EFFECTS OF INJURY BY PARTIAL DEFOLIATION ON CHLOROPHYLL LEVELS IN LEAVES OF GLYCINE MAX (L.) MERR.

by

Dianne Pauline Pacolt

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APPROVED BY THE GRADUATE COMMITTEE OF:

Dr. Virgil A. Thiesfeld, committee chairman
Professor of Biology

Dr. C. Edward Gasque
Associate Professor of Biology

Dr. Joseph B. Harris
Professor of Biology

PREFACE

This work began in 1982 as a research project designed for measuring soybean plant injury caused by environmental factors. This investigation, including my Junior Scientist salary, was supported by a grant awarded to W. L. Koukkari and B. W. Kennedy from the Minnesota Soybean Research and Promotion Council. I was involved in the designing and planning of the field experiments while employed by W. L. Koukkari. The greenhouse experiments were conducted at the University of Minnesota under my supervision while I was enrolled as a student at the University of Wisconsin-Stevens Point. At this time, the project had produced significant results and portions of it were presented at the World Soybean Conference in Ames, Iowa on 11 January, 1983. Authors included W. L. Koukkari, B. W. Kennedy, L. Carlson, D. P. Pacolt, and M. Couderchet. The stage-of-development portion of my research was conducted at the University of Minnesota during December-January, 1984. At this time I was on Christmas break from the University of Wisconsin-Stevens Point. This research is being continued at the University of Minnesota under the direction of W. L. Koukkari.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to W. L. Koukkari, Department of Botany, University of Minnesota, for his suggestions, guidance, encouragement, and criticisms during both the course of study and the preparation of this manuscript. I gratefully acknowledge the assistance provided by B. W. Kennedy, Department of Plant Pathology, University of Minnesota, as well as the technical assistance of LeAnne Carlson and Roxanne Denny. I am indebted to Charolette Kisner, Department of Applied Statistics, University of Minnesota, for her help with the statistical analysis of the data. I thank Virgil A. Thiesfeld, C. Edward Gasque, and Joseph B. Harris for serving as my thesis advisors and for reading and suggesting improvements in the manuscript.

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ABSTRACT

Unifoliolate and trifoliolate leaves of Glycine max (L.) Merr. (soybean, cv. Evans) plants at five stages of development were sampled for chlorophyll content. Unifoliolates showed significantly higher chlorophyll levels than trifoliolates in the vegetative three (V_3) and reproductive one (R_1) stages, and lower levels in the last three reproductive (R_2, R_3, R_4) stages. Trifoliolates showed no significant differences between different stages or nodes. Injury by partial defoliation to trifoliolate leaves was determined by comparison with that of both unifoliolates and trifoliolates which were not subjected to partial defoliation. Following defoliation, the chlorophyll content of the unifoliolate and trifoliolate leaves from mildly and severely defoliated plants increased significantly compared to leaves of plants not subjected to defoliation in three out of four cases. Significant differences in chlorophyll levels between mild and severe defoliation were not evident in greenhouse experiments, but field experiments showed significant increases in chlorophyll levels of severely defoliated (compared to mildly defoliated) top trifoliolates under drought conditions and unifoliolates when the field was irrigated. Results using the chlorophyll assay indicated that older leaves of a of plant, compared to younger leaves, were the most sensitive with respect to changes in chlorophyll levels in plants subjected to partial defoliation.

INTRODUCTION

Chlorophyll levels are known to decline during the aging process in plants and these levels can be affected by injury. Injury by decapitation or partial defoliation increases many constructive or synthetic processes of the remaining shoot material. This enhancement is manifested by increased growth rates (Hogdkinson et al. 1972, Hodgkinson 1974, Carmi and Koller 1979, Binnie and Clifford 1980), photosynthetic activity (Wareing et al. 1968, Meidner 1970, Carmi and Koller 1979), as well as protein and chlorophyll production (Wareing et al. 1968, Carmi and Koller 1979, Katagiri and Tsuji 1980, Van Staden and Carmi 1982). The most dramatic of these is chlorophyll production, and because of this, it is widely used as an index of the effect of injury to aging plants (Leopold 1961, Noodén 1980a). A sensitive biological assay for measuring the effects of injury caused by mechanical, chemical, and biological agents has been developed based on chlorophyll levels of the remaining shoot material after partial defoliation (Koukkari et al. 1984).

Since defoliation is known to increase chlorophyll levels (Van Staden and Carmi 1982), it should be possible to study the effects of defoliation on senescence by monitoring the chlorophyll content of the remaining attached leaves. This possibility was tested in the present study.

The effects of defoliation on chlorophyll content of the remaining shoot material may depend on the severity of injury. Therefore, experiments were designed to determine whether the chlorophyll levels were also dependent upon the severity of defoliation.

REVIEW OF LITERATURE

Senescence is described as an internally programmed, degenerative process leading to the death of an organism, and is a normal process of plant aging (Leopold 1961, Noodén and Leopold 1978, Noodén 1984). Senescence in soybeans involves the loss of the older organs accompanied by continued production of new organs while the plant is growing. The first organ to senesce is normally the cotyledon (Leopold 1961).

Senescence of one plant part may be influenced or controlled by another. Healthy growing regions, such as shoot apices with young leaves, may induce leaf senescence of older, basal leaves (Noodén and Thompson 1984). Removal of these actively growing regions delays senescence of older leaves (Molisch 1938, Noodén and Leopold 1978). In soybeans, removal of the apex prevents cotyledon senescence entirely (Leopold 1961). The same apical influence applies to leaves. Removal of the apex before development of four or five trifoliolate leaves appears to delay senescence. As the plant switches from vegetative to reproductive growth, there is a mobilization and redistribution of resources (nutrients) from vegetative to reproductive parts (Denholm 1975, Noodén 1984). The developing flowers and pods become the final sinks for phloem translocation. Removal of reproductive parts delays senescence of the vegetative parts (Leopold 1961). The delays in senescence are reflected in increased chlorophyll levels of older leaves as compared to younger leaves.

Regulatory signals that are exchanged between parts of the plant during development may play a role in the increased chlorophyll levels

of the remaining shoot material following defoliation (Nooden 1984). The nature of these signals and their effects on chlorophyll levels throughout the plant following injury is uncertain, however, they may possibly involve phytohormones and nutrient fluxes (Wareing et al. 1968, Leopold and Nooden 1984).

Foliar senescence appears to involve phytohormones that both retard and induce senescence (Nooden and Leopold 1978). The primary phytohormones involved in the delay of senescence are auxin and cytokinins. Cytokinins have been shown to delay senescence-related processes such as chlorophyll, protein, and RNA degradation (Nooden 1984). Cytokinin is synthesized in the root tips and transported to the leaves throught the xylem via the transpiration stream (Letham 1978, Van Staden and Davey 1979, Nooden and Thompson 1984). These cytokinins may then be metabolized to other products at relatively high rates in the leaf tissues (Sexton and Woolhouse 1984). The supply of cytokinin traveling upward from the roots seems to be shared among the shoot organs, since removal of one, by partial defoliation or decapitation, leads to a greater accumulation and availability of cytokinins to the remaining shoot material (Thimann 1980, Van Staden and Carmi 1982). The longevity of excised leaves is known to be greatly extended by the formation of roots on these leaves (Molisch 1938, Chibnall 1939). Richmond and Lang (1957) found that cytokinin application greatly extended the life span of detached leaves by delaying chlorophyll and protein degradation. The similarity of the responses brought about by decapitation and cytokinin application support the hypothesis that partial defoliation reduces the competition between the shoot organs for cytokinins from the roots,

and hence, increases the availability of cytokinins to the remaining shoot material (Sitton et al. 1967). Partial excision of the roots causes a foliar yellowing, which may be relieved by applications of cytokinin (Carmi and Koller 1978, Noodén 1980b, Neumann et al. 1983). Delaying of leaf senescence is controlled not only by a decreased demand for cytokinins in the shoot, but also an increased supply of cytokinins from the roots (Van Staden and Carmi 1982).

Observers have noted that conservation of chlorophyll is not completely restricted to the action of cytokinins. Auxin also delays senescence in soybean leaves, however, its effects differ somewhat from those of cytokinins (Osborne 1959, Osborne and Hallway 1960). The combination of the two may be much more effective than either alone (Noodén et al. 1979). In Nicotiana rustica leaves, both cytokinin and auxin are required for maximal retardation of chlorophyll degradation (Engelbrecht and Conrad 1961).

Abscisic acid (ABA) is among the known senescence-inducing hormones present in leaves. ABA appears to have little effect on young or non-senescent leaves, but does accelerate chlorophyll loss in older leaves already induced to senesce (Lindoo and Noodén 1978). Evidence suggests that it may not be the concentration of a single plant hormone that is important in delaying or initiating senescence, but rather concentrations of hormones relative to each other (Oritani and Yoshida 1973). Lindoo and Noodén (1978) found a decline in cytokinin-like activity with concomitant increases in ABA-like inhibitors in older senescing leaves. High concentrations of cytokinins prevent the senescence accelerating effect of low concentrations of ABA (Back and Richmond 1971).

