

**MONOCARPIC SENESENCE IN**  
***BIDENS PILOSA* L.**

by

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## PREFACE

The experimental work described in this thesis was carried out in the Research Centre for Plant Growth and Development, School of Botany and Zoology, University of Natal, Pietermaritzburg and in the Department of Botany, University of Zululand, Kwadlangezwa, under the supervision of Prof J. van Staden.

These studies have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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I certify that the above statement is correct.

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Professor J van Staden  
Supervisor

## ABSTRACT

Senescence was examined in the economic weed *Bidens pilosa*, with the objectives to a) determine the effects of deflowering and defruiting on growth, chlorophyll content, photosynthesis and transpiration; b) to identify the stage of development of the head at which the flowers, seeds/fruit produce senescence signals; and c) to test for senescence activity in plant extracts made from the receptacles and leaves of *Bidens pilosa*.

Total chlorophyll content in the controls, in association with the development of fruit, was lower in the final harvests when compared with earlier harvests in both pot and field-grown plant experiments. Deflowered *Bidens pilosa* plants had a higher chlorophyll concentration than both defruited and control plants in both pot and field-grown plants. Stem death of the control plants was higher than that of deflowered plants in both field and pot experiments. The present results suggest that deflowering is essential if the leaves are to be harvested commercially because it retards senescence and maintains growth. Fruit and flower heads were responsible for the reduction in leaf and stem growth after flowering in *Bidens pilosa*. Removing these organs slowed plant decline, suggesting that the flower head and especially the fruit are responsible for senescence. In contrast, the fruit were the main organs responsible for the decline in leaf chlorophyll concentration.

In pot-grown plants in full sunlight, photosynthesis and transpiration were low in deflowered plants compared with the control and defruited plants 45 days after treatment, and it coincided with a low stomatal conductance. These results suggest that stomatal conductance played a role in lowering photosynthesis in deflowered plants. In contrast, the control plants had a higher stomatal conductance than deflowered plants

75 days after treatment, yet photosynthesis and transpiration rates were the same in both treatments. Thus stomatal conductance alone does not successfully explain differences in photosynthesis in these treatments.

The dry weight of head with mature dry fruit was higher in plants grown at high light intensities than at medium or low light intensities. It coincided with a greater decline in chlorophyll concentration in the leaf nearest to the head and fruit. In contrast, photosynthesis was the same at all light intensities in the leaf nearest to the head and fruit. This suggests that high light accelerated the process of fruit maturation of the fruit which then influenced senescence in the leaf nearest to the flower head.

Ethanollic and water extracts of senescent receptacles purified using paper chromatography, induced senescence of leaves in light but not in the dark. In ethanollic extracts, activity was detected in  $R_f$ s 0.1, 0.2 and 0.3. In water extracts, activity was detected in  $R_f$  0.1.

Senescent leaf extracts purified using column chromatography also induced senescence in light under greenhouse conditions. At high concentrations, activity was detected in fraction 10 eluted with ethyl acetate : methanol (55:45); fraction 11 eluted with ethyl acetate : methanol (50:50); fraction 12 eluted with methanol (100%) and in fraction 13 eluted with ethylacetate : isopropanol : water : acetic acid (52:28:28:4). Under growth room conditions, activity was detected in fractions 12, eluted with methanol (100%) and 13, eluted with ethyl acetate : isopropanol : water : acetic acid (52:28:28:4) in the presence of light.

Fraction 1 ( $R_f$  0.00-0.10) from senescent receptacles, non-senescent and senescent leaves, obtained following thin layer chromatography of ethanollic extracts induced senescence under light. Fraction 1 was eluted with methanol. This fraction lacked activity when eluted with ethyl acetate.

Fraction 4 ( $R_f$  0.25 - 0.35) from non-senescent leaf extracts, which co-chromatographed with 4-chloroindole acetic acid, gave activity in bean cuttings kept under continuous low light. Senescent leaf extracts showed no activity.

Fraction 7 ( $R_f$  0.9 - 1.0) from non-senescent leaf extracts, also induced senescence in bean cuttings under light. The same Fraction from senescent leaf extracts lacked activity.

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## INTRODUCTION

*Bidens pilosa* L. (Asteraceae, tribe Heliantheae) is an annual, erect, branched herb 1.5 m high with yellow flowers 5-15 mm in diameter (HUTCHINSON & DALZIEL, 1963). It is commonly known as blackjack. Although it is a native of South America, it is today widely distributed in tropical and subtropical regions (MAPPI, 1988). It occurs in Cameroon along paths and in crop fields, and close to the dwellings in central and western regions. In South Africa, it is found mainly in waste places, abandoned crop fields and along road sides. It is a common weed.

Blackjack has a wide range of medicinal applications. Its uses in traditional medicine have been described in various areas in Africa (ADJANOHOUN, AHYI, AKE ASSI & BANIAKINA, 1988; KERHARO & GADAM, 1973), China and central America (BASTIEN, 1987; GIRAULT, 1984). Its leaves are used as a treatment for rheumatism, sore eyes, abdominal disorders, ulcers, swollen glands and toothache (ZULUETA, TADA & RAGASA, 1995). Infusions are used for dysentery and diarrhoea (WATT & BREYER-BRANDWIJK, 1962). It also has antibacterial activity (McCORMICK, BOHM & GANDERS 1984; RABE & VAN STADEN, 1997).

Several compounds have been isolated from this species. These include sterols (CHEN, LIN & HONG, 1975); monoterpenes, sesquiterpenes, flavones, flavonoids, glucosides (HOFFMANN & HOLZL, 1988; McCORMICK, BOHM & GANDERS 1984; SASHIDA, OGAWA, KITADA, KARIKONE, MIMAKI & SHIMOMURA, 1991); hydrocarbons (BONDARENKO, PENTRENKO, ACEZENMAN & ENSEENKO, 1985; CHEN, LIN & HONG, 1975) and diterpenes (ZULUETA, TADA & RAGASA, 1995).

Whole plant senescence results in the loss of leaves which are the economically important organs. Several studies have shown that whole plant senescence is induced

by fruits. Deflowered sunflower plants had a higher leaf dry weight than control plants (HO & BELOW, 1989). Senescence of deflowered sunflower plants was significantly lower than that of the controls (PUROHIT, 1982). However, the leaves of sunflower plants are not economically important. In soybeans, deflowering retarded senescence (NOODÉN, 1988c).

A wide range of environmental factors and hormones can initiate/accelerate senescence. These diverse signals probably operate through different signal transduction mechanisms. These signals probably feed into a single pathway which may consist of parallel processes. Environmental factors, including temperature (ATKIN, BARTON & ROBINSON, 1973), pH (BANKO & BOE, 1975), nutrient stress (SALANIA & WAREING, 1979), salinity (ITAI, RICHMOND & VAADIA, 1968), photoperiod (VAN STADEN & WAREING, 1972), growth regulator application (BANKO & BOE, 1975) and nematode infestation (VAN STADEN & DIMALLA, 1977) diminish cytokinin production by the roots and thereby cytokinin flux to the shoot. This decrease in turn promotes shoot senescence. Formation of new roots increases cytokinin levels in the leaves (FORSYTH & VAN STADEN, 1981) and delays chlorophyll loss. Root growth is severely reduced during the reproductive phase of a plant's life cycle (NOODÉN, 1980), and this would influence its cytokinin production (VAN STADEN & DAVEY, 1979). During whole plant senescence, cytokinin levels decline in the leaves of soybean (LINDOO & NOODÉN, 1978, but not in the leaves of white lupin (DAVEY & VAN STADEN, 1978).

Leaf senescence requires viable cells and new gene expression (SMART, 1994). Mature fruits do not drain sugar from the leaves. Thus sugar accumulates in the leaves and suppresses the expression of some genes related to photosynthesis (GAN & AMASINO, 1997). Various stages of monocarpic senescence are discussed in Chapter

1. In the present study, field-grown *Bidens pilosa* plants were watered regularly to avoid stress related processes. Nutrients were also applied during transplanting. Deflowering and defruiting experiments were done in order to determine the role of fruits in whole plant senescence. Plant extracts were tested for senescence activity. Thus, the overall objective of this study was to search for senescence factors in extracts made from the receptacles and leaves of *Bidens pilosa*.

## CHAPTER ONE

### 1. LITERATURE REVIEW

The processes of deterioration that accompany ageing and that lead to death of an organ or organism are called senescence (LEOPOLD, 1975). Monocarpic plants are characterised by a single reproductive phase, followed by senescence and death, attributed to flower and fruit formation, believed due to the release a senescence signal (LEOPOLD, 1975; KULKARNI & SCHWABE, 1985). Monocarpic senescence can be seen in many annuals e.g., soybean [*Glycine max*], all biennials e.g., carrot [*Daucus carota*] and some perennials e.g., century plants [most *Agave* spp] (WILSON, 1997). NOODÉN (1988c) divides monocarpic senescence into various stages, namely: **correlative controls; cessation of vegetative growth; declining assimilatory processes; assimilate partitioning** and **hormonal controls**. These are briefly discussed below.

#### 1.1. CORRELATIVE CONTROLS

Correlative controls (that is, control by specific plant organs) play an important regulatory role in monocarpic senescence and appear to be mediated by hormones (LEOPOLD & NOODÉN, 1984). Various parts of a plant influence each other in ways that serve to achieve a co-ordination of their developmental processes. The flower is the earliest example of correlative control. In orchid and other flowers, pollination causes the female structure to produce a stimulus, probably ethylene, that is transmitted to the petals and

other parts, where it induces senescence (HOEKSTRA & WEGES, 1986). Senescence in some flowers is controlled by factors other than pollination. Plumule removal rejuvenates the senescing cotyledons or leaves below (NEUMANN & STEIN, 1986), implying that the apex is responsible for senescence of cotyledons or older leaves. According to KRUL (1974), decapitation can cause resynthesis of protein after an 85% loss in bean leaves. LEOPOLD and KAWASE (1964) observed that in bean seedling cuttings with two opposite leaves, application of cytokinin to one leaf promoted senescence of the non-treated leaf. In pea plants, a graft-transmissible substance, apparently gibberellin from the roots, delays apex senescence (SPONSEL, 1985). Xylem differentiation illustrates correlative controls operating in the senescence of the cell (SHINNINGER, 1979). Thus individual cells may die because they are targeted to do so as part of the differentiation process (NOODÉN, 1988a). Monocarpic senescence can be delayed by removal of certain plant parts, usually reproductive parts. The prevention of rapid death of soybeans by depodding is well known (LINDOO & NOODÉN, 1977). Depodding may prevent rapid death, but it may not prevent the decline in photosynthesis and other parameters associated with senescence (MONDAL, BRUN & BRENNER 1978). For wheat, head removal retarded some, but not all the changes associated with the senescence syndrome (FELLER, 1979; PATTERSON & BRUN, 1980). The seeds are responsible for the rapid degeneration and death in pea, red kidney bean, soybean and sunflower (NOODÉN, 1988c and references therein). Removal of flowers on male spinach plants can cause a delay in monocarpic senescence; removal of flowers of male hemp plants does not prevent the death of the plant; deflowering does not prevent senescence in cocklebur (NOODÉN, 1988c). In maize and barley, defruiting promotes leaf senescence (ALLISON &

WEINMANN, 1970; MANDAHAR & GARG, 1975). The metabolic decline in defruited plants may have other explanations, but in many cases, the reproductive structures, particularly the seeds, control or induce monocarpic senescence. Root/shoot grafting experiments and photoperiod manipulations of soybeans indicate that leaf senescence causes plants to die during pod maturation (NOODÉN, 1988c and references therein). The roots decline due to a lack of assimilates and hormones from the leaves. The exertion of the influence (senescence signal) by the fruits on the leaves seems to occur during late podfill (LINDOO & NOODÉN, 1977; NOODÉN & LINDOO, 1978). The senescence signal is exerted mainly on the leaf closest to an individual pod or pod cluster, beyond this, the signal travels downwards. The main stimulus on the nearest leaf is not blocked by steam girdling of the petiole, thus it is apparently transported via the xylem (NOODÉN & MURRAY, 1982). The signal moving downwards is blocked by steam girdling, thus it is apparently transported via the phloem (MURRAY & NOODÉN, 1982). In *Kleinia articulata*, the stimulus originates in the leaves where it is induced by long-day photoperiod (KULKARNI & SCHWABE, 1985). This (signal) stimulus is less mobile than the senescence signal of soybean. This indicates that there can be more than one type of signal.

## 1.2. CESSATION OF VEGETATIVE GROWTH

Cessation of vegetative growth is important in monocarpic senescence, but in itself it is not a primary cause (NOODÉN, 1988c and references therein). Monocarpic senescence can be induced in healthy organs. The decreased vegetative growth does not seem to be caused by a limited mineral nutrient supply, especially in soybean plants (DERMAN,

RUPP & NOODÉN, 1978). The decline in vegetative growth prevents renewal of leaves and sets the stage for a decline in photosynthesis. The production of new root apices and the growth of roots decline early in the reproductive phase. The exact timing of cessation varies with the species. Shoot parts excised from ageing or senescent plants are less able to form roots (TRIPPI & BRULFERT, 1973b). In soybean, defruiting does not reinstate shoot elongation, although it may promote root growth (DERMAN, RUPP & NOODÉN, 1978). In the common pea, apex senescence may occur in the absence of flowering (LOCKHART & GOTTSCHALL, 1961). Thus, the apex senescence factor and the flowering factor seem to be different. Apex removal does not promote monocarpic senescence in soybean. On the other hand, unfavourable light-dark cycles (6hr/6hr or 24hr/24hr) cause not only termination of growth, but premature death of tomato plants. However, these plants do not die as a consequence of apex senescence (NOODÉN, 1988c and references therein).

### **1.3. DECLINE OF ASSIMILATORY PROCESSES**

The uptake of different minerals may decline at different times and stages of plant development (NOODÉN & MAUK, 1987). The reduction of cytokinin production by roots and its flux up the xylem into the leaves may occur later in reproductive development (HEINDL, CARLSON, BRUN & BRENNER, 1982). A reduction in mineral flux through the xylem does not seem to be the primary cause of senescence, though it may be part of a preparative process (NOODÉN, 1988c). There are differences in the relative timing of the decline in synthesis and maintenance in chlorophyll, photosynthesis and total nitrogen levels in the leaves of senescing soybean plants under different conditions

(MONDAL, BRUN & BRENNER, 1978; WITTENBACH, ANDERSON, GIAGUINTA & HERBERT, 1980; OKATAN, KAHANAK & NOODÉN, 1981; SECOR, SHIBLES & STEWART, 1983).

The decline in photosynthetic rate seems to be more closely linked to photosynthetic electron transport than to enzymes for synthesis and maintenance of chlorophyll or photosynthetic enzymes (THOMAS & STODDART, 1980). There may be differences among species on when the decline in photosynthesis occurs relative to reproductive development. In soybean, the decline occurs during podfill (DORNHOFF & SHIBLES, 1970). In sugarcane, wheat and flax, the decline in photosynthesis begins during flowering. In the case of *Brassica napus* and *B. campestris*, the leaves senesce before pod growth becomes rapid; foliar photosynthate is stored in the stems and then redistributed to the seeds. Root assimilation may decline ahead of photosynthesis in the leaves due to changes in translocation of photosynthate and priority to reproductive structures at the expense of the roots. However, hormonal controls are also involved. Photosynthetic rate may be regulated by sink activity (HEROLD, 1980). Removal of fruits in pepper or maize may decrease the photosynthetic rate in leaves (CHRISTENSEN, BELOW & HAGEMAN, 1981). Rapidly growing fruits require large quantities of assimilate and they seem to stimulate photosynthesis in a variety of species including soybean, pepper and barley. The stimulatory effect of sink demand may also apply to uptake of some minerals by roots (DERMAN, RUPP & NOODÉN, 1978; NOODÉN & MAUK, 1987). In monocarpic plants vegetative growth is severely curtailed during the reproductive phase and cannot be restored by removing the reproductive structures. Since photosynthesis may be regulated by consumption of its products, defruiting could be expected to cause a decline in assimilatory metabolism.

Thus, the decline in photosynthetic rate in depodded plants is probably the result of a loss in active sinks rather than senescence *per se* (NOODÉN, 1988c). Photosynthetic rate represents the functioning of the photosynthetic system whereas chlorophyll content and the levels of photosynthetic enzymes provide a measure of photosynthetic capacity. It is possible for photosynthetic rate to decline without a concomitant decrease in the components of its capacity. Foliar chlorophyll (DERMAN, RUPP & NOODÉN, 1978; MONDAL, BRUN & BRENNER, 1978; WITTENBACH, 1982) and ribulose biphosphate carboxylase (MONDAL, BRUN & BRENNER, 1978; SCHWEITZER & HARPER, 1985) do not decline substantially in several podless plants, whereas these decline in numerous other cases (HUBER, WILSON & BURTON, 1983; WITTENBACH, 1983; BURKE, KALT-TORRES, SWAFFORD, BURTON & WILSON, 1984; CRAFT-BRANDNER, BELOW, HARPER & HAGEMAN, 1984a, b; ISRAEL, BURTON & WILSON, 1985). Depodding also prevents the rapid death of soybean plants as long as pod development is not allowed to occur too long (LINDOO & NOODÉN, 1977; DERMAN, RUPP & NOODÉN, 1978; NOODÉN & LEOPOLD, 1978) but this does not happen in all the cases cited above in connection with photosynthesis.

In sunflower, deheading retards many changes in the leaves of green-house-grown plants but not in field-grown plants (HO, BELOW & HAGEMAN, 1987). Similarly, depodding may prevent a decline in foliar chlorophyll in chamber-grown soybean plants (WITTENBACH, 1982) but not in field-grown plants (WITTENBACH, 1983). The decrease in photosynthesis and that of chlorophyll synthesis may be different phenomena. In depodded plants, photosynthesis may decline due to sink loss, whereas in podded plants, the decrease may be caused by loss of photosynthetic components. In podded plants, the photosynthetic components appear to decline as part of the

senescence syndrome (NOODÉN, 1988c). Photosynthetic rate should be used as a measure of the progression of the senescence syndrome in normal plants, but not when the sinks are removed.

#### 1.4. PARTITIONING AND REDISTRIBUTION OF ASSIMILATES

The cessation of vegetative growth concomitant with the rapid increase in reproductive growth indicates that a change in the distribution of newly assimilated materials has occurred. This shift away from vegetative parts and to the developing fruits has been considered to play a causal role in monocarpic senescence (NOODÉN & LEOPOLD, 1978). In addition, nutrients stored in one part of the plant may be redistributed to another part, particularly the fruit. Developing reproductive structures are strong sinks (WOLSWINKEL, 1993). There is also competition between the leaves and fruits for mineral nutrients assimilated by the roots. The control of assimilate movement plays an important role in redistribution.  $^{14}\text{C}$ -compounds derived from  $^{14}\text{C}$ - $\text{CO}_2$  through photosynthesis are distributed differently during the reproductive phase, with more going to the fruit and less to the vegetative structures. Labelled sucrose travels mainly to the fruits at the expense of the roots in soybean plants (HUME & CRISWELL, 1973). In wheat, field pea, lupin and cowpea, this shift is less dramatic (NOODÉN, 1988c). It is not clear whether nutrient diversion is the causal factor in monocarpic senescence. However, hormonal controls are likely to be involved. Labelled phosphate and  $^{86}\text{Rb}$  introduced into the xylem goes directly into the leaf blade (NEUMANN & NOODÉN, 1984; MAUK, PELKII & NOODÉN, 1985); both  $^{32}\text{P}$  and  $^{86}\text{Rb}$  are then redistributed to the pods. Thus the explanation of competition between fruits and leaves in monocarpic

senescence is less popular. Studies with  $^{14}\text{C}$ -amino acids and amines revealed that certain compounds (asparagine, glutamine, serine) tend to be redistributed from the leaves or transferred from xylem to phloem; while others (aspartate, glutamate) are retained in the leaves (MCNEIL, ATKINS & PATE, 1979). When isolated oat leaves senesce, the amino acids released are actively transported basipetally. However, if the leaves are attached to the plant, amino acids travel down to the roots (THIMANN, TETLEY & THANH, 1974). Thus, the nature of nitrogenous materials synthesised by the roots may determine their distribution. The movement of  $^{32}\text{P}$  and  $^{86}\text{Rb}$  to pods via the leaves implies that the leaves may be responsible for the regulation of assimilate movement. To support this, neither pod dose nor excision of the seeds greatly influence movement of  $^{32}\text{P}$  from leaves to seeds in the short term (NEUMANN & NOODÉN, 1984). Likewise, the leaves of depodded soybean plants continue to export  $^{32}\text{P}$ , but the export is to the stem rather than the pods (CIHA & BRUN, 1978). Noodén (1988c) recognised five mechanisms for controlling solute movement.

**First:** The xylem flux could be regulated by stomatal aperture; which may control transpiration. In unstressed plants, stomatal resistance does not increase markedly until quite late, after redistribution has progressed quite far and yellowing is about to start; therefore drought is unlikely to regulate xylem flux as a prelude to monocarpic senescence. Stomatal closure may promote senescence, however, since it may accompany delayed senescence in intact plants (THORNE & KOLLER, 1974), other factors must also be involved.

**Second:** The decrease in functional phloem and even xylem due to non-renewal as a result of diminished vegetative growth, would influence transport quantitatively and possibly influence distribution patterns.

**Third:** The physiological function of transfer cells suggest that they could regulate solute partitioning through changes in their location or in the activity of already differentiated transfer cells (PATE & GUNNING, 1972).

**Fourth:** The loading and unloading of xylem and phloem through routes other than transfer cells may also be important. Hormones, pH, other solutes and turgor are likely to play a key role here and in transfer cells (THORNE, 1985).

**Fifth:** The partitioning of nitrogen could be regulated by the nature of amino acids formed. The same could apply to hormones and other compounds. The mobile elements (N & P) are redistributed to a greater extent than the less mobile elements such as Ca and Fe (NOODÉN, 1988c). Redistribution of mobile materials such as N and P may be inhibited by their deficiency or promoted by their abundance. Neither application of NPK to soybean leaf surfaces (SCHREYER & NOODÉN, 1975), nor force feeding via the xylem (MAUK & NOODÉN, 1983), prevent losses of these minerals from leaves. Similarly, foliar application of urea did not retard chlorophyll loss in maize (BELOW, CRAFT-BRANDNER & HAGEMAN, 1985). Where redistribution from leaves to fruits is blocked by defruiting, starch is accumulated in the stem (CIHA & BRUN, 1978) and ribulose-biphosphate carboxylase is converted to seed storage proteins within the leaves (FRANCESCHI, WITTENBACH & GIAQUINTA, 1983). The release of minerals from the leaves of soybean plants is controlled in part by the cytokinin flux up the xylem (MAUK & NOODÉN, 1983).

Monocarpic senescence is viewed as exhaustion death because constituents lost in the leaves appear in the fruit. The theory of exhaustion death is criticised because of the occurrence of monocarpic senescence in spinach with male flowers. Removal of male flowers delayed senescence in these plants. Monocarpic senescence is independent

of pod number in soybean (LINDOO & NOODÉN, 1977; NOODÉN & LINDOO, 1978). The nutritional requirements of the developing soybean seeds can be satisfied without sacrificing the plant if the assimilatory capacities are maintained. A surgical experiment which modified plants so that the leaves are not contiguous with the pods separated seed development and leaf senescence. A non-senescing mutant seems to produce a normal load of pods without the usual breakdown of the plant's assimilatory apparatus (ABU-SHAKRA, PHILLIPS & HUFFAKER, 1978). Application of foliar spray with a combination of auxin and cytokinin can prevent monocarpic senescence (NOODÉN, KAHANAK & OKATAN, 1979). *Vicia faba* shows little redistribution of its foliar N to the fruit (COOPER, HILL-COTTINGHAM & LLOYD-JONES, 1976). Feeding mineral nutrients to the leaves via their surface or through the xylem does not prevent their senescence (CRAFTS-BRANDNER, 1992). Thus, monocarpic senescence may occur without foliar mineral depletion. Nutrient demand by the seeds does not in itself cause monocarpic senescence (NOODÉN, 1988c).

The senescence signal is not nutrient diversion or withdrawal because it is less mobile within the soybean plant than the nutrients required to support pod development. The senescence signal is exerted quite late in seed development after most of the dry matter accumulation is finished. Destruction of the phloem reveals that even though the withdrawal route is blocked, the pods are still able to trigger senescence (yellowing) of the nearest leaf (NOODÉN & MURRAY, 1982). The interpretations of the behaviour of the senescence signal was criticised by some workers (WANG & WOOLHOUSE, 1982; SEXTON & WOOLHOUSE, 1984) primarily on the basis of the differences in age of the leaves involved. The assertion that age of the leaves is the primary determinant of when

the leaves in fruiting soybean plants senesce ignores the well-known fact that the main leaves senesce more or less together in monocarpic senescence of several species, including soybean (NOODÉN, 1988c). Senescence of excised leaves in pod bearing explants is determined by the development of the pods, not the age of the leaf. Nutrient withdrawal and diversion does not seem to account adequately for monocarpic senescence in soybeans.

### 1.5. HORMONAL CONTROLS

The process of monocarpic senescence is probably hormonally-controlled, and involves changes in gene expression (LOHMAN, GAN, JOHN & AMASINO, 1994). There is also considerable direct evidence for a death hormone:

**First:** Monocarpic senescence occurs in male plants of spinach [*Spinaceae oleraceae*] and hemp [*Cannabis sativa*] (LEOPOLD, NIEDERGANG-KAMIEN & JANICK, 1959). In such plants, fruits do not act as sinks. Monocarpic senescence sometimes occurs in plants where the ovules have not been fertilised and in plants where reproductive sinks have been removed (MACKOWN, VAN SANFORD & ZHANG, 1992).

**Second:** The senescence signal is localised, often to the leaf nearest the fruit (LINDOO & NOODÉN, 1977).

**Third:** Hormones, especially cytokinins, can postpone monocarpic senescence (NEUMANN, TUCKER & NOODÉN, 1983; NOODÉN, GUIAMÉT, SINGH, LETHAM, TSUJI & SCHNEIDER, 1990).

**Fourth:** Organic materials are exported, in small amounts, from developing fruits and seeds (BOURBOULOUX & BONNEMAIN, 1972; NITSCH, 1972; TAMAS, ENGELS,

KAPLAN, OZBUN & WALLACE, 1981; GIANFAGNA & DAVIES, 1983). Some work indicates that water may flow out of the fruit (OPARKA & GATES, 1981; PATE, PEOPLES, VAN BEL, KUO & ATKINS, 1985) and outward transport across the seed coat can occur in soybeans (NOODÉN, 1988c and references therein). In an attempt to explain how the senescence factor descended from the fruits to the leaf, PATE, PEOPLES, VAN BEL, KUO & ATKINS, 1985, related the process to a water stress. A decline in water potential at midday is implicated in the descent of senescence factors because it is accompanied by a backflow of the xylem contents from the fruits to the peduncle. In this way, a senescence signal is exported out of the fruits to the nearest leaf.

The evidence from male plants and from defruited plants, indicates that the death hormone can be produced by organs other than fruits. In soybeans, the seeds are the control centers and they exert their effect on the leaves. The senescence signal (stimulus) is probably produced by the fruit (seeds) in soybeans. However, nutritional factors may probably be part of the senescence process (LINDOO & NOODÉN, 1977). Several hormones are known to promote senescence. Ethylene promotes foliar senescence in some species (NOODÉN & LEOPOLD, 1978; MATTOO & AHARONI, 1988), however, it does not seem to play a role in monocarpic senescence because of its limited mobility (spreading by diffusion), and the inability of 2% v/v CO<sub>2</sub> to delay monocarpic senescence of soybeans significantly (SCHREYER & NOODÉN, 1975; LINDOO & NOODÉN, 1978). By contrast 2% CO<sub>2</sub> drastically delayed senescence of the soybean flower petals (SCHREYER & NOODÉN, 1975; LINDOO & NOODÉN, 1978); a tissue whose senescence is generally controlled by ethylene (NOODÉN, 1988c). Anti-senescence constructs that inhibit ethylene formation and mutants that block the

ethylene response do delay leaf senescence, indicating that ethylene does promote leaf senescence (GRBIC & BLEECKER, 1995; JOHN, DRAKE, FARREL, COOPER, LEE, HORTON & GRIERSON, 1995; BUCHANAN-WOLLASTON, 1997; GAN & AMASINO, 1997; NAM, 1997).

Absciscic acid promotes senescence in a wide range of detached leaves (NOODÉN & LEOPOLD, 1978); however, like cytokinin, it is often inactive on attached leaves (NOODÉN & LEOPOLD, 1978). Foliar ABA treatments do accelerate monocarpic senescence of soybean (LINDOO & NOODÉN, 1978). Reduction in pod load (50%) which does not alter foliar senescence (leaf yellowing and abscission) kinetics (NOODÉN, RUPP & DERMAN, 1978) actually produces an increase in foliar ABA. Several inconsistencies exist here;

**First:** Young leaves may contain more ABA than mature green leaves.

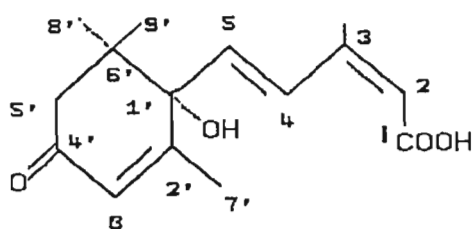
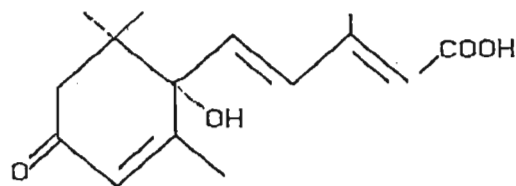
**Second:** Complete depodding which prevents foliar senescence in soybean plants, increases foliar ABA (SETTER, BRUN & BRENNER, 1980).

**Third:** Foliar ABA applications fail to induce senescence of depodded soybeans (LINDOO & NOODÉN, 1978). High levels of senescence retarding hormones (eg cytokinin) which counteract ABA in the leaves of younger or depodded plants, could be the cause of these discrepancies. When injected into the seed cavity of soybean pods,  $^{14}\text{C}$ -ABA penetrates the seeds; but only very small amounts move from the injected pods into the target leaves, even during the phase when the seeds induce senescence of the leaves. Thus ABA may not be the senescence signal for monocarpic senescence in soybean, but may play some role in the senescence process (NOODÉN, 1988c). The chemical structures of ABA and ethylene are presented in **Figure 1.1**.

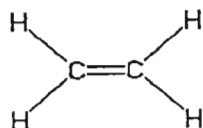
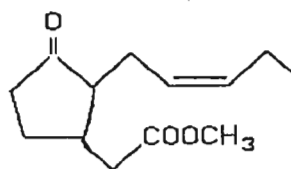
Some natural products have been reported to promote senescence. These include methyl jasmonate, serine, capillin, capillene, aliphatic alcohols and fatty acids such as linolenic acid (NOODÉN, 1988b). Methyl jasmonate which occurs widely in plants, can promote chlorophyll loss in oat leaf segments (UEDA & KATO, 1980). It also promotes amino acid release, increases respiration and causes stomatal closure (STATLER & THIMANN, 1981). However, no information is available on its hormonal role. Its chemical structure is presented in **Figure 1.1**.

Capillin and capillene, which contain aromatic rings with aliphatic side chains, promote chlorophyll loss in oat leaf segments in light, but in darkness, they cause chlorophyll retention (UEDA, KOJIMA, NICHIMURA & KATO, 1984). Their chemical structures are presented in **Figure 1.1**.

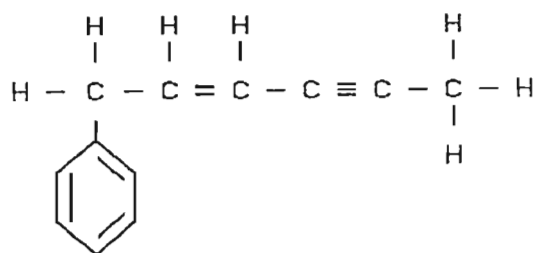
L-Serine accelerates yellowing in oat leaf segments at concentrations above  $10^{-3}$  M (THIMANN, SHIBAOKA & MARTIN, 1972). Serine is more active in darkness than in light (VEIERSKOV, STATLER & THIMANN, 1985). D-Serine is inactive (SHIBAOKA & THIMANN, 1970). L-Serine also inhibits  $^{14}\text{C}$ -leucine uptake but increases protein degradation, proteolytic activity and free amino acid levels (MARTIN & THIMANN, 1972; VEIERSKOV, STATLER & THIMANN, 1985). It counteracts the chlorophyll retaining effects of kinetin, IAA and adenine. The role of serine *in vivo* is unknown. It is a byproduct of photosynthesis and a mobile component of photosynthate (PATE, 1980). In senescing tissues where the different amino acids have been analysed, the serine concentration may not increase much more than that of other amino acids (MALIK, 1982), or it may rise disproportionately (LECKSTEIN & LLEWELLYN, 1975; PATE, SHARKEY & ATKINS, 1977). The structure of serine is presented in **Figure 1.2**.

*cis*-Absciscic acid (ABA)*trans*-Absciscic acid (*t*-ABA)

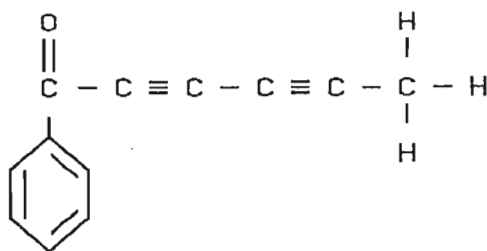
The numbers in the formula for ABA identify the carbon atoms.

**Ethylene**

(±)-methyl jasmonate  
(Ueda & Kato, 1980)

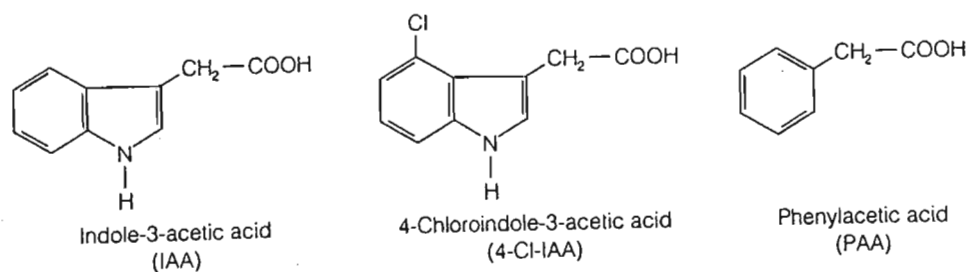


Capillene (1-Phenyl-hex-2-ene-4-yne)  
(Gibbs, 1974)



Capillin (1-Phenyl-hexa-2, 4-diyne-1-one)  
(Gibbs, 1974)

Figure 1.1: The chemical structures of absciscic acid, methyl jasmonate, capillene, capillin and ethylene.



