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Response of Soybean Growth and Dinitrogen-Fixing Ability to Insect-Induced Defoliation.

Maurice Blake Layton Jr
Louisiana State University and Agricultural & Mechanical College

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Response of soybean growth and dinitrogen-fixing ability to insect-induced defoliation

Layton, Maurice Blake, Jr., Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1988
RESPONSE OF SOYBEAN GROWTH AND DINITROGEN-FIXING ABILITY TO INSECT-INDUCED DEFOLIATION

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University
Agricultural and Mechanical College
in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Entomology

by

Maurice Blake Layton, Jr.
B.S., Mississippi State University, 1977
M.S., Louisiana State University, 1983
May 1988
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A series of greenhouse experiments was conducted to investigate the effects of insect-induced defoliation on growth and \( N_2 \)-fixing ability of soybean. This research had three major objectives: 1) to examine the effects of different levels of defoliation, 2) to determine the impact of progressive defoliation, and 3) to study the ability of soybeans to recover following defoliation. All experiments were conducted in a similar manner. Larvae of the soybean looper, *Pseudoplusia includens* (Walker), were used to defoliate soybeans in the vegetative stage of development and the acetylene-ethylene reduction assay was used to provide an indirect measure of \( N_2 \)-fixing ability of defoliated plants relative to control plants.

When soybeans were subjected to varying levels of defoliation (0 to 85%), \( N_2 \)-fixing ability and total nodule dry weight decreased linearly as defoliation increased. Nodule specific activity (NSA), a measure of \( N_2 \)-fixing ability per gram of dry nodule weight, was not affected by levels of defoliation less than 47%, but decreased sharply as defoliation exceeded ca. 47%. Stem and root dry weight also decreased linearly as defoliation increased.

In soybeans that were sampled during progressive defoliation, NSA was significantly reduced as early as 10 days post-infestation (DPI) at which time defoliation had
reached 37%. This decrease in NSA resulted in a significant decrease in $N_2$-fixing ability. By 14 and 16 DPI (62 & 73% defoliation), there was a significant decrease in total nodule dry weight which exacerbated the continued decrease in NSA, causing even greater loss of $N_2$-fixing ability.

Soybeans that were defoliated ca. 70% were able to recover their $N_2$-fixing ability as early as three weeks post-defoliation (WPD). Recovery of $N_2$-fixing ability was due to full recovery of NSA and partial recovery of total nodule dry weight. Leaf area, and stem and root dry weight of defoliated plants were significantly less than those of controls for as long as 5 WPD, at which time the experiment was terminated. Therefore, defoliated plants recovered their $N_2$-fixing ability despite prolonged effects of defoliation on plant growth.
INTRODUCTION

Nitrogen is the most abundant element in the atmosphere; however, it is also, with the exception of water, the essential nutrient that most often limits plant growth. This is because higher plants are unable to utilize the diatomic N of the atmosphere and instead, must absorb N from the soil as \( \text{NO}_3^- \) or \( \text{NH}_4^+ \). Soil has an inherently low N content, and it is only by the conversion of atmospheric \( \text{N}_2 \) into forms that plants can utilize and the transfer of this N into the soil, that soils can accumulate and maintain enough N to support plant development (Tisdale & Nelson 1975).

Tisdale and Nelson (1975) list four ways in which atmospheric \( \text{N}_2 \) is converted, or fixed, into forms that plants can use. These are: 1) industrial fixation, 2) fixation by electrical discharge, 3) asymbiotic biological fixation, and 4) symbiotic biological fixation. In agricultural production, the most important of these are industrial fixation and symbiotic fixation.

Within the past century, agriculture has become increasingly more dependent on the use of industrially fixed N. Production of most non-legume crops relies heavily on the use of synthetic N fertilizers. While there is no doubt that the use of synthetic N fertilizers has greatly increased agricultural productivity, one must also realize that there are costs associated with the manufacture,
transport, and application of this N. Therefore, the monetary cost of synthetic N fertilizer is an important component of total production costs.

Legume crops form a mutualistic association with N₂-fixing bacteria that allows them to take advantage of biologically fixed N. Thus, most legumes are able to meet all of their N requirement via a combination of symbiotically fixed N, and N that is already present in the soil (Nutman 1965). Hardy and Havelka (1975) estimated that the annual production of symbiotically fixed N by grain legumes was equal to that produced industrially. Symbiotically fixed N replaces N that would otherwise have to be supplied by synthetically fixed N and therefore, has monetary value to the producer. Thus, the N produced by symbiotic fixation can be considered as a second component of yield (Newsom & Boethel 1986).

Soybean is the most important of the grain legumes; its seeds contain ca. 20% oil and 40% protein (Martin et al. 1976), and thus, it is an important source of two major food products. These products enter our diet directly, as components of a wide variety of foods, and indirectly, as animal protein that was produced using soy protein (Cowan 1973). Like other grain legumes, soybean is able to meet its high requirement for N via a combination of soil and symbiotically fixed N (deMooy et al. 1973).