Redistribution of nutrients has figured very prominently in research directed towards the causes of senescence in the soybean (Nooden 1980b). Given the limiting supply of many minerals in the soil, it may be necessary for old assimilates as well as new assimilates such as amino acids (nitrogen compounds), carbon compounds (sugars) and certain minerals (e.g. phosphate, potassium, magnesium, and iron) to be redistributed from older senescing leaves (sources) to younger leaves (sinks) causing a nutrient deficiency in the older leaves. Evidence strongly indicates that high concentrations of cytokinins regulate mineral export from leaves by establishing sinks, or areas that preferentially attract and concentrate nutrients (ie. growing tips and expanding leaves), while source organs (older leaves) loose the capacity to accumulate cytokinins and nutrients, thereby causing changes in chlorophyll synthesis, as well as protein and RNA synthesis (Witham and Miller 1965). When introduced into the xylem through the cut base of an explant, cytokinins in combination with mineral nutrients are able to substitute for the products of the root system in delaying foliar yellowing by merely promoting uptake of mineral ions from solution (Garrison et al. 1984).

Of the individual nutrients, nitrogen may have the greatest effect on chlorophyll levels since it is an important compound involved in chlorophyll structure (Devlin and Witham 1983). The redistribution of minerals, especially in times of injury (defoliation) represents an important retrieval or recovery of nutrients from senescing organs and allows the plants to cope with injury more effectively (Nooden and Leopold 1978).

It is apparent that upon injury, many processes rather than a

single primary process accounts for the delay in chlorophyll degradation in the remaining leaves of soybean plants (Nooden 1984).

MATERIALS AND METHODS

Plant Material

Stage-of-Development (SOD) Experiment

Once a week, for five consecutive weeks, seeds of Glycine max (L.) Merr. cv. Evans were planted in five round plastic pots (diameter ca. 15.2 cm) containing a mixture of soil and vermiculite (3:1) at a depth of 2 cm. Seedlings were watered daily with distilled water and maintained in controlled environment chambers at ca. 25 degrees C, 70% relative humidity (RH), and on a LD 12:12 regime of 12 h light followed by 12 h darkness. Illumination was supplied by 32 fluorescent lamps (F96T12-CW-HO, G.E.) supplemented with twenty 60-watt incandescent lamps (G.E. code-60A21/TS Traffic Signal Type) and providing 300-450 $mE/m^2 \cdot s$ of photosynthetically active radiation at plant height. Leaf chlorophyll was assayed in these plants after three to seven weeks. The first group of plants had reached the reproductive stage referred to as R_4 (ca. seven weeks old) while the plants started five weeks later were in the vegetative V_3 stage. The system selected for grouping soybean plants into various vegetative and reproductive stages was based upon a standard soybean procedure (Fehr and Caviness, 1977). Five of these stages of development and their characteristics are shown in Table 1.

Greenhouse Experiments

Seeds (cv. Evans) were dusted with Captan fungicide and planted at a depth of 2 cm in 12 x 12 cm plastic flats containing perlite. After 15 days, 98 plants were transplanted to round plastic pots (15.2 cm, 1 plant/pot) containing a mixture of soil, peat and expanded

Table 1. Description of vegetative and reproductive stages in soybean plants (modified slightly from Fehr and Caviness, 1977).

Stage no.*	Abbreviated Stage title	Description
V_3	Third-node	Fully developed leaves at the unifoliolate and next two trifoliolate nodes.
R_1	Beginning bloom	Main stem has one open flower.
R_2	Full bloom	Open flower on main stem of one of the two uppermost nodes having a fully developed leaf.
R_3	Beginning pod	Pod 5 mm long on the main stem of one of the four uppermost nodes having a fully developed leaf.
R_4	Full pod	Pod 2 cm long on the main stem of one of the four uppermost nodes having a fully developed leaf.
R_5	Beginning seed	Seed 3 mm long in a pod on the main stem of one of the four uppermost nodes with a fully developed leaf.

*Refer to Figure 3 for illustrations of plants in each developmental stage.

polystyrofoam (1:1:1). They were watered daily with distilled water and maintained in the greenhouse (St. Paul, MN, USA, 45 degrees N) under natural illumination (experiment A started 11 April, 1984, and experiment B started 12 July, 1984). The plants were subjected to defoliation (mechanical injury, subsequently described) when they were five weeks of age.

Field Experiments

Seeds were sown with a mechanical field planter in 16 rows, 126 feet long in St. Paul, MN (45 degrees N). After two weeks the plant population was thinned to one plant every 2 cm in 14 of the rows, leaving two outside rows as border rows. When the plants were four weeks old they were subjected to defoliation (mechanical injury).

Experimental Design

In the greenhouse experiment, 72 plants were selected for uniformity of development and arranged in a 9 x 9 random block design, with six plots, each containing four rows of three plants each and 20 replications of each treatment (control, mild and severe). The field experiment was divided into six plots, each plot containing six 7 foot subplots, each subplot containing four rows with 48 replications of each treatment (control, mild and severe).

Two methods were selected for statistical analysis of the data: the analysis of variance F-test and the T-test (cf. Snedecor and Cochran, 1967). The T-test was performed on the stage-of-development and greenhouse experiment data and the F-test for the field experiment data using IVAN, a statistical package available on the University of

Minnesota computer systems. Treatment means were compared using two different methods; contrasts and the Newman-Keuls Studentized Range method.

Defoliation (Mechanical Injury)

In both the mild and severe treatments, the leaflets were cut with a small scissors, leaving only the bottom and top fully expanded leaflets to be used for chlorophyll sampling. Mild treatment consisted of removing the two lateral leaflets of the trifoliolate leaf and severe treatment consisted of removing the entire trifoliolate leaf at the base of all three leaflets (Figure 1).

Leaf Samples and Chlorophyll Assays

In the stage-of-development experiment, five plants from each stage were sampled by cutting one disc with a 7 mm cork borer from each half of both unifoliolate leaves (four dics total) as well as each half of the two lateral trifoliolate leaflets on every node up to and including the top fully expanded trifoliolate. The vegetative three (V_3) stage cotyledons were cut in half lengthwise. Chlorophyll sampling in the greenhouse plants was similar to that of the stage-of-development experiments, except that only the bottom and top trifoliolate leaves were sampled ten days after injury.

In the field experiments, chlorophyll samples consisted of the right half of one of the unifoliolate leaves (only in 1983) and the right half of the terminal leaflet on the bottom and top fully expanded trifoliolate leaf, excluding the midvein, ten days after injury (Figure 2, A and B). The left half of the leaf was saved for

Figure 1. A control (uninjured) soybean trifoliolate leaf (A) and one subjected to mild (B) or severe (C) injury (defoliation). Mild injury consisted of removing two lateral leaflets of the trifoliolate leaf and severe injury consisted of removing the entire trifoliolate leaf at the base of all three leaflets.