Linolenic acid (18:3)  $\text{CH}_3\text{CH}_2\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_7\text{CO}_2\text{H}$

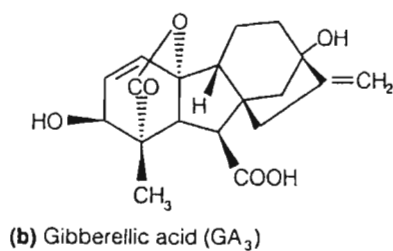
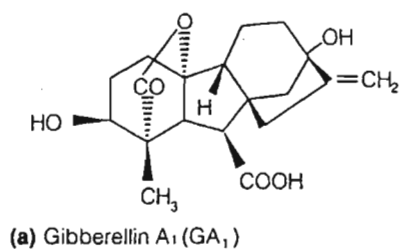
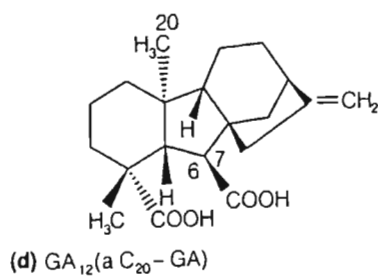
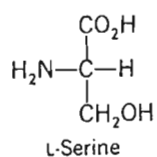


Figure 1.2: The chemical structures of linolenic acid, serine and natural plant hormones.

The implication of free fatty acids in senescence seem to have grown mainly out of the observation that they increase during senescence and they are physiologically active e.g. inhibit mitochondrial oxidations. The breakdown of cell membranes, particularly the thylakoid membranes, in senescing tissues, release large quantities of linolenic acid (THOMAS, 1982). Free linolenic acid inhibits photosynthesis in isolated chloroplasts (MCCARTY & JAGENDORF, 1965; MVÉAKAMBA & SIEGENTHALER, 1979).

Exogenous gibberellins can inhibit processes such as RNA and protein breakdown that may be associated with senescence. It may also delay senescence in petals and petioles. It promotes fruit ripening (MCGLASSON, WADE & ADATO, 1978). In some species e.g. *Rumex*, GA<sub>3</sub> retards chlorophyll loss in leaves, while in others e.g. peanut, bean and *Taxodium* it promotes chlorophyll loss. In others, like tobacco and barley, there is no effect (NOODÉN, 1988b).

Endogenous GA activity declines prior to or during senescence in a variety of tissues (CHIN & BEEVERS, 1970; AHARON & RICHMOND, 1978). For tissues showing a decline in GA activity, e.g. lettuce leaves, *Nasturtium* leaves, *Dandelion* leaves, exogenous GA delays senescence, thus supporting the argument that for these tissues and these species, a decline in GA plays a role in senescence. Exogenous GA seems to be more effective when the endogenous GA is low (FLETCHER, OEGEMA & HORTON, 1969). The chemical structures of some gibberellins are presented in **Figure 1.2**. By injecting high specific activity [<sup>3</sup>H]-acetate into pods on soybean explants, it was demonstrated that [<sup>3</sup>H]-labelled metabolites may travel from the pods to the leaves. More <sup>3</sup>H moves from the pods to the leaves at mid- and late-pod fill than at other stages before or after, and this corresponds to the behaviour of the senescence signal

(NOODÉN & MAUK, 1987). By injecting  $^{14}\text{C}$ -compounds into the pods of pea, Gianfagna and Davies (1983) observed the transport of labelled substances out of the developing pods to the shoot apex in relation to induction of apex senescence. The labelled compound was neither abscisic acid nor phaseic acid and remains unknown. Thus, solutes do move out of the fruits to the vegetative parts where they play a regulatory role, including induction of senescence (NOODÉN, 1988c). The roots supply minerals to the leaves, and the leaves provide photosynthate for roots. Hormones play a role in mediating root-shoot interactions. Exogenous auxins induce adventitious root development (GOODWIN, 1978). Auxin may also stimulate formation of lateral roots on the main root (WIGHTMAN & THIMANN, 1980). Auxin generally inhibits, though sometimes it may promote, root elongation (BATTEN & GOODWIN, 1978; GOODWIN, 1978; PILET & SAUGY, 1985). These root-promoting auxins are probably produced by the leaves. Radioactive label from IAA applied to the leaves is translocated to the roots and to the fruit (BOURBOULOUX & BONNEMAN, 1972; 1979; MORRIS & KADIR, 1972; MORRIS, KADIR & BARRY, 1973; MORRIS, 1977) but the changes in partitioning patterns of IAA fruiting is unknown (NOODÉN, 1988c). Auxin applied to the shoots generally promotes root growth, supporting the idea that the leaves produce auxin that is transported to the roots (McDAVID, SAGAR & MARSHALL, 1972; GOODWIN, 1978). Some data implicate auxin in monocarpic senescence. Diffusible auxin activity from the leaf blades decreases with age (NOODÉN, 1988c). This decrease in downward flux of auxin from the leaf blade probably controls leaf abscission (NOODÉN & LEOPOLD, 1978). The IAA levels in common bean and soybean leaves decrease as they mature and during pod development (ROBERTS & OSBORNE, 1981; HEIN, BRENNER & BRUN, 1984). Depodding does not inhibit this early decline of IAA in soybean leaves

(HEIN, BRENNER & BRUN, 1984). However, it could prevent further decrease later during the abscission induced period. In many species including soybean, auxin applied to the leaves delays leaf yellowing, which suggests that a decrease in endogenous auxin levels does play a causal role in foliar senescence beyond its effect on abscission. By contrast, the level of auxin activity in hemp plants appear to rise during leaf yellowing. Auxin flux from pods to leaves has been implicated as a factor regulating photosynthesis in beans (TAMAS, ENGELS, KAPLAN, OZBUN & WALLACE, 1981) but wheat and soybean may be different (KING, WARDLAW & EVANS, 1967; NOODÉN & NOODÉN, 1985). Thus the chemicals that mediate the influence of leaves on the roots are: auxin, thiamine, ABA and photosynthate. There is evidence that the leaves are the major sources of thiamine and ABA; that these compounds are translocated from the leaves to the roots and that they can promote root growth. Derivatives of 4-chloroindoleacetic acid (ENGVILD 1989) may be death hormones in *Pisum*, *Vicia* and *Lathyrus*. Chloroindoleacetic acid (CIIAA) induces very strong, almost irreversible ethylene production. CIIAA (**Figure 1.2**) induces death of the apical meristem in pea cuttings. Auxin in lanolin placed in deseeded bean pods induce leaf senescence (TAMAS, ENGELS, KAPLAN, OZBUN & WALLACE, 1981). CIIAA is the strongest natural auxin known (ENGVILD, 1989). The problem with chloroindoles is the postulation of two distinct auxin groups with opposite physiological properties: growth inducers and endogenous herbicides. Moreover, chloroindoles have been found in peas and related species, but not in soybean or kidney bean (ENGVILD, 1989). The cytokinin flux from the roots to the shoots is well established (LETHAM, 1978; VAN STADEN & DAVEY, 1979; LETHAM & PALNI, 1983). The chemical structures of some cytokinins are presented in **Figure 1.3**. Root apices appear to be the major, though probably not

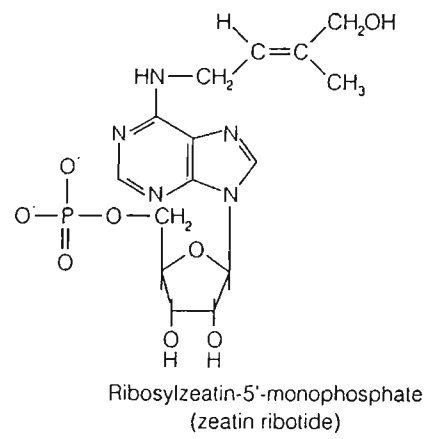
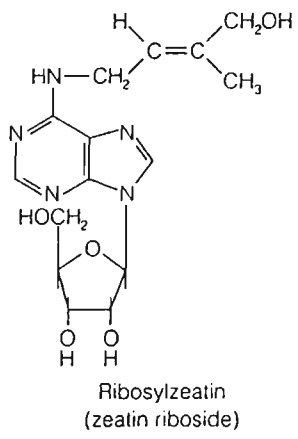
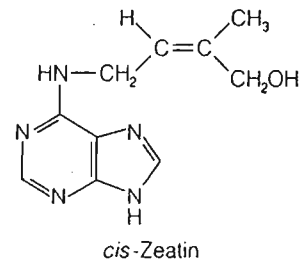
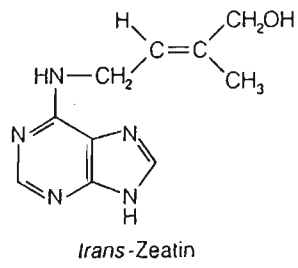
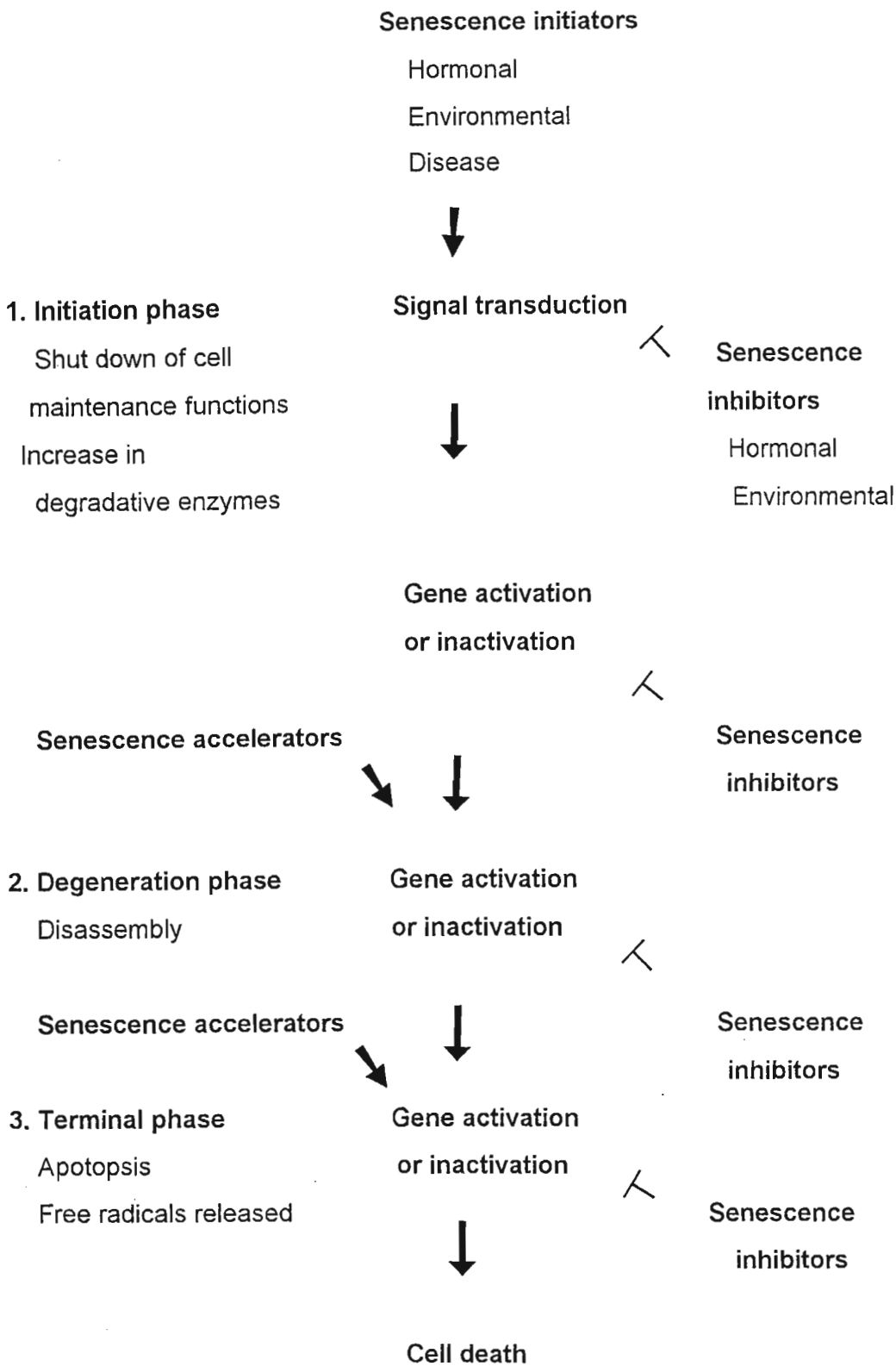


Figure 1.3: The chemical structures of certain cytokinins.

the only sites of cytokinin synthesis in the whole plant. Because the young apices are generally more active than the older ones in the production of cytokinins and assimilation of mineral nutrients for supply to the foliage and pods, the cessation of root growth is important (LETHAM, 1978; VAN STADEN & DAVEY, 1979). Mineral uptake and cytokinin production decrease during podfill when root growth would also be declining. One mechanism proposed for fruit induction of monocarpic senescence holds that the developing fruits monopolise the supply of cytokinins flowing from the roots to the leaves, thereby creating a deficiency in the leaves and hence senescence. Using explants,  $^3\text{H}$  derived from [ $^3\text{H}$ ]zeatin riboside (ZR) or [ $^3\text{H}$ ]zeatin (Z) were applied as a pulse (1 hour) through the base of explants at early podfill or just before podfill. Relatively little  $^3\text{H}$  travelled to the fruit (2.1%) and most of that was in the carpels, with very little in the seeds, minute amounts in the seed coats and no detectable quantities in the embryo. This is not the flux pattern which one would expect if the pods were competing with the leaves for cytokinin fluxing up the stem (NOODÉN, 1988c). Analysis of the nature of soluble  $^3\text{H}$  indicated that the  $^3\text{H}$ -Z and ZR were rapidly metabolised, so little of the  $^3\text{H}$  in the pods was still cytokinin. This metabolism of Z and ZR does not seem to change as monocarpic senescence progresses. It seems probable that the roots maintain the shoot (leaves) function through production of cytokinin and gibberellins. In peas, a decline in gibberellin flux from the roots seems to be important in promoting monocarpic senescence (PROEBSTING, DAVIES & MARX, 1978).

Soluble sugar concentration often goes up in senescing leaves (NOODÉN, GUAMÉT & JOHN, 1997). Since senescence is an active process, an adequate supply of carbohydrate is probably needed to support senescence. It has been proposed that elevated sugar concentrations may cause senescence (CRAFTS-BRANDNER, BELOW,

WITTENBACH, HARPER & HAGEMAN, 1984). Senescence can be regulated by cytokinin, sugar and light. Accumulation of sugars during senescence can block the effect of cytokinin especially in low light (WINGLER, VON SCHAEWEN, LEEGOOD, LEA & QUICK, 1998).



**Figure 1.4:** Proposed outline of the central pathways of leaf senescence (NOODÉN, GUIAMÉT & JOHN 1997).

Senescence can be initiated by a wide variety of factors (**Figure 1.4**) and these may feed into the same pathway. It is not clear which of these factors are central and which are peripheral in the senescence process (NAM, 1997). Many of the enzymes that increase during senescence are peripheral to senescence (NOODÉN, GUIAMÉT & JOHN, 1997). These include the enzymes required to metabolize the amino acids and lipids released and to convert them to transportable forms.

In addition to the objectives which were stated in the introduction, monocarpic senescence of *Bidens pilosa* was studied in order to:

- a) determine when to harvest leaves from plants which are grown for commercial purposes;
- b) determine how to improve yield; and
- c) test for the presence of senescence factors and if possible, isolate and identify such factor(s).

## CHAPTER TWO

### 2. THE EFFECTS OF DEFLOWERING AND DEFRUITING ON GROWTH AND SENESCENCE OF *BIDENS PILOSA*

#### 2.1 INTRODUCTION

Senescence represents endogenously controlled degenerative processes (NOODÉN, GUIAMÉT & JOHN 1997), but is also influenced by environmental factors. Monocarpic senescence is characterised by a single reproductive phase, followed by senescence and death, and it has been postulated that a senescence signal is released by the flowers or fruit. It is common for many flowering annuals. While much research has been initiated, the process is not well understood in most plants, except perhaps for soybeans (NOODÉN 1988c). Although not universally accepted as the best method to measure senescence, chlorophyll degradation is often used to follow changes in leaf ageing (GUIAMÉT, SCHWARTZ, PICHERTSKY & NOODÉN 1991).

There exists a close linear correlation between stomatal conductance and leaf ABA content (STEUER, STUHLFAUTH & FOCK 1988). Reduced levels of ABA have been shown to greatly enhance both transpiration and photosynthetic CO<sub>2</sub> assimilation in leaves of *Vitis* (LOVEYS, 1991).

Chloroplasts undergo ordered sequential changes of their photosynthetic capability from maturity through the process of senescence. Stroma thylakoids vesiculate and thereafter lose their integrity. Coincident with thylakoid disintegration, a gradual increase in the volume and or number of plastoglobuli occurs. Subsequent to loss of

integrity of stroma thylakoids, the grana thylakoids swell and then undergo gradual disintegration (GREENING, BUTTERFIELD & HARRIS, 1982). Since PSII is localised mainly in the grana and PSI is distributed both in the grana and stroma lamellae (ANDERSON, 1981), the earlier loss of stroma lamellae explains why PSI declines faster than PSII (BRICKER & NEUMAN, 1982). The chloroplast envelope retains its integrity until late when the internal membranes are already completely broken. The chloroplast envelope has a major role in the process of export of materials. In the present study, photosynthesis and transpiration were measured in order to determine whether these processes could be used as indicators of senescence.

Black jack (*Bidens pilosa*) is used in traditional medicine to treat malaria and it has anti-inflammatory activity (GEISSBERGER & SEQUIN 1991). Because the leaves are the economically important organs, understanding their chlorophyll loss is essential in determining the correct time for harvesting them. Thus, chlorophyll was chosen as a measure of senescence in this study.

Defruiting experiments in some annuals including pea (*Pisum sativum*), red kidney bean (*Phaseolus vulgaris*), soybean (*Glycine max*) and sunflower (*Helianthus annuus*) showed a delay in leaf yellowing. Senescence was examined in the economic weed *Bidens pilosa*, with the objectives to determine the effects of deflowering and defruiting on growth, chlorophyll content, photosynthesis and transpiration, and to identify the stage of development of the head at which the flowers, seeds/fruit produce senescence signals.

## 2.2. AN EXPERIMENT TO INVESTIGATE THE EFFECT OF DEFLOWERING AND DEFRUITING ON GROWTH AND SENESCENCE OF POT-GROWN PLANTS

### 2.2.1. Materials and Methods

#### 2.2.1.1. Plant material

Seeds of *Bidens pilosa* L. were placed in trays for germination in a nursery. When the seedlings were one month old with two pairs of leaves, they were transplanted into 35 cm pots (1 plant per pot). Three sets were placed under shade cloth with varying solar radiation (300, 600 and 800  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ). The fourth set was placed outside the shade cloth at maximum solar radiation. Plants in each treatment were placed in a randomised block design. The experiment was carried out at Kwadlangezwa (28°51'S, 31°51'E) where the climate is subtropical. The seedlings were planted in loam soil lined at the bottom of each 35 cm pot by a layer of stones. Compost (10 g per plant) and 4 grams N:P:K fertiliser (2:3:2 + 0.5% Zn) were applied to each pot. The pots were placed 65 cm apart with rows 72 cm apart, and watered regularly. When flowering commenced at 44 days after transplanting, plants were divided into three blocks: left to flower (control); deflowered as ray florets emerged, leaving the peduncle; or fruit removed from heads with wilted ray florets.

After 15, 45 and 75 days five plants were harvested from each treatment, and divided into roots, stems and leaves. Heads with fruits were excluded from the stems, and

weighed separately to determine fruit maturity. During the final harvest, live and dead stems were separated in order to determine the degree of stem necrosis. In the control, the heads were sorted into: immature fruit (diameter of receptacle 4 mm, fruit 5 mm long); mature green fruit (diameter of receptacle 5 mm, fruit 9 mm long), and mature dry fruit. All the samples were dried in an oven at 70°C.

Dead leaves were separated from live ones in order to determine total fresh weight. Two discs (1 cm<sup>2</sup>) were punched from the terminal leaflets from five plants per treatment, giving ten samples per treatment. Each sample was weighed accurately to obtain 1 g fresh leaf weight. Five of these were used for chlorophyll extraction, and the other five to calculate dry weight for the chlorophyll determinations. The mobility of the senescence signal was determined by analysing total chlorophyll content in the first and second pair of leaves from the top of the plant at day 45 and 60. This was done using 0.1 g fresh weight of the control and deflowered plants. Leaf samples were homogenised in a mortar and pestle and extracted overnight in 20 ml N,N-dimethylformamide. The resultant homogenate was filtered through Whatman No. 1 filter paper.

The absorbance of aliquots of the extracts was then measured in a Beckman DU-64 spectrophotometer at 647 nm (maximum for chl b) and 664 nm (maximum for chl a) using a 10 mm cuvette. Absolute chlorophyll concentrations (chl a and chl b) expressed in mg, were calculated according to INSKEEP AND BLOOM (1985). The data are presented as means with standard errors.

### 2.2.1.2. Gas exchange measurements




Rates of CO<sub>2</sub> and H<sub>2</sub>O exchange by attached leaf parts were measured using a portable combined infra-red gas analysis system (Ciras-1 PP Systems Unit 2, Glovers Court, Bury Mead Rd Hitchin, UK). The flow rate in the air supply unit was 300 ml per min. Air entering the air supply unit was dried by passing through a pair of absorbing columns connected in series. The chemical used was silica gel. The window area of a Parkinson leaf cuvette was 2.5 cm<sup>2</sup>. Adjacent to the chamber window was a sensor for monitoring photosynthetic active radiation. Additionally, there were sensors for humidity and temperature within the chamber. The chamber was positioned so that during measurement the lamina portion being measured was in the same location within the canopy as during growth, but perpendicular to the direction of radiation.

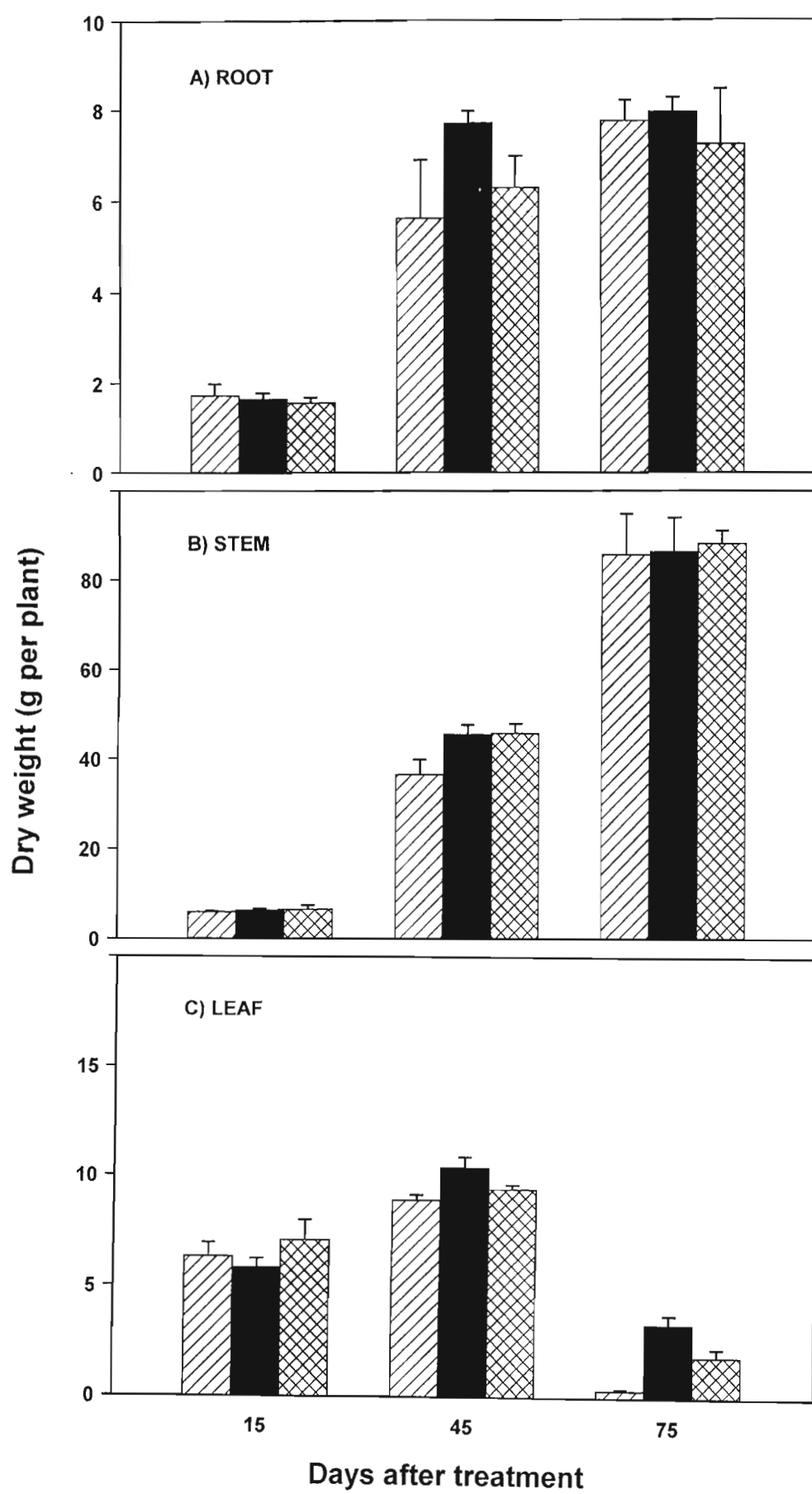
Measurements were made between 9H30 and 11H30 under shade cloth conditions with varying solar radiation (300, 600 and 800  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ). Photosynthesis and evaporation were measured in the terminal leaflet of the first and second pair of each compound-leaf from the head. Ten measurements were made per plant. There were five plants per treatment.

### 2.2.2. Results

#### 2.2.2.1. Dry weight

The root, stem and leaf dry weight for control, deflowered and defruited plants are presented in **Figure 2.1**. Deflowering and defruiting did not result in a significant difference in root and stem dry weight. The treatments showed differences in terms of

Figure 2.1: Effect of deflowering and defruiting on growth expressed as dry weight of pot-grown *Bidens pilosa* plants. Control (  ); deflowered (  ); defruited (  ).



leaf dry weight. Deflowered plants had a significantly higher leaf dry weight than the controls 45 and 75 days after treatment. There were no significant differences between deflowered and defruited plants at 45 days after treatment. At 75 days after treatment, the leaf dry weight of the controls was significantly lower than for both deflowered and defruited plants. Furthermore, deflowered plants had a higher leaf dry weight than defruited ones.

After 75 days,  $36\pm4\%$  of stems were dead in the controls, compared with  $31\pm6\%$  after defruiting and  $5\pm1\%$  after deflowering.

#### 2.2.2.2. Plant height

There were no significant differences between the treatments in terms of plant height (**Figure 2.2**).

#### 2.2.2.3. Chlorophyll content and fruit development

The chlorophyll content of the control plants was significantly lower than that of both deflowered and defruited plants at 45 and 75 days after treatment (**Figure 2.3**). At 75 days after treatment, deflowered plants had a higher chlorophyll content than defruited ones. At 60 days after treatment, the control plants showed a decline in chlorophyll in the first and second leaf from the top, whereas in deflowered plants chlorophyll content remained the same in both leaves (**Figure 2.4**). A low leaf dry weight and a low chlorophyll content in the control plants (**Figure 2.1 & 2.3**) coincided with a high dry weight of mature ripe fruits (**Figure 2.5**).

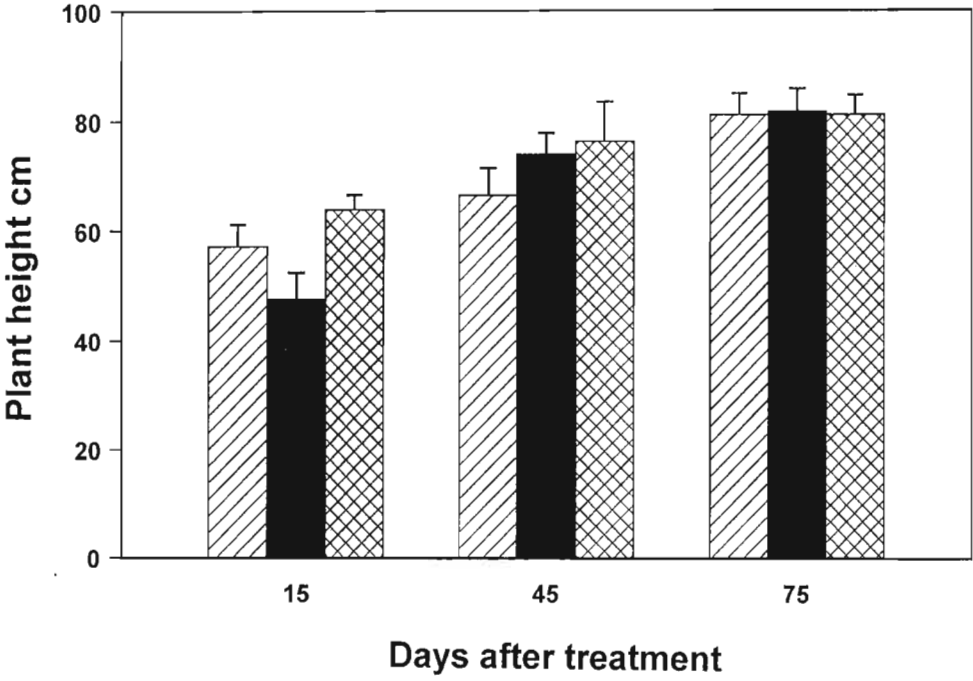





Figure 2.2: Effect of deflowering and defruiting on plant height of pot-grown *Bidens pilosa* plants. Control (  ); deflowered (  ); defruited (  ).

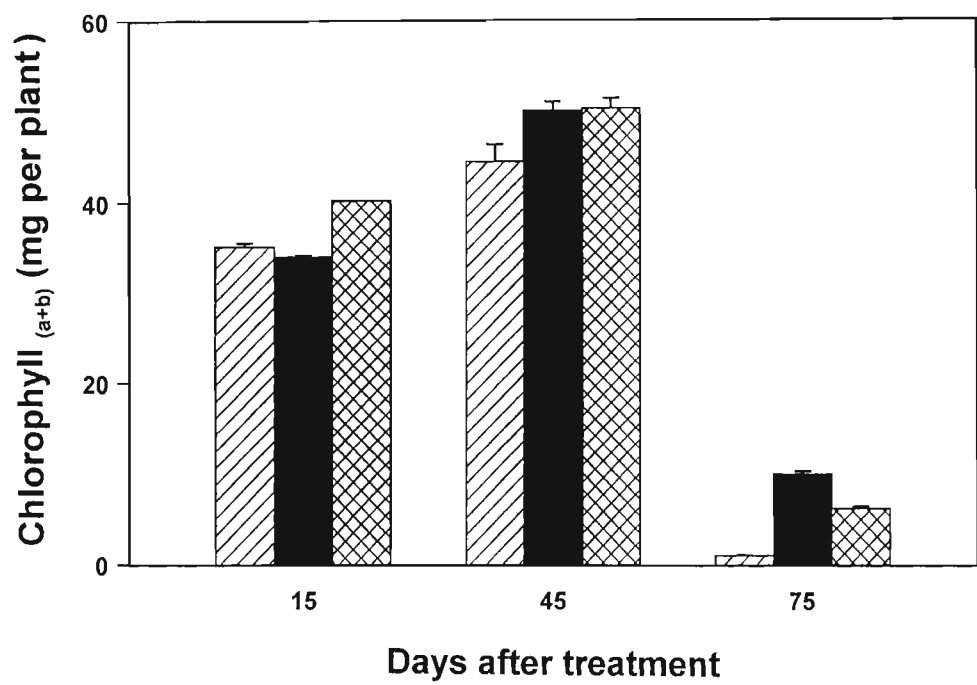





Figure 2.3: Effect of deflowering and defruiting on chlorophyll content of pot-grown *Bidens pilosa* plants. Control (  ); deflowered (  ); defruited (  ).

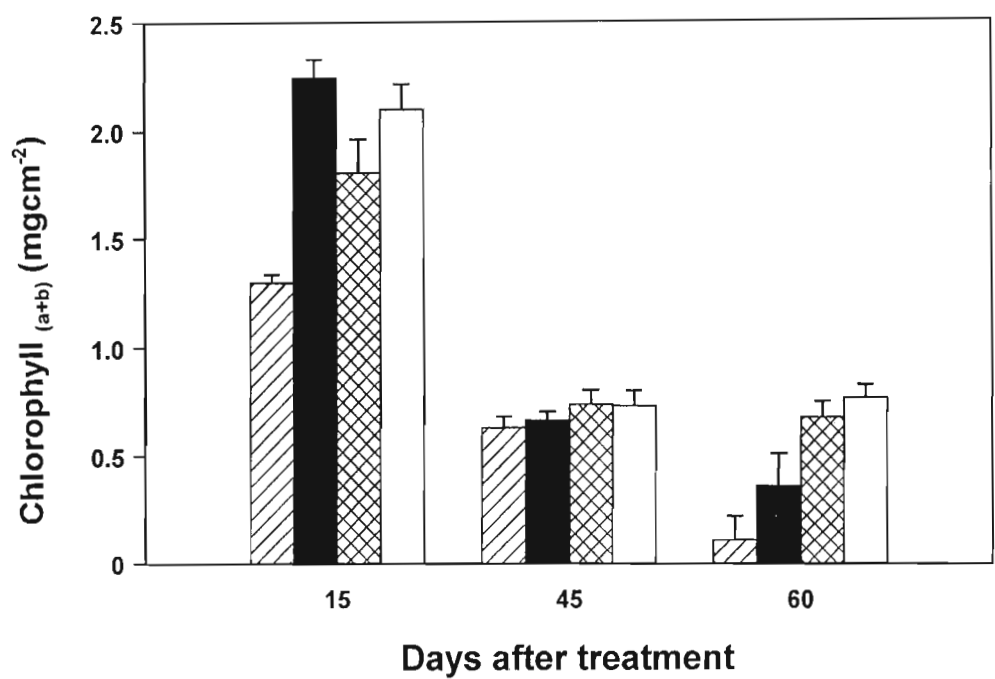






Figure 2.4: Effect of deflowering and position of the leaf on chlorophyll content in pot-grown *Bidens pilosa* plants. Control plant, leaf 1 (  ); control plant, leaf 2 (  ); deflowered plant, leaf 1 (  ); deflowered plant, leaf 2 (  ).