The presence of combined N in the soil tends to inhibit symbiotic N₂ fixation in soybean (Harper 1976, Lie 1974,
Pate 1977, Tanner & Anderson 1964). Thus, soybeans grown in soil containing large amounts of combined N rely largely on this soil N for their N supply, while soybeans grown on N poor soils rely largely on symbiotically fixed N. Therefore, the relative amount of N that soybeans derive from symbiotic N$_2$ fixation varies with soil N content.

Weber (1966) reported that the contribution of symbiotically fixed N to the total N content of a soybean crop was increased from 13% to 74% when soil N was immobilized by the addition of ground corn cobs. Estimates of the average percentage of the N requirement of soybean derived by symbiotic fixation vary widely (LaRue & Patterson 1981). Hardy and Havelka (1975) estimated 25%, while Weber (1966) estimated 40%. Criswell et al. (1976) suggested 33% as a compromise.

Because high temperature accelerates the rate of decomposition of soil organic matter, which is the major source of soil N; the organic matter content, and therefore the N content, of soils in the southern U. S. tends to be lower than that of northern soils (Brady 1974). This means that, in general, soybeans grown in the South will be more dependent on symbiotically fixed N. Hiltbold and Thurlow (1986) determined the percent contribution of symbiotically fixed N to the total N economy of Lee soybeans on a wide range of Alabama soils, and found that, on the average, 74% of the N was derived from symbiotic fixation. Also, the
relative dependence on biologically fixed N tends to increase as the growing season progresses (Harper 1976).

These figures emphasize that symbiotic fixation provides soybean with a substantial portion of its N supply and is thus an important component of soybean production. Therefore, it is important to identify and understand factors that may limit N₂ fixation in soybean.

In soybean, biological N₂ fixation occurs because of the symbiotic association with the N₂-fixing bacteria, *Bradyrhizobium japonicum* (Kirchner) Buchanan. These bacteria are able to survive saprophytically in the soil (Vest et al. 1973), but when soybeans are planted in soil containing these bacteria, they invade the growing root hairs and stimulate the development of root structures, known as nodules, that house the N₂-fixing bacteria. The end result is an organ composed of both plant cells and bacteria cells that is capable of reducing N₂ to a form that the plant can use (Carlson 1973, Dart 1977).

The biochemistry of N₂ fixation is quite complex and has been reviewed by Bergersen (1977) and Evans and Russell (1971). The rhizobium bacteria growing within the nodules produce an enzyme, nitrogenase, that reduces N₂ to NH₃ (Eady & Smith 1979, Vest et al. 1973, Yates & Eady 1980). The reduction of N₂ to NH₃ requires a large amount of chemical energy. From 10 to 12 ATP molecules are required for each N₂ molecule that is reduced (Gutschick 1978). This ATP is produced by the oxidation of photosynthate, primarily
sucrose, within the bacteriods (Evans & Russell 1971, Ogren & Rinne 1973, Vest et al. 1973). Minchen and Pate (1973) found that greenhouse grown peas transported 32% of the carbon gained by photosynthesis to the nodules. Approximately half of this was used for nodule growth and respiration while the remainder was returned to the plant as amino acids.

Ryle et al. (1979a) compared the respiratory cost of N₂ fixation in soybean, cowpea, and white clover. They found a similar cost for all three legumes; 6.3 to 6.8 mg of C was respired for each mg of N fixed. Ryle et al. (1979b) also reported that soybean plants that were dependent on N₂ fixation for all of their N supply respired 11% more of their photosynthetically fixed C than plants that received all of their N from fertilizer. Thus, it is apparent that the energy that drives biological N₂ fixation is ultimately derived from photosynthate. Indeed, many researchers have indicated that photosynthate supply is a major factor limiting the rate of N₂ fixation (Farnham et al. 1986, Hardy & Havelka 1976, Quebedeaux 1979, Streeter et al. 1979).

Because of the importance of photosynthate to N₂ fixation, any factor that alters the supply of photosynthate to the root system will affect the rate of fixation. When photosynthate supply was increased by growing plants in an enhanced CO₂ environment and thus increasing photosynthesis (Hardy & Havelka 1974), by grafting two shoots onto one root (Streeter 1973), or by increasing light intensity
(Schweitzer & Harper 1985), the rate of $N_2$ fixation increased. Conversely, abiotic factors that reduce photosynthate supply, such as shading or artificial defoliation (Lawn & Brun 1974, Riggle et al. 1984), reduced $N_2$-fixing ability.