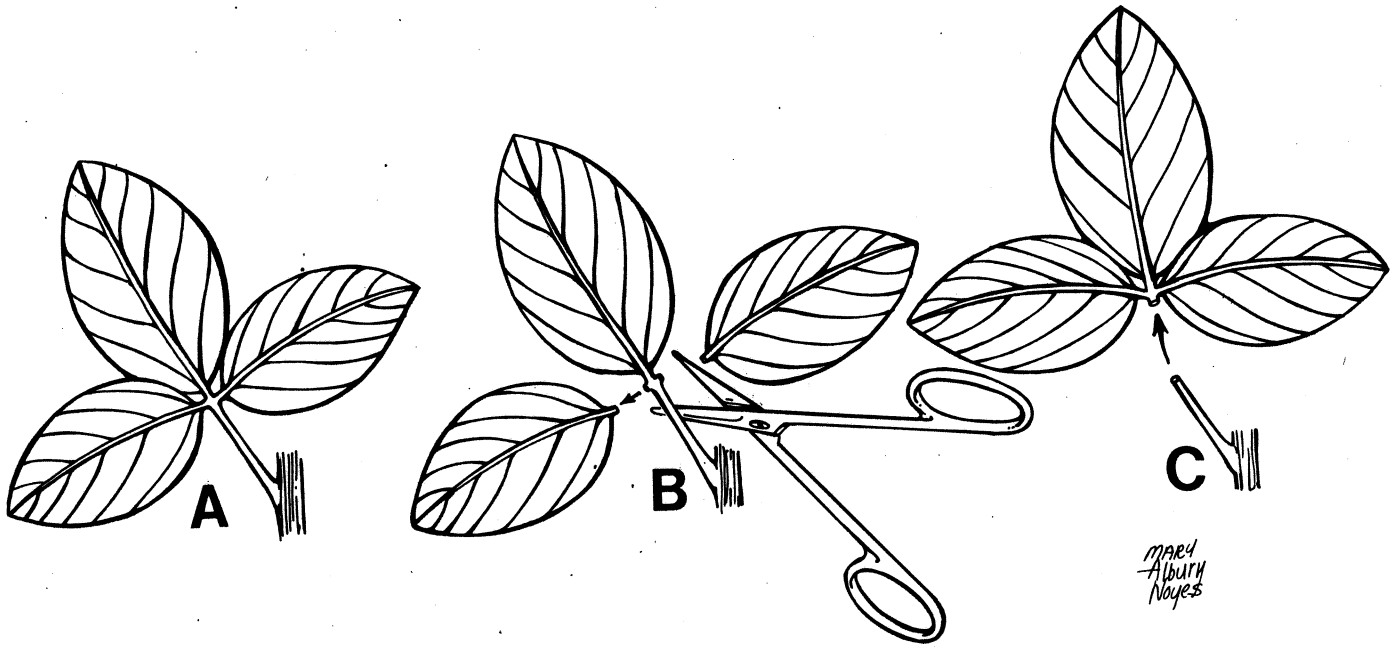
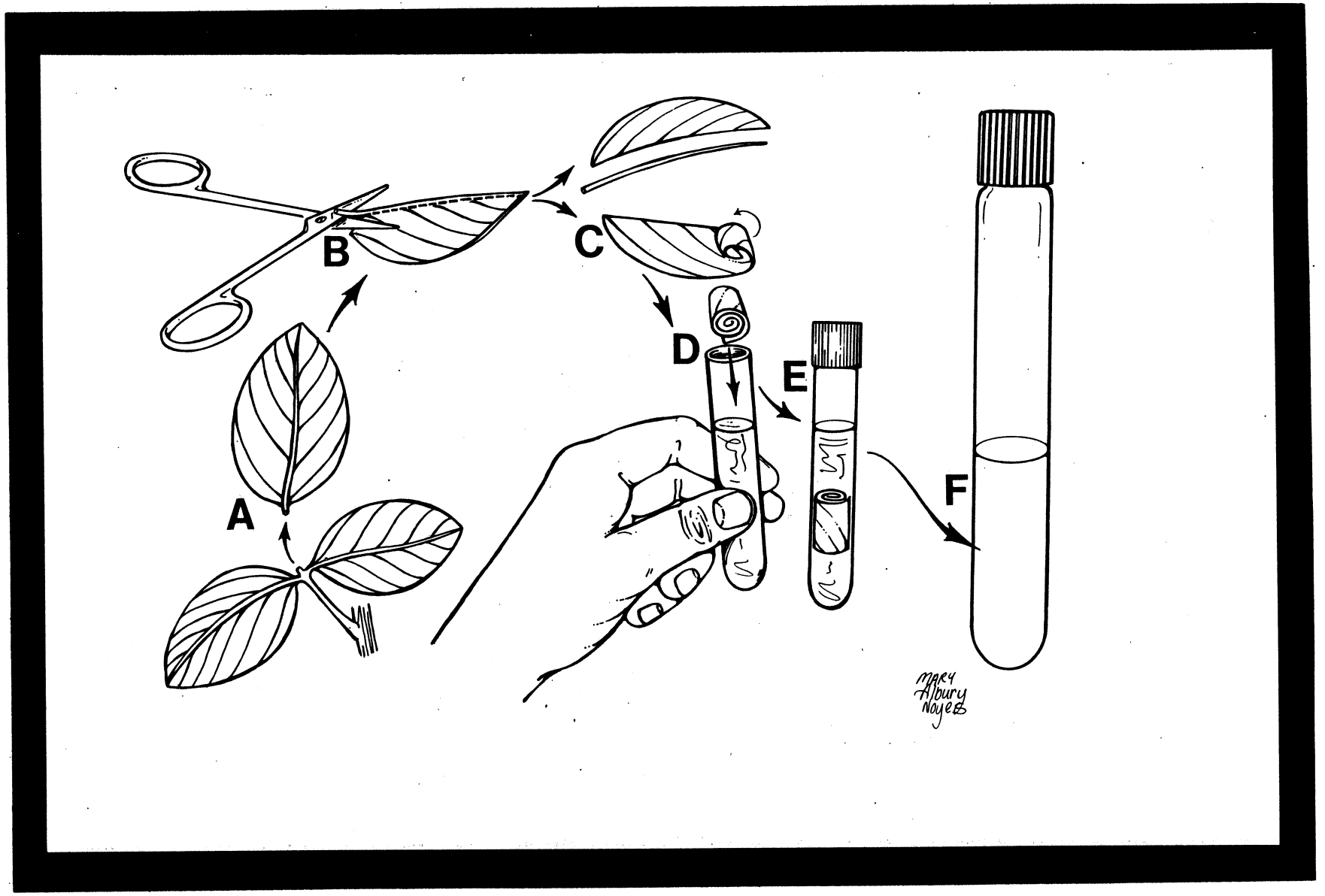

A
B
C
Mary Albury Noyes

Figure 2. Stepwise procedure for collecting leaf samples and extracting chlorophyll in the field experiments. Leaf samples consisted of the right half of the terminal leaflet (A) of the bottom and top fully expanded trifoliolate leaf, excluding the midvein (B), ten days after injury. Leaf samples were rolled (C) to fit into an extraction tube containing 3.5 ml of 95% ethanol (D), capped, and kept in darkness for 24 hours (E), then decanted into a collection tube (F) (modified slightly from Knudson et al. 1977). Samples were rinsed with 2.5 ml of ethanol and decanted into the same collection tube. The extraction tube with the leaf material was filled with 3.5 ml of ethanol and kept in darkness, along with the collection tube, for 24 hours. The procedure was repeated two more times for a total volume of 18 ml in the collection tube (see Materials and Methods).


A
B
C
D
E
F
Mary Albury Noyes

future studies on protein and potassium.

The general procedures for chlorophyll extraction in the SOD and greenhouse experiments were very similar to those published elsewhere (Knudson et al. 1977, Koukkari et al. 1984). Leaf discs from SOD and greenhouse experiments were placed in 12 x 75 mm plastic extraction tubes containing 4 ml of 95% ethanol, capped, and maintained in darkness for ca. 24 hours. After 24 hours the liquid, excluding the leaf material, was decanted in a 20 x 150 mm glass collection tube. The leaf discs were then rinsed with 2 ml of 95 % ethanol, and this was decanted into the same collection tube. The extraction tubes with the leaf discs were refilled wth 4 ml of 95% ethanol and kept in darkness, along with the collection tube, for 24 hours. This procedure was repeated two more times for a combined volume of 18 ml in the collection tube.

Briefly, the procedures for collecting leaf samples and extracting chlorophyll in the field experiments are illustrated in Figure 2, A-F). Field leaf samples were rolled to fit into the extraction tube (Figure 2, C and D). Because of the larger sample size, the chlorophyll extraction procedure was modified to use 3.5 ml of ethanol for each of three extractions, 2.5 ml rinses, for a total volume of 18 ml. Following the final decanting procedure, the remaining leaf material was dried at 70 degrees C for 3 days and dry weight in mg was determined. The absorbances of the chlorophyll extracts for all experiments were measured using a spectrophotometer (Beckman, model 25) at 649 nm and 665 nm. A combined equation was used to express total chlorophyll as µg/mg dry weight of tissue (Wintermans and Demots, 1965).

RESULTS

Chlorophyll Levels in Relation to Stage of Development

The chlorophyll levels in the leaves of soybean plants were related to the age or stage of development (Figure 3, Table 2) and to the extent of injury (defoliation) applied to the plant (Tables 3,4). In young vegetative plants (V_3) the unifoliolate leaves had a chlorophyll content of 48.0 ± 9.7 µg/mg dry weight (d.w.) compared to a level of 33.72 ± 3.6 µg/mg d.w. in the first trifoliolate leaf. A similar difference existed between the unifoliolate leaves and upper trifoliolate leaves in the early reproductive stage (R_1). As the plant matured, the chlorophyll levels in the unifoliolate leaves decreased. This decrease was very pronounced between the R_1 stage (41.34 ± 6.7 µg) and the R_2 stage 23.12 ± 7.99 µg). Trifoliolate leaf chlorophyll within each stage showed a gradual increase up to the two youngest nodes with a slightly lower value for the youngest node. Chlorophyll levels varied in the individual nodes across the stages as well (Figure 3). For example, in the second trifoliolate leaf, chlorophyll increased from the V_3 (26.46 ± 10.29 µg) to R_1 stage (37.03 ± 4.49 µg) and then gradually decreased as the leaf matured. Similar differences in chlorophyll levels between unifoliolate and trifoliolate leaves for the R_2 stage were observed in the two greenhouse (Table 3) and two field experiments (Table 4).

Changes in Chlorophyll Levels Following Defoliation (Injury)

The chlorophyll levels of mildly and severely treated unifoliolate and bottom and top trifoliolate leaves differed from the

Figure 3. Total chlorophyll (μg/mg d.w.) levels in cotelydonary (c), unifoliolated (u), and trifoliolated (t) leaves of soybean plants ranging from early vegetative stages (V_3) to reproductive stages (R_1 to R_4). Each value represents the mean ± standard error of the mean at five replications.