#### 2.2.2.4. Photosynthesis and transpiration

There was a decline in photosynthesis in all the treatments from 15 to 75 days after treatment (**Figure 2.6A**). Deflowered plants had a significantly lower photosynthetic rate than defruited ones at 45 and 75 days after treatment.

The results for transpiration rate are shown in **Figure 2.6B**. Transpiration rate for deflowered plants was significantly lower than that of defruited ones from 15 to 75 days after treatment. Except for 75 days after treatment, the control plants had a higher transpiration rate than deflowered plants.

There was a decline in stomatal conductance in all the treatments from 15 to 75 days after treatment (**Figure 2.6C**). Stomatal conductance in the controls was significantly higher than in deflowered plants at 45 and 75 days after treatment. Defruited plants also had a higher stomatal conductance than deflowered ones at 45 and 75 days after treatment. The controls and defruited plants did not differ significantly in stomatal conductance.

#### 2.2.2.5. Influence of light intensity on growth and senescence

There were no significant differences in root dry weight between the treatments at light intensities of 300, 600 and 800  $\mu\text{molm}^{-2}\text{s}^{-1}$  respectively (**Figure 2.7A**).

Plants which were grown at light intensities of 300 and 600  $\mu\text{molm}^{-2}\text{s}^{-1}$  had a higher stem dry weight than the ones grown at 800  $\mu\text{molm}^{-2}\text{s}^{-1}$  in all the treatments (**Figure**

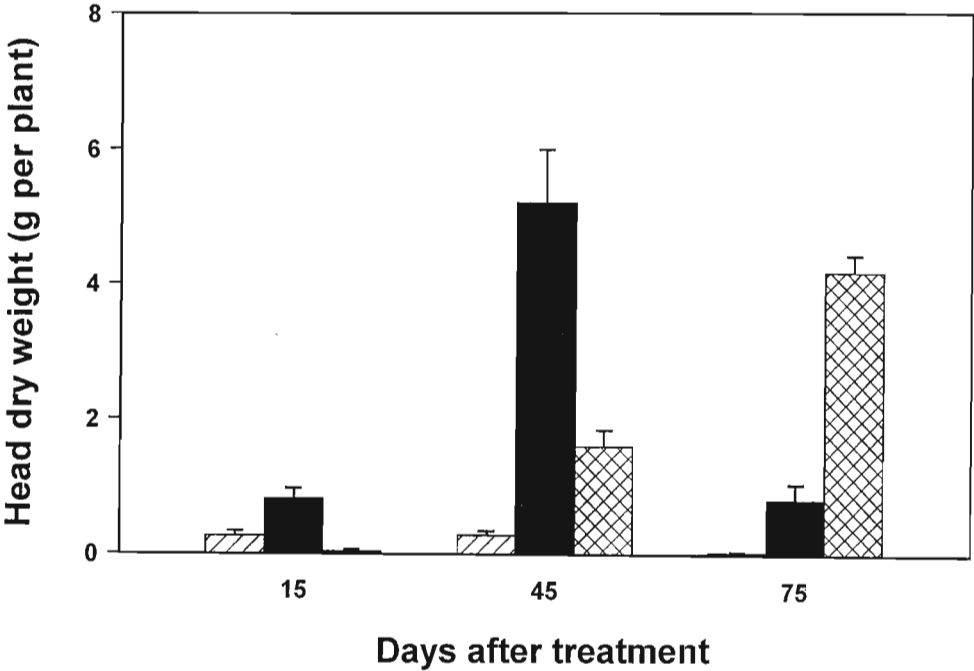





Figure 2.5: Growth of the flower heads and fruits in pot-grown control plants of *Bidens pilosa*. Heads with young fruits (  ); heads with mature green fruits (  ); heads with mature ripe (dry) fruits (  ).



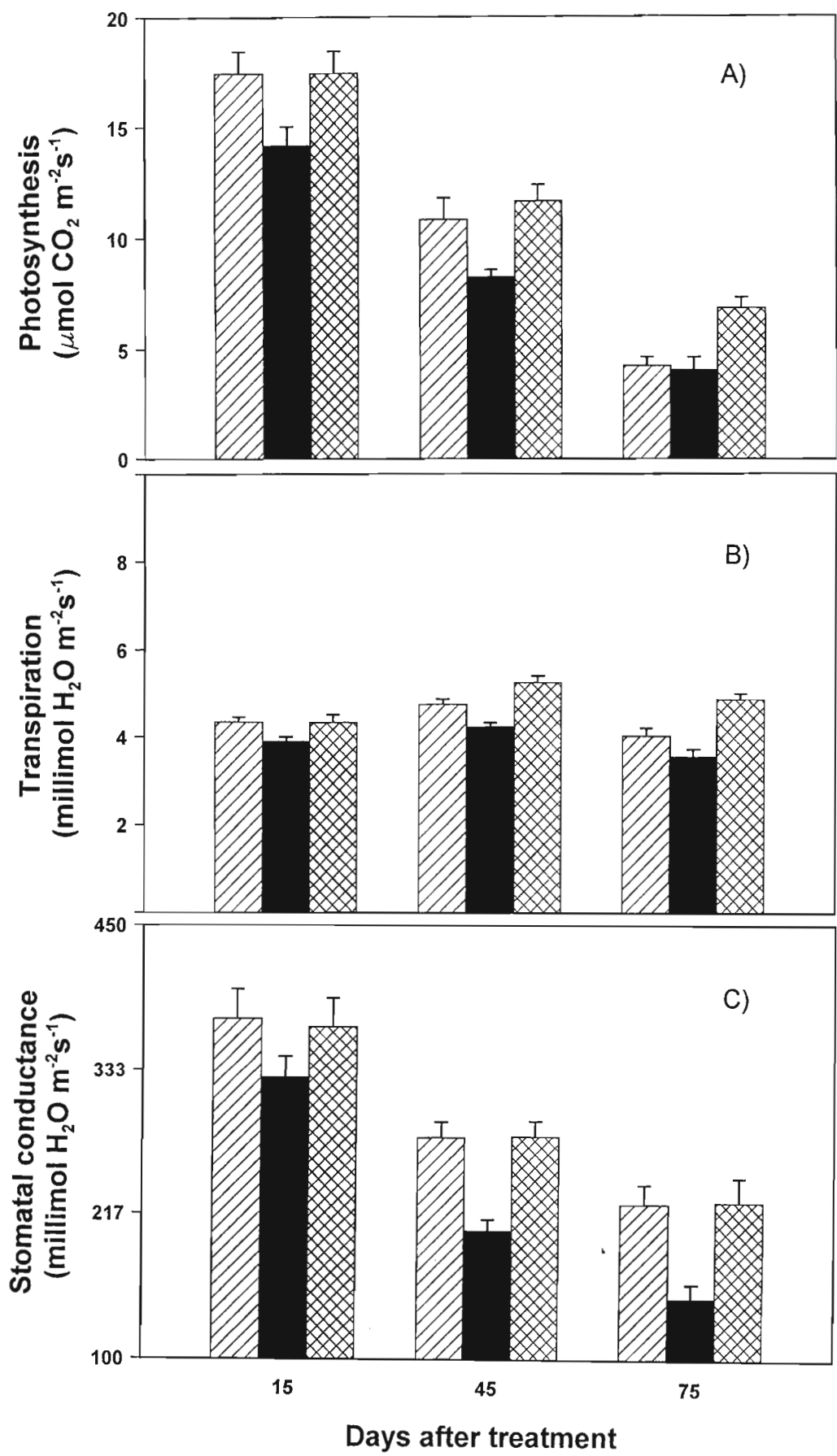



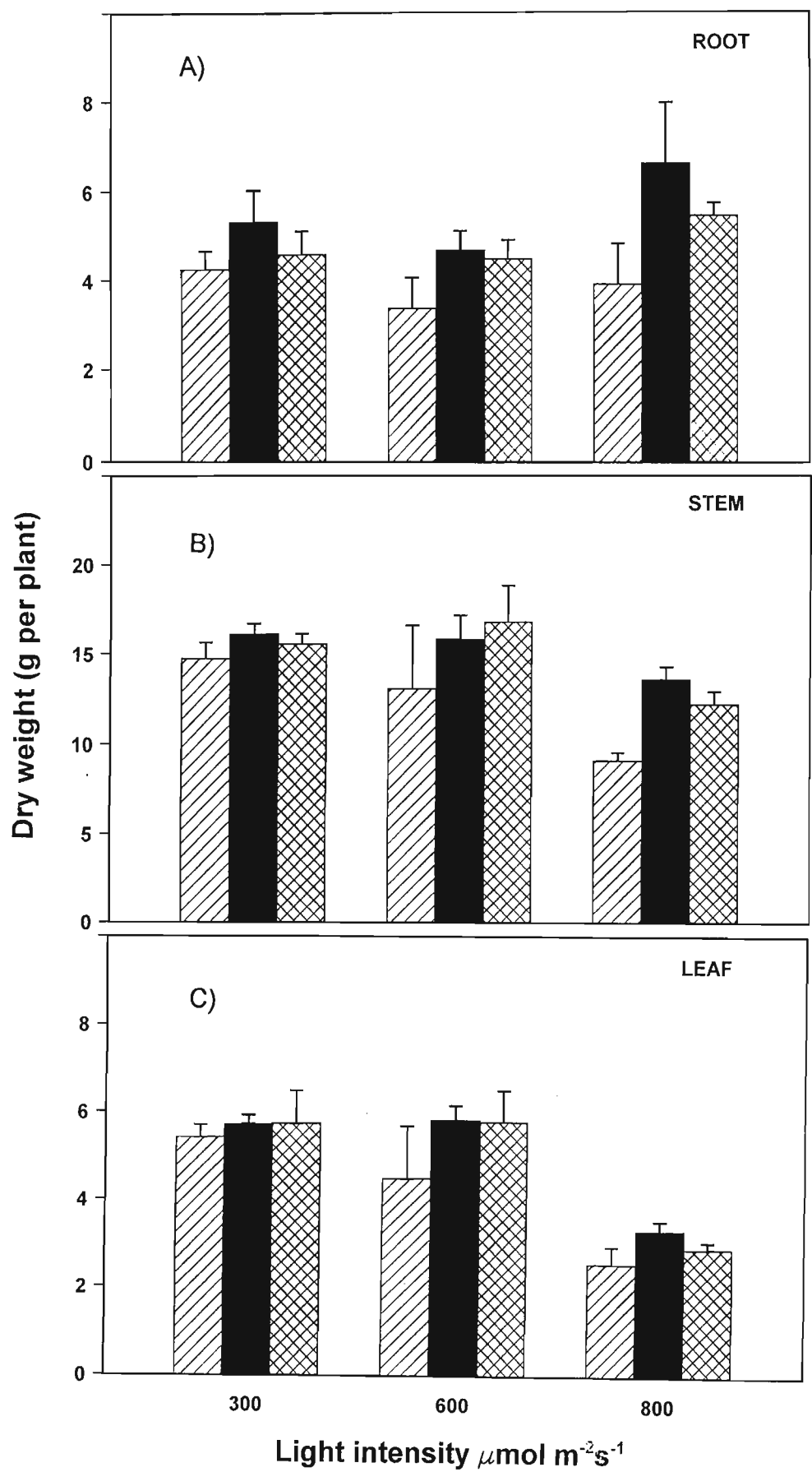


Figure 2.7: Effect of deflowering and defruiting on growth expressed as dry weight of pot-grown *Bidens pilosa* plants at different light intensities. Control (  ); deflowered (  ); defruited (  ).



**2.7B).** The control plants at a light intensity of  $800 \mu\text{molm}^{-2}\text{s}^{-1}$  were significantly lower than deflowered and defruited ones in terms of dry weight.

Plants that were grown at a light intensity of  $800 \mu\text{molm}^{-2}\text{s}^{-1}$  had a lower leaf dry weight than the ones grown at light intensities of 300 and  $600 \mu\text{molm}^{-2}\text{s}^{-1}$  (**Figure 2.7C**). There were no significant differences between the treatments at each light intensity. The leaf area showed no significant difference between the treatments at all light intensities (**Figure 2.8A**). Plants grown at a light intensity of  $800 \mu\text{molm}^{-2}\text{s}^{-1}$  had a lower leaf area than the ones grown at 300 and  $600 \mu\text{molm}^{-2}\text{s}^{-1}$  in all the treatments.

Plants grown at a light intensity of  $800 \mu\text{molm}^{-2}\text{s}^{-1}$  were slightly shorter than the ones grown at light intensities of 300 and  $600 \mu\text{mol m}^{-2}\text{s}^{-1}$  in all the treatments (**Figure 2.8B**).

**In the first pair of leaves**, the control plants did not differ significantly in photosynthetic rate at all light intensities (**Figure 2.9A**). Deflowered plants had a higher photosynthetic rate than the control plants at all light intensities. Deflowered plants had a significantly higher photosynthetic rate than defruited ones at light intensities of 600 and  $800 \mu\text{molm}^{-2}\text{s}^{-1}$ . The control and defruited plants did not differ in photosynthetic rate at light intensities of 600 and  $800 \mu\text{molm}^{-2}\text{s}^{-1}$ .

**In the second pair of leaves**, the control and deflowered plants had a lower photosynthetic rate at a light intensity of 800 than at 300 and  $600 \mu\text{molm}^{-2}\text{s}^{-1}$  (**Figure 2.9B**). In defruited plants, photosynthetic rate was higher at a light intensity of 300 than at 600 and  $800 \mu\text{molm}^{-2}\text{s}^{-1}$ . There was a general decline in photosynthetic rate in all the treatments at a light intensity of 800 compared to  $300 \mu\text{molm}^{-2}\text{s}^{-1}$ .

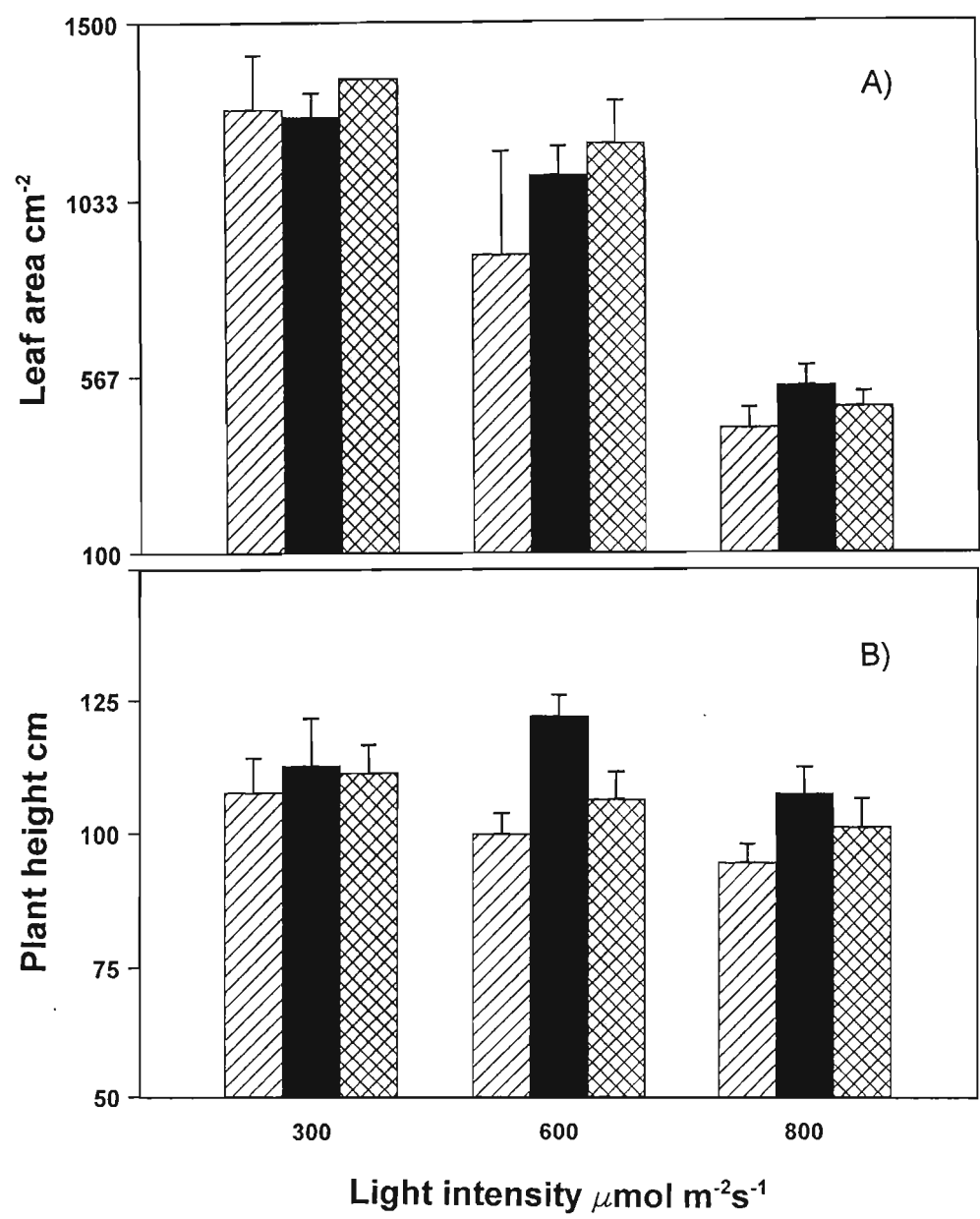





Figure 2.8: Effect of deflowering and defruiting on leaf area and plant height of pot-grown *Bidens pilosa* plants at different light intensities. Control (  ); deflowered (  ); defruited (  ).

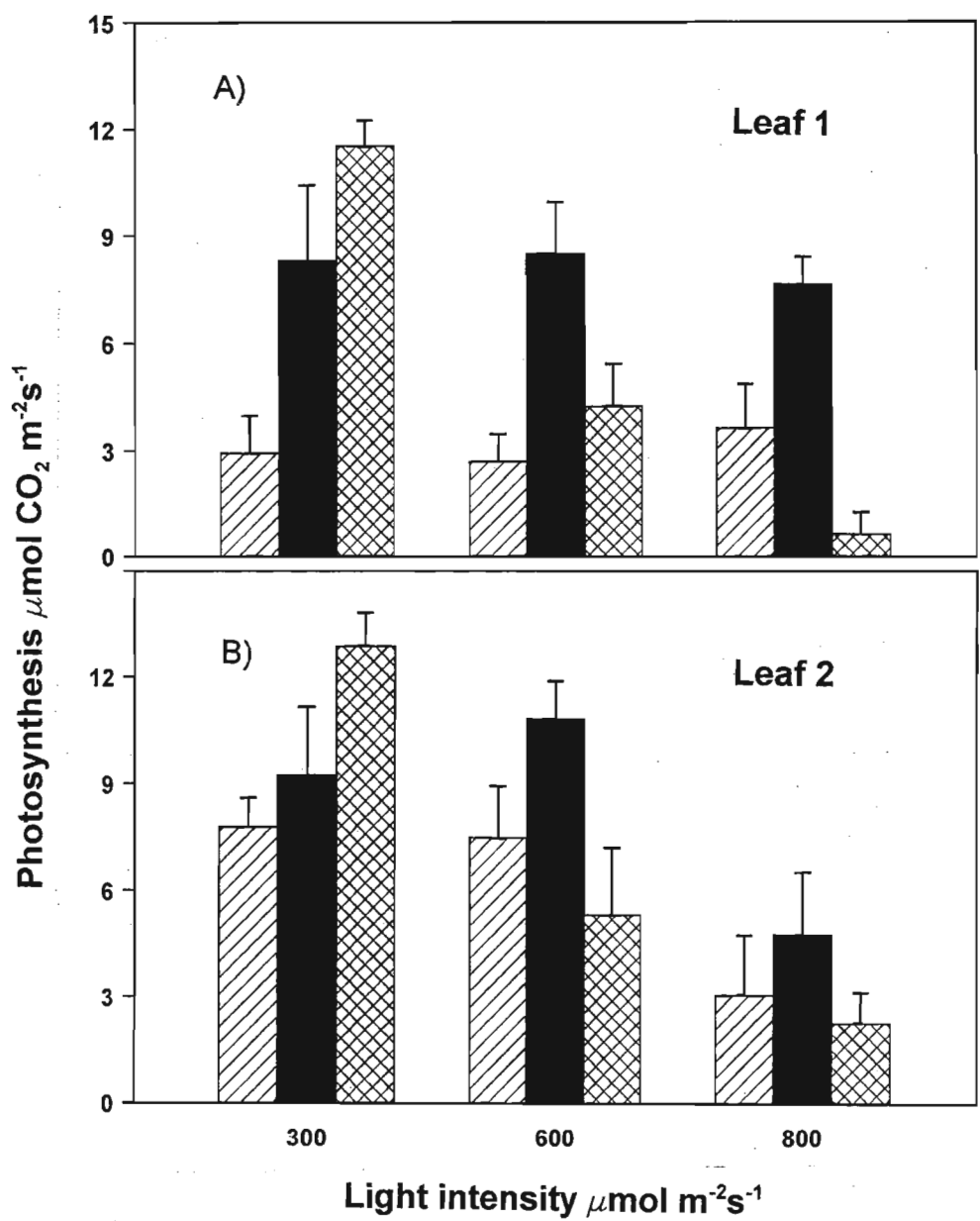





Figure 2.9: Effect of deflowering, defruiting and position of the leaf on photosynthetic rate of pot-grown *Bidens pilosa* plants at different light intensities. Control (  ); deflowered (  ); defruited (  ).

**In the first pair of leaves**, total chlorophyll declined in all the treatments at all light intensities (**Figure 2.10A**). Control plants had a significantly lower chlorophyll content than deflowered ones at 300 and 600  $\mu\text{molm}^{-2}\text{s}^{-1}$ . The chlorophyll content of deflowered plants was significantly higher than that of defruited ones at 800  $\mu\text{molm}^{-2}\text{s}^{-1}$ .

**In the second pair of leaves**, total chlorophyll content declined in all the treatments at all light intensities (**Figure 2.10B**). At light intensities of 300 and 600  $\mu\text{molm}^{-2}\text{s}^{-1}$ , there were no significant differences between the treatments. At light intensity of 800  $\mu\text{molm}^{-2}\text{s}^{-1}$ , the chlorophyll levels of the control and defruited plants were slightly lower than in deflowered plants. However, there were no significant differences between the treatments.

The dry weight of mature ripe fruits above the first pair of leaves was  $0.25 \pm 0.03$  g at a light intensity of 300;  $0.28 \pm 0.05$  g at a light intensity of 600; and  $0.43 \pm 0.09$  g at a light intensity of 800  $\mu\text{molm}^{-2}\text{s}^{-1}$ . A low chlorophyll content in the first pair of leaves of control plants at high light intensity (**Figure 2.10A**) coincided with a high dry weight of mature ripe fruits above the first pair of leaves. The dry weight of mature ripe fruits per plant did not differ significantly at all light intensities (**Figure 2.11**).

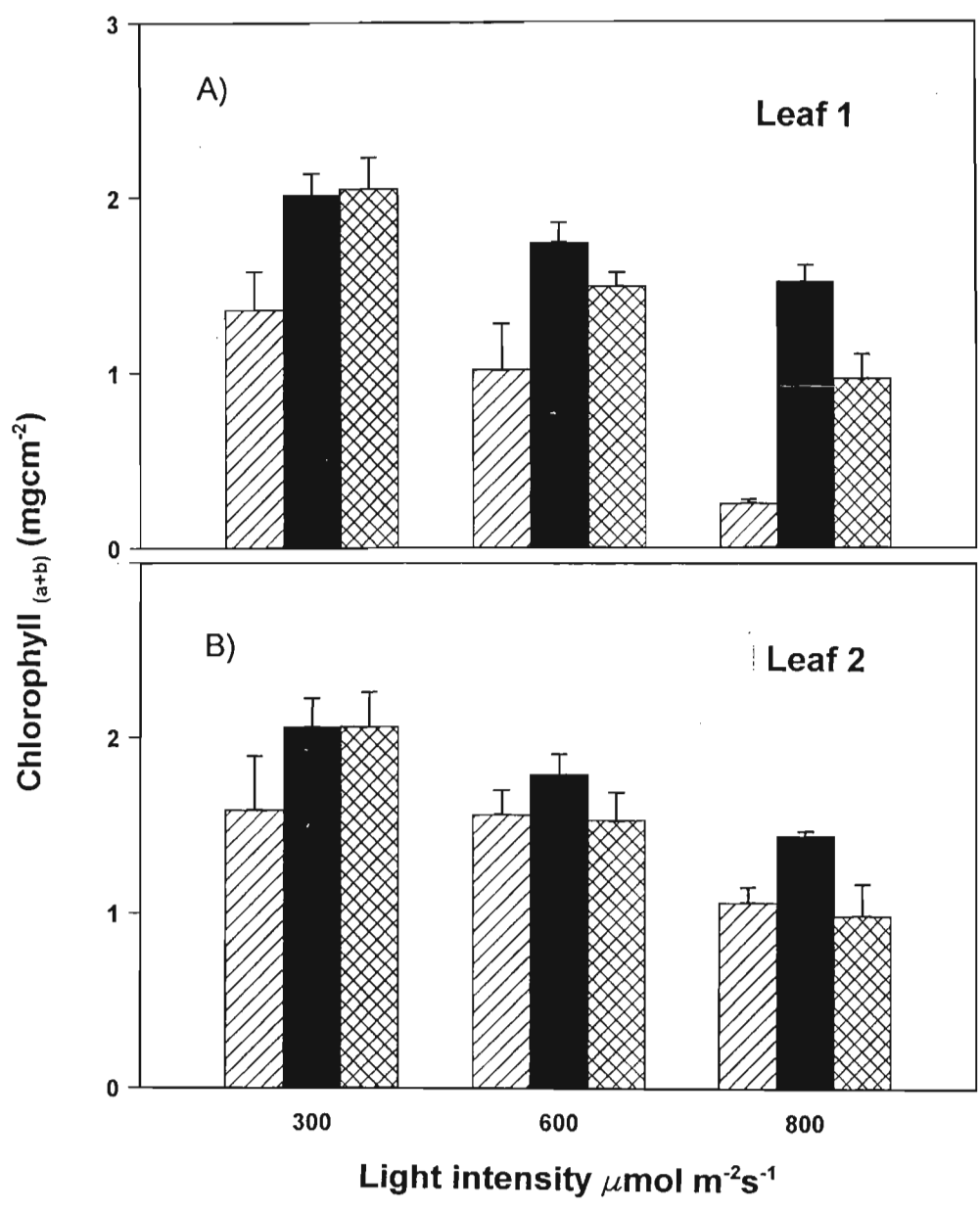


Figure 2.10: Effect of deflowering and position of the leaf on chlorophyll content in pot-grown *Bidens pilosa* plants at different light intensities. Control ( ▨ ); deflowered ( ■ ); defruited ( ▩ ).

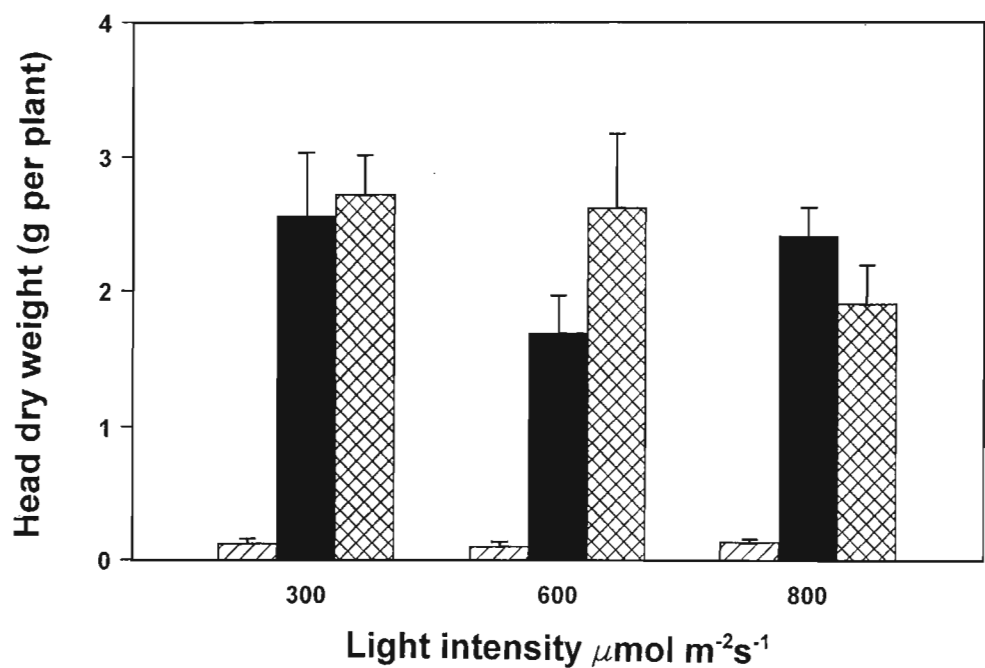





Figure 2.11: Growth of the flower heads and fruits in pot-grown control plants of *Bidens pilosa* at different light intensities. Heads with young fruit (  ); heads with mature green fruits (  ); heads with mature ripe (dry) fruits (  ).

## **2.3. AN EXPERIMENT TO INVESTIGATE THE EFFECTS OF DEFLOWERING AND DEFUITING ON GROWTH AND SENESCENCE OF FIELD-GROWN *BIDENS PILOSA* PLANTS**

### **2.3.1 Materials and Methods**

#### **2.3.1.1. Plant Material**




Seeds of *Bidens pilosa* were placed in trays for germination in a nursery as in the pot experiment described earlier. When the seedlings were one month old with two pairs of leaves, they were planted out in the field. The seedlings were placed 65 cm apart with rows 72 cm apart and watered regularly. When flowering commenced at 44 days after transplanting, plants were divided into three blocks: left to flower (control); deflowered as ray florets emerged, leaving the peduncle; or fruit removed from heads with wilted ray florets.

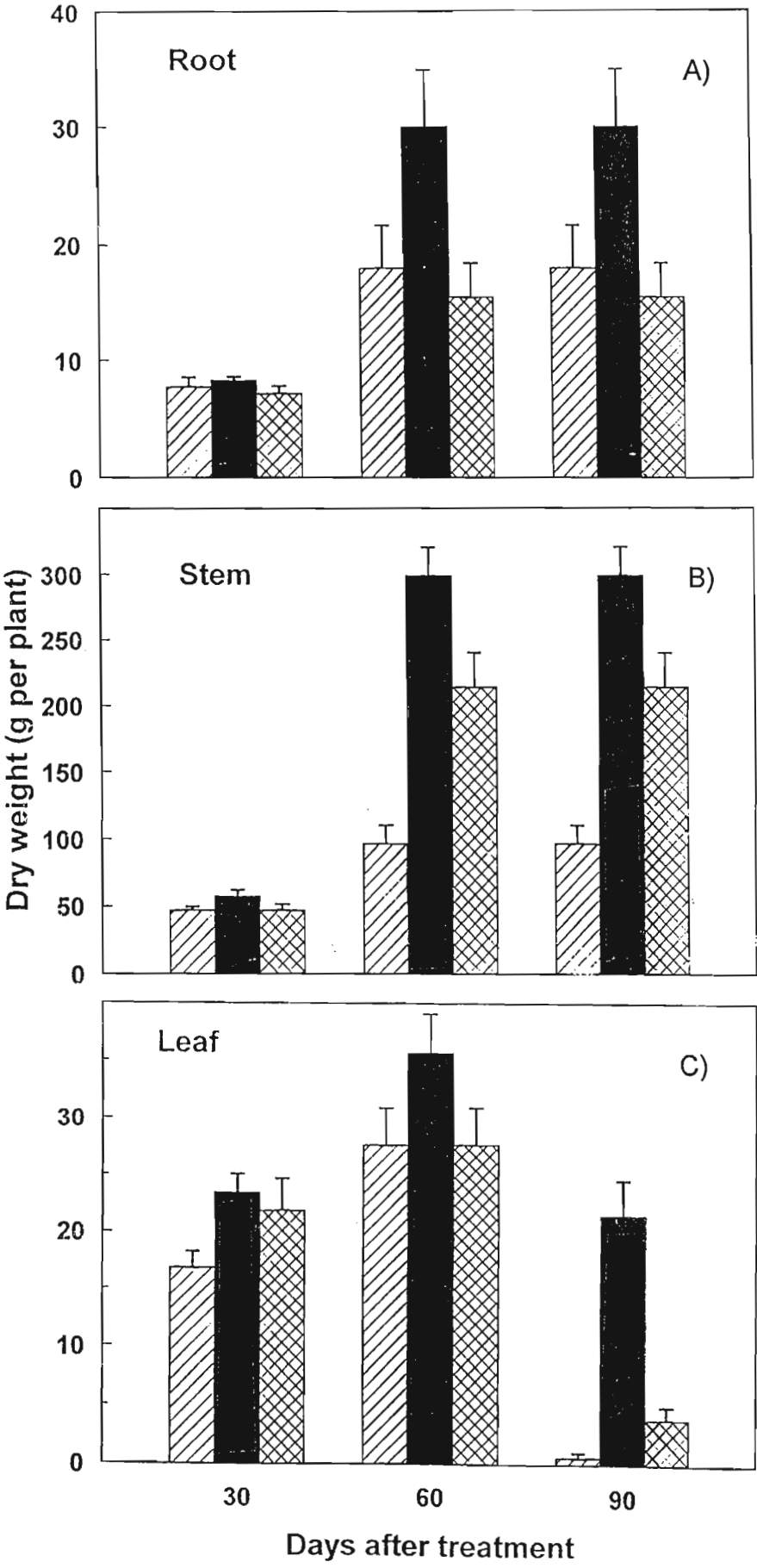
After 30, 60 and 90 days five plants were harvested from each treatment, and divided into roots, stems and leaves. Dry weight, chlorophyll content and plant height were analysed as described earlier. There were five observations per treatment. The data are presented as means with standard errors.

### **2.3.2 Results**

#### **2.3.2.1. Dry weight**

Deflowered *Bidens pilosa* plants had a greater leaf weight at 90 days, and greater

Figure 2.12: Effect of deflowering and defruiting on growth expressed as dry weight of field-grown *Bidens pilosa* plants. Control (  ); deflowered (  ); defruited (  ).



stem and root weight after 60 and 90 days compared with control plants (**Figure 2.12**). Defruited plants also had a greater stem weight for the last two harvests. In contrast, there was no effect of defruiting on root weight (**Figure 2.12**).