This relationship between photosynthate supply and $N_2$ fixation is important from an entomological point of view because insects damage soybeans in two ways that interfere with photosynthate supply. The threecornered alfalfa hopper, *Spissistilus festinus* (Say), feeds in the phloem of soybean stems often causing a ring of damaged tissue that completely encircles the stem. This stem girdling interferes with the translocation of photosynthate to the nodules and causes reduced $N_2$-fixing ability (Hicks et al. 1984). Sirur and Barlow (1984) reported reduced $N_2$ fixation in peas in response to infestation by aphids, because the aphids competed with the nodules for photosynthate.

The second way in which insects damage soybeans and cause reduced photosynthate supply to the nodules is by defoliation. Soybean is attacked by a large number of defoliating insect pests and defoliation is one of the most common types of insect damage sustained by soybeans (Turnipseed 1973). Clearly, plants that have lost a large portion of their leaf area to defoliating insects will not produce as much photosynthate as undefoliated plants. Hutchinson (1979) studied the effects of insect-induced
defoliation on \( N_2 \)-fixing ability and found that defoliation greatly reduced the rate of fixation.

Because of the negative impact of insect-induced defoliation on \( N_2 \) fixation in soybean and the realization that symbiotically fixed \( N \) represents a component of yield, this research project was devoted to further study of the effects of defoliation on soybean \( N_2 \) fixation. Hutchinson's (1979) proof that defoliation reduces \( N_2 \)-fixing ability stimulated new questions about how soybeans respond to defoliation. What is the effect of varying levels of defoliation? How do soybeans respond to progressive defoliation? Can soybeans recover all or part of their \( N_2 \)-fixing ability subsequent to defoliation? The following chapters address these questions and, in doing so, attempt to supply more information about the response of soybean growth and \( N_2 \)-fixing ability to insect-induced defoliation.
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CHAPTER I

Reduction In N₂ Fixation by Soybean
In Response to
Insect-induced Defoliation

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Nitrogen-fixing ability (measured by \( \text{C}_2\text{H}_2 - \text{C}_2\text{H}_4 \text{ assay} \)) of greenhouse-grown, vegetative soybean, *Glycine max* (L.) Merrill, decreased linearly in response to increased levels of defoliation by the soybean looper, *Pseudoplusia includens* (Walker) [Lepidoptera: Noctuidae]. Acetylene reduction rate of control plants was 5-fold greater than that of 77% defoliated plants. This decrease was the result of reduced number, weight, and efficiency (nodule specific activity) of nodules. Nodule specific activity was unaffected by defoliation of < 47%, but decreased sharply as defoliation exceeded 47%.

**INTRODUCTION**

Soybean is unique among the important grain crops grown in the United States in that it has the ability to obtain much of its N requirement via symbiotic \( \text{N}_2 \) fixation. Estimates of the amount of total N derived via \( \text{N}_2 \) fixation range from 33% (Criswell et al. 1976) to 80% (Hardy & Havelka 1975). It is generally agreed that well nodulated soybeans are capable of meeting all of their N requirement via symbiotic \( \text{N}_2 \) fixation and uptake of available soil N. Applications of N fertilizer are not needed in the commercial production of soybean (deMooy et al. 1973), but
other grain crops require additions of large amounts of N fertilizer for profitable yields.

Because of the importance of N\textsubscript{2} fixation to soybean production, there is a critical need to understand the impact of biotic and abiotic stresses on the N\textsubscript{2} fixation process. The effects of abiotic stresses have been rather extensively studied (Hardy & Silver 1977, Hardy et al. 1979), but only recently has the impact of biotically induced stress been examined.

Three types of insect-induced stress have been reported to adversely affect N\textsubscript{2} fixation of soybean. Nodule destruction by larvae of the bean leaf beetle, *Cerotoma trifurcata* Forster, can reduce N\textsubscript{2} fixation by as much as 2-fold (Layton 1983). This represents direct damage to the N\textsubscript{2}-fixing organs, but insects may affect N\textsubscript{2} fixation by indirect damage also. Hicks et al. (1984) demonstrated that stem girdling by the threecornered alfalfa hopper, *Spissistilus festinus* Say, caused significant reductions in N\textsubscript{2}-fixing ability by restricting translocation of photosynthate to the root system. Hutchinson (1979) demonstrated that insect-induced defoliation, which decreases photosynthate production, also caused significant reductions in N\textsubscript{2} fixation rate.

The recognition that such stresses affect N\textsubscript{2} fixation adversely is the first step toward quantifying the impact of these stresses. Relatively little is known about the degree of reduction in N\textsubscript{2} fixation, the amount of injury necessary
to cause reduction, the ability of the plant to recover or
to compensate for injury, or the interactive effects of
these biotic stresses with other biotic or abiotic stresses.
The objective of this study was to examine the relationship
between degree of insect-induced defoliation and N\textsubscript{2}-fixing
ability in order to define better the impact of defoliation
on N\textsubscript{2} fixation.