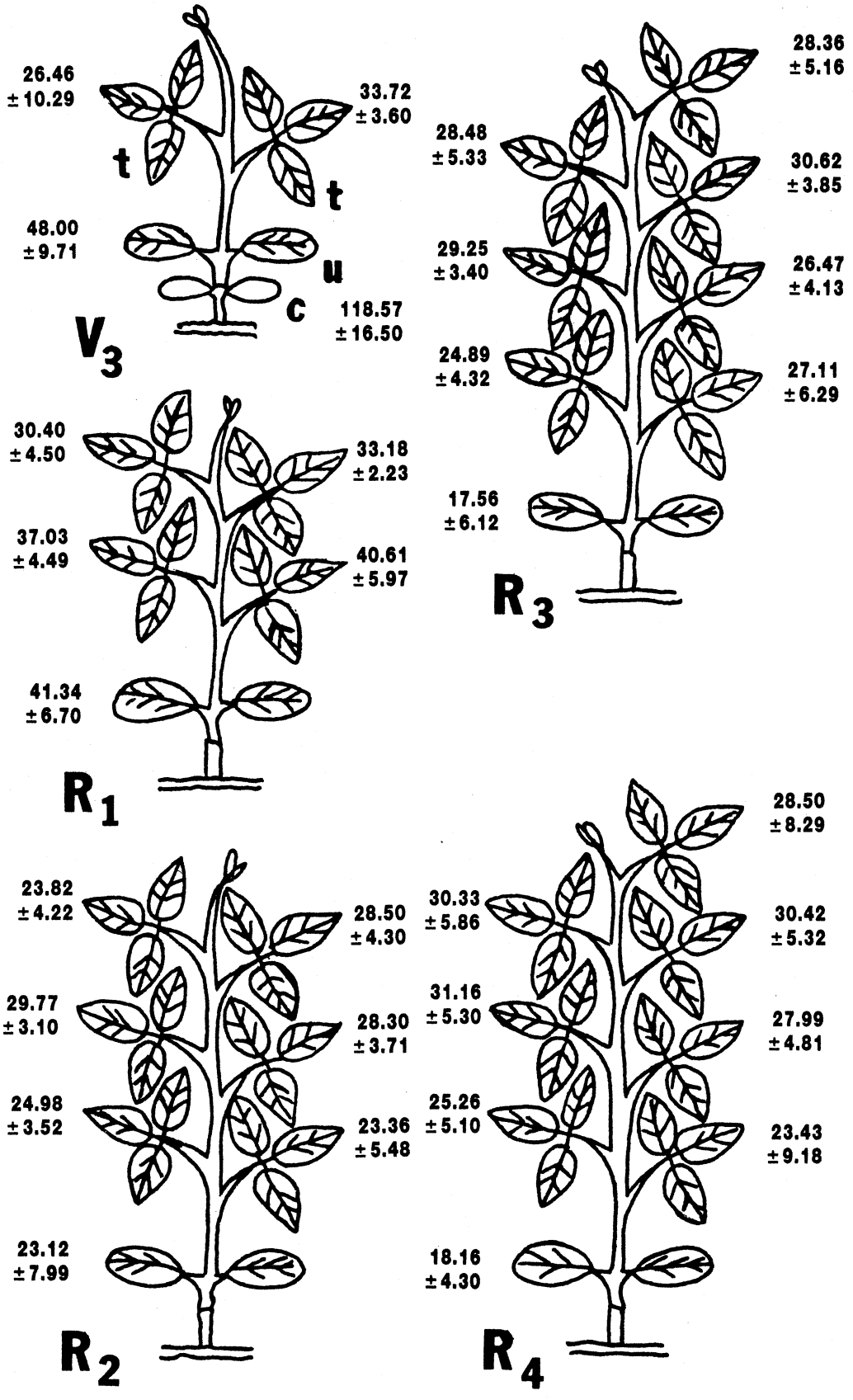

26.46
±10.29
33.72
±3.60
t
t
48.00
±9.71
u
c
118.57
±16.50
V3
28.36
±5.16
28.48
±5.33
30.62
±3.85
29.25
±3.40
26.47
±4.13
24.89
±4.32
27.11
±6.29
17.56
±6.12
R3
30.40
±4.50
33.18
±2.23
37.03
±4.49
40.61
±5.97
41.34
±6.70
R1
28.50
±8.29
30.33
±5.86
30.42
±5.32
31.16
±5.30
27.99
±4.81
25.26
±5.10
23.43
±9.18
18.16
±4.30
R4
23.82
±4.22
28.50
±4.30
29.77
±3.10
28.30
±3.71
24.98
±3.52
23.36
±5.48
23.12
±7.99
R2

Table 2. Total chlorophyll (μg/mg d.w.) levels of cotyledonary, unifoliolated, and trifoliolated leaves of soybean plants ranging from early vegetative stages (V_3) to reproductive stages (R_1 to R_4). Each value represents the mean ± standard error of the mean at five replications (refer to Figure 3).

Stage-of-Development Experiment

Leaf	Stage Number V_3	R_1	R_2	R_3	R_4
Cotyledon	118.57±16.50	---	---	---	---
Unifoliolate	48.00 ± 9.71	41.34±6.70	23.12±7.99	17.56±6.12	18.16±4.30
1st Trifoliolate	33.72 ± 3.60	40.61±5.97	23.36±5.48	27.11±6.29	23.43±9.18
2nd Trifoliolate	26.46±10.29	37.03±4.49	24.98±3.53	24.89±4.32	25.26±5.10
3rd Trifoliolate	---	33.18±2.23	28.30±3.71	26.47±4.13	27.99±4.81
4th Trifoliolate	---	30.40±4.50	29.77±3.10	29.25±3.40	31.16±5.30
5th Trifoliolate	---	---	28.50±4.30	30.62±3.85	30.42±5.32
6th Trifoliolate	---	---	23.82±4.88	28.48±5.33	30.33±5.86
7th Trifoliolate	---	---	---	28.36±5.16	28.50±8.29

Table 3. Total chlorophyll (μg/mg d.w.) levels of unifoliolate and trifoliolate soybean, Glycine max (L.) Merr., leaves in two greenhouse experiments (A and B). Leaf samples consisted of either one half of a middle leaflet from a trifoliolate leaf or one half of the unifoliolate leaf (Figure 1). Samples harvested ten days following the excision of two (mild) or all three (severe) leaflets from all expanded leaves (2-3) above the oldest trifoliolate leaf of plants in R_2 stage of development. Each value represents the mean ± standard error of the mean at 20 replications.

Experiment A	Injury		
Leaf	None	Mild	Severe
Top Trifoliolate	8.67±2.33	9.61±2.89*	11.31±3.33*
Bottom Trifoliolate	6.08±1.12	7.12±1.63*	8.99±2.00*
Unifoliolate	5.28±0.97	5.83±1.44	6.77±1.57

*significantly different from control (uninjured) (P-value≤.05).

Experiment B	Injury		
Leaf	None	Mild	Severe
Top Trifoliolate	20.40±2.61	21.38±3.00	21.17±3.30
Bottom Trifoliolate	12.35±2.70	13.28±2.30	14.06±2.63
Unifoliolate	9.17±3.88	11.34±2.86	11.76±4.44

*significantly different from control (uninjured) (P-value≤.05).

Table 4. Total chlorophyll (µg/mg d.w.) levels of unifoliolate and trifoliolate soybean leaves from field experiments conducted in 1982 (A) and 1983 (B). Leaf samples consisted of either one half of a middle leaflet from a trifoliolate leaf or one half of the unifoliolate leaf (Figure 1). Samples harvested ten days following excision of two (mild) or all three (severe) leaflets from all expanded leaves (5-6) above the oldest trifoliolate leaf of plants in R_2 stage of development. Each value represents the mean ± standard error of the mean at 48 replications.

Experiment A (drought conditions)

Leaf	Injury: None	Mild	Severe
Top Trifoliolate	7.99 ± 1.79	8.50 ± 3.54	12.35 ± 1.47**
Bottom Trifoliolate	7.44 ± 1.72	10.95 ± 5.98*	11.22 ± 2.76*
Unifoliolate	---	---	---

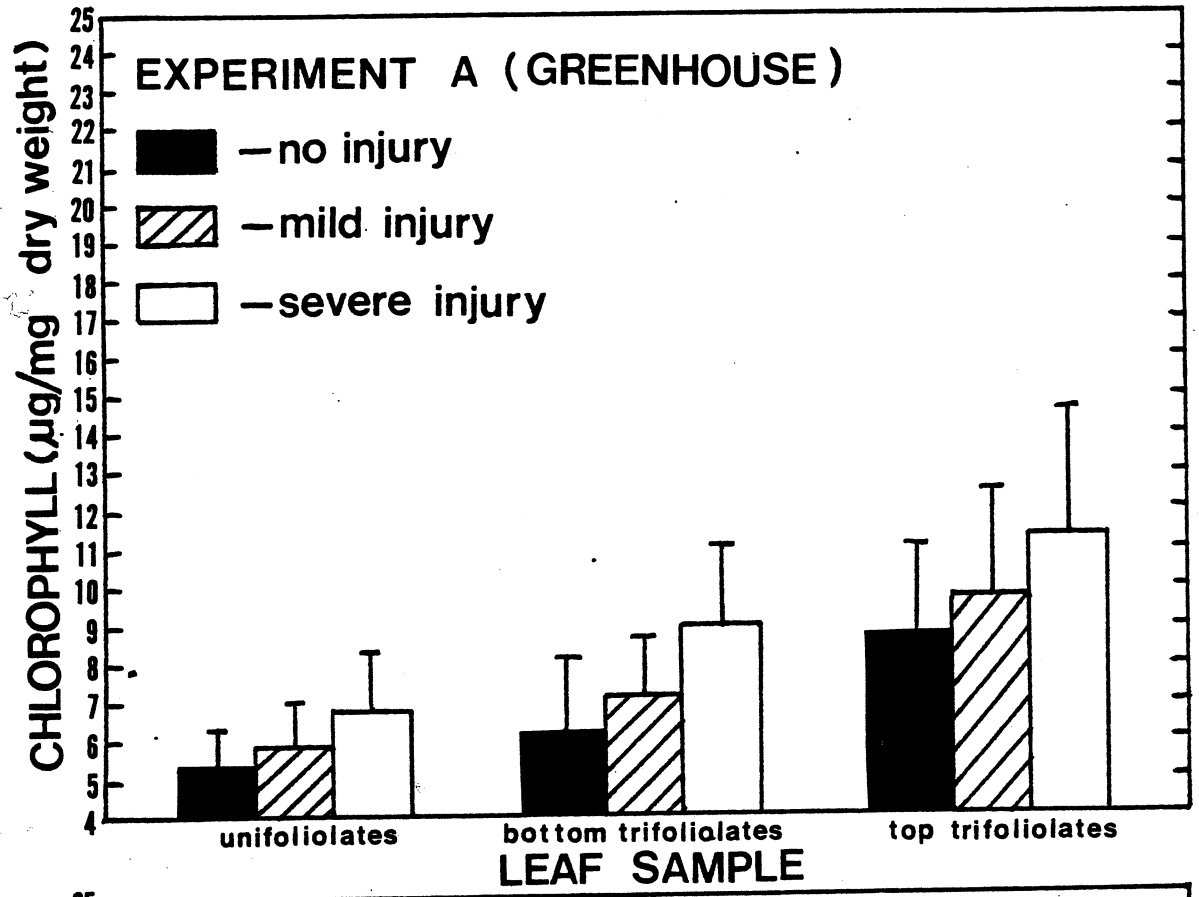
* significantly different from control (uninjured) (P-value ≤ 0.5).
** significantly different from mild injury (P-value ≤ 0.5).