#### 2.3.2.2. Plant height

Deflowered plants were taller than the controls and those that were defruited (**Figure 2.13**).

#### 2.3.2.3. Chlorophyll content and fruit development

Total chlorophyll content in the controls, in association with the development of fruit, was lower at 90 days compared with earlier harvests (**Figure 2.13** & **Figure 2.15**). There was also an effect of treatment, with the deflowered plants having higher chlorophyll levels than the other two treatments after 90 days (**Figure 2.13**).

Data was also collected on the chlorophyll content of leaves 1 and 2 from the controls and deflowered plants (**Figure 2.14**). Leaf chlorophyll decreased from day 30 to 60 in the control, but remained stable or increased slightly in the deflowered plants. This gave rise to higher chlorophyll levels in the deflowered plants compared with controls in the second harvest. The loss of chlorophyll seems to be related to the presence of fruit, although there is also an impact of the stalk. After 90 days,  $86 \pm 7\%$  of stems were dead in the controls, compared with  $35 \pm 4\%$  after defruiting and  $9 \pm 2\%$  after deflowering. These results suggest that the fruit, and to a lesser degree the flower heads, contributed to mortality of the stems.

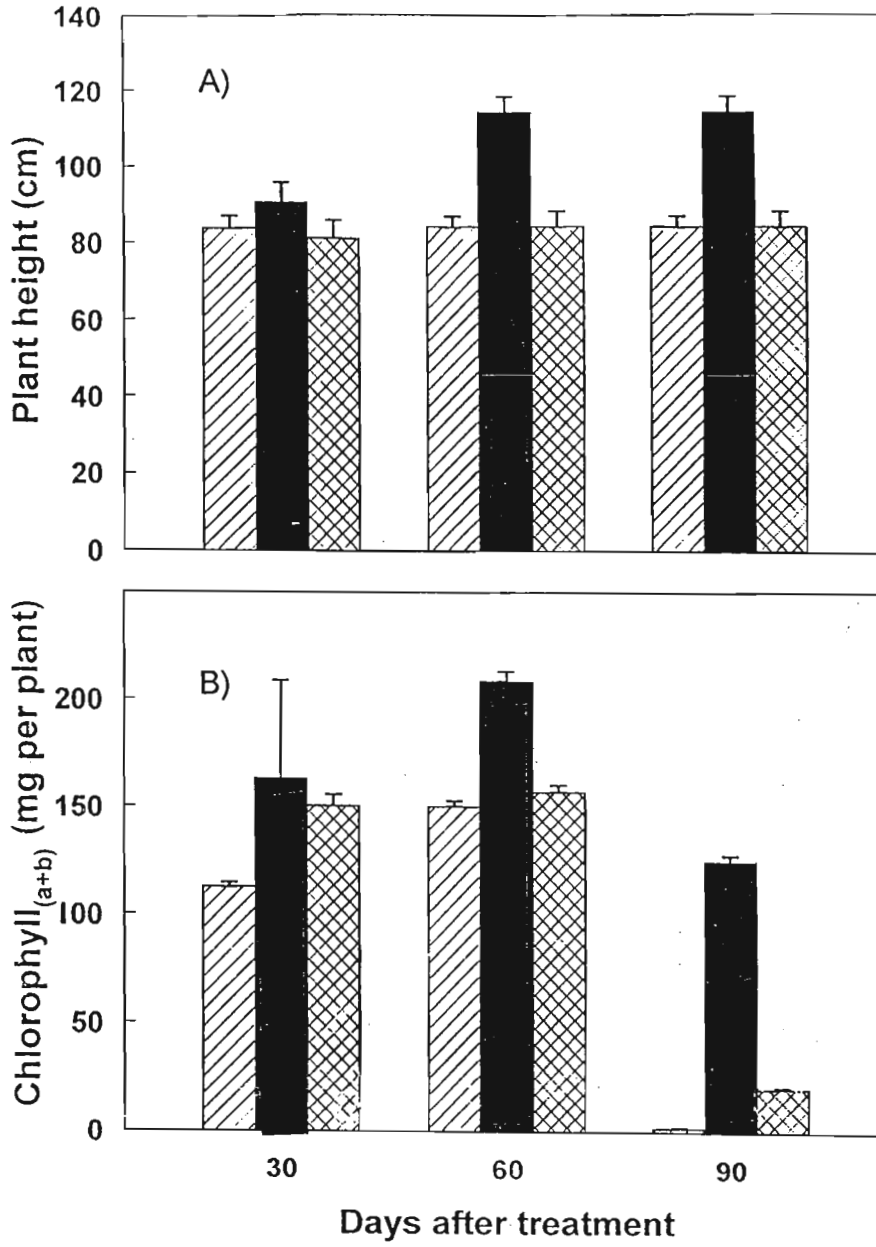





Figure 2.13: Effect of deflowering and defruiting on plant height and chlorophyll content of field-grown *Bidens pilosa* plants. Control (  ); deflowered (  ); defruited (  ).

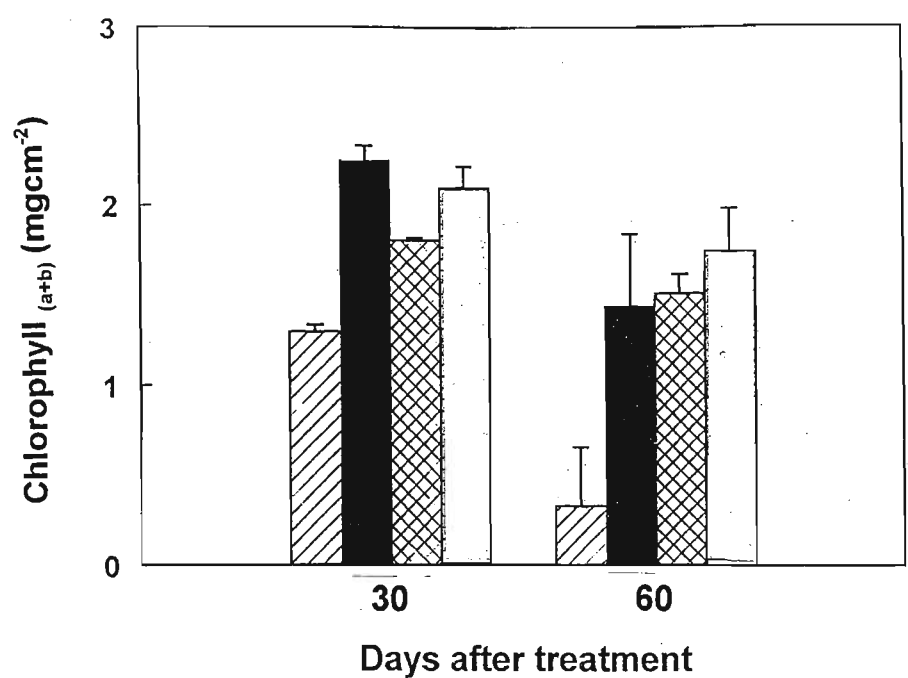


Figure 2.14: Effect of deflowering and position of the leaf on chlorophyll content in field-grown *Bidens pilosa* plants. Control plant, leaf 1 ( ▨ ); control plant, leaf 2 ( ■ ); deflowered plant, leaf 1 ( ▩ ); deflowered plant, leaf 2 ( □ ).

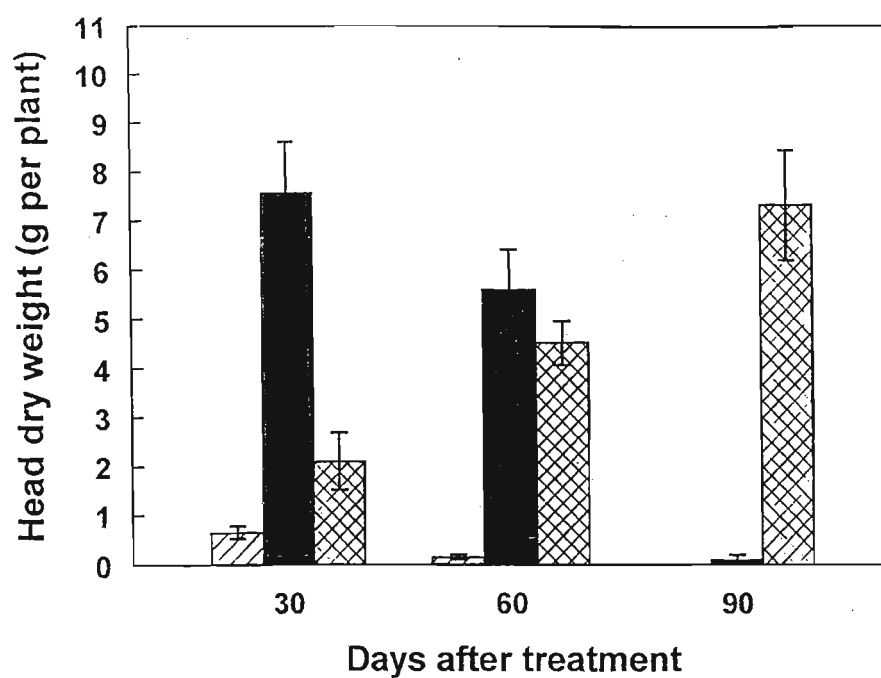





Figure 2.15: Growth of the flower heads and fruits in field-grown control plants of *Bidens pilosa*. Heads with young fruit (  ); heads with mature green fruits (  ); heads with mature ripe ( dry) fruits (  ).

## 2.4. DISCUSSION

The results for the pot and field plant experiments showed similarities as well as differences in terms of growth and senescence.

In the pot plant experiment, there were no significant differences between the treatments in terms of root and stem dry weight. With respect to the leaf dry weight, deflowered plants were significantly higher than the controls at 45 and 75 days after treatment.

In the field plant experiment, there were significant differences between the treatments in terms of root, stem and leaf dry weight. Deflowered *Bidens pilosa* plants had a greater leaf weight at 90 days, and a greater stem and root weight after 60 and 90 days compared with control plants. Other workers reported a higher stem and leaf dry weight in deflowered sunflower plants (HO & BELOW, 1989). In rice plants, removal of the panicle caused a significant increase in root and stem dry weight (NAKANO, MAKINO & MAE, 1995). Bean plants whose pods were harvested had a higher root, stem and leaf dry weight than the controls (KOHASHI-SHIBATA, BACA-CASTILLO & SANTOS-VIGIL, 1997). Vegetative growth slows once plants flower and fruit and cannot be restored to pre-flowering rates (NOODEN, 1988c).

There were no significant differences between the treatments in terms of plant height of the pot plant experiment. This was in contrast to the report by LINDOO & NOODÉN (1976), where deflowered soybean plants were taller than the controls. This implies that growth was suppressed in pots.

In the field plant experiment, deflowered *Bidens pilosa* plants were taller than the controls and those that were defruited. Thus, growth was not suppressed in field-grown plants.

Total chlorophyll content in the controls, in association with the development of fruit, was lower in the final harvests when compared with earlier harvests in both pot and field plant experiments. A rapid loss of chlorophyll with fruit maturation has been reported in other species (OKATAN, KAHANAK & NOODÉN 1981). There were also differences between the treatments in chlorophyll content. Deflowered *Bidens pilosa* plants had a higher chlorophyll level than both defruited and control plants in both pot and field experiments. Deflowered *Triticum aestivum* plants had a higher leaf fresh weight and chlorophyll content than the controls (FELLER, 1979). In rice plants, removal of the panicle caused a significant increase in root and stem dry weight (NAKANO, MAKINO & MAE, 1995). Bean plants whose pods were harvested had a higher root, stem and leaf dry weight than the controls (KOHASHI-SHIBATA, BACA-CASTILLO & SANTOS-VIGIL, 1997).

In both pot and field experiments, the controls showed a decrease in chlorophyll content from earlier harvests to day 60, whereas in deflowered plants the chlorophyll content remained stable in both the first and second leaves. The positions of the first and second leaves, and the stages of maturation of the fruits, are presented in **Figure 2.16 & 2.17**. In *Tagetes patula* and *Chrysanthemum coronarium*, the first leaf always senesced earlier than the third leaf. Removal of reproductive organs retarded senescence in the first leaf (GOSH & BISWAS 1994).

In the pot experiment, stem death was highest in both the control and defruited plants than in deflowered plants. In the field experiment, stem death was highest in the control and lowest in deflowered plants. Defruited plants were intermediate. These results suggest that the fruit, and to lesser degree the flower heads, contribute to the death of

Figure 2.16: Photographs of control (A); deflowered (B) and defruited (C) *Bidens pilosa* plants 15 days after treatment.

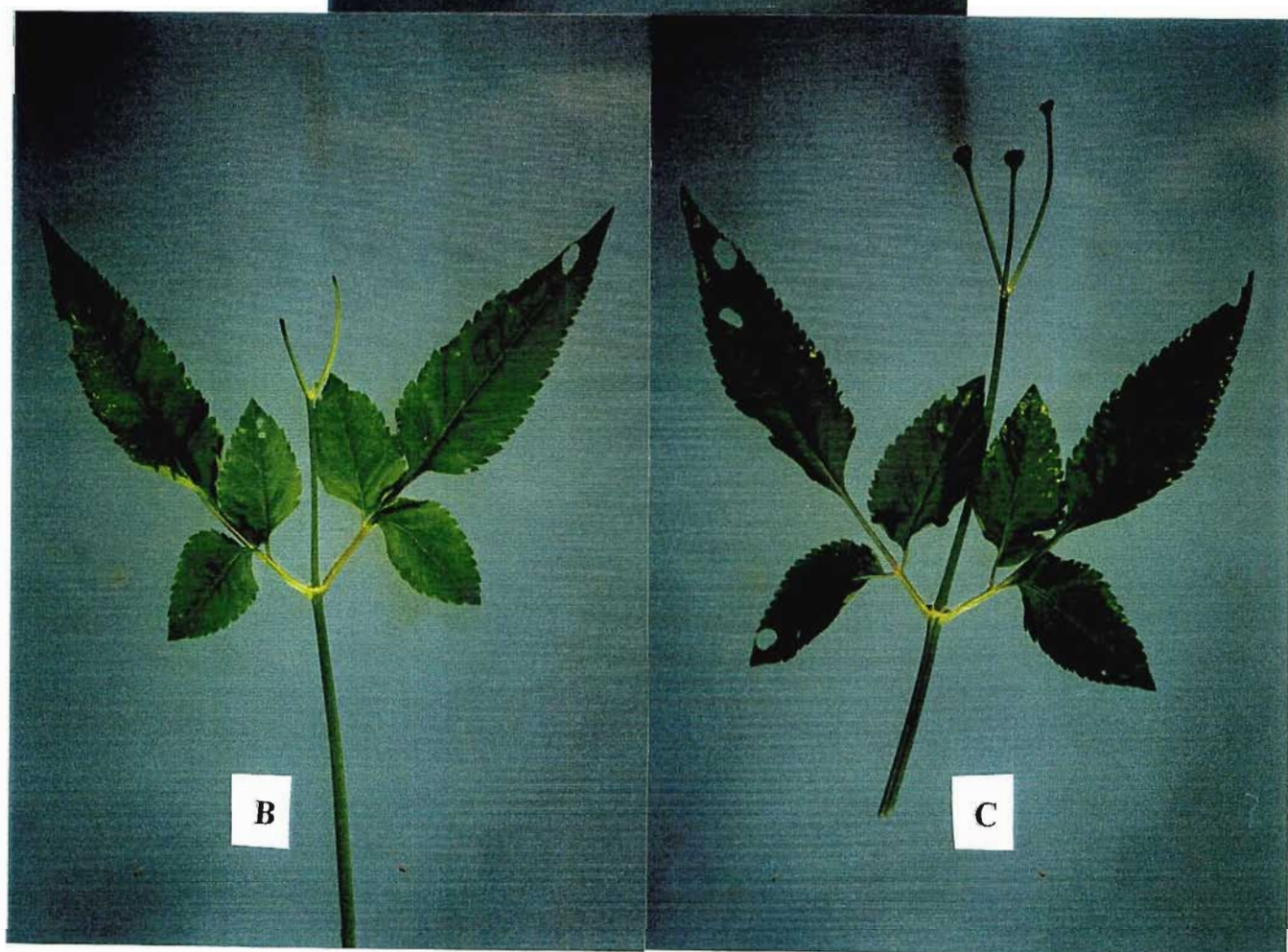
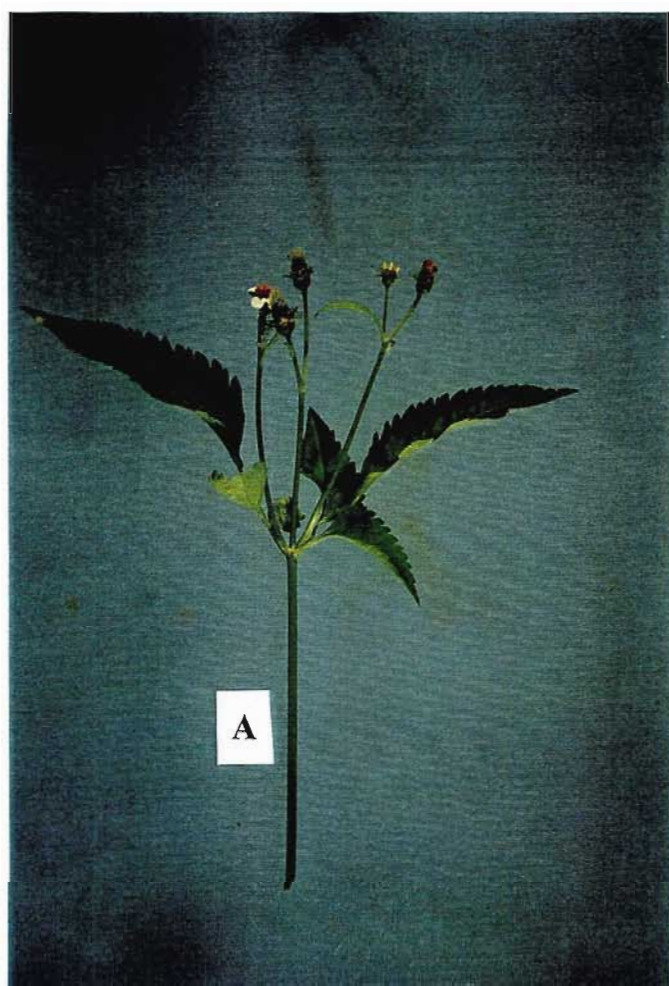
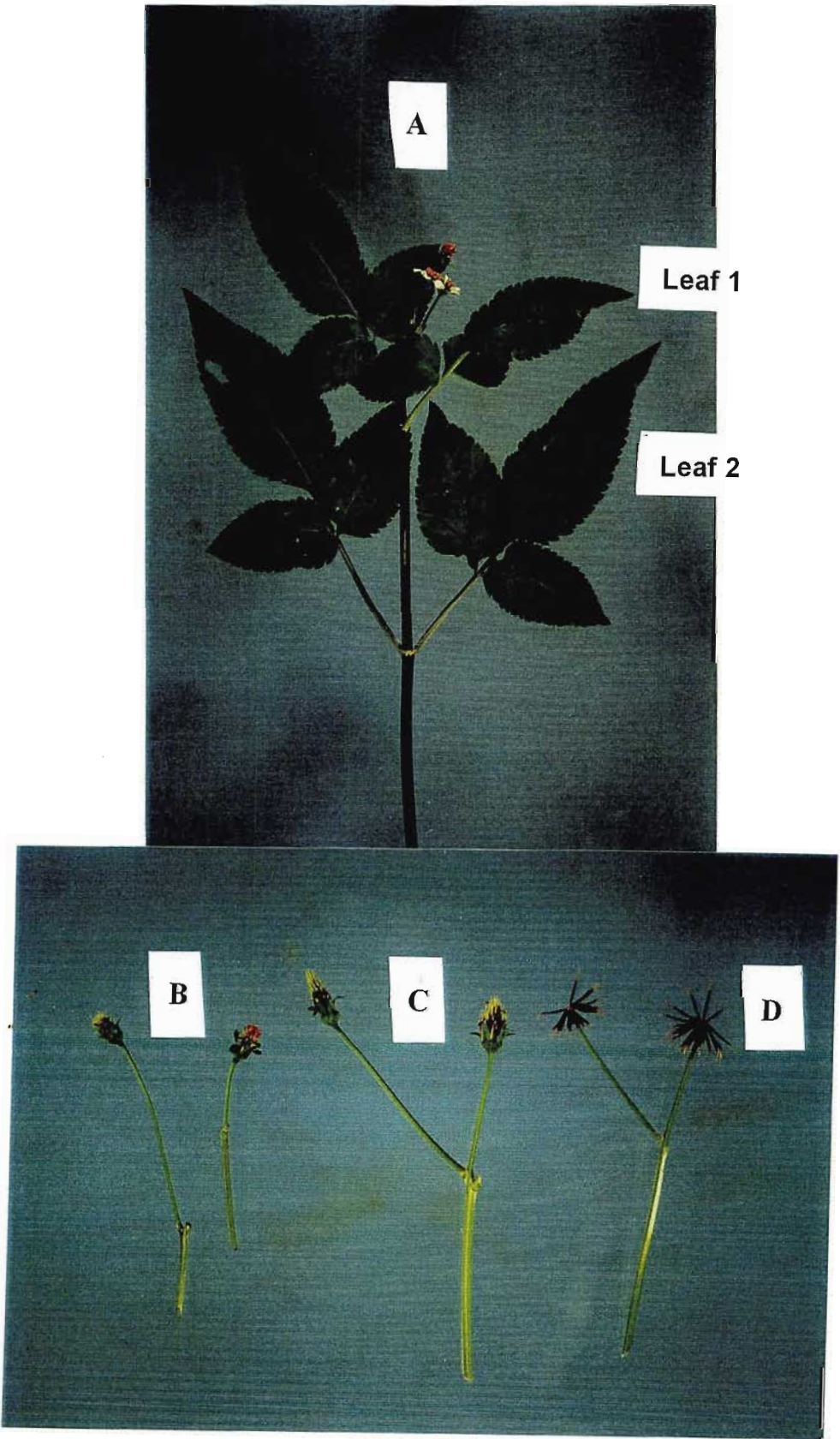


Figure 2.17: Photograph of a control plant of *Bidens pilosa* indicating the positions of the first and the second leaves (A); heads with young fruit (B); heads with mature green fruit (C); heads with mature ripe (dry) fruit (D).



the stems. Depodding prevented rapid death of soybean plants as long as it was not done too late during pod development. It worked even in field-grown plants under favourable conditions (LINDOO & NOODÉN, 1977; DERMAN, RUPP & NOODÉN, 1978; NOODÉN, RUPP & DERMAN 1978). There was a decline in photosynthesis in all the treatments from 15 to 75 days after treatment. The controls and defruited plants had a significantly higher photosynthetic rate than deflowered ones 15 and 45 days after treatment. Photosynthesis, may be coupled with, or regulated by, consumption of its products. Thus, defruiting could be expected to cause a decline in assimilatory metabolism in monocarpic plants (NOODÉN, 1988c). Photosynthesis declines during pod development in soybeans, and blocking pod development by depodding does not prevent this (MONDAL, BRUN, & BRENNER, 1978; WITTENBACH, 1982,1983; HUBER, WILSON & BURTON, 1983; CRAFTS-BRANDNER, BELOW, HARPER & HAGEMAN 1984b; SCHWEITZER & HARPER, 1985). It is possible that the decline in photosynthetic rate in depodded plants is a desinking effect rather than senescence (NOODÉN, 1988c). Thus, in the present study, low photosynthesis in deflowered plants at 15 and 45 days after treatment was probably caused by sink loss.

The decline in photosynthesis, and that in chlorophyll, or other photosynthetic components may be different phenomena. In depodded plants, photosynthesis may decline due to sink loss, whereas in podded plants, this decrease may be caused by a loss of photosynthetic components (NOODÉN, 1988c). It does not seem right to call the decreased photosynthesis in depodded plants senescence, since it does not cause death. In podded plants, the photosynthetic components appear to decline as part of senescence. They may or may not be lost in depodded plants, but where they are lost, that process seems to be driven by stress rather than pods (NOODÉN, 1988c). The death and the final stages of monocarpic senescence are prevented by depodding in

soybean if grown under favourable conditions and before the pods develop too far. Depodding prevents the collapse of the permeability barrier in the leaf cells (ARTIS, MISCHÉ & DHILLON, 1985), an important terminal event (NOODÉN, 1988c). During flowering, flower heads and fruits act as sinks for photosynthetic products. The control and defruited plants had significantly higher photosynthetic and transpiration rates than deflowered plants 15 and 45 days after treatment. Low photosynthesis and transpiration in deflowered plants at 45 days after treatment coincided with low stomatal conductance. Low stomatal conductance in deflowered plants as compared with defruited ones at 45 and 75 days after treatment suggest that it was influential in, or was a cause of low photosynthesis and low transpiration in this treatment. In contrast, the control plants had a higher stomatal conductance than deflowered ones at 75 days after treatment, yet photosynthesis and transpiration rates were the same in both treatments. Thus, stomatal conductance alone does not successfully explain differences in photosynthesis and transpiration in these treatments. It is possible that accelerated ageing in the control plants was influential in lowering photosynthesis and transpiration 75 days after treatment.

In the second pair of leaves, control and deflowered plants did not differ in terms of photosynthesis at low, medium and high light. The dry weights of mature ripe fruits above the first pair of leaves were  $0.25 \pm 0.03$  g at low light;  $0.28 \pm 0.05$  g at medium light; and  $0.43 \pm 0.09$  g at high light. Thus, a decline in chlorophyll content in the first leaf rather than in the second leaf from the head, at high light intensity, coincided with a high weight of mature ripe fruit. This suggests that high light speeded the process of maturation of the fruits which were then influential in the senescence of the first leaf. A lack of correlation between photosynthesis and dry weight of mature ripe fruits at high light in the control plants, suggests that photosynthesis is not a good index for

monitoring the influence of fruits on senescence, especially if plants are grown under reduced light where there is less chlorophyll loss.

The results of both pot and field experiments have shown that fruits accelerate senescence. Removing these organs retards chlorophyll loss. Fruits and flower heads are attached to the receptacle. Thus, substances which move from the fruits to the nearest leaf will move via the receptacle. In *Bidens pilosa*, senescence of the leaf nearest to the fruit was preceded by the drying of the receptacle and the peduncle. Senescence of the leaves nearest to the flower heads suggests the presence of a senescence factor which moved from the fruit and flower heads to the nearest leaf. Plant extracts contain growth regulators, some of which may induce senescence. These aspects are investigated in Chapter 3.

## CHAPTER THREE

### 3. SENESCENCE ACTIVITY OF PLANT EXTRACTS MADE FROM THE RECEPTACLES AND LEAVES OF *BIDENS PILOSA*

#### 3.1. INTRODUCTION

Monocarpic senescence which follows the reproductive phase of plants, is one of the most dramatic and complex forms of senescence (NOODÉN, GUIAMÉT & JOHN, 1997). During senescence, the colour change is due both to preferential degradation of chlorophylls compared with carotenoids, and to synthesis of new compounds such as anthocyanins and phenolics (MATILE, 1992). Senescence might be triggered to occur at a time of the year when the environment is becoming limiting due to factors such as cold, drought or competition from other plants (LEOPOLD, 1961). Monocarpic plants escape the effects of seasonal adversity by undergoing complete senescence, leaving resistant structures such as fruits and seeds to survive stressful conditions (LARCHER, 1973). Senescence seems to be imposed on the chloroplast by the nucleus (NOODÉN, 1988). Leaf senescence requires viable cells and new gene expression (SMART, 1994).

Sugar accumulation in the leaves suppresses the expression of some genes related to photosynthesis (JANG, LEON, ZHOU, & SHEEN, 1997; GAN & AMASINO, 1997). According to GRBIC and BLEECKER (1993), ethylene hastens the progress of senescence by activating senescence-associated genes while suppressing photosynthesis associated genes. The use of an anti-senescence gene to produce tomato plants in which the synthesis of ethylene-forming enzymes is inhibited has been

reported by other workers (PICTON, BARTON, BOUZAYEN, HAMILTON, GRIERSON, 1993). In their plants, the onset of visible yellowing was delayed by one week. According to THIMANN (1980), the production of ethylene can be stimulated by auxin, provided its concentration is slightly above the physiological level. Light seems to play an important role in senescence. In the genus *Craterostigma*, light and dehydration of the leaves trigger the expression of desiccation-induced genes. These genes encode either chloroplastic (dsp 22, dsp 34, dsp 21) or cytosolic (dsp 16, dsp 15) proteins (ALAMILLO & BARTELS, 1966; BARTELS, HANKE, SCHNEIDER, MICHAEL, SALAMINI, 1992; SCHNEIDER, WELLS, SCHMELZER, SALAMINI & BARTELS, 1993). The expression of many of these genes is also induced by exogenous ABA treatment in the presence of light (ALAMILLO & BARTELS, 1996). In *Craterostigma*, desiccation leads to the accumulation of specific gene products and high concentrations of sucrose (BARTELS, SCHNEIDER, TERSTAPEN, PIATKOWSKI & SALAMINI, 1990; PIATKOWSKI SCHNEIDER, SALAMINI & BARTELS, 1990).

Recently, we have shown that deflowering of *Bidens pilosa* retards senescence (Chapter 2). Several workers have hypothesised that a senescence signal is produced by fruit and seeds in monocarpic plants (NOODÉN 1988 and references therein). If a senescence factor is produced by seeds of *Bidens pilosa*, it would pass via the receptacle on its way to the nearest leaf. The objective of this study was to test whether extracts made from receptacles of *Bidens pilosa* L. could induce leaf senescence in monocarpic plants. During the course of the study, more receptacles were needed for preparation of plant extracts. Due to the small size of the receptacles, it became difficult to obtain adequate material for preparation of plant extracts. Thus, leaves were included in the assays.

## 3.2. MATERIALS AND METHODS

### 3.2.1. Plant material

Seeds of *Phaseolus vulgaris* L., *Rumex obtusifolius* L. and *Bidens pilosa* L. were germinated in 10 cm pots in the nursery. *Rumex obtusifolius* seedlings were transplanted in the garden. The experiment was carried out at Kwadlangezwa (28° 51'S, 31°51'E) where the climate is subtropical.

#### 3.2.1.1. *Bidens pilosa* bioassay

In this section, assays were done on cuttings, pot-grown plants and on leaf discs of *Bidens pilosa*.

##### Leaf disc experiment

Leaf discs (1cm<sup>2</sup>) were punched from the 3rd pair of leaves from the flower heads. Crude water extracts were made from non-senescent and senescent receptacles of *Bidens pilosa* (Figure 2.17C & Figure 2.17D respectively) . Fruits were removed from the receptacles. In each case, 4.5 grams dry weight samples were crushed and extracted with 5 ml distilled water. Extracts used in leaf disc experiments were diluted to 50 ml. Leaf discs were placed in petri-dishes lined with filter paper. The petri-dishes which were moistened with plant extracts were kept in the growth room at 25°C (adapted from WHYTE & LUCKWILL, 1966). Distilled water was used as control. There were five observations per treatment.

### Pot-grown plants and cuttings experiment

Cuttings and pot-grown plants were deflowered and defruited below the first pair of leaves from the flower heads (Chapter 2). Crude water extracts from senescent and non-senescent receptacles of *Bidens pilosa* were prepared as described earlier. The filtrates were mixed with 5 g anhydrous lanolin (adapted from TAMAS, ENGELS, KAPLAN, OZBUN & WALLACE, 1981). From the mixture of lanolin and extracts, 0.04 g was applied to the cut end of the shoot, defruited receptacle and to the tips and margins of leaves. Cuttings and pot-grown plants which were treated with a mixture of lanolin and plant extracts were kept under a shade cloth (maximum temperature 30°C). Lanolin mixed with water was used as a control. The treatment was applied to the third pair of leaves. There were five observations per treatment.

#### 3.2.1.2. Bean bioassay

This bioassay was necessary to replace the one for *Bidens pilosa* because *Bidens pilosa* needed 3 months of growth before being ready for use, whereas *Phaseolus vulgaris* needed one month. The receptacles for preparation of plant extracts were selected as described earlier (**Figure 2.17C & Figure 2.17D**). Non-senescent leaf extracts were prepared using green leaves (**Figure 2.17A**). Leaves greater than half yellow were used to prepare senescent leaf extracts (LINDOO & NOODÉN, 1976). Bean leaves which were treated with lanolin mixed with water (controls) senesced at the same time as the ones treated with lanolin mixed with plant extracts. Because bean leaves were sensitive to lanolin, experiments were conducted with extracts made from the receptacles and leaves of *Bidens pilosa* which were not mixed with lanolin. Two assays were done in this section namely cuttings and pot-grown plants. In both assays,

there were 8 observations per treatment.

#### Bean cuttings experiment

Plants with two pairs of trifoliate leaves were cut at soil level, sterilised with 1% sodium hypochlorite for 1 min and placed in distilled water in the growth room for 24 h as pretreatment. The upper leaf and primary leaves were removed from each plant. Two drops of 0.05% Tween20 and 4 drops of the plant extract were placed on the abaxial side of both opposite leaflets on one side of the midrib. The mixture was then spread on the leaflets (adapted from LINDOO & NOODÉN, 1978). Plants were kept in distilled water in the growth room (continuous low light, 25°C) until senescence commenced. Distilled water mixed with 0.05% Tween20 was used as a control. After 3 days a fresh cut was made at the base of the cutting and any roots which had grown out were removed (LEOPOLD & KAWASE, 1964).

#### Pot-grown bean experiment

Plants with two pairs of trifoliate leaves were used in this assay. The upper leaf and primary leaves were removed from each plant. The leaves were treated as in cutting's bioassay described earlier. The treated plants were kept in the green house where they received solar radiation. The maximum temperature was 40°C. Senescence was observed on the whole leaflet.

#### 3.2.1.3. *Rumex* bioassay

Leaves of bean cuttings which were treated with plant extracts and kept in the dark senesced at the same time as the controls. *Rumex* assay was thus included in order to investigate the effect of plants extracts on senescence under dark conditions. The

broad leaves of *Rumex* provided a more uniform material for the bioassay, as there were many Fractions to be tested for senescence activity. *Rumex* leaves were harvested from plants grown in the garden. The leaves were kept in the dark for 24 h as a pretreatment. Leaf discs (1 cm<sup>2</sup>) were removed on either sides of the midrib in the middle of a leaf. Leaf discs were punched from older leaves (4<sup>th</sup> or 5<sup>th</sup> from the apex). The number of discs per treatment was equivalent to 0.1 g fresh weight. Leaf discs were placed in small bottles with their abaxial sides in contact with the filter papers lining the bottles. Into each bottle, 0.5 ml extract made from the receptacles and leaves of *Bidens pilosa* was mixed with 0.1 ml of 0.05% Tween20 dissolved in water. Distilled water and Tween20 were used as control. Each bottle received discs from seven leaves. Bottles were incubated in the dark for 7 days at 25°C (adapted from WHYTE & LUCKWILL, 1966). Other bottles were illuminated for 3 days prior to dark incubation. There were 6 bottles per treatment.