MATERIALS AND METHODS

'Davis' soybean seeds were inoculated with a commercial
preparation of a mixture of strains of N\textsubscript{2}-fixing bacteria,
\textit{Bradyrhizobium japonicum} (Kirchner) Buchanan, and planted on
17 August 1985, in 7.6-liter pots containing 6 kg of
air-dried, sandy loam soil. Soil nitrogen content was not
determined, but the organic matter content was 0.24% which
suggests low N content (Brady 1974). Because combined soil
N is known to inhibit nodulation and N\textsubscript{2} fixation (Lie 1974,
Pate 1977), it was desirable to use soil low in N in order
to maximize N\textsubscript{2} fixation. A total of 22 mg/kg of P
(monocalcium phosphate) and 40 mg/kg of K (potassium
chloride) was added to the soil in two applications, the
first at 18 days after planting and the second 16 days
later.

Initial seeding rate was five seeds per pot, but
seedlings were thinned to a uniform stand of two per pot at
1 week post-emergence. Thus, an experimental unit consisted of a single pot containing two plants. Seedlings were grown in the greenhouse under supplemental lighting (photoperiod of 16:8 [L:D]) from planting to growth stage V5 (Fehr et al. 1971). Supplemental lighting was supplied by 40-W fluorescent lamps (Gro-Lux®) suspended ca. 70 cm above the tops of plants.

At 28 days post-planting (V5), the 40 pots were randomly assigned to one of five treatments and infested with 0, 6, 12, 18, or 24 neonate soybean looper larvae, *Pseudoplusia includens* (Walker), per pot. The pots were arranged in a completely random design with eight replications per treatment. Movement of the larvae was not restricted by cages but rather by spacing the pots widely apart so that the leaves did not touch. Supplemental lighting was terminated at this time because it was no longer possible to adequately light all of the area covered by the plants. Larvae that wandered off the plants during the defoliation period were placed back on plants based on visual estimates of the amount of defoliation an experimental unit had sustained relative to others in the same treatment. A barrier of petroleum jelly on the first internode of each plant minimized contamination of control plants by wandering larvae. Plants were inspected twice daily, and any larvae found on controls were removed, thus defoliation of controls was negligible.
Larvae were allowed to feed for 17 days, by which time most had developed into pupae or prepupae. At the end of the defoliation period the number of insects present on each experimental unit was ca. 25% of the initial infestation level. The others perished as a result of various undefined mortality factors. This high mortality was anticipated and accounted for in the high initial infestation rates.

At 17 days post-infestation (V10), the plants were harvested. The two shoots of a pot were cut at the cotlyledonary node and placed in a labeled plastic bag. The roots of both plants were carefully shaken free of soil, placed together in a 473-ml glass jar, and subjected to the acetylene-ethylene ($C_2H_2-C_2H_4$) reduction assay of Hardy et al. (1968). This assay provides an indirect measure of relative $N_2$-fixing ability. Fifty ml of air was removed from the jar through a rubber septum installed in the lid and replaced with an equal volume of $C_2H_2$. After a 1-h incubation period, a 7-ml sample of gas was removed from the jar and stored in a 7-ml evacuated tube. The $C_2H_4$ content of each gas sample was later determined using a gas chromatograph (Perkin Elmer Model 3920) equipped with dual flame ionization detectors and two, 80-100 mesh, 1.8 m by 3.2 mm stainless steel columns (Porapak®). The carrier gas was $N_2$.

The roots and shoots were brought to the laboratory where the leaves were removed and leaf area per pot was determined using an area meter (LI-COR® LI-3100). Nodules
were removed from roots and counted. Dry weight of stems, roots, and nodules was determined after these plant parts were dried to constant weight in a forced air oven at 60°C. All measurements were made on a two plant basis because an experimental unit consisted of two plants.

Seven parameters were measured: number of nodes, leaf area, nodule dry weight, stem dry weight, root dry weight, number of nodules, and \( C_2H_4 \) production per two plants per h. An additional six parameters were calculated from these data: weight per nodule, \( C_2H_4 \) per nodule per h, nodule specific activity (\( C_2H_4 \) production per g of dry nodule weight per h), \( C_2H_4 \) per 1000 cm\(^2\) of leaf area per h, nodule to root ratio (dry weight:dry weight), and percent defoliation \( \{[(\bar{X} \text{ leaf area of control} - \text{leaf area of experimental unit}/\bar{X} \text{ leaf area of control}] \times 100\} \).

Data were analyzed using PROC REG (SAS 1985). Regression equations were calculated from the 40 individual data points, rather than the treatment means, with percent defoliation as the independent variable. Variables that did not fit a linear, quadratic, or cubic model with an \( R^2 > 0.60 \) were analyzed using PROC ANOVA and Duncan's multiple-range test at \( P = 0.05 \) (SAS 1985).