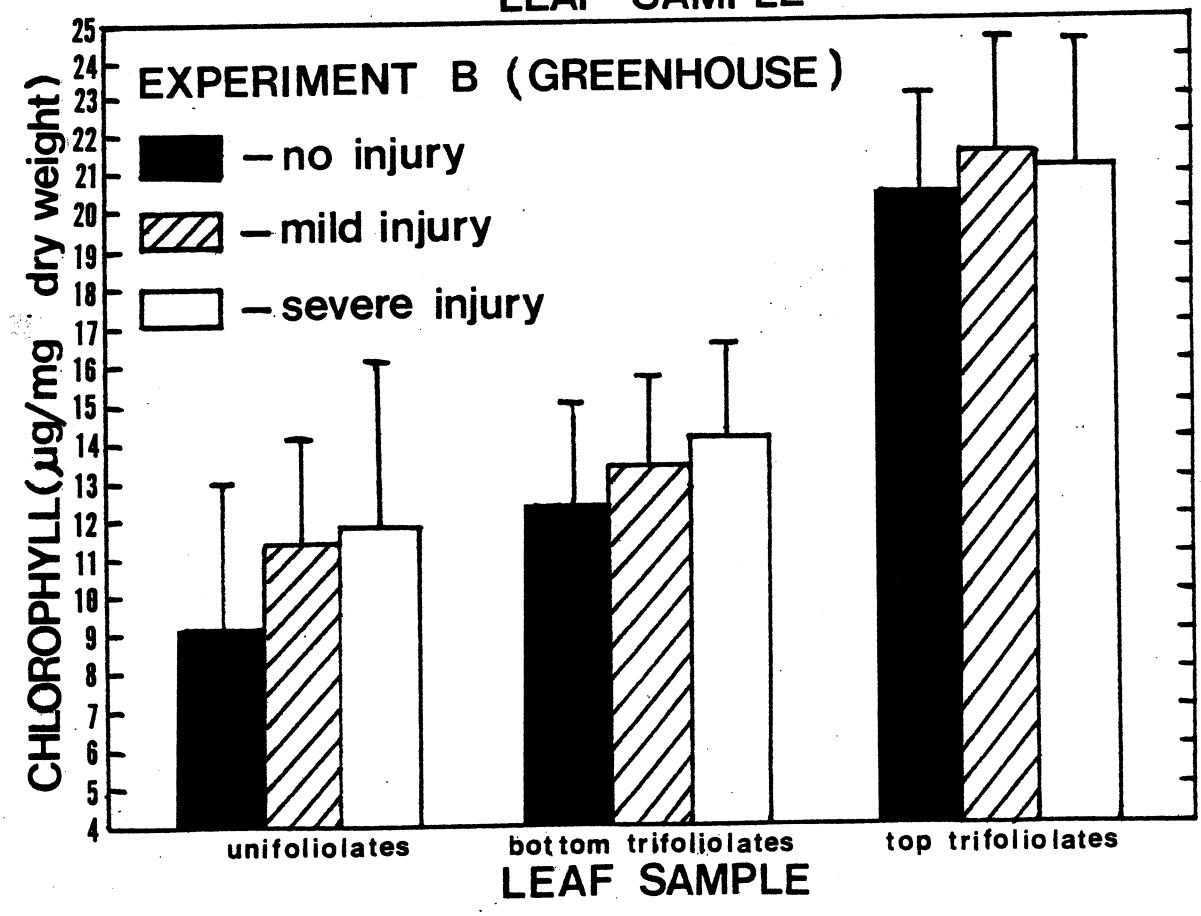
Experiment B (irrigated)

Leaf	Injury: None	Mild	Severe
Top Trifoliolate	14.81 ± 3.73	13.97 ± 3.79	12.95 ± 2.70*
Bottom Trifoliolate	13.5 ± 4.22	11.62 ± 3.91*	10.58 ± 4.62*
Unifoliolate	3.56 ± 3.54	4.31 ± 1.97	5.97 ± 1.44**

* significantly different from control (uninjured) (P-value ≤ .05).
** significantly different from mild injury (P-value ≤ .05).

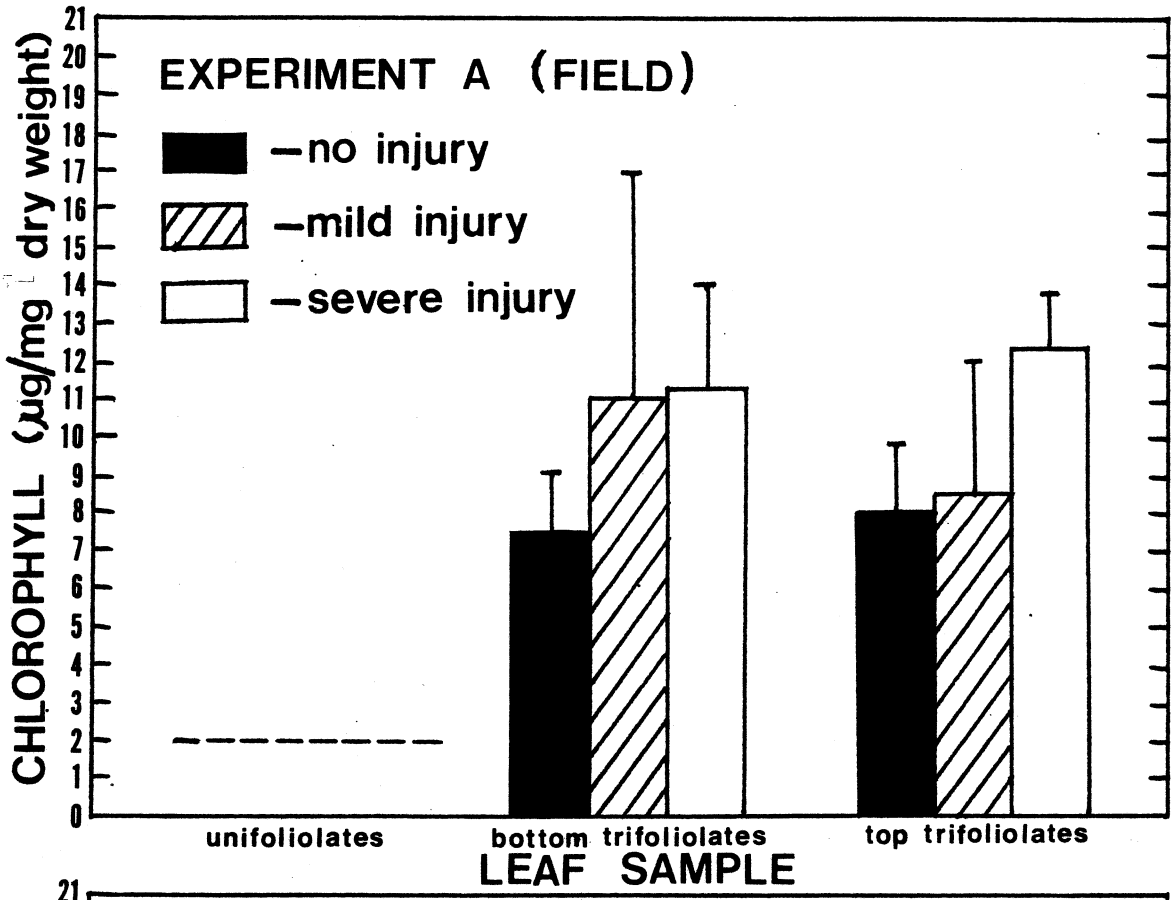
Figure 4. Effects of mild and severe injury (defoliation) on chlorophyll levels (µg/mg d.w.) in unifoliolate, bottom trifoliolate, and top trifoliolate soybean leaves. Each box represents a separate greenhouse experiment (A and B). Values represent the mean ± the standard error of the mean at 20 replications (refer to Table 3 for actual values).