#### 3.2.1.4. Chlorophyll analysis

Leaf discs (1 cm<sup>2</sup>) were weighed accurately to obtain 0.1 g fresh weight. Leaf samples were homogenised in a mortar and pestle and extracted overnight in 5 ml N,N-dimethylformamide. Chlorophyll content was determined as described earlier (Chapter 2).

#### 3.2.2. Crude ethanolic and water extracts

Ethanolic and water extracts were prepared by mixing 4.5 g crushed senescent receptacles with 250 ml 80% ethanol or water respectively overnight. Ethanolic and water filtrates concentrated to 10 ml, were subjected to a standard serial dilution (10<sup>-1</sup>

to  $10^{-5}$ ).

### 3.2.3. Paper chromatography

Ethanollic and water filtrates obtained from senescent receptacles of *Bidens pilosa* were concentrated to 5 ml. In each case, 1 ml was strip-loaded as a 10 mm wide band onto a sheet of Whatman No.1 chromatography paper for descending chromatographic separation. The 60 ml of solvent poured into the trough consisted of isopropanol : 25% ammonium hydroxide : distilled water in the ratio 10:1:1 (v/v). After the  $R_f$ s were cut, ethanolic extracts were eluted with methanol while water extracts were eluted with water. The eluants were dried and resuspended with 5 ml water. A standard serial dilution was done to obtain lower concentrations.

### 3.2.4. Column chromatography

Ethanolic extracts were loaded onto a 3.5 x 17.0 cm Sephadex LH-20 column and eluted with varying proportions of ethyl acetate and methanol (**Figure 3.1A**). Each fraction was resuspended with 10 ml  $H_2O$  and tested for activity using the bean bioassay. Fractions were subjected to a serial dilution.

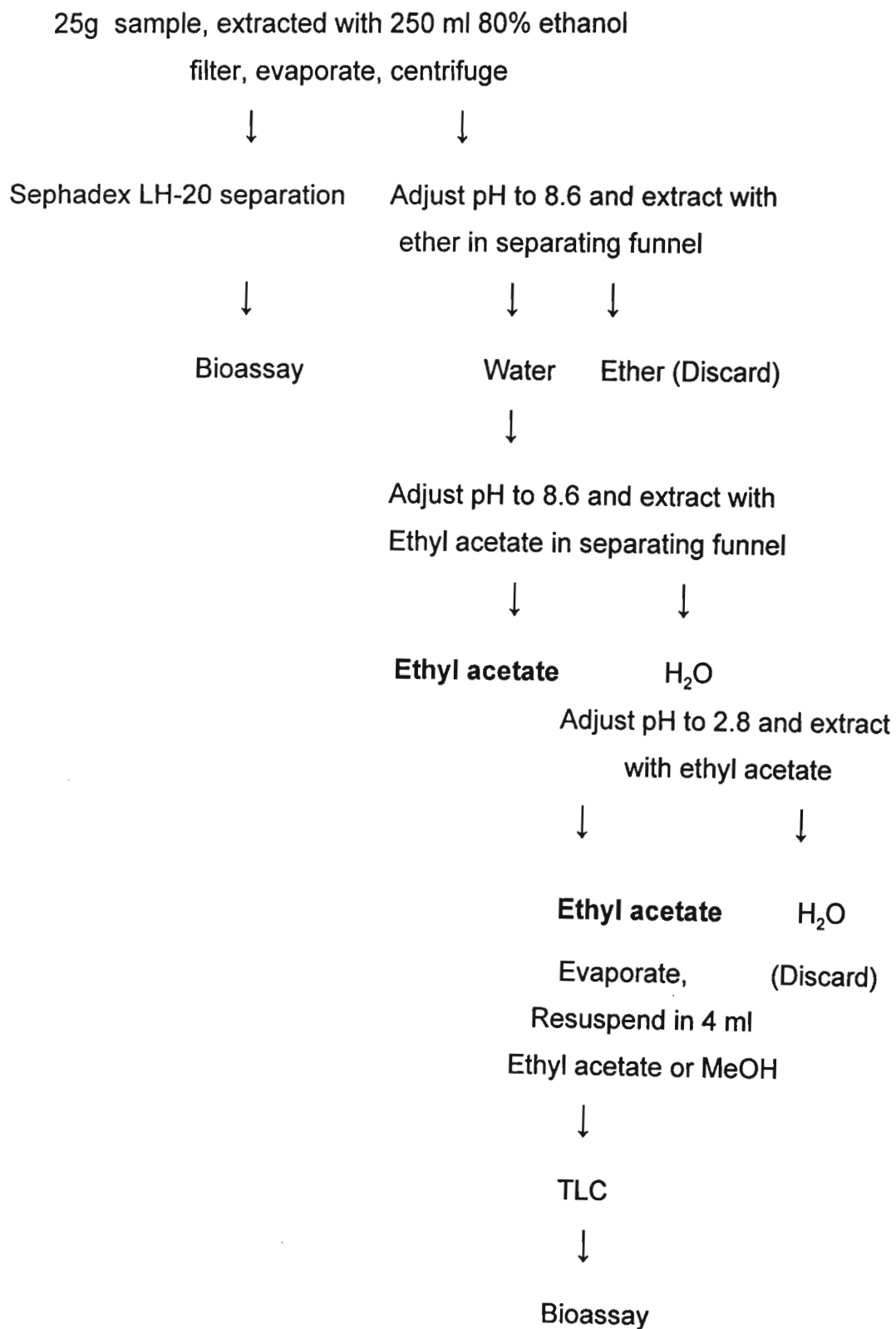
Fractions	Solvent mixtures	
	Ethyl acetate : Methanol	
A	100	: 0
B	95	: 5
C	90	: 10
D	85	: 15
E	80	: 20
F	75	: 25
G	70	: 30
H	65	: 35
I	60	: 40
J	55	: 45
K	50	: 50
L	0	: 100
M	Ethyl acetate : isopropanol : water : acetic acid (52:28 :28:4 v/v)	
N	Ethyl acetate : Methanol (0:100)	

**Figure 3.1A:** Solvent systems for eluting leaf and receptacle extracts of *Bidens pilosa* through column chromatography.

### 3.2.5. Thin layer chromatography

The pH of the extract was first adjusted to pH 8.6 using 1% sodium hydroxide. The extract was then partitioned three times against petroleum ether using 50 ml of solvent, followed by ethyl acetate in a separating funnel. The pH of the aqueous phase was then adjusted to pH 2.8 with HCl, and partitioned against ethyl acetate. The acidic ethyl acetate fraction was purified via TLC using a mixture of benzene : ethyl acetate : acetic acid (50:5:2). Plant extracts (750  $\mu$ L) were strip-loaded onto a 20 x 20 cm Merck UV

fluorescent 60F<sub>254</sub> silica gel TLC plate using a pasteur pipette. Four standards viz ABA, IAA, GA<sub>3</sub> and JA were run together with the extracts. The procedure of SHINDY and SMITH (1975), presented in **Figure 3.1B**, was used. The silica gel associated with the R<sub>f</sub>s were scraped from the plates, eluted with 100 ml ethyl acetate or methanol which were later evaporated. Some TLC plates were stained using p-anisaldehyde. Each R<sub>f</sub> was resuspended with 5 ml methanol or ethyl acetate, from which 1 ml was poured into an empty bottle, dried, resuspended with 5 ml water and tested for activity. To obtain lower concentrations, a standard serial dilution was done.



**Figure 3.1B:** Procedure for analysis of leaf and receptacle extracts of *Bidens pilosa*.

### 3.3. RESULTS

#### 3.3.1 Effect of water extracts made from non-senescent and senescent receptacles of *Bidens pilosa* on its senescence

Water extracts from non-senescent and from senescent receptacles of *Bidens pilosa* did not induce senescence in *Bidens pilosa* leaf discs in the dark. The control leaf discs had a lower chlorophyll content ( $1.41 \pm 0.05$  mg per  $\text{cm}^2$ ) than both non-senescent ( $2.29 \pm 0.07$  mg per  $\text{cm}^2$ ) and senescent ( $2.18 \pm 0.04$  mg per  $\text{cm}^2$ ) receptacle extracts. Thus, extracts retarded chlorophyll loss in the dark. Under shade cloth conditions, extracts made from senescent receptacles of *Bidens pilosa* induced senescence in *Bidens pilosa* cuttings and in pot-grown plants in the presence of light (**Table 3.1**).

Table 3.1: Effect of crude water extracts made from the receptacles of *Bidens pilosa* on its senescence 26 days after treatment on sunny days. Lanolin on receptacle: lanolin mixed with water and applied to a defruited receptacle and to tips and margins of leaves; Lanolin on shoot: lanolin mixed with water and applied to a deflowered shoot tip and to the margins and tips of leaves; Non-senescent receptacle: lanolin mixed with plant extract made from non-senescent receptacles and applied to a deflowered shoot tip and to margins and tips of leaves; Senescent receptacle: lanolin mixed with plant extract made from senescent receptacles, applied to deflowered shoot tip and to the margins and tips of leaves.

Treatment	Chlorophyll <sub>(a+b)</sub> (mg per cm <sup>2</sup> )	
	Pot-grown	Cuttings
Lanolin on receptacle	1.974 ± 0.094b	1.896 ± 0.135b
Lanolin on shoot	1.924 ± 0.099b	1.840 ± 0.063b
Non-senescent receptacle	1.982 ± 0.088b	1.582 ± 0.033b
Senescent receptacle	1.000 ± 0.142a	1.036 ± 0.190a

Shade cloth conditions did not have artificial light and were not protected from rain. As a result, only experiments which coincided with continuous sunny days gave positive results (Table 3.1). Experiments which coincided with rainy days gave negative results. Thus extracts from non-senescent and from senescent receptacles retarded chlorophyll loss (Table 3.2). Non-senescent receptacle extracts retarded senescence to a greater extent than senescent receptacle extract after 46 days.

At 26 days after treatment, there were no significant differences between the values for lanolin on receptacle and lanolin on shoot (Table 3.1). There was a greater decline in

the value of lanolin on receptacle than that of lanolin on shoot 36 days after treatment (Table 3.2). The treatment 'lanolin on shoot' was deflowered. These results suggest that the receptacle exerted an influence on leaf senescence (Chapter 2).

Table 3.2: Effect of crude water extracts made from the receptacles of *Bidens pilosa* on its senescence on rainy/cloudy days. Lanolin on receptacle: lanolin mixed with water and applied to a defruited receptacle and to tips and margins of leaves; Lanolin on shoot: lanolin mixed with water and applied to a deflowered shoot tip and to the margins and tips of leaves; Non-senescent receptacle: lanolin mixed with plant extract made from non-senescent receptacles and applied to a deflowered shoot tip and to margins and tips of leaves; Senescent receptacle: lanolin mixed with plant extract made from senescent receptacles, applied to deflowered shoot tip and to the margins and tips of leaves.

Treatment/Application	Chlorophyll <sub>(a+b)</sub> (mg per cm <sup>2</sup> )		
	Pot-grown		Cuttings
	Days after treatment		
	36	46	36
Lanolin on receptacle	1.22±0.02a	0.00±0.00a	1.54±0.24a
Lanolin on shoot	1.70±0.01b	0.00±0.00a	1.84±0.22ab
Non-senescent receptacle	1.97±0.11c	1.51±0.04c	1.74±0.15ab
Senescent receptacle	1.81±0.08bc	1.16±0.01b	2.20±0.23b

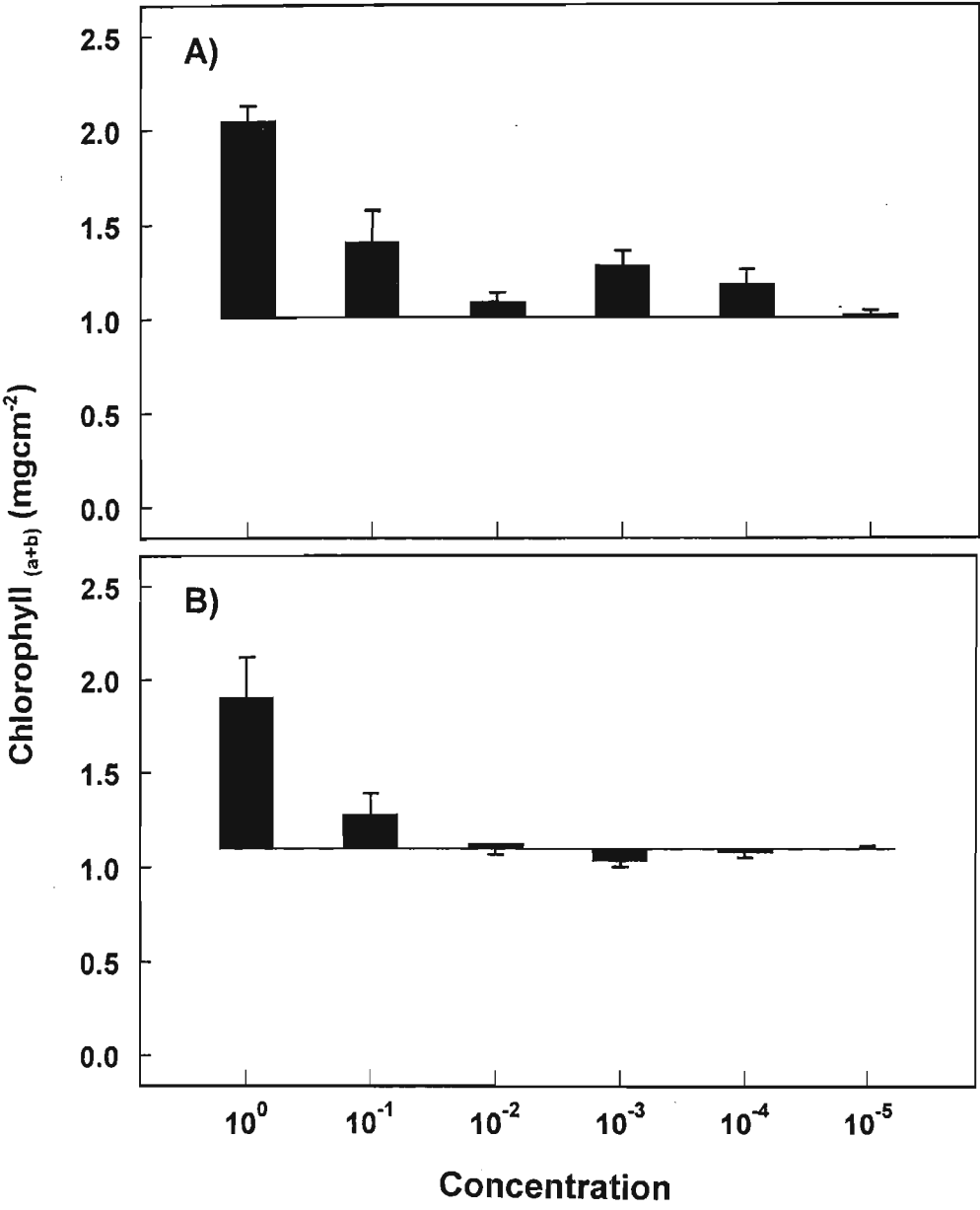
### 3.3.2. Effect of crude ethanolic and water extracts made from senescent receptacles of *Bidens pilosa* on the senescence of *Rumex* leaf discs

Crude ethanolic extracts made from senescent receptacles of *Bidens pilosa* were initially tested on *Rumex* leaf discs (dark) and on pot-grown beans (light). These extracts did not induce senescence under either light or dark conditions, instead, they retarded chlorophyll loss significantly at higher concentrations (**Figure 3.2**). Crude water extracts retarded chlorophyll loss from *Rumex* leaf discs in the dark and induced senescence in pot-grown beans in the light at higher concentrations (**Figure 3.3**).

### 3.3.3. Effect of fractions obtained following paper chromatography of ethanolic and water extracts of senescent receptacles of *Bidens pilosa* on the senescence of *Rumex* leaf discs and pot-grown beans

Extracts made from senescent receptacles were fractionated using paper chromatography to obtain different fractions. The  $R_f$ s from both ethanolic and water extracts did not induce senescence in *Rumex* test in the dark at low or high concentrations (**Figure 3.4A, B, Figure 3.5A, B**). In ethanolic extracts, five Fractions prevented chlorophyll breakdown significantly at higher concentrations (0.1 mg per ml). These were  $R_f$  0.1; 0.2; 0.3; 0.5; 0.9 and 1.0. In water extracts, five Fractions prevented chlorophyll breakdown from *Rumex* leaf discs in the dark significantly. These were  $R_f$  0.1; 0.2; 0.3; 0.4 and 0.9. In the presence of light,  $R_f$  0.1, 0.2 and 0.3 from ethanolic extracts induced senescence of pot-grown beans (**Figure 3.6**). In water extracts, senescence activity of pot-grown beans was detected in  $R_f$  0.1 in the light (**Figure 3.6**).

Figure 3.2: Effect of a crude ethanolic extract made from senescent receptacles of *Bidens pilosa* on senescence of pot-grown beans under light (A); and on *Rumex* leaf discs under dark conditions (B). In this and other graphs, the second X-axis from the bottom represents the value of the control. Plant extract induces senescence: the histogram projects downwards; plant extract retards senescence: histogram projects upwards.



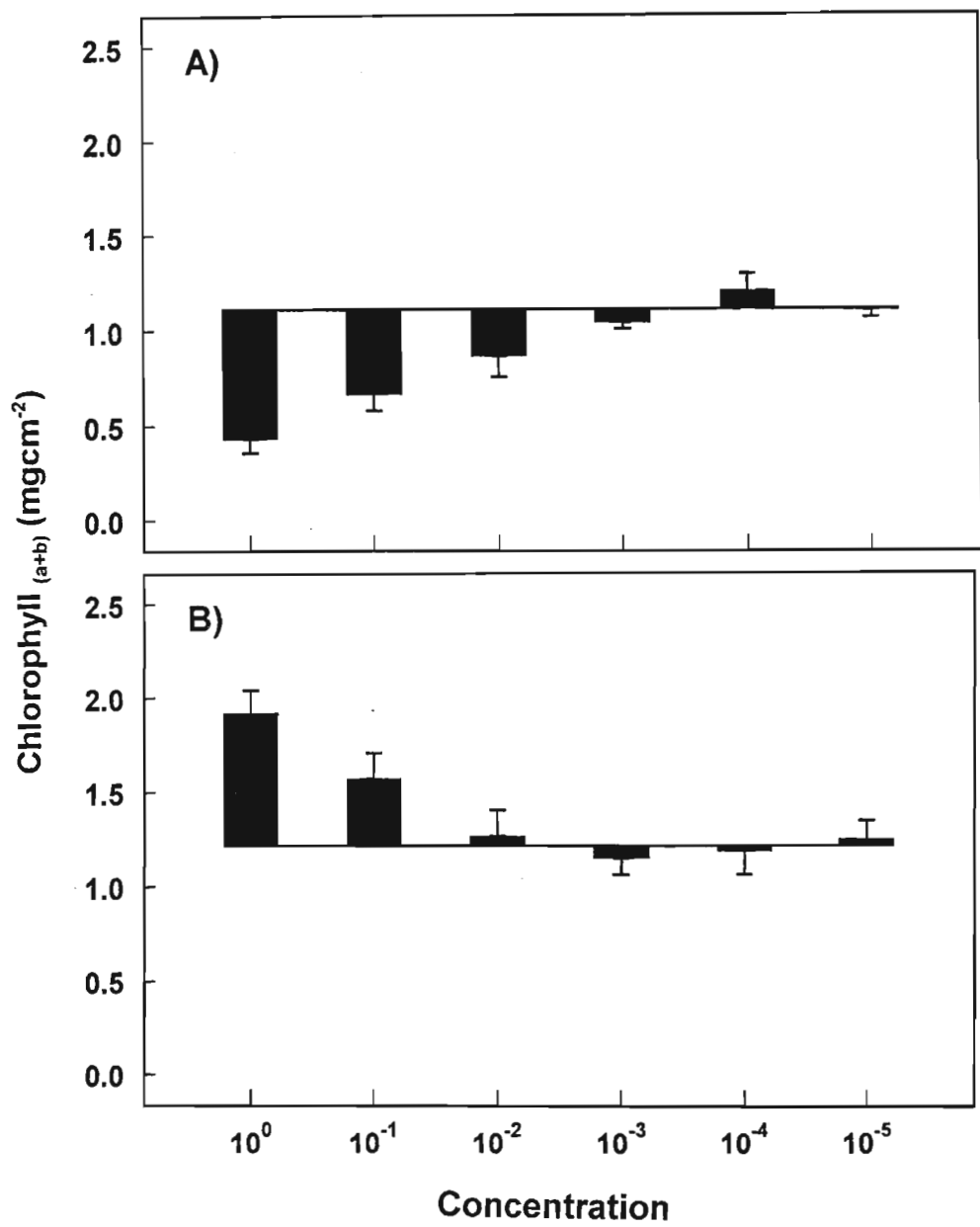


Figure 3.3: Effect of a crude water extract made from senescent receptacles of *Bidens pilosa* on senescence of pot-grown beans under light (A); and on *Rumex* leaf discs under dark conditions (B).

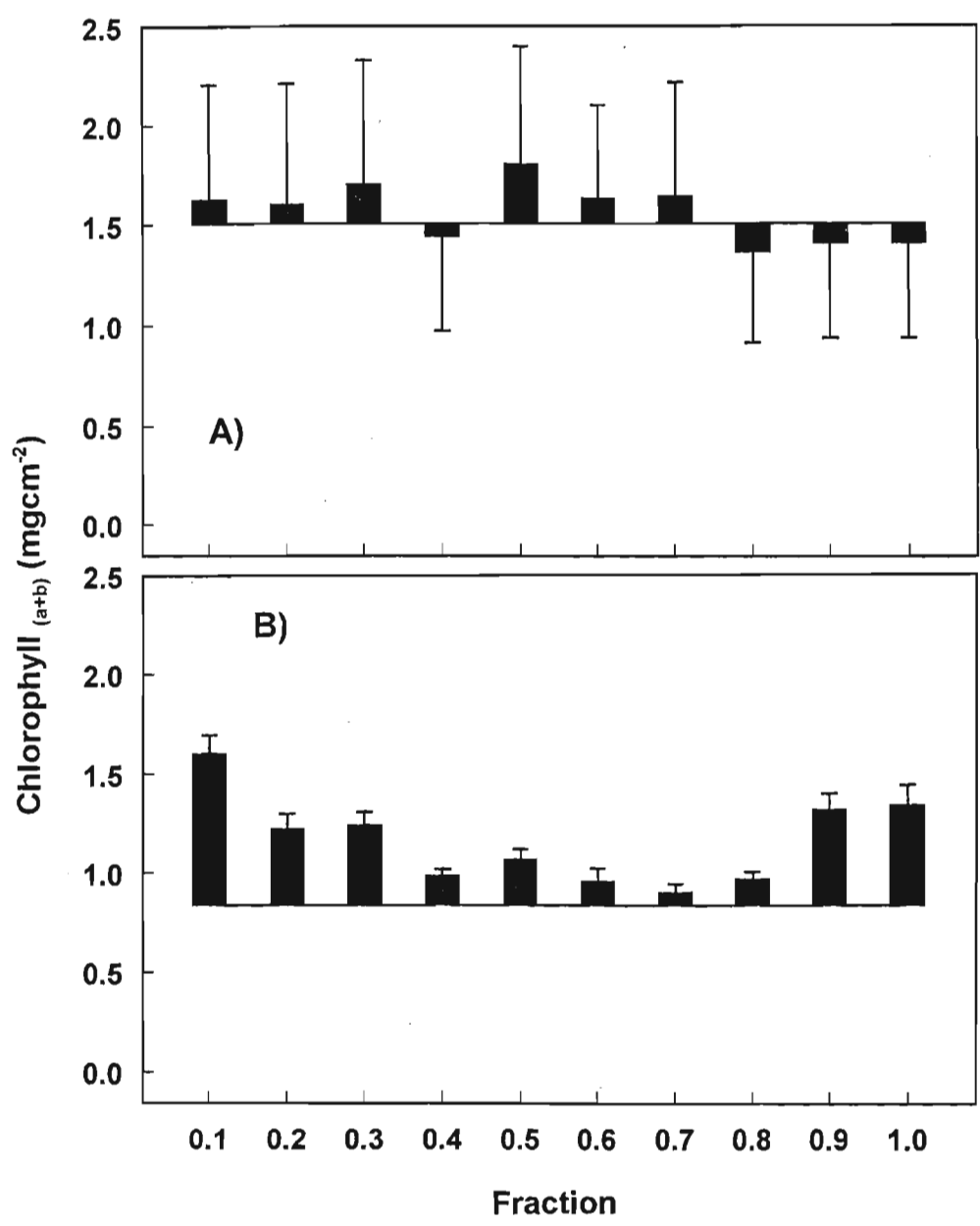


Figure 3.4: Effect of Fractions obtained following paper chromatography of an ethanolic extract of senescent receptacles of *Bidens pilosa* on senescence of *Rumex* leaf discs in the dark at low (A); and at high concentrations (B).

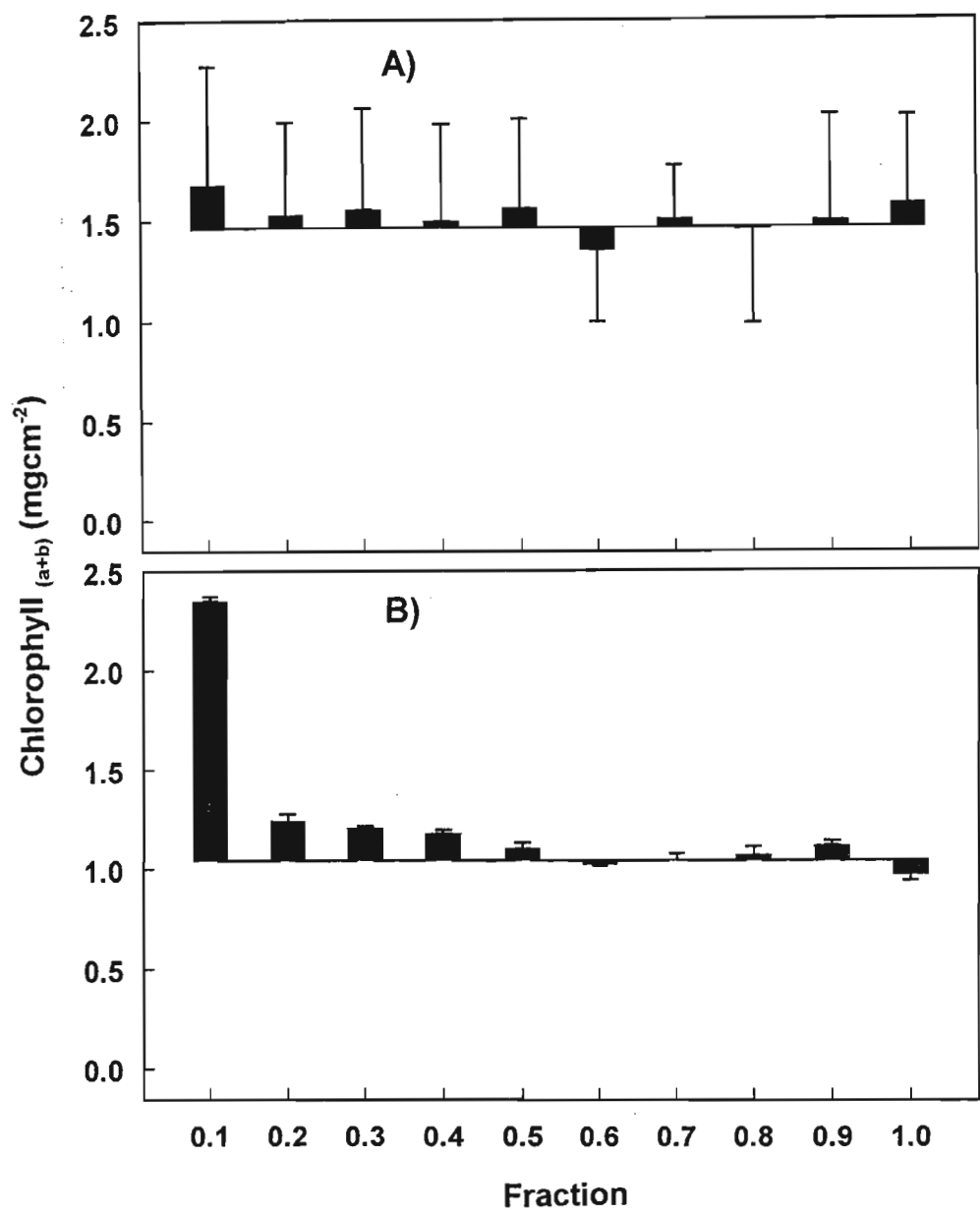


Figure 3.5: Effect of Fractions obtained following paper chromatography of a water extract of senescent receptacles of *Bidens pilosa* on senescence of *Rumex* leaf discs in the dark at low (A); and at high concentrations (B).

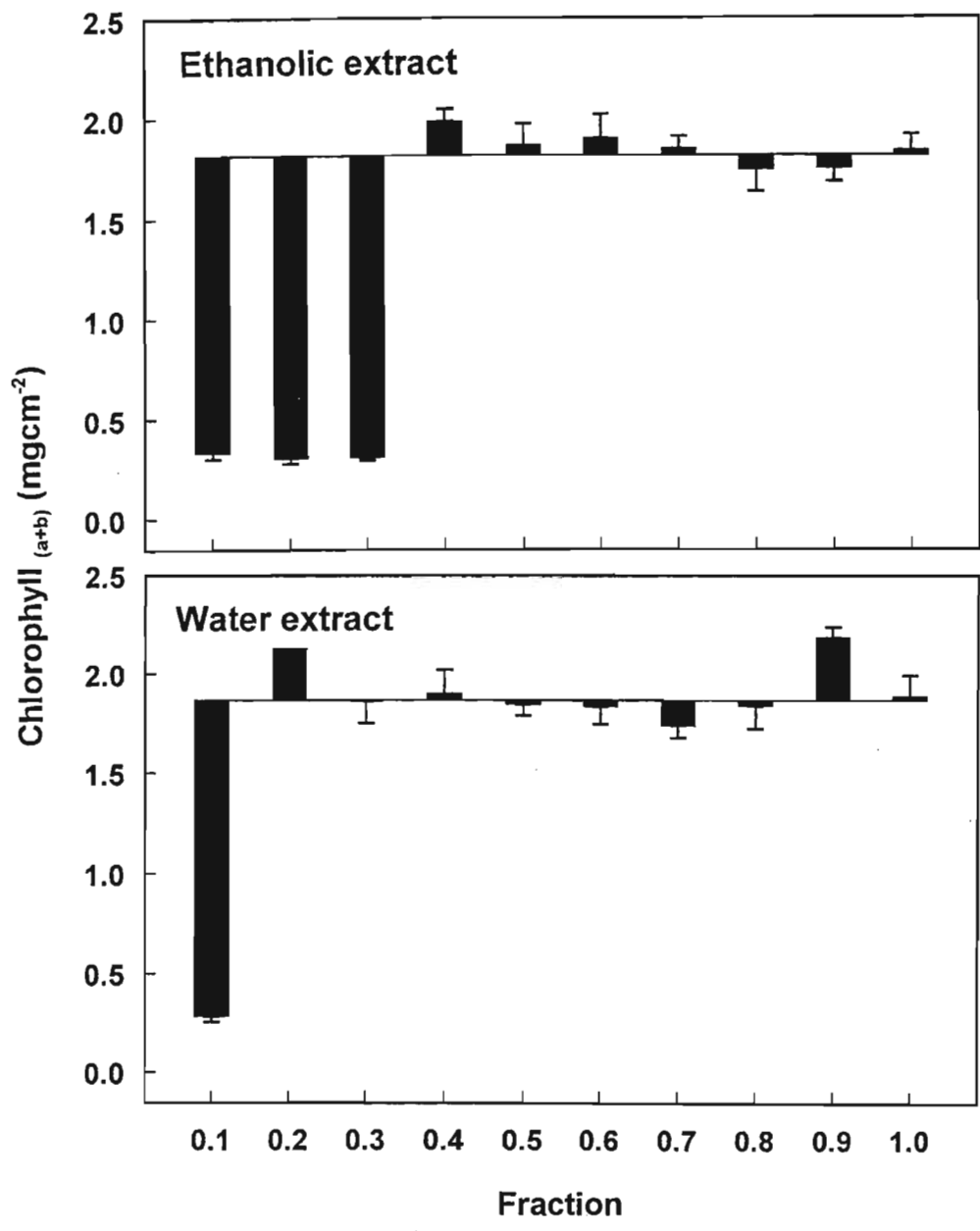


Figure 3.6: Effect of Fractions obtained following paper chromatography of ethanolic and water extracts of senescent receptacles of *Bidens pilosa* on senescence of pot-grown beans under light conditions.

### **3.3.4 Effect of Fractions obtained from ethanolic extracts of senescent receptacles and leaves of *Bidens pilosa* through column chromatography on the senescence of cuttings and pot-grown beans**

Senescent receptacle extracts which were fractionated using column chromatography induced senescence in the leaves of pot-grown beans in light under greenhouse conditions. Activity was detected in Fractions 12 and 13 at a concentration of  $10^0$  (Figure 3.7).

Senescent leaf extracts also induced senescence in the leaves of pot-grown beans in light under greenhouse conditions. At high concentrations ( $10^0$  or 0.1 mg per ml), activity was detected in Fractions 10, 11, 12 and 13 (Figure 3.8). At intermediate concentration ( $10^{-0.25}$ ), activity was detected in Fractions 8, 9, 10, 11, 12 and 13. There was no senescence activity at a concentration of  $10^{-1}$  (0.01mg per ml). Using bean cuttings, under growth room conditions, activity was detected at the highest concentration ( $10^0$ ) in Fractions 1, 7, 12 and 13 in light (Figure 3.9).

### **3.3.5. Effect of Fractions obtained following thin layer chromatography of ethanolic extracts of senescent receptacles, senescent and non-senescent leaves of *Bidens pilosa* on the senescence of *Rumex* leaf discs and bean cuttings**

Ethanolic extracts of senescent receptacles, senescent and non-senescent leaves were purified using TLC to obtain a number of Fractions which were eluted with both methanol and ethyl acetate. In the senescent receptacles extract, Fraction 1 contained the most senescence-inducing activity. Fraction 1 ( $R_f$  0.0-0.1) gave activity at

Figure 3.7: Effect of Fractions obtained from an ethanolic extract of senescent receptacles of *Bidens pilosa* through column chromatography on senescence of pot-grown beans under green-house conditions.