RESULTS AND DISCUSSION

Mean levels of 0, 35, 47, 66, and 77% defoliation were obtained for the five treatments. Percent defoliation of
individual, infested experimental units ranged from 16 to 85%. Fig. 1 illustrates the relationship between these mean levels of defoliation and amount of leaf area per two plants and, more importantly, also shows the distribution of the individual data points about these means. In summarizing the results of a series of experiments, Turnipseed (1972) reported that 33 and 67% defoliation at bloom resulted in only 2.9 and 10.5% reductions in seed yield, respectively. The levels of defoliation in this experiment were comparable to those in the study by Turnipseed, but in this study the defoliation occurred earlier, while the plants were still in the vegetative stages of development.

Defoliation caused a reduction in the mean number of nodes per plant (Table 1). Because the plants were not blooming at harvest, number of nodes corresponds to the vegetative development stages of Fehr et al. (1971). At the higher levels of defoliation, 66 & 77%, plant development was delayed an entire growth stage.

A significant linear reduction in weight of dry stems ($F = 235.6; \text{df} = 1,38; P < 0.0001$) and roots ($F = 92.2; \text{df} = 1,38; P < 0.0001$) occurred in response to increased levels of defoliation (Figs. 2A & 2B). Mean weights for the 77% defoliation level were ca. half those of undefoliated plants for both variables.

The reduction in root weight adversely affects $N_2$ fixation in two ways. First, reduced root development is indicative of reduced photosynthate supply to the root
Fig. 1. Relationship of mean percent defoliation (■) to leaf area per experimental unit and distribution of individual data points (x).
Table 1. Effects of increasing levels of defoliation in vegetative 'Davis' soybean; selected parameters.

<table>
<thead>
<tr>
<th>Mean percent defoliation</th>
<th>Number nodes per plant</th>
<th>nodule:root ratio</th>
<th>Weight per nodule (mg)</th>
<th>$\text{C}_2\text{H}_4$ per 1000 cm$^2$ leaf area (µMoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.2 (0.16) a</td>
<td>0.69 (0.048) a</td>
<td>6.0 (0.22) a</td>
<td>14.7 (0.75) a</td>
</tr>
<tr>
<td>35</td>
<td>9.5 (0.09) b</td>
<td>0.60 (0.036) ab</td>
<td>5.5 (0.31) ab</td>
<td>15.7 (1.02) a</td>
</tr>
<tr>
<td>47</td>
<td>9.5 (0.09) b</td>
<td>0.49 (0.030) b</td>
<td>5.6 (0.29) ab</td>
<td>14.7 (0.89) a</td>
</tr>
<tr>
<td>66</td>
<td>9.1 (0.18) c</td>
<td>0.52 (0.054) b</td>
<td>4.8 (0.28) bc</td>
<td>13.3 (0.80) ab</td>
</tr>
<tr>
<td>77</td>
<td>9.1 (0.06) c</td>
<td>0.47 (0.037) b</td>
<td>4.6 (0.31) c</td>
<td>11.0 (1.43) b</td>
</tr>
</tbody>
</table>

Column means not followed by the same letter are significantly different based on a post-ANOVA ($F > 2.64$; df = 4,35; $P < 0.05$) Duncan's multiple range test ($F = 0.05$) (SAS 1985). Number in parenthesis is standard error of mean.
Fig. 2. Effect of increasing defoliation on dry stem (A) and root (B) weight per experimental unit; squares (■) represent means. Regression equations were calculated from individual data points.
system and therefore, to the nodules. The link between N\textsubscript{2} fixation rate and photosynthate supply is well established (Farnham et al. 1986). Photosynthesis supplies both the energy for N\textsubscript{2} fixation and the carbon skeletons that combine with the resulting NH\textsubscript{3} (Evans & Russell 1971; Vest et al. 1973). Photosynthate supply is the primary factor limiting N\textsubscript{2} fixation (Quebedeaux 1979). Second, reduced root development results in less root mass to support nodule formation and development.

The nodule:root ratio (nodule dry weight/root dry weight) decreased significantly in the 47, 66, and 77% defoliation treatments relative to the control (Table 1). This indicates a reduction in nodule load per g of root weight with increasing defoliation. Thus, defoliation caused a reduction not only in root mass, but also in the relative nodule mass supported by the root.

The previous discussion largely explains the strong linear reduction in number of nodules ($F = 148.9$; $df = 1,38$; $P < 0.0001$) in response to increased defoliation (Fig. 3A). The defoliation period lasted only 17 days and the amount of leaf area removed by the early instar larvae during the first 7 days was of little consequence. Thus, reduction in number of nodules was due to stress experienced in the last 10 days before harvest. Prior to this, all treatments would have had an equal number of nodules, which would represent a lower limit to any reduction in number of nodules.
Fig. 3. Effect of increasing defoliation on nodule number (A) and weight (B) per experimental unit; squares (■) represent means. Regression equations were calculated from individual data points.
Average nodule size was also significantly reduced as a result of defoliation but not in the linear fashion seen for other parameters. However, a sharp reduction in weight per nodule was observed at the 66 and 77% defoliation levels (Table 1). Apparently at these high levels of defoliation, plants were not producing enough photosynthate to meet other, more immediate, needs and still support normal nodule development.

