD_{0.01}$ = 1 057, SE = 0 362
Population $LSD_{0.05}$ = 1 085, $LSD_{0.01}$ = 1 496, SE = 0 512

Within the column, means followed by the same letters (a,b) and within the rows, means followed by the same letters (x, y) are not significantly different at 5% level of significance according to Least Significant Difference Test

Appendix 21 Plant height differences of the two biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand), P_2 (25% B_1 75% B_2), P_3 (50% B_1 50% B_2), and P_4 (75% B_1 25% B_2) at flowering stage

	Biotype			
Population	B1	B2	Total	Mean
P1	46 20	9 80	21 43	10 715
P2	43 73	9 93	19 73	9 865
P3	46 20	9 27	20 44	10 220
P4	47 71	10 97	23 74	11 870
Mean	45 965x	40 783y		43 374

Biotype: $LSD_{0.05}$ = 3 640, $LSD_{0.01}$ = 5.015, SE = 1 717

Within the columns means followed by the same letter of x, y, are not significantly different at 5% level of significance according to the Least Significant Difference Test

Appendix 22: Differences of number of leaves of the two biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand) P_2 25% B_1 75% B_2), P_3 (50% B_1 50% B_2), and P_4 (75% B_1 25% B_2) at 3 weeks after transplanting

	Biotype			
Population	B1	B2	Total	Mean
P1	8 53	6 20	14 73	7 365
P2	8 67	7 93	17 14	8 670
P3	9 73	7 73	19 46	9 730
P4	7 87	6 53	14 40	7 200
Mean	8 70x	7 098y		7 890

Biotype $LSD_{0.05}$ = 0 917, $LSD_{0.01}$ = 1 338, SE 0 458

Within the columns means followed by the same letter of x, y, are not significantly different at 5% level of significance according to the Least Significant Difference Test

Appendix 23: Differences of number of leaves of the two biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand) P_2 25% B_1 75% B_2), P_3 (50% B_1 50% B_2), and P_4 (75% B_1 25% B_2) at flowering stage

	Biotype			
Population	B1	B2	Total	Mean
P1	13.33	11.80	25.13	12.565
P2	11.60	13.00	24.60	12.300
P3	13.87	12.40	26.27	13.135
P4	16.00	12.73	28.73	14.365
Mean	13.70	12.489		13.094

Appendix 24: Differences in leaf area of two *B. pilosa* biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand) P_2 25% B_1 75% B_2), P_3 (50% B_1 50% B_2); and P_4 (75% B_1 25% B_2) at 3 weeks after transplanting

	Biotype			
Population	B1	B2	Total	Mean
P1	12 53	11 78	24 31	12 150
P2	3 95	11 79	25 74	12 870
P3	10 77	14 79	25 56	12 780
P4	15 75	12 62	28 35	14 175
Mean	13 243	12 745		12 995

Appendix 25: Differences in leaf area of the two *B. pilosa* biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand) P_2 25% B_1 75% B_2), P_3 (50% B_1 50% B_2), and P_4 (75% B_1 25% B_2) at flowering stage

	Biotype			
Population	B1	B2	Total	Mean
P1	39 62	34 39	74 01	37 005
P2	39 53	36 85	76 38	38 190
P3	47 88	41 48	89 36	44.678
P4	26 50	16 25	72 75	36 375
Mean	38 383	39 743		39 063

Appendix 26: Differences in number of branches of the two biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand) P_2 25% B_1 75% B_2); P_3 (50% B_1 50% B_2), and P_4 (75% B_1 25% B_2) at flowering stage

	Biotype			
Population	B1	B2	Total	Mean
P1	5 200	3 333	8 533	4 267
P2	5 467	4 133	9 600	4 800
P3	6 333	2 867	9 200	4 600
P4	6 000	3 133	9 133	4 567
Mean	5 750x	3 367y		4 557

Biotype $LSD_{0.05}$ = 1 122; $LSD_{0.01}$ = 1 545, SE = 0 529

Within the columns means followed by the same letter of x, y, are not significantly different at 5% level of significance according to the Least Significant Difference Test

Appendix 27: Differences in number of inflorescences per plant of the two biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand) P_2 25% B_1 + 75% B_2), P_3 (50% B_1 + 50% B_2), and P_4 (75% B_1 + 25% B_2) at flowering stage

	Biotype			
Population	B1	B2	Total	Mean
P1	5.73	3.67	9.40	4.700b
P2	5.20	4.47	9.67	4.835a
P3	8.27	4.80	13.07	6.535a
P4	7.20	4.53	11.73	5.865ab
Mean	6.60x	4.367y		5.484

Biotype $LSD_{0.05}$ = 0.920; $LSD_{0.01}$ = 1.268, SE = 0.434
Population $LSD_{0.05}$ = 1.300; $LSD_{0.01}$ = 1.793, SE = 0.614

Within the column, means followed by the same letters (a,b,) and within the rows, means followed by the same letters (x, y) are not significantly different at 5% level of significance according to Least Significant Difference Test

Appendix 28: Differences in fresh weight of the two *B. pilosa* biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand) P_2 25% B_1 75% B_2), P_3 (50% B_1 50% B_2), and P_4 (75% B_1 25% B_2) a week after flowering

	Biotype			
Population	B1	B2	Total	Mean
P1	8.54	9.17	17.71	8.855
P2	9.26	8.74	18.00	9.000
P3	13.19	9.74	22.93	11.465
P4	9.42	8.06	17.48	8.740
Mean	10.103	8.927		9.515

Appendix 29: Differences in dry weight of the two *B. pilosa* biotypes (B_1 & B_2) planted in four different ratios P_1 (purestand) P_2 25% B_1 : 75% B_2), P_3 (50% B_1 : 50% B_2), and P_4 (75% B_1 : 25% B_2) a week after flowering

	Biotype			
Population	B1	B2	Total	Mean
P1	0 96	1 00	1 96	0 980
P2	1 01	1 07	2 08	1 037
P3	1 64	1 12	2 76	1 383
P4	1 25	1 01	2 26	1 127
Mean	1 215	1 05		1 133