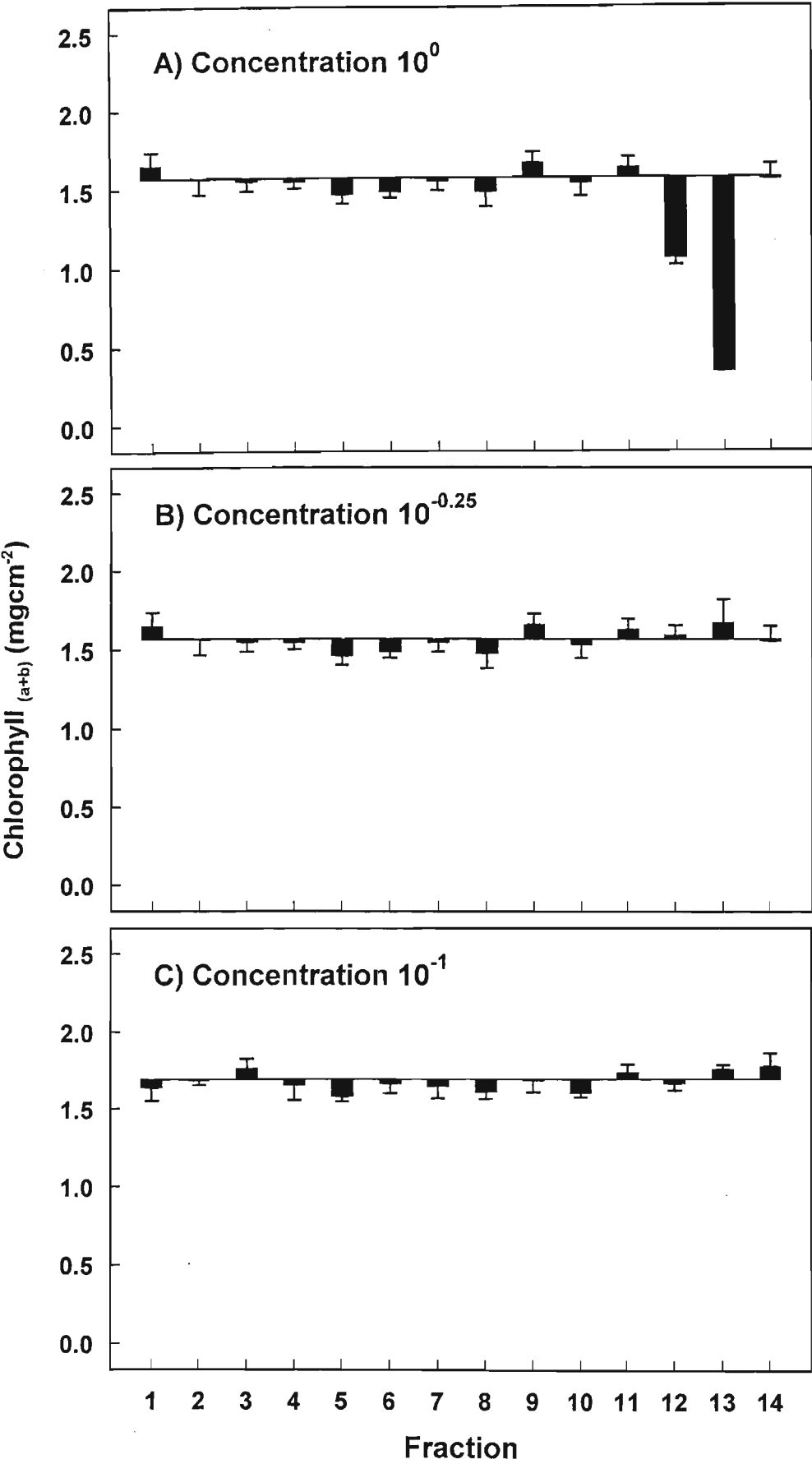


Figure 3.8: Effect of Fractions obtained from an ethanolic extract of senescent leaves of *Bidens pilosa* through column chromatography on senescence of pot-grown beans under green-house conditions.

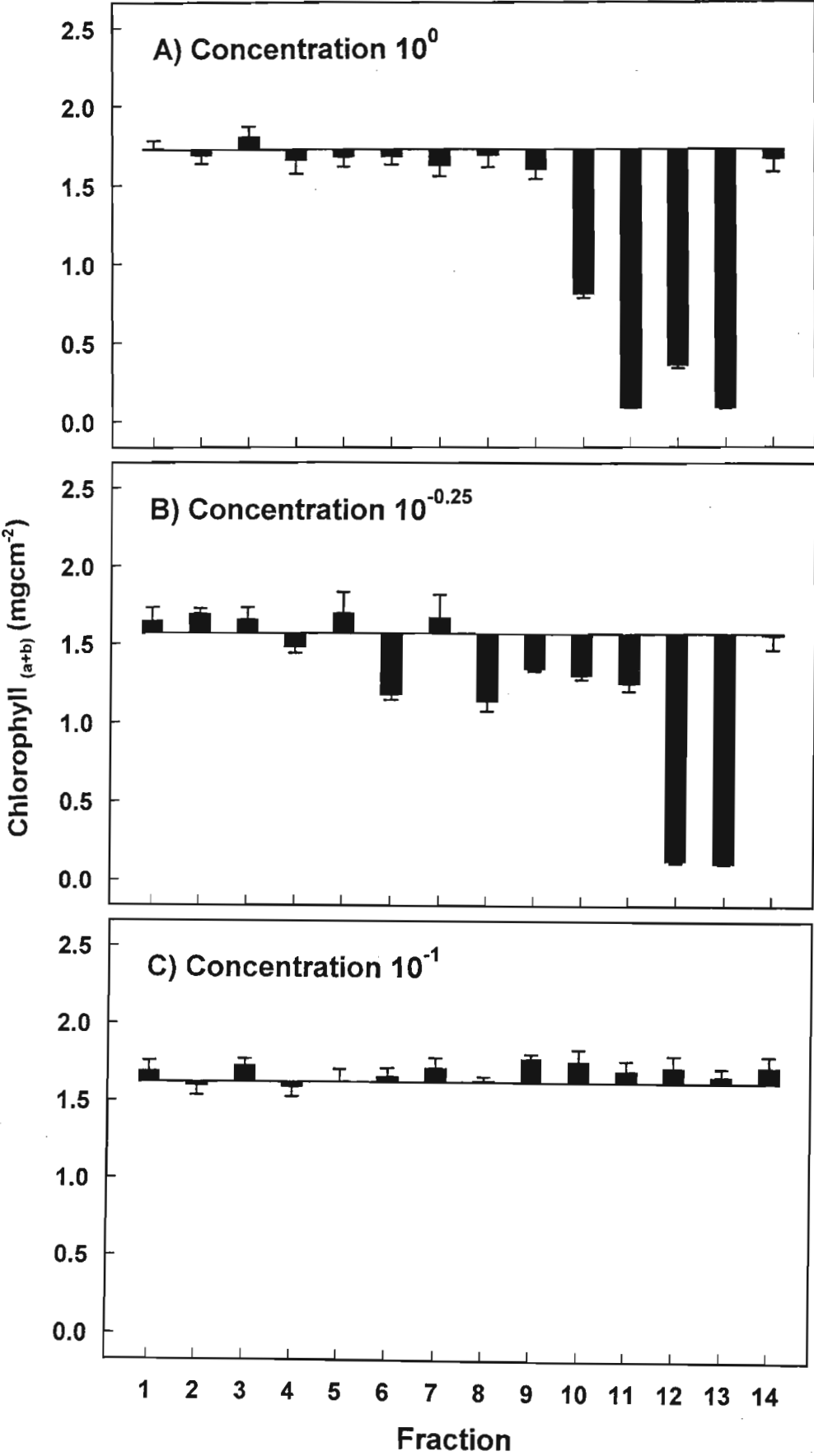
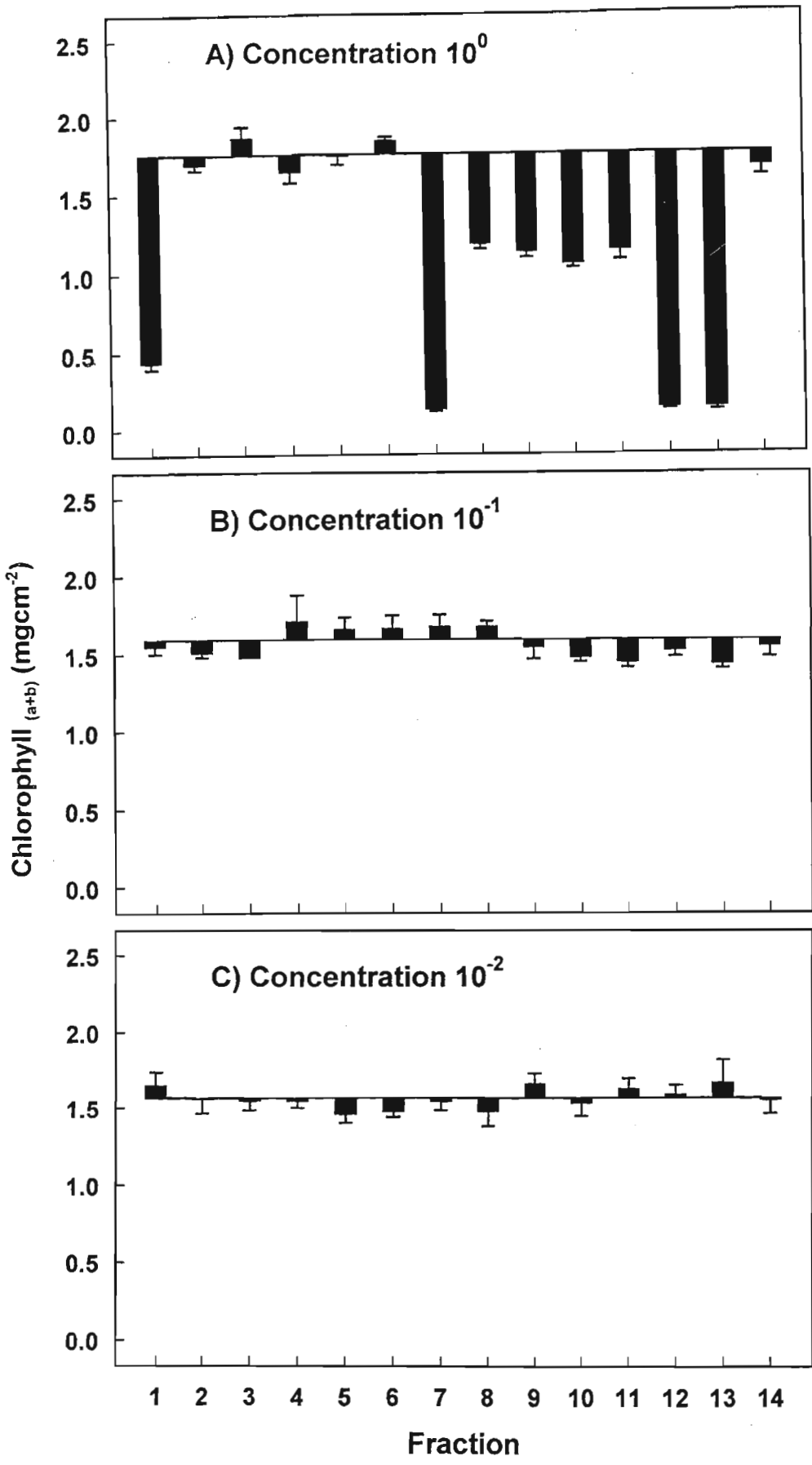


Figure 3.9: Effect of Fractions obtained from an ethanolic extract of senescent leaves of *Bidens pilosa* through column chromatography on senescence of bean cuttings under light in a growth room.



concentrations  $10^0$  (0.1 mg per ml) to  $10^{-2}$  (0.001 mg per ml) under light conditions (**Figure 3.10A, B & C; Figure 3.11**). Fraction 1 was eluted with methanol, dried, re-suspended in water and applied to cutting leaves at different concentrations.

Fraction 1 ( $R_f$  0.0 - 0.1) from senescent leaf extracts also induced senescence of bean cuttings in light. Its activity declined as the concentration decreased from  $10^0$  to  $10^{-2}$  (**Figure 3.12A, B, C & D; Figure 3.13; Figure 3.14**). Fraction 1 was eluted with methanol, dried, re-suspended in water and applied to cutting leaves at different concentrations. Senescence started from the treated spots and progressed towards the entire leaflet.

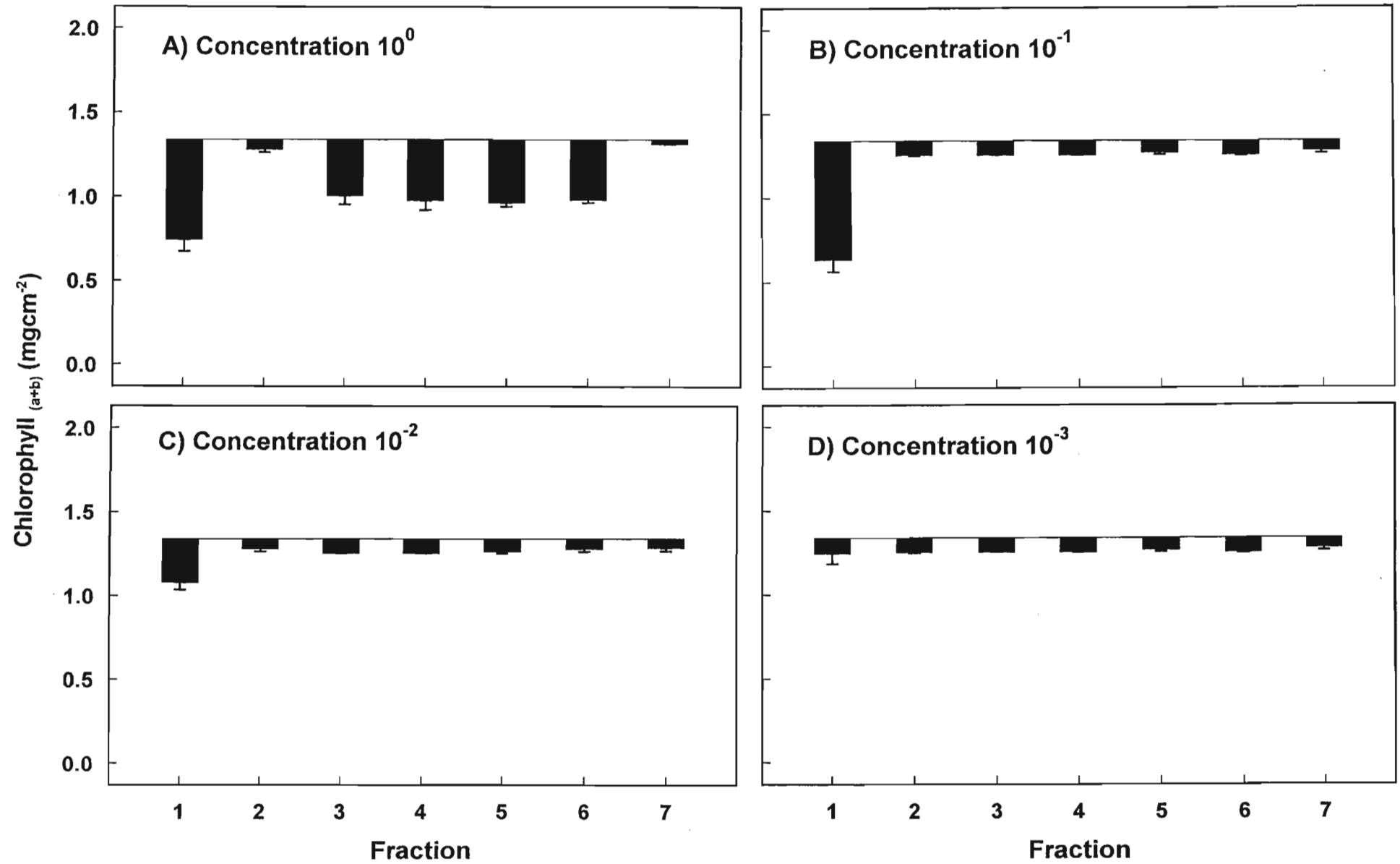
From senescent leaf extracts, Fractions which were eluted with ethyl acetate, dried, re-suspended in water and applied to cutting leaves at different concentrations, did not induce senescence of bean cuttings in light (**Figure 3.15; Figure 3.16**).

Fractions from non-senescent leaf extracts, eluted with ethyl acetate, did not induce senescence in *Rumex* leaf discs in the dark. Fractions 1, 2 and 3 prevented chlorophyll breakdown significantly at a concentration of  $10^0$  (**Figure 3.17**). Fraction 1 ( $R_f$  0.0 - 0.1) from non-senescent leaf extracts, eluted with methanol, dried, re-suspended in water and applied to cutting leaves at different concentrations, induced senescence in light (**Figure 3.18**).

Fractions 4 ( $R_f$  0.25 - 0.35) and 7 ( $R_f$  0.9 - 1.0), eluted with ethyl acetate, dried, re-suspended in water and applied to cutting leaves at different concentrations, showed activity at concentrations around  $10^{-3}$  in light (**Figure 3.19; Figure 3.20**).

All the extracts were tested in the *Rumex* test and the results were negative. Attempts to illuminate the discs for 48 hours prior to dark incubation also failed. *Rumex* test retarded chlorophyll breakdown significantly at higher concentrations. The pattern of the

Figure 3.10: Effect of Fractions obtained following thin layer chromatography of an ethanolic extract of senescent receptacles of *Bidens pilosa* on senescence of bean cuttings under light conditions. Fractions were eluted with methanol.



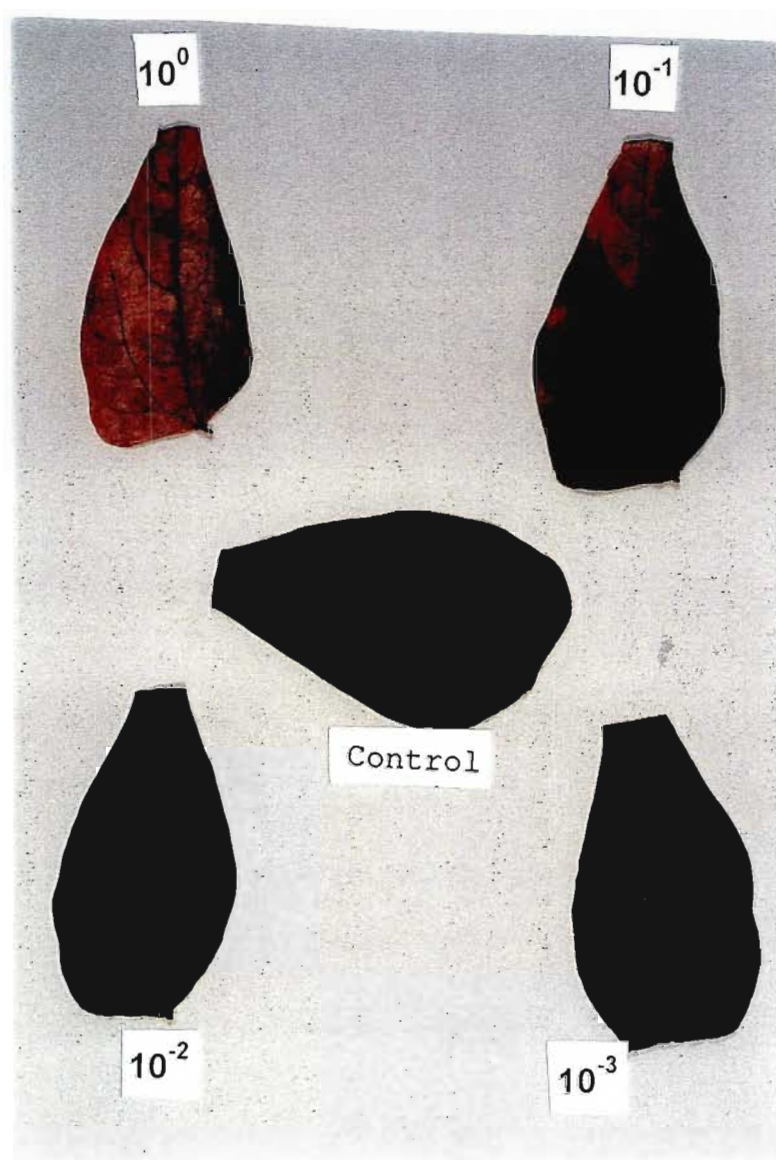
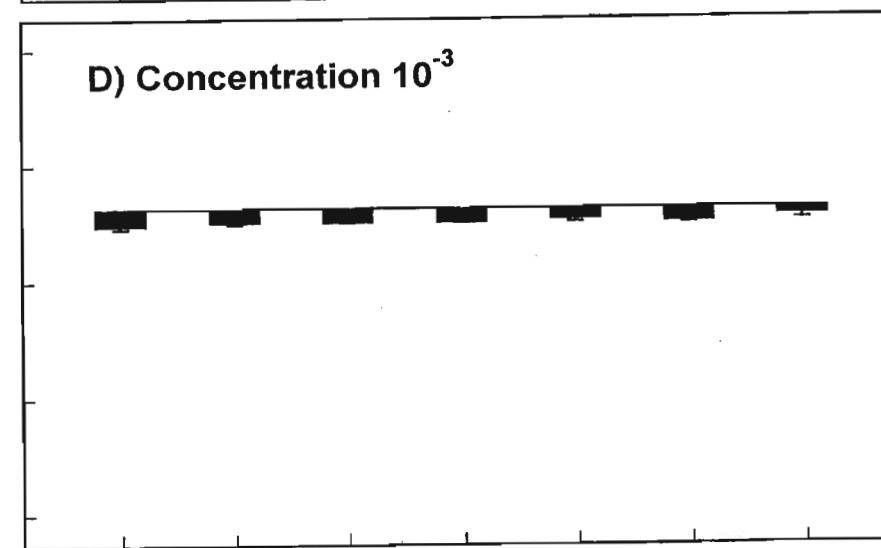
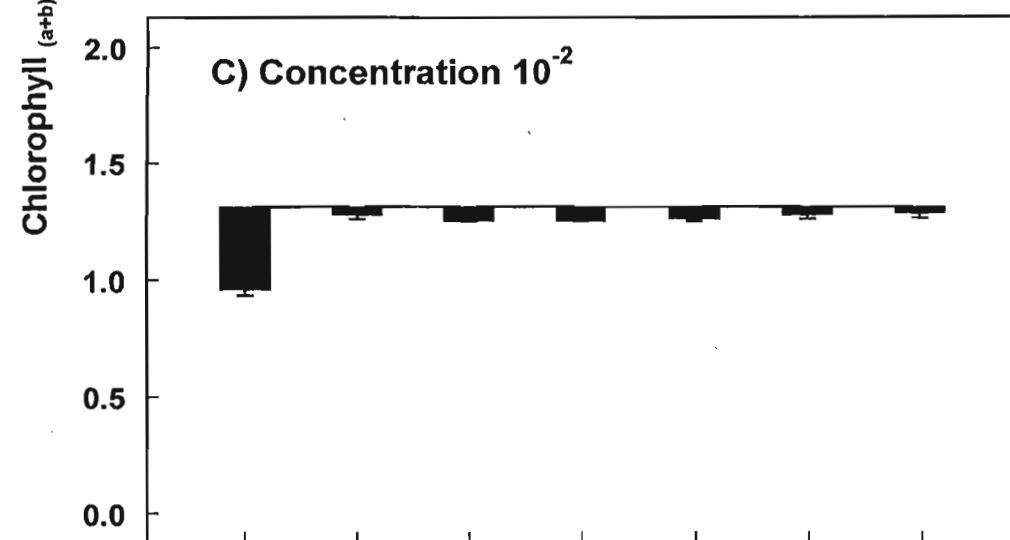
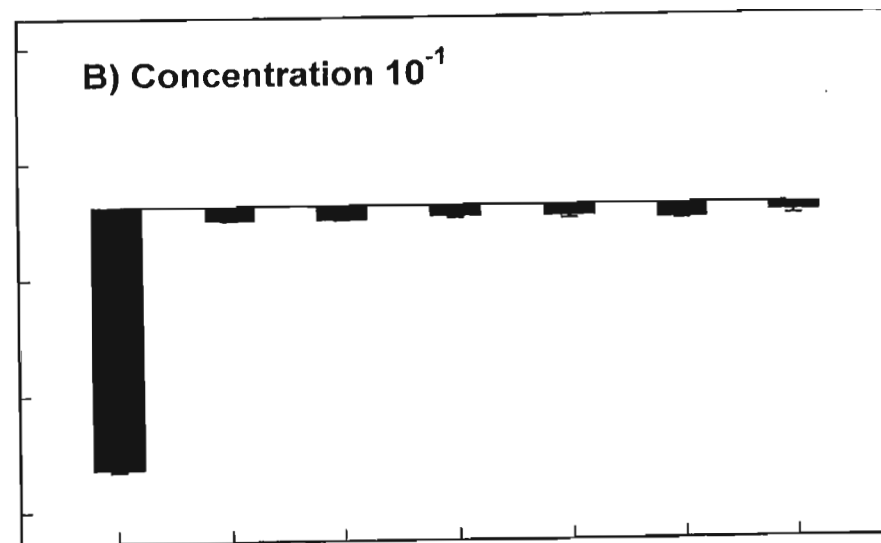
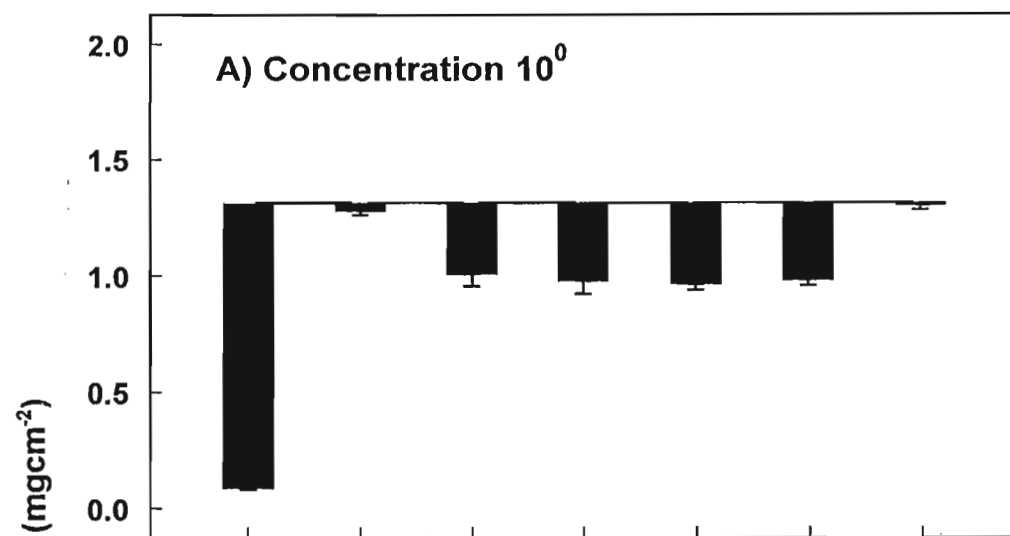


Figure 3.11: Photographs of leaflets of bean cuttings which were treated with various concentrations of Fraction 1 obtained following thin layer chromatography of ethanolic extracts of senescent receptacles of *Bidens pilosa*. Fraction 1 was eluted with methanol.

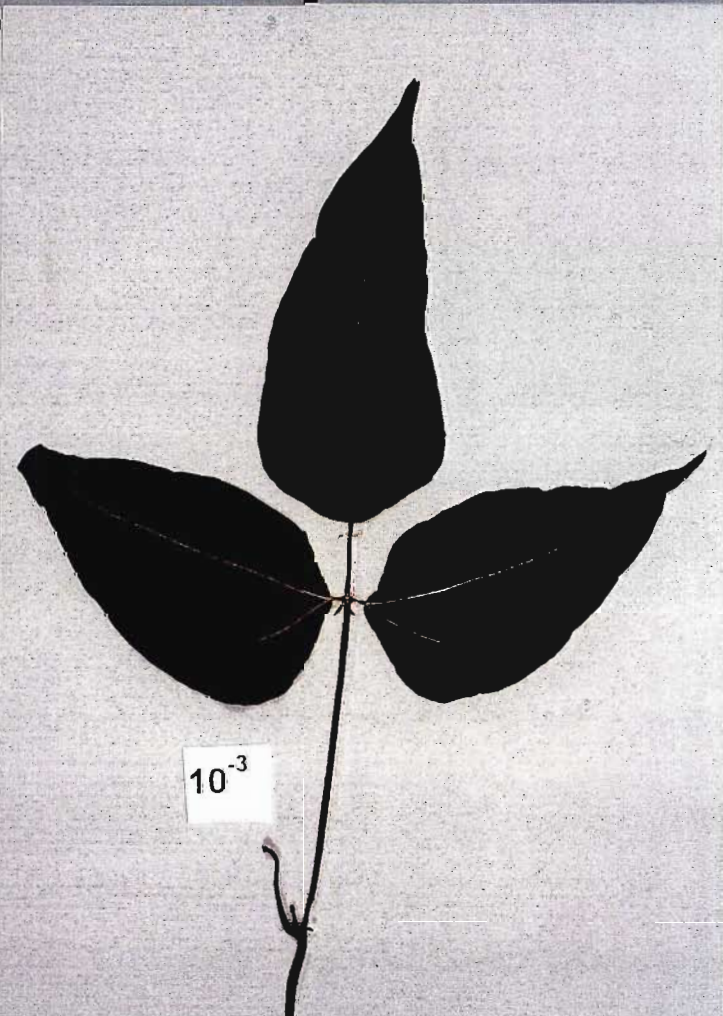
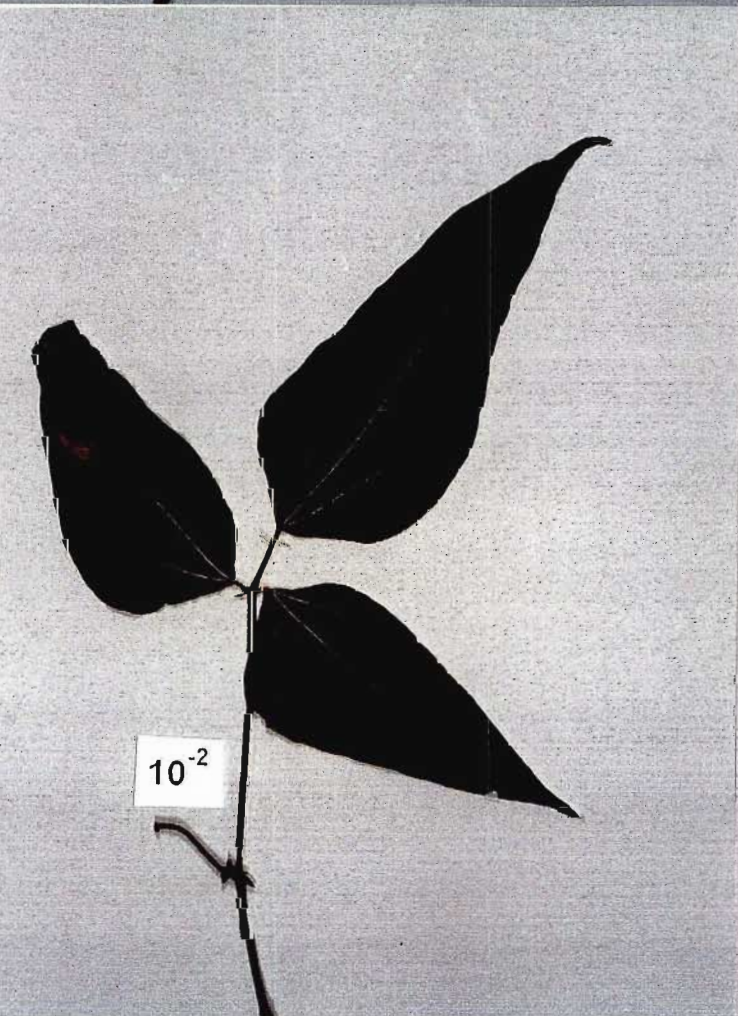
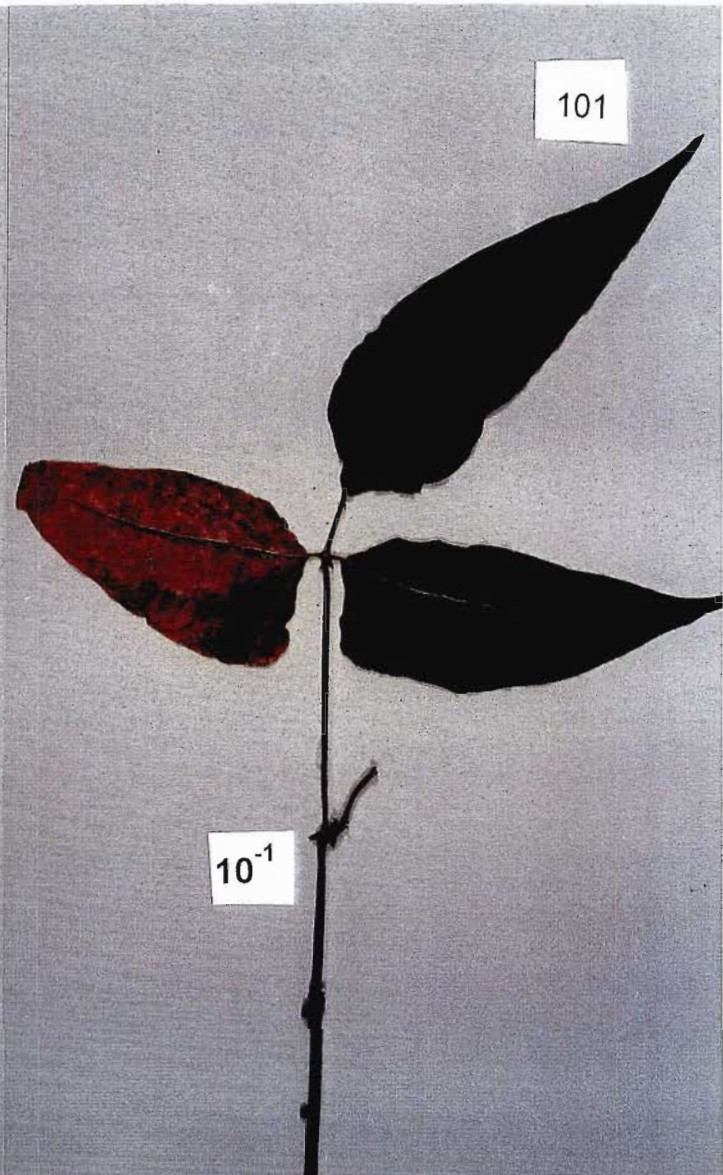
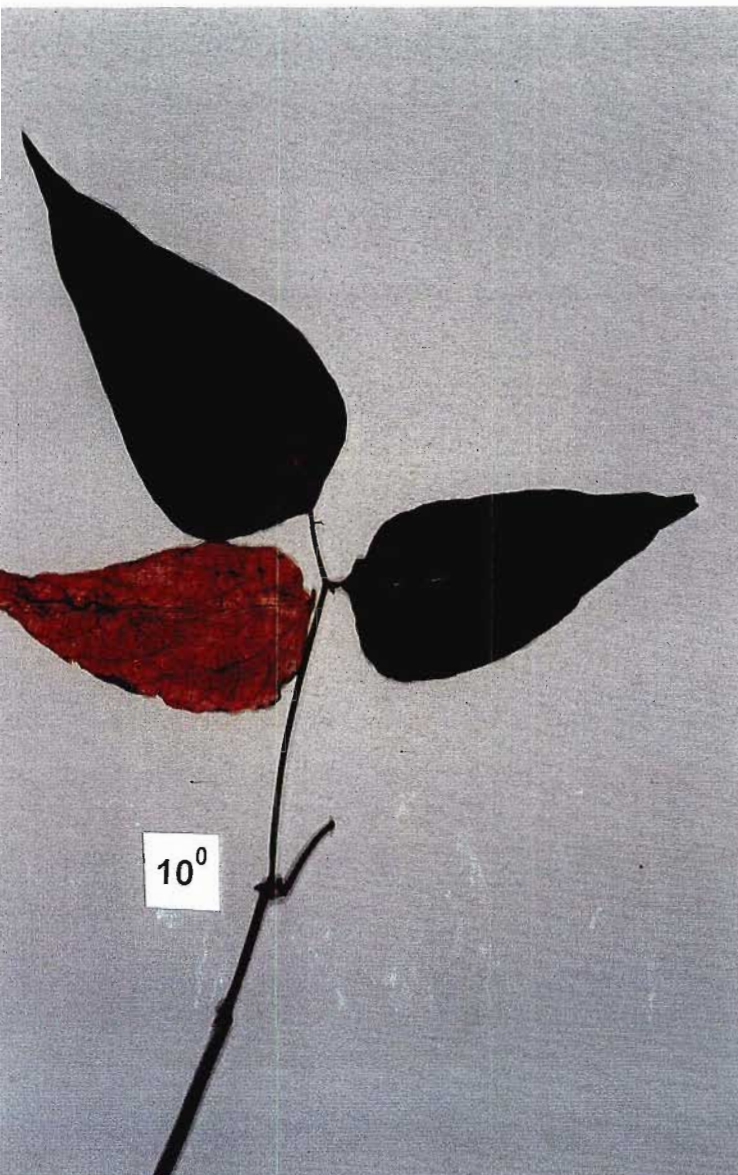
Figure 3.12: Effect of Fractions obtained following thin layer chromatography of an ethanolic extract of senescent leaves of *Bidens pilosa* on senescence of bean cuttings under light conditions. Fractions were eluted with methanol.