Thus, the linear reduction ($F = 301.4; \text{df} = 1,38; P < 0.0001$) in total nodule weight (Fig. 3B) was the result of decreases in both number of nodules and average nodule size. Control plants had 1.4 and 2.6 times as much total nodule mass as the 35 and 77% defoliated treatments, respectively.

Nodule specific activity is a measure of $\text{C}_2\text{H}_4$ production per g of dry nodule weight and is thus a measure of relative efficiency of $\text{N}_2$ fixation for the nodule mass that is present on the plant. When the data for nodule specific activity were subjected to regression analysis, the model that best described the results was a cubic polynomial ($F = 24.8; \text{df} = 3,36; P < 0.0001$). The regression line, shown along with the 5 treatment means (Fig. 4A), demonstrates that defoliation of 0 to ca. 47% had little effect on nodule specific activity, but any defoliation beyond 47% caused a sharp reduction. This suggests the existence of a critical level of defoliation, ca. 47%, beyond which nodule specific activity is reduced.
Fig. 4. Relationship of (A) nodule specific activity (μMol ethylene per gram dry nodules) to percent defoliation \( (Y = 39.4 - 0.2872X + 0.01524X^2 - 0.000191859X^3) \) and (B) ethylene per nodule to percent defoliation \( (Y = 0.233 - 0.002X + 0.0008545X^2 - 0.000000108X^3) \); squares (■) represent means. Regression equations were calculated from individual data points.
C2H4 production per two plants and dry nodule weight were both reduced by increased defoliation. Nodule specific activity is essentially a ratio of C2H4 production to nodule dry weight and would not be expected to change if both the numerator variable and denominator variable were similarly reduced. It appears that as defoliation exceeds ca. 47% plants must use a greater proportion of the photosynthate produced, simply for maintenance. This would mean that less photosynthate would be available for N2 fixation, which would explain the reduction in nodule specific activity at defoliation levels greater than 47%.

Examination of C2H4 production per nodule per h (Fig. 4B) showed a cubic response ($F = 24.1; df = 3,36; P < 0.0001$) similar to that observed for nodule specific activity. Again, there was essentially no change in C2H4 production until defoliation exceeded ca. 47%.

Ethylene produced per 1000 cm$^2$ of leaf area per h was not significantly reduced until defoliation exceeded 66% (Table 1). Again, this is not surprising because this variable is simply a ratio of C2H4 production per two plants and leaf area, both of which decreased at relatively similar rates.

Of the parameters used to estimate N2 fixing ability, C2H4 per two plants per h is the most comprehensive. There was a strong, linear reduction ($F = 344.0; df = 1,38; P < 0.0001$) in C2H4 production per two plants in response to increased levels of defoliation (Fig. 5). Defoliation of
Fig. 5. Effect of increasing defoliation on ethylene production ($N_2$-fixing ability) of 'Davis' soybean; squares (■) represent means. Regression equation was calculated from individual data points.
35% caused a 32% decrease in \( \text{C}_2\text{H}_4 \) production, whereas 77% defoliation resulted in an 82% decrease.

Results presented herein show that \( \text{N}_2 \)-fixing ability decreased linearly with increasing levels of defoliation. Rate of \( \text{C}_2\text{H}_4 \) production in control plants was 5-fold that of plants that had sustained 77% defoliation (Fig. 5). Although the deleterious effects of defoliation on \( \text{N}_2 \) fixation can simplistically be attributed to reduced photosynthate supply, this reduction in photosynthate actually affects \( \text{N}_2 \) fixation in two ways. First, reduced photosynthate production results in depressed development of roots and nodules. Number of nodules and weight of individual nodules were both reduced, with the combined result of reduced nodule weight per experimental unit. Control plants had 2-fold more nodules and 2.6-fold greater total nodule weight than plants that were 77% defoliated. Nodule weight and number are directly related to rate of \( \text{N}_2 \) fixation (Weber 1966). Second, photosynthesis supplies the \( \text{N}_2 \)-fixing bacteria with the energy needed to drive the \( \text{N}_2 \)-fixing process (Evans & Russell 1971, Vest et al. 1973). When defoliation is severe enough, photosynthate supply is sufficiently reduced to cause decreased nodule specific activity. Nodule specific activity of control plants was over 2-fold greater than that of the 77% defoliated plants. These results are similar to those of Riggle et al. (1984) who found that 60% artificial defoliation of reproductive soybean reduced nodule specific activity by 54%. It is the
combined effect of these two consequences of reduced photosynthesis that results in defoliation having so great an impact on N₂-fixing ability.