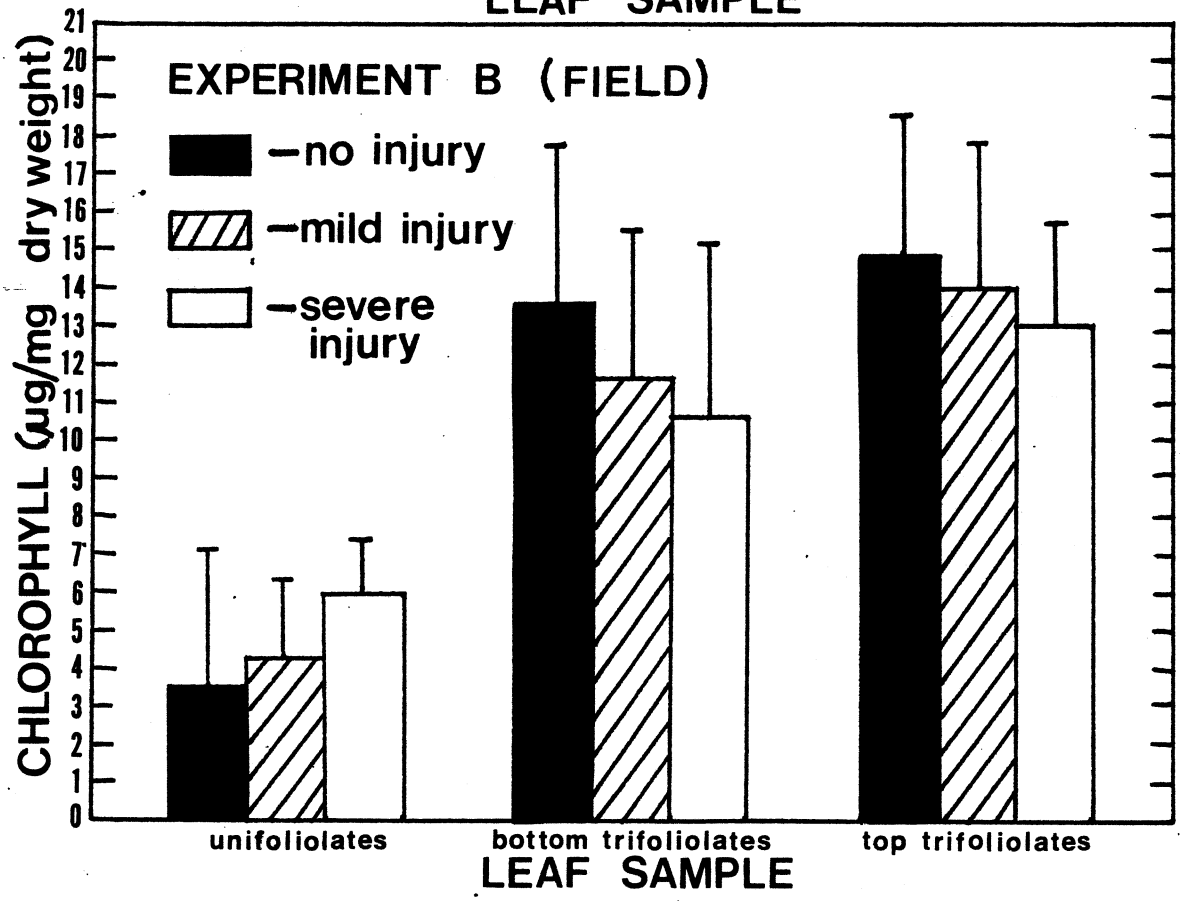

EXPERIMENT A (GREENHOUSE)
—no injury
—mild injury
—severe injury
CHLOROPHYLL (µg/mg dry weight)
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
unifoliolates
bottom trifoliolates
top trifoliolates
LEAF SAMPLE


EXPERIMENT B (GREENHOUSE)
—no injury
—mild injury
—severe injury
CHLOROPHYLL (µg/mg dry weight)
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
unifoliolates
bottom trifoliolates
top trifoliolates
LEAF SAMPLE

control (uninjured) in all experiments. In the greenhouse experiments (A and B) there was an increase in chlorophyll upon defoliation (injury) (Table 3, Figure 4). For example, the chlorophyll levels of the control (uninjured) top trifoliolates in experiment A (8.67 ± 2.33 µg) were significantly lower than those of both mildly (9.61 ± 2.89 µg) and severely injured (11.31 ± 3.33 µg) plants. These trends were also evident in field experiment A and experiment B unifoliolates (Table 4, Figure 5). Lower chlorophyll levels occurred in the bottom and top trifoliolates with increasing defoliation or injury. Within the treatments themselves, trifoliolate leaf chlorophyll increased from the unifoliolates as the leaves matured, similar to that observed in the control (uninjured) plants.

Figure 5. Effects of mild and severe injury (defoliation) on chlorophyll levels (µg/mg d.w.) in unifoliolate, bottom trifoliolate, and top trifoliolate soybean leaves. Each box represents a separate experiment (A and B) conducted in the field. Values represent the mean ± the standard error of the mean of 48 replications (refer to Table 4 for actual values).


EXPERIMENT A (FIELD)
—no injury
—mild injury
—severe injury
CHLOROPHYLL (µg/mg dry weight)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
unifoliolates
bottom trifoliolates
top trifoliolates
LEAF SAMPLE


EXPERIMENT B (FIELD)
—no injury
—mild injury
—severe injury
CHLOROPHYLL (µg/mg dry weight)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
unifoliolates
bottom trifoliolates
top trifoliolates
LEAF SAMPLE

DISCUSSION

In the SOD experiment, cotyledons showed high chlorophyll levels following seedling emergence, then decreasing levels as new growing unifoliolate leaves emerged (Table 2, Figure 3). When trifoliolate leaves emerged, unifoliolate leaves exhibited decreases in chlorophyll levels. Similarily, as new trifoliate leaves emerged, older trifoliolates showed decreases in chlorophyll levels. This progressive pattern continued as new leaves were produced by the apex. These findings support those of other authors suggesting declines in chlorophyll levels as well as other leaf constituents and processes to be among the earliest signs of soybean leaf senescence (Leopold 1961, Noodén 1984). Lower chlorophyll levels are reflected in the progressive pattern of senescence of older organs (leaves) as compared to younger parts of the soybean plant (Leopold 1961, Koukkari et al. 1984).

Injury (defoliation) to a portion of a plant may very well affect chlorophyll levels in other portions of the plant. Unifoliolate and trifoliolate leaves of control (uninjured) plants exhibited chlorophyll decreases as the plants matured (Table 2, Figure 3). The highest chlorophyll levels existed in leaves on the younger portions of the plant. This can be seen in the control (uninjured) plants in Tables 3 and 4 as well. Upon defoliation, however, chlorophyll levels of injured plants were higher as compared to the control (uninjured) plants in both the greenhouse (Table 3, Figure 4) and field (Table 4, Figure 5) experiments. Chlorophyll levels increased as the severity of injury increased in field experiment A top trifoliolates and unifoliolates of experiment B, in particular. These findings are in

basic agreement with those of Noodén (1980b) and Koukkari et al. (1984). Noodén (1980b) found that decapitation (removal of younger leaves and apex) can prevent chlorophyll loss in older leaves and can even rejuvenate them. Koukkari et al. (1984) showed that mild and severe injury by partial defoliation to leaves of Helianthus and Albutilon plants increased the total chlorophyll of the cotyledons, with the highest chlorophyll in the cotyledons of plants injured severely.

Environmental stress conditions (other than injury by defoliation) can be expected to alter senescence processes including chlorophyll content (Samet and Sinclair 1980, Noodén and Thompson 1984). Fluctuating environmental factors such as moisture, wind, and soil-borne organisms, may account for the decreased chlorophyll levels on the average in the greenhouse (Table 3) and field (Table 4) experiments in comparison to the controlled environmental conditions in the SOD experiment (Table 2). In the 1982 (A) field experiment plants, drought conditions may have affected root physiology, resulting in a decreased level of cytokinins in the xylem sap, as well as nutrient deficiencies resulting in chlorophyll loss (Richmond and Lang 1957, Burrows and Carr 1969, Noodén and Leopold 1978) as compared to 1983 (B) irrigated conditions.

Large differences existed in the standard errors of the mean in chlorophyll levels of unifoliolate and trifoliolate leaves within and between experiments. Large standard errors were evident in the SOD experiment (Table 2) compared to greenhouse (Table 3) and field (Table 4) experiments. These differences may have been due to the variation in the chlorophyll sampling methods (refer to materials and methods).

Because the SOD experiment involved the use of discs, the variability in weighing the samples in mg may have contributed to a greater possibility of error. Secondly, the replication size varied between each experiment. The SOD experiment involved five replications, whereas the greenhouse and field experiments used 20 and 48 replications, respectively. Because statistical calculation of the standard error of the mean includes division by the square root of the sample size, the higher the number of replications, the less chance of error if the sample is representative of the population (Snedecor and Cochran 1967). The purpose of this paper is to reach appropriate conclusions about each population despite sample variation.