Fraction

Fraction

Figure 3.13: Photographs of bean cuttings indicating the positions of the leaflets which were treated with various concentrations of Fraction 1 obtained following thin layer chromatography of ethanolic extracts of senescent leaves of *Bidens pilosa*. Fraction 1 was eluted with methanol.



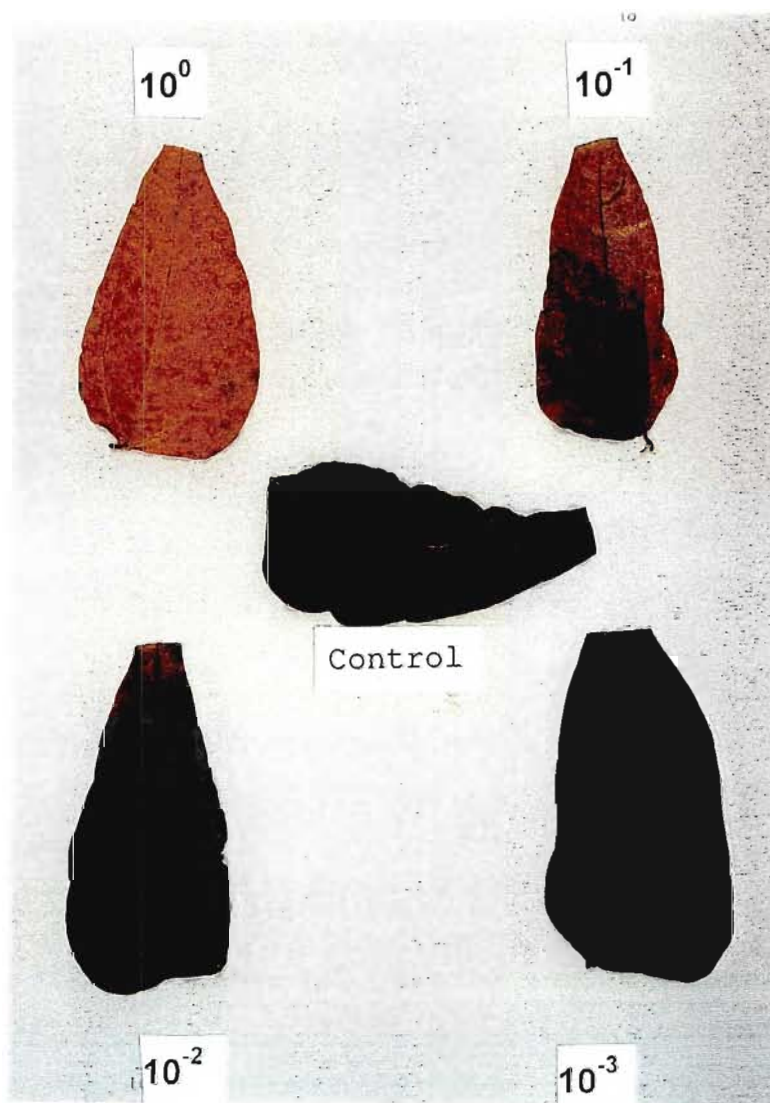


Figure 3.14: Photographs of leaflets of bean cuttings which were treated with various concentrations of Fraction 1 obtained following thin layer chromatography of ethanolic extracts of senescent leaves of *Bidens pilosa*. Fraction 1 was eluted with methanol.

Figure 3.15: Effect of Fractions obtained following thin layer chromatography of an ethanolic extract of senescent leaves of *Bidens pilosa* on senescence of bean cuttings under light conditions. Fractions were eluted with ethyl acetate.

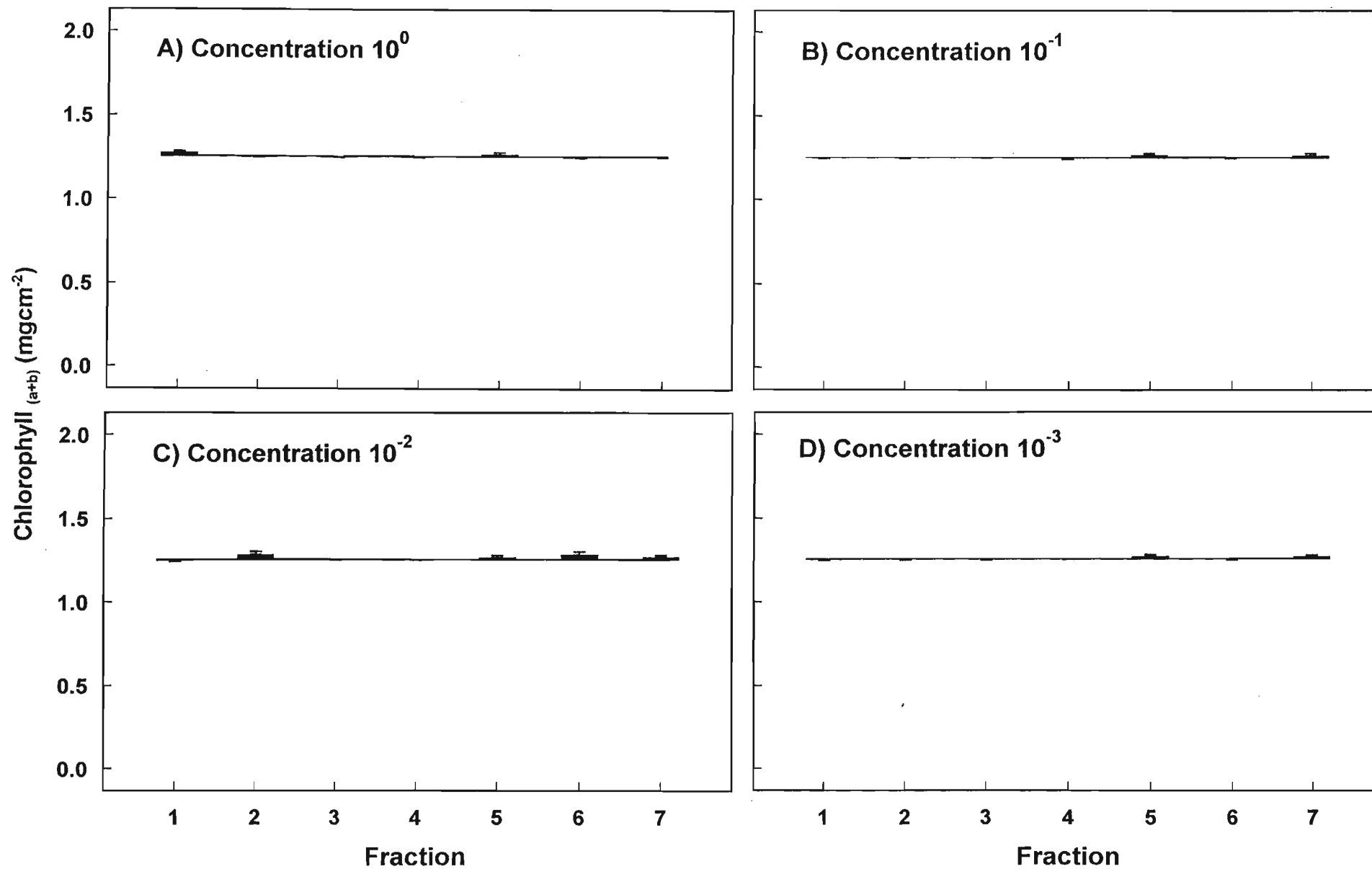


Figure 3.16: Photographs of leaflets of bean cuttings which were treated with various concentrations of Fractions obtained following thin layer chromatography of ethanolic extracts of senescent leaves of *Bidens pilosa*. Fractions were eluted with ethyl acetate.

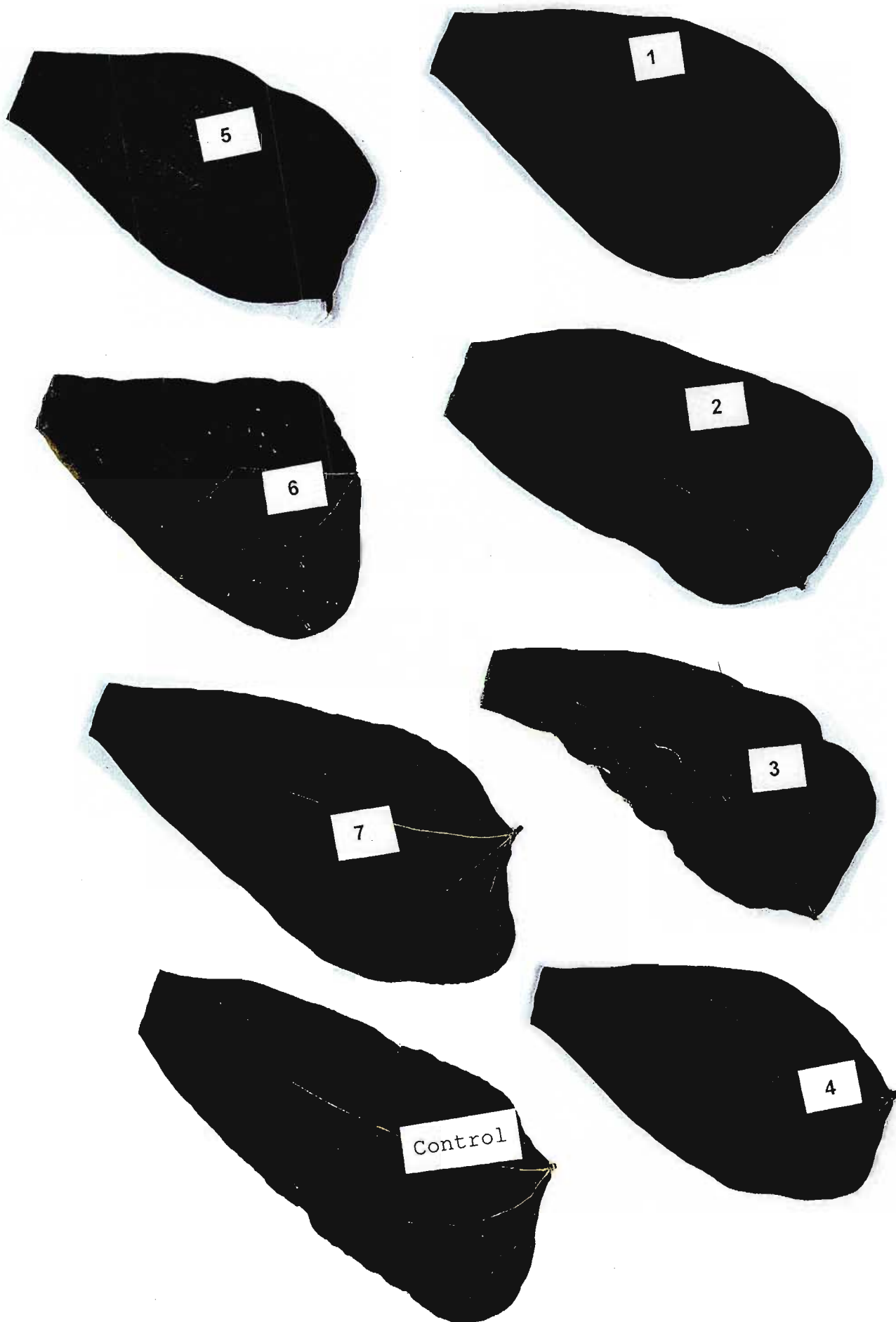


Figure 3.17: Effect of Fractions obtained following thin layer chromatography of an ethanolic extract of non-senescent leaves of *Bidens pilosa* on senescence of *Rumex* leaf discs under dark conditions. Fractions were eluted with ethyl acetate.

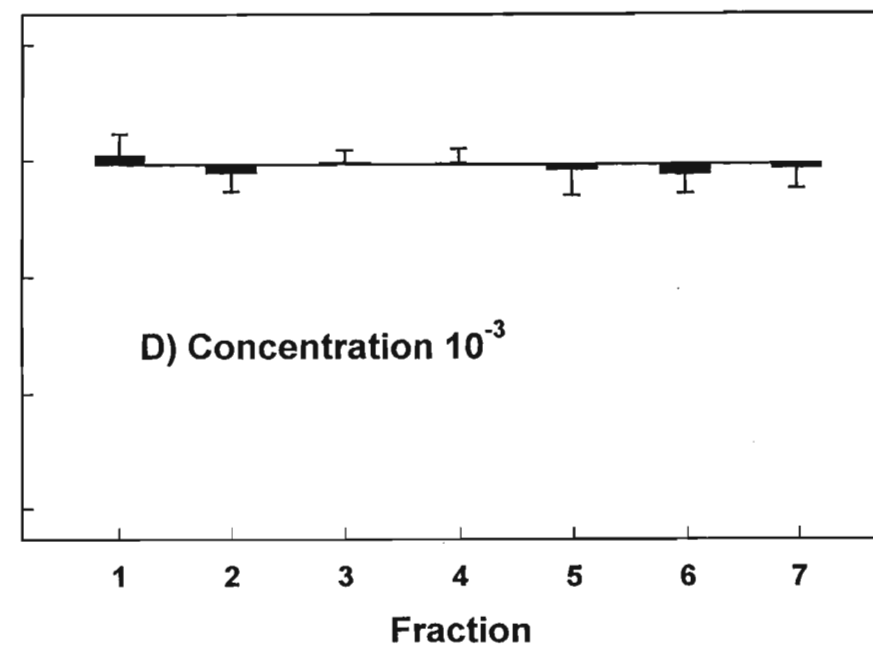
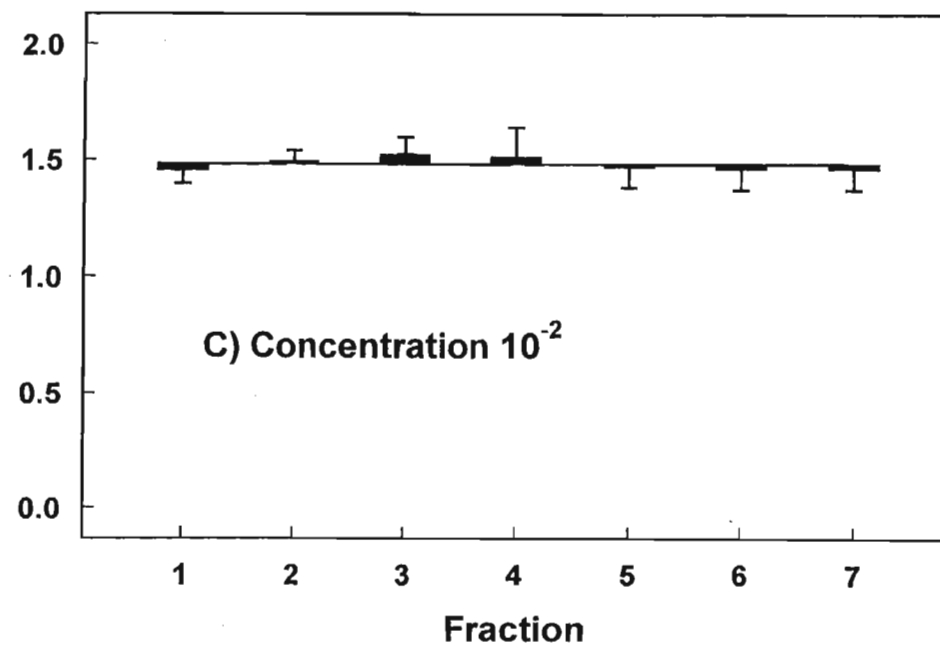
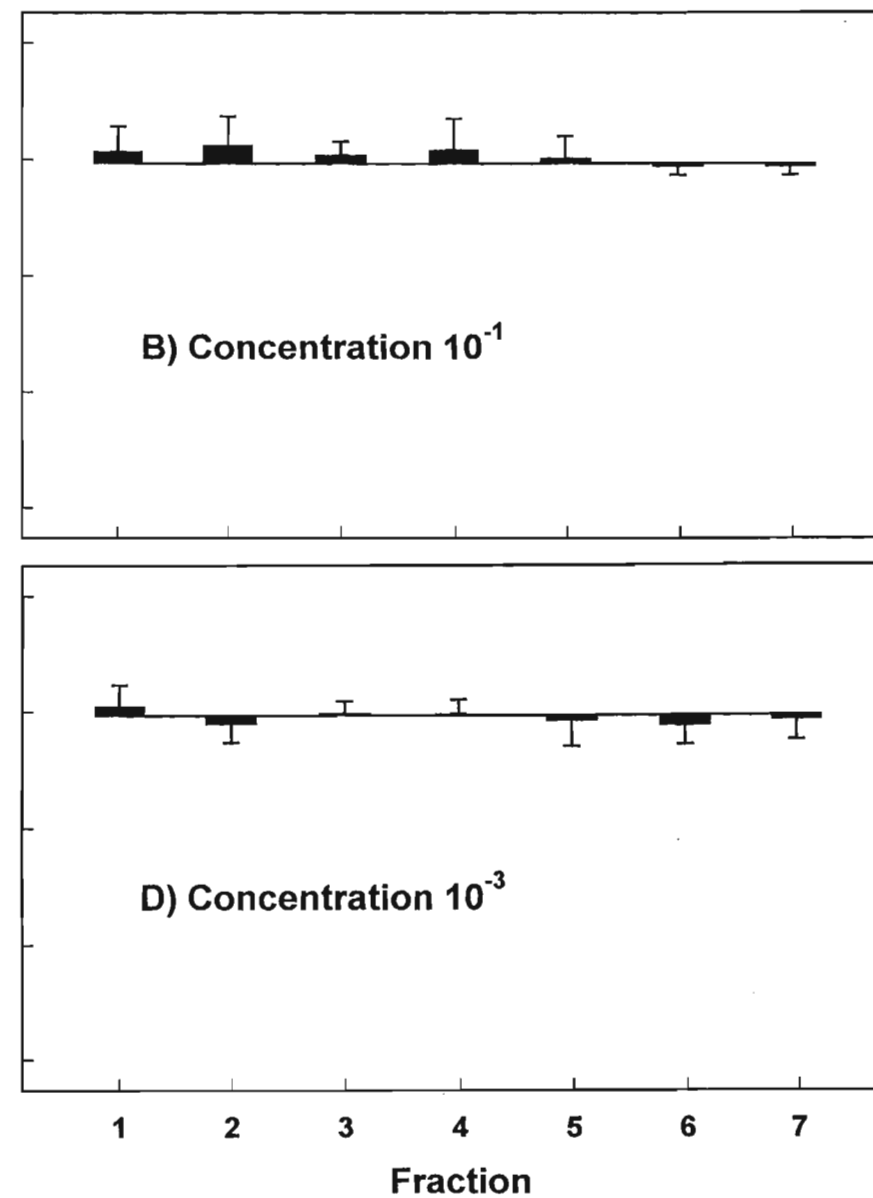
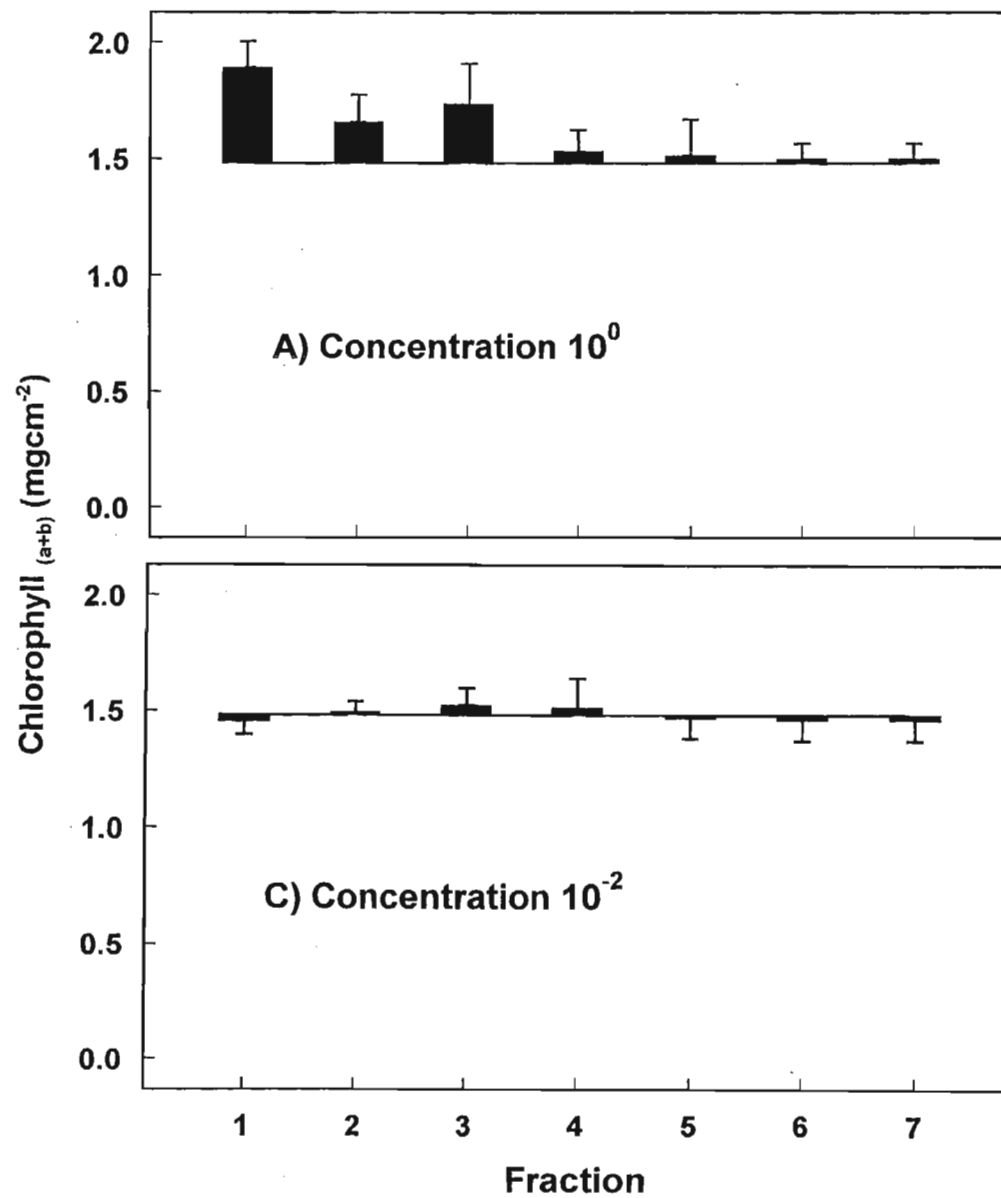


Figure 3.18: Effect of Fractions obtained following thin layer chromatography of an ethanolic extract of non-senescent leaves of *Bidens pilosa* on senescence of bean cuttings under light conditions. The Fractions were eluted with methanol.

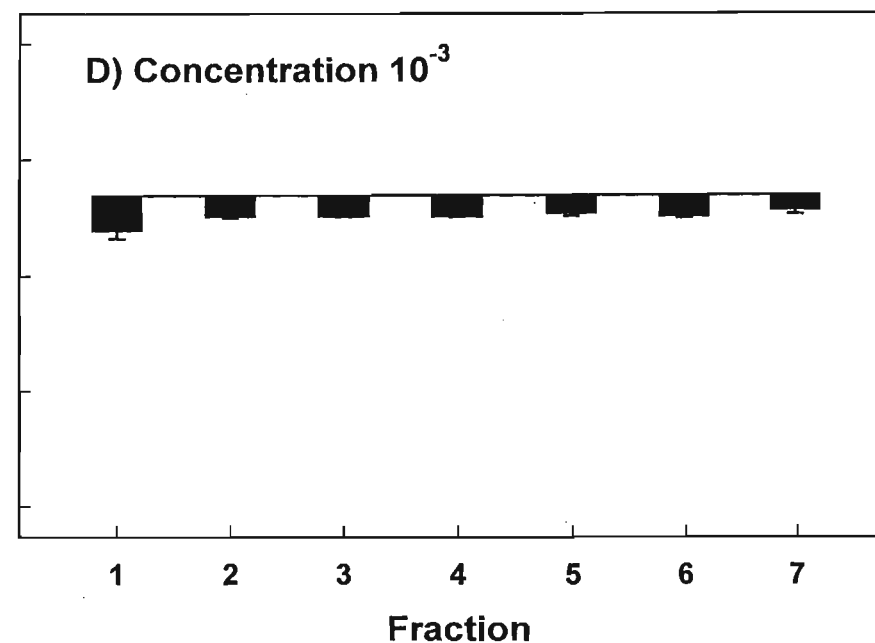
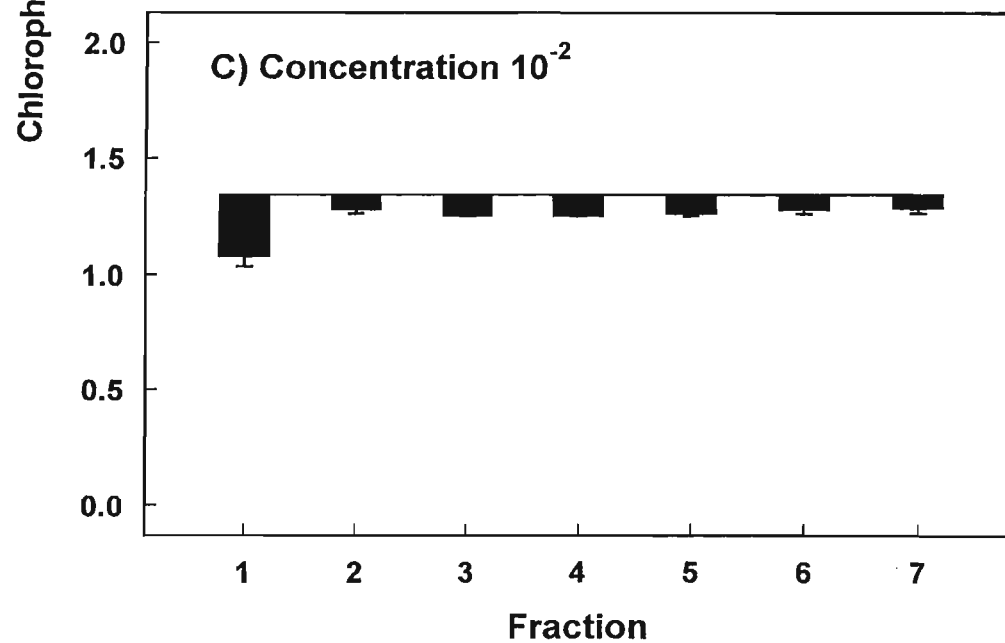
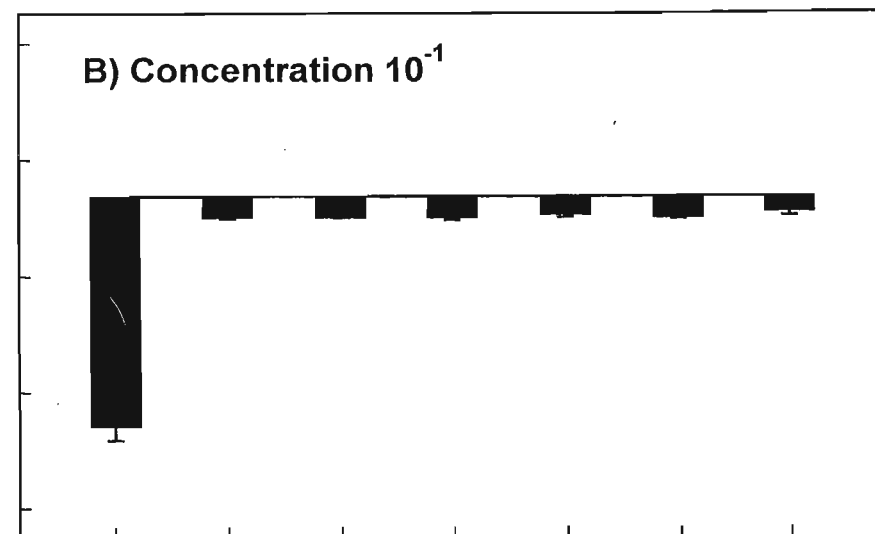
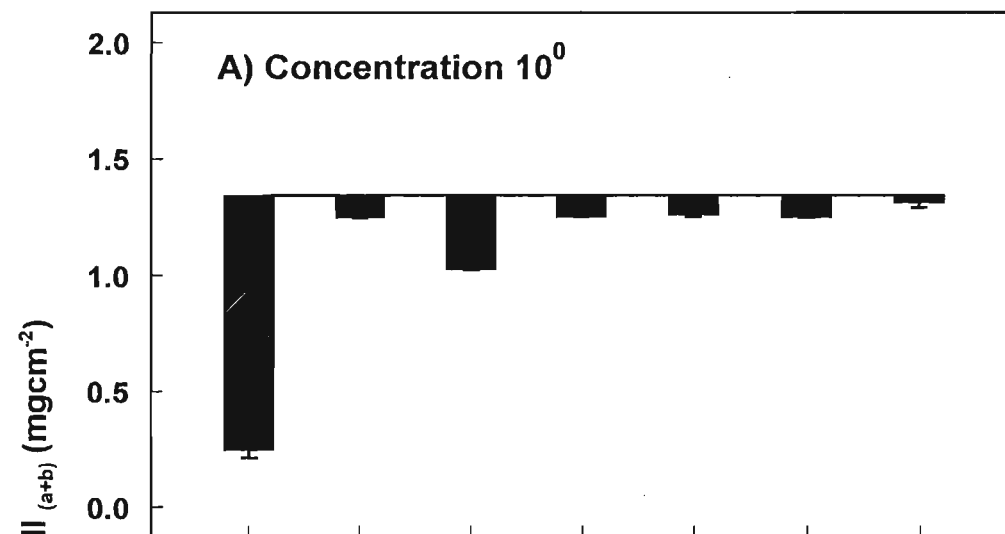


Figure 3.19: Effect of Fractions obtained following thin layer chromatography of an ethanolic extract of non-senescent leaves of *Bidens pilosa* on senescence of bean cuttings under light conditions. The Fractions were eluted with ethyl acetate.