The N gained through symbiotic N₂ fixation has a value to the soybean plant and, consequently, to the soybean producer. Based on current prices for inorganic N fertilizer (NH₄NO₃), exclusive of application cost, a kg of fixed N is worth $ 0.51. Thus the N derived from symbiotic N₂ fixation can be viewed as a second component of yield (Newsom & Boethel 1986). These results confirm that insect-induced defoliation has a negative effect on this component of yield and illustrate the degree of this effect over a range of defoliation levels.
REFERENCES CITED


CHAPTER II

Response of Soybean Growth and $N_2$-fixing Ability to Progressive Insect-induced Defoliation

This chapter is written in the style of the Journal of Economic Entomology and has been submitted for publication in this journal.
ABSTRACT

Greenhouse-grown, vegetative soybean plants (cv 'Davis') were infested with neonate larvae of the soybean looper, *Pseudoplusia includens* (Walker), which were allowed to progressively defoliate the plants for 16 days. At 7, 10, 12, 14, and 16 days post-infestation (DPI), plants were destructively sampled to measure plant growth and N$_2$-fixing ability (measured using C$_2$H$_2$-C$_2$H$_4$ reduction assay). On the respective sample dates, defoliated plants suffered 13, 37, 43, 62, and 73% defoliation relative to controls. This progressive defoliation caused significant reductions in dry weight of stems, by 10 DPI (37% defoliation), and of roots and nodules, by 14 DPI (62% defoliation). Number of nodules and mean dry weight per individual nodule were not affected during the course of the experiment. N$_2$-fixing ability was reduced significantly by 32% defoliation at 10 DPI, largely because of a significant decrease in nodule specific activity (NSA). Further decreases in N$_2$-fixing ability at 14 and 16 DPI (66 & 73% defoliation) were due to a combination of decreased nodule weight and decreased NSA.
INTRODUCTION

Soybean is an important source of both oil and protein. Soy products enter our diet directly, as components of a wide variety of foods, and indirectly, as animal protein produced using soy protein (Cowan 1973). As with other crops, nitrogen is a primary limiting factor in soybean production, but in soybean the requirement for N is particularly high because of the high protein content (ca. 40%) of the seeds (Ogren and Rinne, 1973).

Soybean has adapted to meet its high requirement for N in two ways: 1) it is an exceptionally efficient scavenger for soil N (Johnson et al. 1975) and 2) it enters into a symbiotic association with the N₂-fixing bacteria, *Bradyrhizobium japonicum* (Kirchner) Buchanan. The ability of soybean to use N from these two sources means that soybeans are generally self-sufficient in meeting their N requirements (deMooy et al. 1973). Although the contribution of symbiotically fixed N to the overall N economy of a soybean crop varies considerably (Criswell et al. 1976, LaRue & Patterson 1981), biologically fixed N is quite important to soybean production, and, in most fields, means the difference between N sufficiency and N deficiency.

The N derived by symbiotic fixation is not without cost to the plant which must support the N₂-fixing process by supplying nutrients, in the form of photosynthate, to the N₂-fixing bacteria housed within the root nodules. Thus,
photosynthate supply to nodules is an important limiting factor to $N_2$ fixation, and any stress that interferes with this supply will affect $N_2$-fixing ability (Hardy & Havelka 1976, Quebedeaux 1979, Streeter et al. 1979).

It is therefore intuitively clear that insect-induced defoliation will adversely affect $N_2$-fixing ability. This has been proven by Hutchinson (1979), who used the acetylene-ethylene ($C_2H_2-C_2H_4$) reduction assay (Hardy et al. 1968) to show that insect-induced defoliation greatly reduced $N_2$-fixing ability in soybean. The relationship between insect-induced defoliation and $N_2$ fixation was examined further by Layton and Boethel (1987), who demonstrated that $N_2$-fixing ability decreased linearly as percent insect-induced defoliation increased. Further research has shown that soybean has some capacity to recover from this effect. Vegetative soybeans that suffered large reductions in $N_2$-fixing ability immediately following ca. 70% defoliation were able to recover $N_2$-fixing ability by three weeks post-defoliation (Layton and Boethel 1988). This recovery in $N_2$-fixing ability occurred despite prolonged effects of the defoliation on leaf area, stem dry weight, and nodule mass.

Thus, previous studies have examined the effects of various levels of defoliation on $N_2$-fixing ability, as well as the recovery of $N_2$ fixation following defoliation. In all cases, effects of defoliation on plant growth and $N_2$-fixing ability were not measured until defoliation was
complete and had reached its maximum level. No study has examined the effects of defoliation on plants as they are being defoliated by an insect. Therefore, the objective of the present study was to examine the response of soybean growth and $N_2$-fixing ability to progressive, insect-induced defoliation.