Senescence involves decreases in chlorophyll levels and this loss has been selected as a criterion for measuring senescence. It should not, however, be the only acceptable measure. The use of only one parameter to the exclusion of others would be inappropriate, since senescence almost certainly involves a complex of may constituents and processes (Nooden 1980b). Any of several other parameters indicative of senescence, such as protein levels, nucleic acid levels (RNA an DNA), nitrogen content, stomatal resistance, or photosynthesis, may also serve as measures of senescence. For example, loss of protein in leaves has been found to correlate with the loss in chlorophyll (Nooden and Leopold 1978). Chlorophyll loss is still, however, one of the most visible indicators of senescence in soybeans, and is a useful measure if cross-checked against other parameters when determining the effects of defoliation (injury) on soybean plants (Lindoo and Nooden 1976, Nooden 1984).

It may be of both practical and theoretical interest to study the

effects of defoliation on chlorophyll levels in relation to final yield. Studies have shown that partial defoliation of soybeans and the effect on final yield is a function of the stage of plant development when defoliation occurs. A specific percentage of defoliation before flowering has less effect on yield than the same level of defoliation during pod and seed development (Fehr et al. 1981). Severe defoliation (80%) in the R_2 stage of development has reduced seed yield compared to mild defoliation (40%), which increased seed yield (McAlister and Krober 1959). It may be possible to correlate chlorophyll increases with yield increases, or chlorophyll decreases with a given reduction in yield. Estimations of the yield may be directly proportional to changes in chlorophyll upon partial defoliation. A practical approach for evaluating hail damage, in which most losses to the plant do occur from defoliation, may allow for estimations of total crop damage and final yield production (Teigen and Vorst 1975) in relation to chlorophyll levels. The prevention of senescence through application of plant growth regulators (i.e. chemicals) or surgical and genetic modifications, may also eventually open a way to yield improvements (Noodén et al. 1979).

REFERENCES

Back, A. and A. E. Richmond. 1971. Interrelations between gibberellic acid, cytokinins and abscisic acid in retarding leaf senescence. Physiol. Plant. 24: 76-79.

Binnie, R. C. and P. E. Clifford. 1980. Effects of some defoliation and decapitation treatments on the productivity of French beans. Ann. Bot. 46: 811-813.

Burrows, W. J. and D. J. Carr. 1969. Effects of flooding on the root system of sunflower plants on cytokinin content in the xylem sap. Physiol. Plant. 22: 1105-1112.

Carmi, A. and D. Koller. 1978. Effects of the roots on the role of photosynthesis in primary leaves of bean (Phaseolus vulgaris L.). Photosynthetica 12: 178-184.

Carmi, A. and D. Koller. 1979. Regulation of photosynthetic activity in the primary leaves of bean (Phaseolus vulgaris L.) by materials moving in the water conducting system. Plant. Physiol. 64: 285-288.

Chibnall, A. C. 1939. Protein Metabolism in the Plant. Yale University Press, New Haven.

Denholm, J. V. 1975. Necessary condition for maximum yield in a senescing two-phase plant. J. Theor. Biol. 52: 251-254.

Devlin, R. M. and F. H. Witham. 1983. Functions of essential mineral elements and symptoms of mineral deficiency. Pages 139-153 in Plant Physiology. William Grant Press, Boston, MA.

Engelbrecht, L. and K. Conrad. 1961. Vergleichende untersuchungen zur wirkung von kinetin und auxin. Ber. Dtsch. Bot. Ges. 74: 42-46.

Fehr, W. R. and C. E. Caviness. 1977. Stages of soybean development. Iowa Agric. Home Econ. Exp. Stn., Iowa Coop, Ext. Serv. Rep. 80.

Fehr, W. R., B. K. Lawrence, and T. A. Thompson. 1981. Critical stages of development for defoliation of soybean. Crop Science. 21: 259-262.

Garrison, F. R., A. M. Brinker, and L. D. Noodén. 1984. Relative activities of xylem-supplied cytokinins in retarding leaf senescence and sustaining pod development. Plant Cell Physiol. 25: 213-224.

Hodgkinson, K. C. 1974. Influence of partial defoliation on photosynthesis, photorespiration and transpiration by Lucerne leaves at different ages. Aust. J. Plant Physiol. 1: 561-578.

Hodgkinson, K. C., N. G. Smith, and G. E. Miles. 1972. The photosynthetic capacity of stubble leaves and their contribution to growth of the Lucerne plant after high level cutting. Aust. J. Agric. Res. 23: 225-238.

Katagiri, K. and H. Tsuji. 1980. Increases in DNA content of primary leaves of Phaseolus vulgaris upon decapitation. J. Exp. Bot. 31: 209-222.

Knudson, L. L., T. W. Tibbits, and G. E. Edwards. 1977. Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol. 60: 606-608.

Koukkari, W. L., M. A. Johnson, C. L. Gilson, S. B. Warde and B. W. Kennedy. 1984. Versatility of a cotyledon chlorophyll assay in measuring the effects of injury. Chronobiologia 11: 149.

Leopold, A. C. 1961. Senescence in plant development. Science 134: 1727-1732.

Leopold, A. C. and L. D. Noodén. 1984. Hormonal regulatory systems in plants. Pages 4-21 in T. K. Scott, ed. Encyclopedia of Plant Physiology, Vol. 10: Hormonal Regulation of Plant Development II. Springer-Verlag, New York.

Letham, D. S. 1978. Cytokinins. Pages 205-263 in P. S. Letham, P. B. Goodwin, and T. J. V. Higgins, eds. Phytohormones and Related Compounds - A Comprehensive Treatise. Vol. 1. Elsevier/North Holland Biomedical Press, Amsterdam.

Lindoo, S. J. and L. D. Noodén. 1976. The interrelation of fruit development and leaf senescence in Anoka soybeans. Bot. Gaz. 137: 218-223.

Lindoo, S. J. and L. D. Noodén. 1978. Correlation of cytokinins and abscisic acid with monocarpic senescence in soybeans. Plant Cell Physiol. (19) 6: 997-1006.

McAlister, D. F. and A. O. Krober. 1959. Response of soybeans to leaf and pod removal. Agron. J. 67: 674-677.

Meidner, H. 1970. Effects of photoperiodic induction and debudding on Xanthium pennsylvanicum and of partial defoliation in Phaseolus vulgaris on rates of net photosynthesis and stomatal conductances. J. Exp. Bot. 21: 164-169.

Molisch, H. 1938. The Longevity of Plants (H. Fulling, Transl.). Science Press, Lancaster, PA.

Neumann, P. M., A. T. and L. D. Noodén. 1983. Characterization of leaf senescence and pod development in soybean explants. Plant Physiol. 72: 182-185.

Noodén, L .D. 1980a. Regulation of senescence. Pages 139-152 in F. T. Corbin, ed. World Soybean Research Conference II: Proceedings. Westview Press, Boulder, CO.

Noodén, L. D. 1980b. Senescence in the whole plant. Pages 219-258 in K. V. Thimann, ed. Senescence in Plants. CRC Press, Boca Raton, FL.

Noodén, L. D. 1984. Integration of soybean pod development and monocarpic senescence. A minireview. Physiol. Plant. 62: 273-284.

Noodén, L. D., G. M. Kahanak and Y. Okatan. 1979. Prevention of monocarpic senescence in soybeans with auxin and cytokinin: an antidote for self-destruction. Science. 206: 841-843.

Noodén, L. D. and A. C. Leopold. 1978. Phytohormones and the endogenous regulation of senescence and abscission. Pages 329-369 in D. S. Letham, P. B. Goodwin and T. J. V. Higgins, eds. Phytohormones and Related Compounds. Vol. 2. Elsevier/North Holland Biomedical Press, Amsterdam.

Noodén, L. D. and J. E. Thompson. 1984. Aging and senescence in plants. Pages 105-127 in C. E. Finch and E. Schneider, eds. Handbook of Aging. 2d ed. Van Nostrand Reinhold Co., Inc., New York.

Oritani, T. and R. Yoshida. 1973. Studies on nitrogen metabolism in crop plants. XIII. Cytokinins and abscisic acid-like substances levels in rice and soybean leaves during their growth and senescence. Proc. Crop Sci. Soc. Jpn. 42: 280-287.

Osborne, D. J. 1959. Identity of the abscission-accelerating substance in senescent leaves. Nature 183: 1593.

Osborne, D. J. and H. M. Hallway. 1960. Auxin control of protein levels in detached autumn leaves. Nature 188: 240-241.

Richmond, A. E. and A. Lang. 1957. Effect of kinetin on protein content and survival of detached Xanthium leaves. Science 125: 650-651.

Samet, J. S. and T. R. Sinclair. 1980. Leaf senescence and abscisic acid in leaves of field-grown soybean. Plant Physiol. 66: 1164-1168.

Sexton, R. and H. W. Woolhouse. 1984. Senescence and abscission. Pages 469-497 in M. B. Wilkins, ed. Advanced Plant Physiology. Pitman Publishing Ltd., London.

Sitton, D., Itai, and H. Kende. 1967. Decreased cytokinin production in the roots as a factor in shoot senescence. Planta 73: 296-300.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods Applied to Experimenting in Agriculture and Biology. 6th ed. Iowa State College Press, Ames, IA. 593pp.

Teigen, J. B. and J. J. Vorst. 1975. Soybean response to stand reduction and defoliation. Agron. J. 67: 813-816.