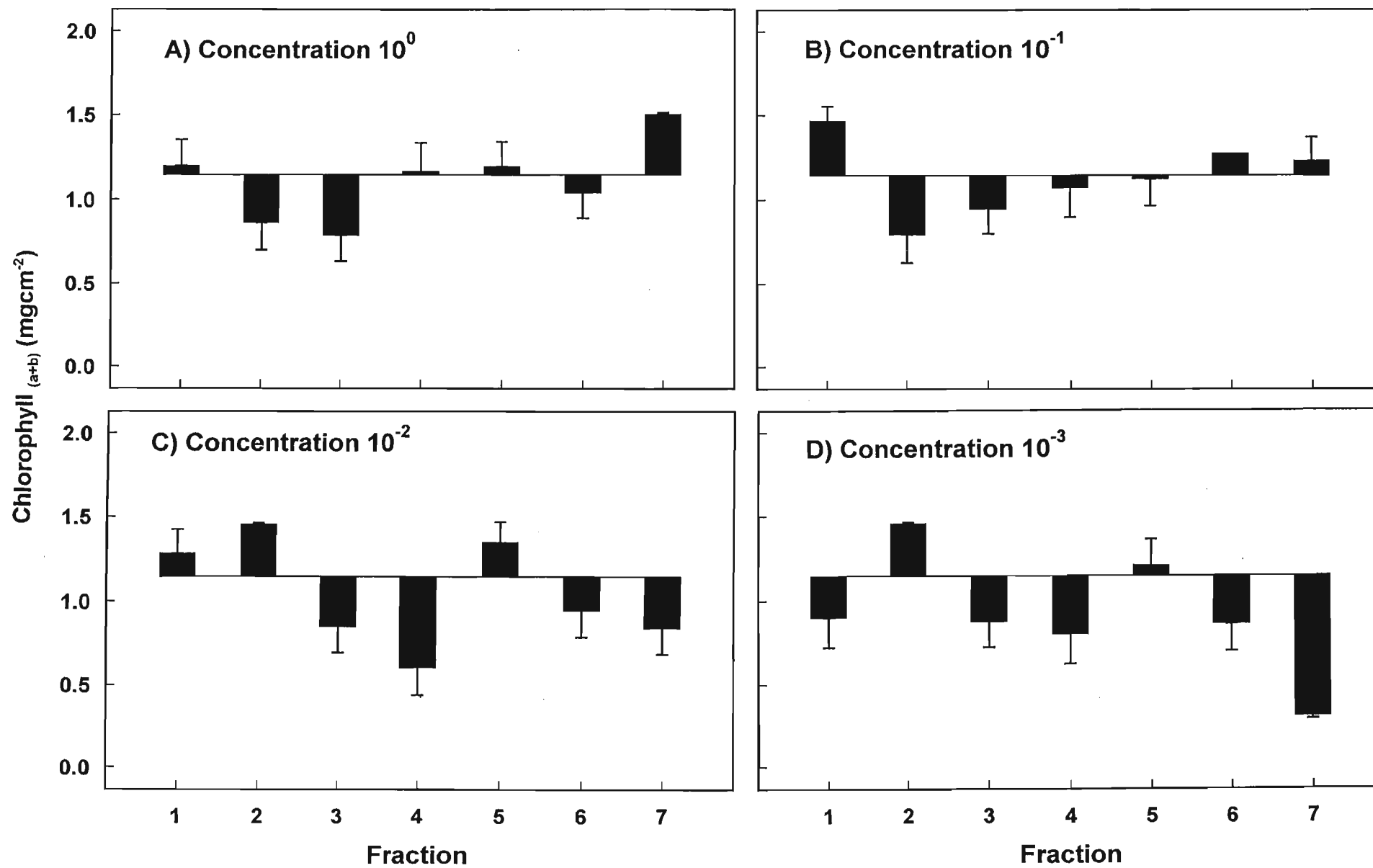
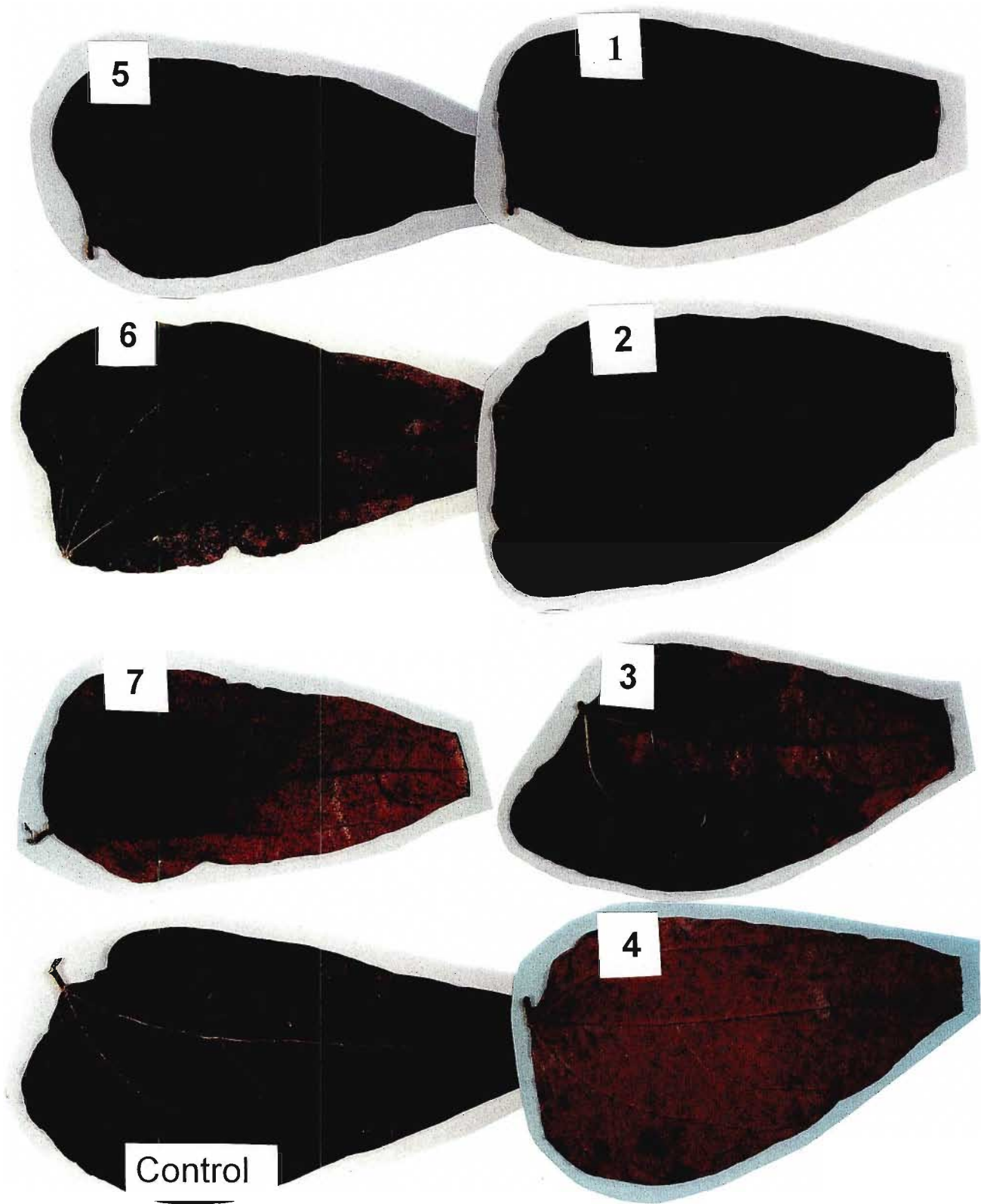


Figure 3.20: Photographs of leaflets of bean cuttings which were treated with various concentrations of Fractions obtained following thin layer chromatography of ethanolic extracts of non-senescent leaves of *Bidens pilosa*. Fractions were eluted with ethyl acetate.



results was the same as in **Figures 3.3; 3.4 and 3.5**. Because the objective was to find a bioassay for demonstrating senescence activity of extracts from the receptacles and leaves of *Bidens pilosa*, some negative results of *Rumex* test are not shown in this thesis.

Solutions of abscisic acid, gibberellic acid, jasmonic acid and 4-chloroindole acetic acid were prepared by dissolving 1 mg in 10 ml of water. These solutions were run on TLC plates together with ethanolic extracts from senescent and non-senescent leaves (**Figure 3.21 & Figure 3.22**).

Fraction 1 ( $R_f$  0.0-0.1) from ethanolic extracts of senescent and non-senescent leaves co-chromatographed with  $GA_3$ .

Fraction 3 ( $R_f$  0.10 - 0.25) from non-senescent leaf extracts (eluted with ethyl acetate) co-chromatographed with ABA (**Figure 3.21**).

Fraction 4 ( $R_f$  0.25 - 0.35) from non-senescent leaf extracts (eluted with ethyl acetate) co-chromatographed with 4-chloroindole acetic acid (**Figures 3.21 & 3.22**).

Fraction 5 ( $R_f$  0.35-0.40) from both senescent and non-senescent leaf extracts (eluted with ethyl acetate) co-chromatographed with jasmonic acid.

A comparison of the appearance of ethyl acetate soluble fractions obtained following thin layer chromatography of senescent and non-senescent leaf extracts, revealed a decline of certain compounds during senescence. Fraction 3 ( $R_f$  0.10 - 0.25) from non-senescent leaf extracts, which co-chromatographed with ABA, was absent in senescent leaf extracts (**Figure 3.21C,D: blue & Figure 3.22**).

Figure 3.21: Photographs of Fractions of ethanolic extracts made from the leaves of *Bidens pilosa*. Extracts were fractionated using Benzene : Ethyl acetate : Acetic acid (50:5:2) (v/v). Non-senescent leaf extracts eluted with methanol (A); senescent leaf extracts eluted with methanol (B); non-senescent leaf extracts eluted with ethyl acetate (C); senescent leaf extracts eluted with ethyl acetate (D). Dark green colour : positions of fractions on TLC plates; blue colour : relative quantities of fractions on TLC plates.

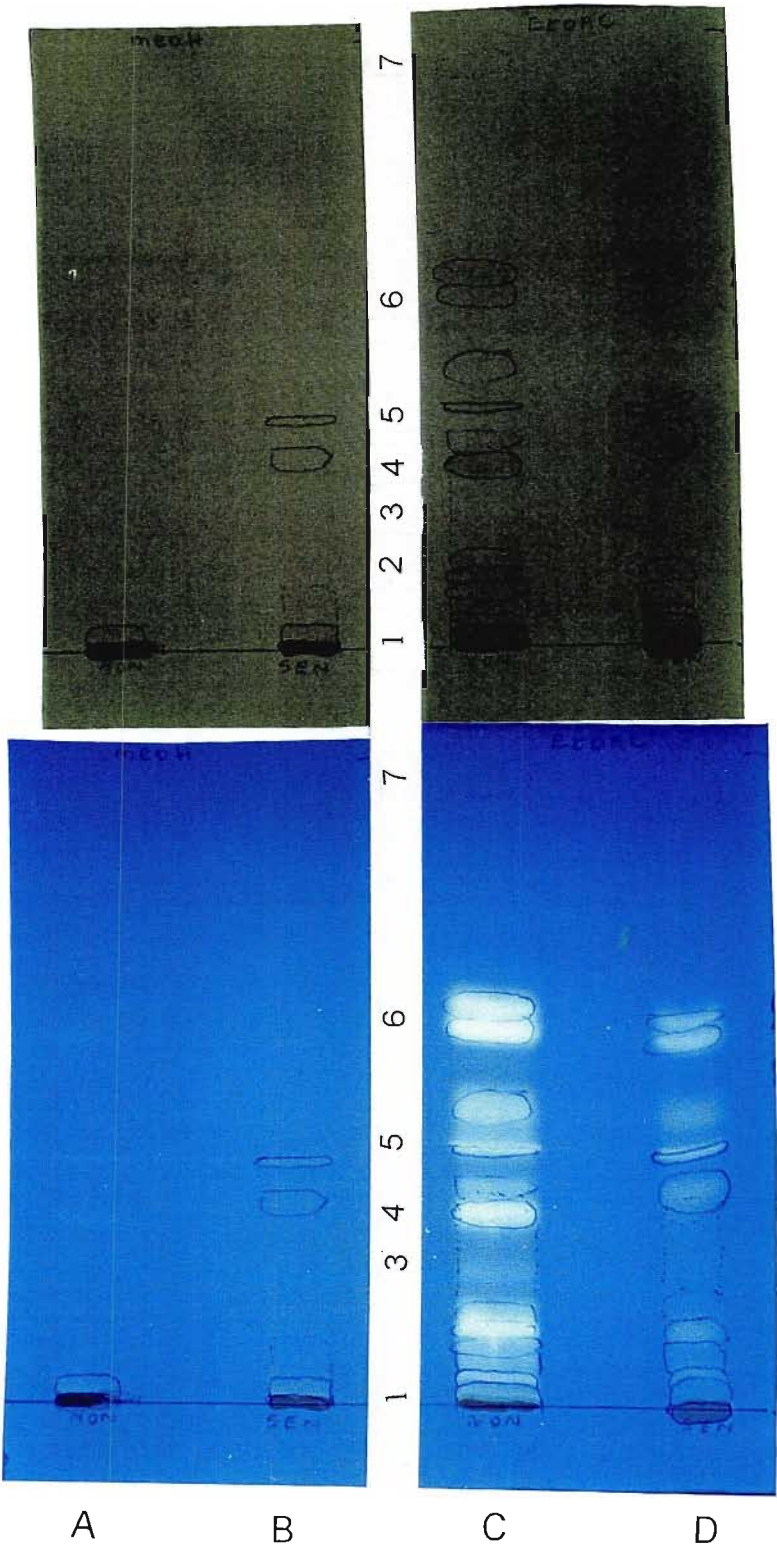
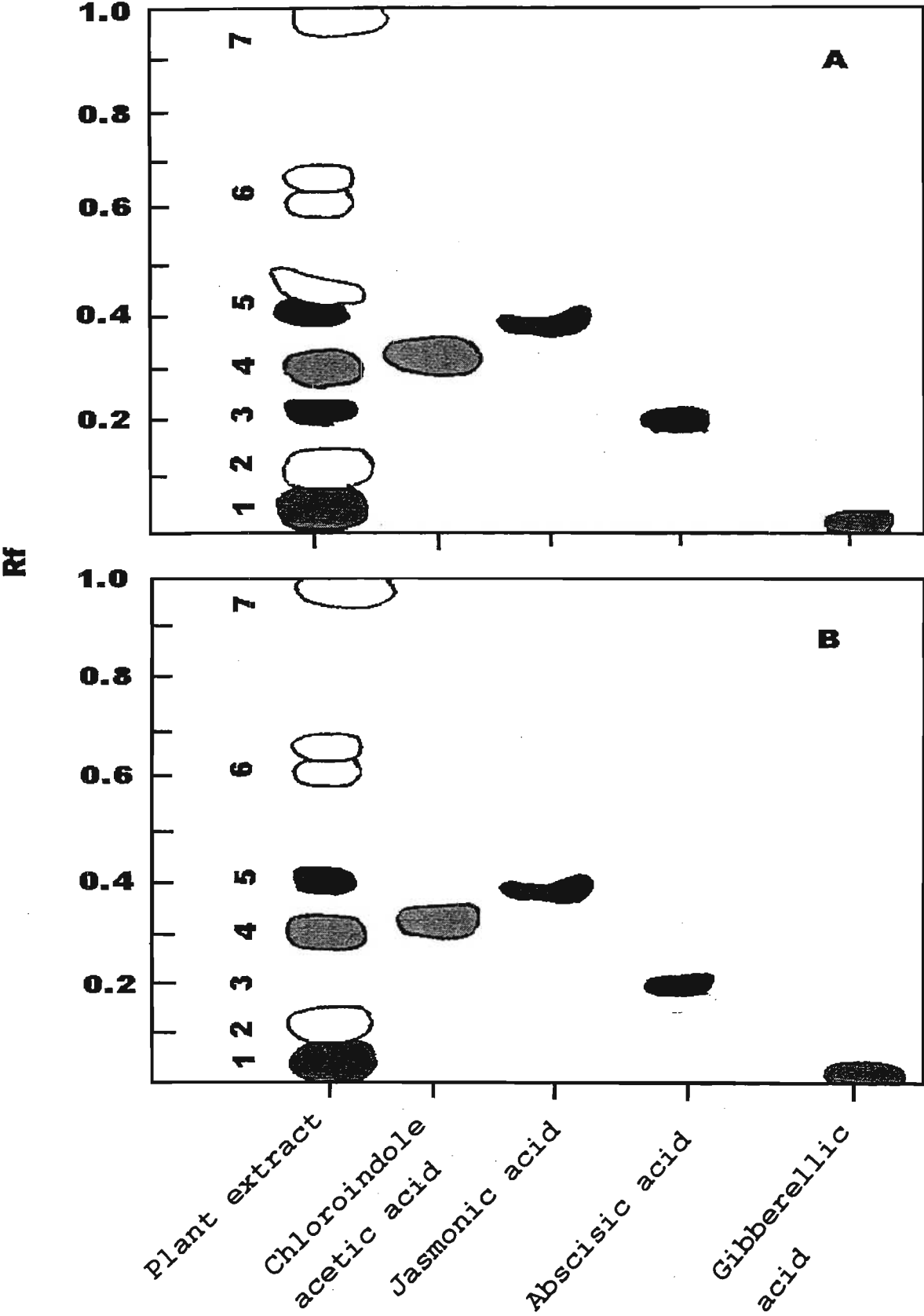


Figure 3.22: Diagrammatic presentation of positions of Fractions on TLC plates made from ethanolic extracts of non-senescent (A); and senescent leaves of *Bidens pilosa* (B). Fractions were eluted with ethyl acetate.



Fraction 4 ( $R_f$  0.25 - 0.35) which co-chromatographed with 4-chloroindole acetic acid, was more prominent in non-senescent than in senescent leaf extracts.

Fraction 7 ( $R_f$  0.9 - 1.0) was more prominent in non-senescent than in senescent leaf extracts when stained with Iodine crystals (**Figure 3.21C, D**: green).

### 3.4. DISCUSSION

Crude water extracts made from non-senescent receptacles did not induce senescence in *Rumex* leaf discs, *Bidens pilosa* cuttings and pot-grown plants in either light or dark (Tables 3.1 & 3.2). On the other hand, crude water extracts made from senescent receptacles induced senescence in cuttings and in pot-grown *Bidens pilosa* plants in light. Under dark conditions, high concentrations ( $10^0$  &  $10^{-1}$ ) of crude water extracts made from senescent receptacles prevented chlorophyll breakdown in *Rumex* leaf discs significantly (Figure 3.3).

These results suggest that extracts made from non-senescent receptacles contained more retardants of senescence than extracts from senescent receptacles. The lack of senescence activity in crude ethanolic extracts made from senescent receptacles (Figure 3.2), could imply that there were both promoters and/or retardants of senescence in the extracts. It is likely that water extracted less retardants of senescence, hence its extracts showed activity (Figure 3.3). Removal of retardants of senescence was achieved using paper chromatography. Thus, activity was detected from ethanolic extracts (Figure 3.6).

Senescence activity was also detected in fractions that were obtained, following column chromatography of ethanolic extracts of senescent receptacles and leaves of *Bidens pilosa*. In senescent receptacle extracts, activity was detected in two fractions, namely, Fractions 12 (eluted with 100% methanol) and 13 (eluted with ethyl acetate : isopropanol : water : acetic acid, in a ratio of 52:28:28:4 (v/v)). In these extracts, only one concentration ( $10^0$ ) induced senescence. The results were obtained under greenhouse light conditions.

In senescent leaf extracts, activity was detected in Fraction 10 (eluted with ethyl acetate

: methanol, 55:45 ) (v/v); Fraction 11 (eluted with ethyl acetate : methanol, 50:50) (v/v); Fraction 12 (eluted with 100% methanol) and in Fraction 13 (eluted with ethyl acetate : isopropanol : water : acetic acid, 52:28:28:4)(v/v), under greenhouse light conditions. In these extracts, two concentrations ( $10^0$  &  $10^{-0.25}$ ) induced senescence. Thus, senescent receptacle extracts had less senescence activity than senescent leaf extracts. If the senescence signals were translocated from the fruit via the receptacle to the leaf, one would expect to find more activity in the sink (leaves) than in the source (receptacles), provided that both organs had senesced. On the other hand, there are more synthetic processes in the leaf than in the receptacle. If senescence factors in the receptacle were synthesised and exported by the leaf, one would expect more activity in the leaf, because the leaf senesced when the receptacle was already dead, removing the sink. The death of the receptacle would block export of these signals to the fruit, leading to their accumulation in the leaves.

Senescence activity was detected in fractions that were obtained following thin layer chromatography of ethanolic extracts of senescent receptacles, non-senescent and senescent leaves.

Fraction 1 ( $R_f$  0.0-0.1) from senescent receptacles, senescent and non-senescent leaf extracts (eluted with methanol) induced senescence of bean cuttings in light (**Figures 3.10; 3.12 & 3.18**). There was no difference in senescence activity of Fractions obtained from senescent and non-senescent leaf extracts. However, senescence activity from senescent receptacle extracts was lower than that of the leaf extracts at concentrations of  $10^0$  and  $10^{-1}$ .

Although the  $R_f$  of Fraction 1 ( $R_f$  0.0-0.1) from ethanolic extracts of senescent and non-

senescent leaves co-chromatographed with GA<sub>3</sub>, other compounds in the base line could have acted together with GA<sub>3</sub> to induce senescence in the light. Fraction 1 from ethanolic extracts of senescent and non-senescent leaves did not induce senescence in the light when eluted with ethyl acetate (**Figure 3.15 & Figure 3.19**).

Fraction 3 ( $R_f$  0.10 - 0.25) from non-senescent leaf extracts (eluted with ethyl acetate), which co-chromatographed with ABA (**Figure 3.21**), induced senescence of bean cuttings under continuous low light. However, the activity was not dramatic. ABA promotes the loss of chlorophyll and protein in light (ZHI-YI, VEIERSKOV, PARK & THIMANN, 1988). Light maintains an effective supply of ATP by increasing respiration rate (THIMANN, TETLEY & KRIVAK, 1977). The mode of action of ABA is linked to a water stress. ABA causes stomatal closure and triggers ethylene production. Senescence activity of this Fraction was not dramatic because the treated plants were kept in distilled water, which eliminated a water stress. Thus, Fraction 3 was ABA-like in both  $R_f$  and behaviour. According to DENT & COWAN, (1994), exogenous ABA caused a decline in Chl and carotenoid content of light-incubated barley leaf segments, and the rate of decline increased with an increase in exogenous ABA concentration of  $10^{-5}$ M to  $10^{-3}$ M. However, in the present study, Fraction 3 did not induce senescence in *Rumex* leaf discs even under continuous low light. It is likely that Fraction 3 contained less ABA which was antagonised by *Rumex*. Furthermore, the leaf discs were not floated in solution, in contrast to the procedures used by other workers.

Fraction 4 ( $R_f$  0.25 - 0.35) from non-senescent leaf extracts (eluted with ethyl acetate), which co-chromatographed with 4-chloroindole acetic acid, induced senescence of bean cuttings at lower concentrations in the light (**Figures 3.21 & 3.22**). Fraction 4 from senescent leaf extracts showed no activity (**Figure 3.15 & Figure 3.19**). The

senescence inducing effect of seeds on bean leaves can be duplicated by replacing the seeds with auxin (TAMAS, ENGELS, KAPLAN, OZBUN & WALLACE 1981). In soybean leaves, IAA decreased during podfill (HEIN, BRENNER, & BRUN, 1984). Since senescent leaves were collected from plants that were undergoing monocarpic senescence, it is likely that their IAA content had declined.

Methyl jasmonate promotes chlorophyll loss in oat leaf segments (UEDA & KATO 1980). Jasmonic acid is less active than its methyl ester (UEDA, KATO, YAMANE & TAKAHASHI, 1981). Fraction 5 ( $R_f$  0.35-0.40) from both senescent and non-senescent leaf extracts (eluted with ethyl acetate), which co-chromatographed with jasmonic acid, did not induce senescence.

Fraction 7 ( $R_f$  0.9 - 1.0) from non-senescent leaf extracts (eluted with ethyl acetate) also induced senescence in bean cuttings in the light. Fraction 7 from senescent leaf extracts lacked senescence activity.

A comparison of the appearance of ethyl acetate soluble fractions obtained following thin layer chromatography of senescent and non-senescent leaf extracts, revealed a decline of certain compounds during senescence. Fraction 3 ( $R_f$  0.10 - 0.25), Fraction 4 ( $R_f$  0.25 - 0.35) and Fraction 7 ( $R_f$  0.9 - 1.0) were present in non-senescent and absent in senescent leaf extracts (**Figures 3.21 & 3.22**).

The loss of senescence activity in Fractions 3, 4 and 7 of senescent leaf extracts was probably caused by the decline of active compounds during senescence. These results suggest that green leaves of *Bidens pilosa* contain promoters of senescence which stimulate ethylene production. This is an active phase of senescence during which

senescence signals are translocated from one organ to another e.g. from fruits to the nearest leaf. Once senescence is in progress, degradation products resulting from the dismantling process are released. Some fatty acids have been shown to accelerate senescence. These fatty acids could be released from lipid catabolism.

The results which were obtained following paper chromatography and thin layer chromatography showed some similarities. In both techniques, senescence-inducing activity was detected in  $R_f$  0.1 (**Figure 3.6 & Figure 3.12**). Fractions 12 & 13 obtained following column chromatography induced senescence (**Figure 3.7**). When these fractions were purified using TLC, senescence activity was detected in  $R_f$  0.1. The active extracts obtained from the three techniques were dark brown and soluble in methanol, suggesting similarities in the compounds involved.

The presence of senescence activity in ethyl acetate soluble fractions from non-senescent leaf extracts, and its absence in senescent leaf extracts, suggests that senescence factors operate during the active terminal growth phase of monocarpic plants. The processes which lead to the decline in senescence factors are discussed in Chapter 4.

## CHAPTER FOUR

### 4. CONCLUSIONS

#### 4.1. Growth and senescence

Deflowered *Bidens pilosa* plants had a greater root, stem and leaf weight compared with control plants. Other workers reported a higher stem and leaf dry weight in deflowered sunflower plants (HO & BELOW, 1989). According to Gosh and Biswas (1989), plant dry weight was highest in deflowered plants, followed by defruited and control plants of *Arachis hypogea*.

In field-grown plants, deflowered *Bidens pilosa* plants were taller than the controls and those that were defruited. Deflowered soybean plants were also taller than the controls (LINDOO & NOODÉN, 1976).

Total chlorophyll in the controls, in association with the development of the fruit, was lower in the final than in earlier harvests of *Bidens pilosa* plants. These results are consistent with senescence pattern in sunflower plants, where leaf chlorophyll content decreased during the development of the seed structure. A further decrease in chlorophyll content occurred at the end of rapid seed growth (ZHDANOVA & KARYAGINA, 1997). The senescence signals from the fruit and flower heads probably caused an increase in mRNA levels of senescence-related genes which in turn

stimulated senescence (VALJAKKA, LUOMALA, KANGASJARVI AND VAPAAVUORI, 1999).

In the present study, a decline in chlorophyll levels in the first leaf rather than in the second leaf from the head, at high light intensity, coincided with a high dry weight of mature ripe fruit. This suggests that high light intensity accelerated the process of maturation of the fruits which were then influential in the senescence of the first leaf. Aerial flowers/fruits of *Arachis hypogea* had a higher capacity than underground fruits in inducing monocarpic senescence (GHOSH & BISWAS, 1995). Aerial flowers/fruits are more subject to fluctuations in temperature and light intensity, suggesting that light plays a role in monocarpic senescence.

There were also differences between the treatments in chlorophyll levels. Deflowered *Bidens pilosa* plants had higher chlorophyll levels than either defruited or control plants. Other workers have observed contrasting patterns of senescence in maize and sunflower. Their results showed that a lack of seed/grain accelerated senescence in maize and delayed senescence in sunflower plants. In sunflower plants, there was no interaction between reproductive treatment and position of leaves on the plant, indicating that the senescence signal was equally effective throughout the plant (SADRAS, ECHARTE & ANDRADE, 2000).

In the control plants of *Bidens pilosa* the senescence signal was more effective in the first than in the second leaf from the flower heads.

In *Bidens pilosa* there was a decline in photosynthetic rates of all the treatments during

senescence. In *Nicotiana tabacum* green leaves had a higher photosynthetic rate than senescent leaves when assessed using a radioactive technique (PHUKAN & PHUKAN, 1999). With increasing senescence, decreases in abundances of Rubisco, Ru5P kinase and chlorophyll were closely correlated with the decline in photosynthetic capacity (CRAFTS-BRANDNER, SALVUCCI & EGLI, 1990; CHERNYARD, 2000). In sugar beet photosynthetic CO<sub>2</sub> assimilation decreased by 50% during leaf senescence. After full expansion of the leaves, there was a decrease in Rubisco protein RbcS, mRNA and processes related to senescence subsequently followed in *Betula pendula*. Control and defruited *Bidens pilosa* plants had significantly higher photosynthetic and transpiration rates than deflowered plants 15 and 45 days after treatment. These results suggest that removal of flower heads affected the sink capacity which in turn lowered photosynthetic rates of deflowered plants. Species which have sink organs for carbohydrate accumulation do not show the suppression of photosynthesis (MAKINO & MAE, 1999). Decreasing the sink intensity (reducing pod number from 2 to 1 per node) reduced the rate of senescence as indicated by chlorophyll concentration in cowpea leaves. The rate of decline of photosynthesis was slowed by removal of pods despite accumulation of carbohydrates in leaves, indicating a feedback inhibition of photosynthesis (SRIVALLI, KHANNA, CHOPRA & GARAB, 1998).

Low stomatal conductance in deflowered plants as compared with defruited ones at 45 and 75 days after treatment, suggest that it was instrumental in lowering of photosynthesis and transpiration rates. In other studies, low photosynthetic rates were associated with reductions in stomatal conductance, chlorophyll levels and carbohydrates (PAL, SENGUPTA, STRIVASTAVA, VANITAN MEENA & JAIN, 1999).

In the present study, stomatal conductance alone did not successfully explain differences in photosynthetic and transpiration rates in the treatments, because the control plants had higher stomatal conductance than deflowered ones 75 days after treatment, yet photosynthetic and transpiration rates were the same in both treatments.

#### 4.2. Senescence activity

Ethanollic extracts made from the receptacles and leaves of *Bidens pilosa*, purified using either paper, column or thin layer chromatography did not induce senescence of *Rumex* leaf discs in the dark. Fraction 1 eluted with methanol co-chromatographed with Gibberellic acid used as a standard. It retarded chlorophyll loss significantly in the dark. Foliar sprays of gibberellins do prevent rapid leaf senescence induced by dark conditions (RANWALA & MILLER, 2000). GA<sub>3</sub> retarded chlorophyll loss significantly in *Polyalthia longifolia* and *Nerium indicum* leaf discs (MAITY, CHOWDHURY, BHAKAT, CHAKRABARTI & BHATTACHARJEE, 1999).

Fraction 1 induced senescence in bean cuttings under light conditions. Senescence activity was low in senescent receptacles compared with senescent leaf extracts. The death of the receptacle preceded senescence of the nearest leaf, suggesting that the signals moved from the fruit and receptacle to the nearest leaf. In *Tagetes patula* and *Chrysanthemoides coronarium*, the first leaf always senesced earlier than the third leaf (GOSH & BISWAS, 1994). There was no interaction between reproductive treatment and position of leaves on the sunflower plants. The senescence signal of sunflower plants induced senescence of all the leaves equally (SADRAS, ECHARTE & ANDRADE, 2000).

Fraction 3 from non-senescent leaf extracts co-chromatographed with ABA. When Fraction 3 was eluted with ethyl acetate, it induced senescence of bean cuttings under continuous light. In cut flowers of *Gladiolus* held in distilled water for 8 days, senescence was correlated with changes in the ratio of cytokinins to abscisic acid in the perianth tissues, suggesting that ABA is a senescence factor (KONDRAT & BELYNSKAYA, 1999). By increasing ammonium level and tissue sensitivity to ethylene, abscisic acid promoted senescence in detached rice leaves, (CHEN, HUNG, & KAO, 1997). In wheat tillers and leaves, there was a decrease in zeatin riboside and an increase in abscisic acid and ethylene content after flowering (YUE, YU & YU, 1996; MA & LIANG 1997). Leafy flowering shoots of carnation cv White sim subjected to a water stress and treated with ABA lost more chlorophyll than the controls (MOHANTY & BEHERA, 1996). In the present study, the treated bean cuttings were kept in distilled water. ABA is a stress hormone, The activity of Fraction 3 under these Experimental conditions was not pronounced. Fraction 3 from senescent leaf extracts did not induce senescence. In genera which shed their leaves, ABA reached its highest level at abscission (HASSANEIN, FODA & EMAM, 1998). The decline in ABA levels in senescent leaves suggest that it is catabolised during monocarpic senescence.

Fraction 4 from non-senescent leaf extracts was eluted with ethyl acetate and it co-chromatographed with 4-chloroindole acetic acid. Fraction 4 induced senescence of bean cuttings in light. Fraction 4 from senescent leaf extracts showed no activity, suggesting its decrease during senescence. Auxins reach their highest levels when leaves are fully expanded and thereafter decline during senescence. Activities of certain enzymes (IAA-oxidase and RN-ase) increase during senescence (HASSANEIN, FODA

& EMAM, 1998). Natural auxins (4-chloroindole acetic acid and its methyl ester) have a strong senescence activity on pea, a plant in which they occur. Naphthalene acetic acid and IAA induce senescence at higher concentrations. While all auxins killed mustard (*Sinapis alba*), none killed barley suggesting that different species may have different senescence factors (ENGVELD, 1996).

Fraction 5 from leaf extracts co-chromatographed with jasmonic acid. Application of methyl jasmonate significantly increased senescence of rice leaves incubated in the dark (WANG & KAO, 1999). Methyl jasmonate and its precursors in the biosynthetic pathway (linolenic and linoleic acids), promoted senescence and increased the level of lipid peroxidation in rice leaves. Methyl jasmonate induced lipid peroxidation is mediated through free radicals (HUNG & KAO, 1998). Jasmonic acid and methyl jasmonate promote fruit ripening, senescence and abscission (Bialecka & Kepezynski, 1998). Fraction 5 did not induce senescence in either light or dark, suggesting that it was neither jasmonic acid nor its methyl ester.

Fraction 7 from non-senescent leaf extracts, eluted with ethyl acetate, also induced senescence in bean cuttings in light. Fraction 7 from senescent leaf extracts lacked senescence activity.

Fractions 3, 4 and 7 which were obtained following thin layer chromatography of ethanolic extracts of senescent leaves, lacked senescence activity when eluted with ethyl acetate. The TLC plates showed that these Fractions declined during senescence. The results of this study have shown that plant extracts contain senescence signals. Non-senescent tissues contained more signals than senescent tissues, suggesting that

they are produced during the active growth phase of the plant. Their decrease in senescent tissues suggests that they are catabolised. Initiation of senescence occurs in the chloroplast. It is characterised by an increase in the number of plastoglobuli and a decrease in the expression of photosynthesis related genes (BUCHNANN-WOLLASTON, 1997; BLEEKER & PATTERSON, 1997). During senescence, there is a loss of compartments in which degradative enzymes are stored. This leads to the exposure of senescence factors to enzymes which degrade them. Senescence factors secreted by developing seeds inhibit and or kill the plant. Nutrients are then mobilized and translocated to the seeds (ENGVELD, 1996). High temperature may interact with senescence factors and accelerate senescence (BENBELLA & PAULSEN, 1998). Membrane disruption is the key event in plant senescence. Phospholipase D plays a role in membrane deterioration (FAN, ZHENG & WANG, 1997). The overall picture is that senescence of *Bidens pilosa* is very similar to that of other annuals. In phylogenetically related species, similar types of senescence factors may be operative.

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