Thimann, K. V. 1980. The senescence of leaves. Pages 85-115 in K. V. Thimann, ed. Senescence in Plants. CRC Press, Boca Raton, FL.

Wareing, P. F., M. M. Khalifa and K. J. Treharne. 1968. Rate limiting processes in photosynthesis at saturating light intensities. Nature 220: 453-457.

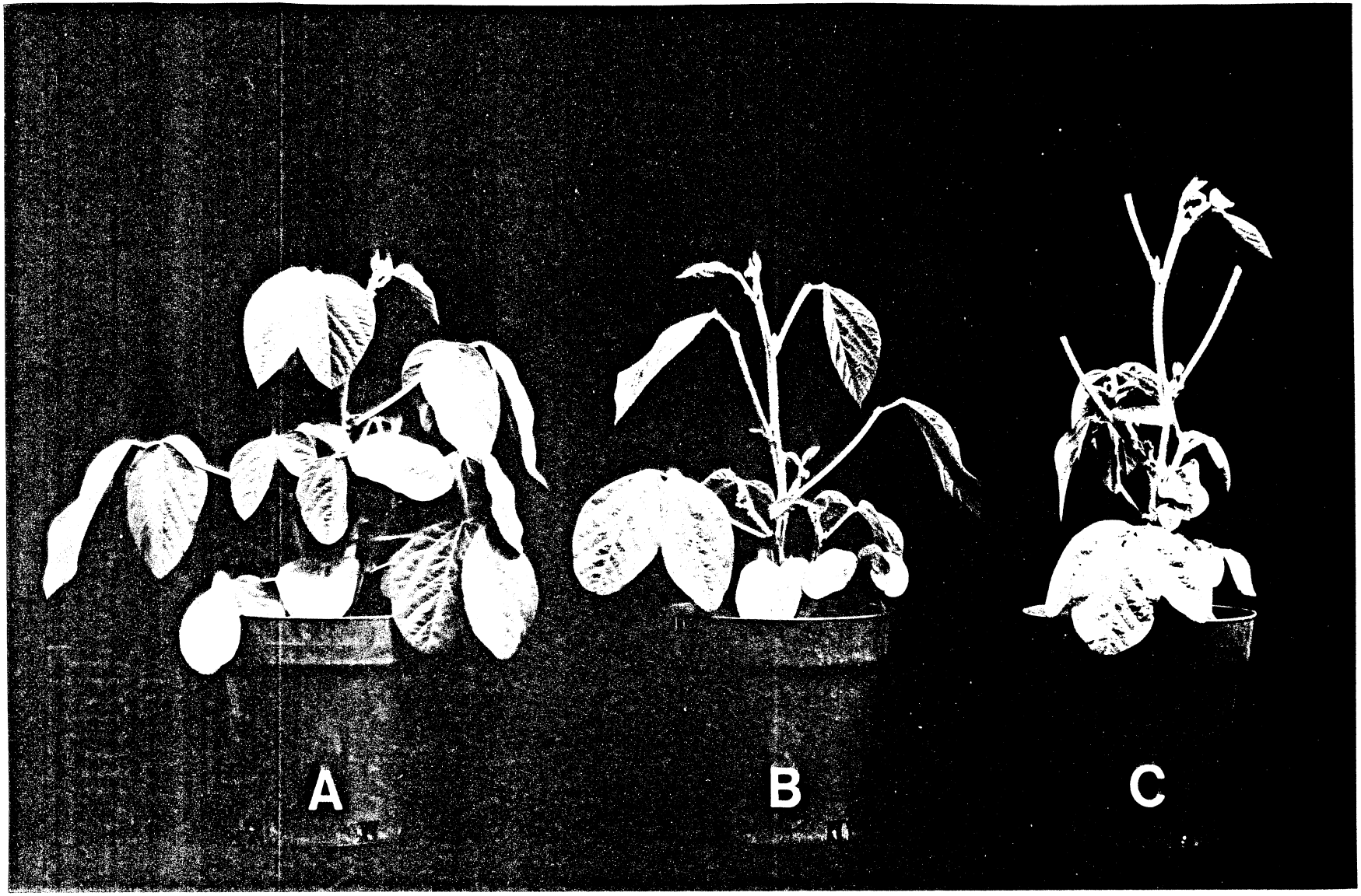
Wintermans, J. F. G. M. and A. De Mots. 1965. Spectrophotometric characteristics of chlorophylls a and b and their pheophytins in ethanol. Biochem. Biophys. Acta 109: 448-453.

Witham, F. H., and C. O. Miller. 1965. Biological properties of a kinetin-like substance occurring in Zea Mays. Plant Physiol. 18: 1007.

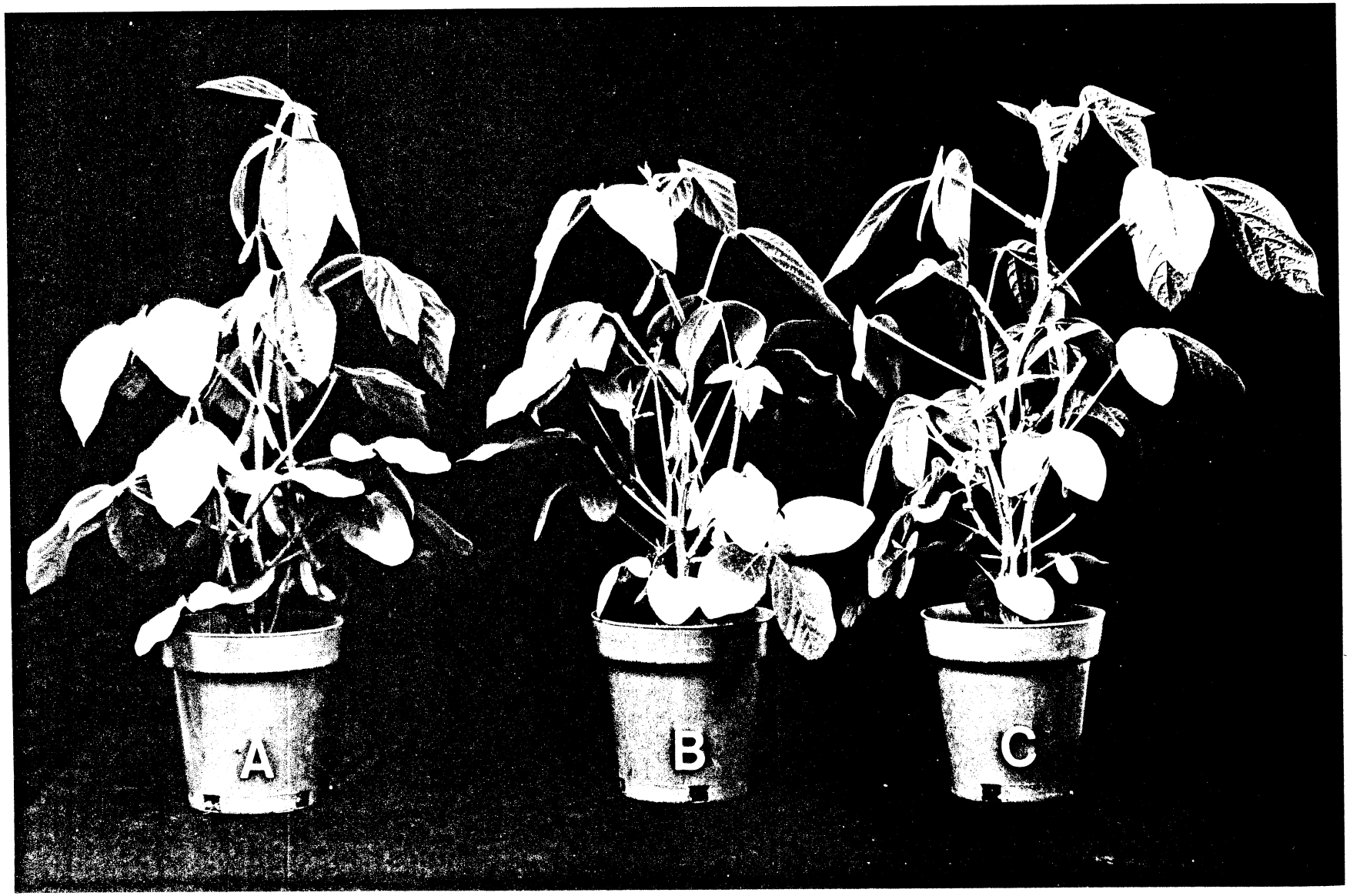
Van Staden, J. and A. Carmi. 1982. The effects of decapitation on the distribution of cytokinins and growth of Phaseolus vulgaris plants. Physiol. Plant. 55: 39-44.

Van Staden, J. and J. E. Davey. 1979. The synthesis, transport and metabolism of endogenous cytokinins. Plant and Cell Environment 2: 93-116.

Appendix Figure 1. Photograph of a control (uninjured) soybean, Glycine max (L.) Merr. (cv. Evans) plant (A) in comparison to plants subjected to mild (B) or severe (C) defoliation. All three plants were five weeks of age.


A
B
C

Appendix Figure 2. Photograph of three soybean plants, control (uninjured) (A), mildly injured (B), and severely injured (C), illustrating the ability of the plants to compensate for defoliation ten days following injury (defoliation) (Appendix Figure 1).


A
B
C