MATERIALS AND METHODS

Experimental Procedure. On 1 April, 1986, five 'Davis' soybean seeds (maturity group VI) were planted in each of 80, 7.6-liter plastic pots. Each pot contained 6 kg of an air-dried sandy loam that had an organic matter content of 0.24%. Prior to planting, seeds were inoculated with a commercial preparation of a mixture of strains of the $N_2$-fixing bacteria, Bradyrhizobium japonicum (Kirchner) Buchanan. Plants were thinned to two per pot at 12 days after planting (DAP); thus an experimental unit consisted of a single pot containing two plants. All pots received 20 mg kg$^{-1}$ K (potassium chloride) and 11 mg kg$^{-1}$ P (monocalcium phosphate) at 21, 38, and 48 DAP. Plants were grown in a greenhouse at ambient photoperiod without supplemental lighting.

At 38 DAP, when plants had developed to the V6 stage (Fehr et al. 1971), pots were assigned randomly to either the defoliated or control treatment. Each of the 40 pots
assigned to the defoliated treatment then was infested with 25 neonate larvae of the soybean looper, *Pseudoplusia includens* (Walker). At 7, 10, 12, 14, and 16 days post-infestation (DPI), eight pots were chosen randomly from each treatment and destructively sampled. The objective in harvesting plants at these times was to allow comparison of plant growth and $N_2$-fixing ability of control plants to that of plants undergoing progressive defoliation.

At 7 DPI, larvae had consumed only a small amount of foliage and in many cases the upper epidermial layer of leaves remained intact over small defoliated areas, creating a 'window pane' effect that is characteristic of feeding by early instar soybean loopers. The area meter used in this experiment could be adjusted to measure these 'window pane' areas as missing foliage, thus allowing accurate determination of percent defoliation. By the 16 DPI sample date, many of the insects had begun to pupate and defoliation had reached the maximum level.

Many larvae disappeared during the defoliation period so that by the 16 DPI sample date, infested plants had only six to seven larvae per pot. This was expected and accounted for by the high initial infestation level. Relatively uniform defoliation of all infested pots within a particular sample date was assured by examining all pots daily and manually adjusting the number of larvae per pot as necessary.
Sampling Procedure. At time of harvest, shoots were severed from the roots at the cotlydenary node and placed in plastic bags to await further processing. After the soil was shaken carefully from the root systems of both plants of an experimental unit (EU), the two root systems then were placed together in a 473-ml glass jar and subjected to the acetylene-ethylene ($C_2H_2$-$C_2H_4$) reduction assay of Hardy et al (1968). This assay measures the rate at which $C_2H_2$ is reduced to $C_2H_4$ by the $N_2$-fixing bacteria within the root nodules and thus provides an indirect measure of relative $N_2$-fixing ability. The methods used in conducting the assay are described in greater detail by Layton & Boethel (1987).

Parameters that were determined for each two-plant EU were: leaf area (measured using a Decagon® area meter); number of nodes; stem dry weight ($60^\circ$ C for 72 h); root dry weight; number of root nodules; total nodule dry weight; mean individual nodule dry weight; $C_2H_4$ two-plants$^{-1}$ h$^{-1}$; $C_2H_4$ nodule$^{-1}$ h$^{-1}$; nodule specific activity (NSA) (calculated as $C_2H_4$ g$^{-1}$ nodule dry weight h$^{-1}$; $C_2H_4$ 1000-cm$^{-2}$ leaf area h$^{-1}$; and nodule:root ratio (nodule dry weight/root dry weight).

Statistical Analyses. Data from each sample date were analyzed separately using PROC TTEST of SAS (SAS Institute 1985). Means for control and defoliated treatments of a particular sample date were declared significantly different when: $|t| > 2.145$, df = 14, $P < 0.05$. Actual $P$ values are reported in the text only when $0.05 < P < 0.1$.
RESULTS AND DISCUSSION

Plant Development. The difference in mean leaf areas for control and defoliated treatments increased from time of infestation to 16 DPI when the experiment ended (Fig 1A). Percent defoliation of the defoliated treatment relative to the control ([X leaf area of control - X leaf area of defoliated]/X leaf area of control) * 100) at each of the five sample dates: 7, 10, 12, 14, and 16 DPI, was: 13, 37, 43, 62, and 73%, respectively. These data provide a measure of the degree of stress that plants suffered at each sample date and also indicate how defoliation progressed as larvae developed.

The effects of this progressive defoliation on plant growth are illustrated by the data for stem (Fig 1B) and root (Fig 1C) dry weight. Defoliated plants had significantly less stem dry weight by 10 DPI (37% defoliation), but root dry weight was not affected until 14 DPI (62% defoliation). Thus it is clear that the stress of defoliation had an impact on growth of both above and below ground plant parts.

Defoliation also appeared to have an effect on the development of new nodes (Table 1). At 10 DPI (37%
Fig. 1. Leaf area (A), stem dry weight (B), and root dry weight (C) per two plants in 'Davis' soybean during progressive insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
Table 1. Effects of progressive insect-induced defoliation on selected parameters in 'Davis' soybean at 7, 10, 12, 14, and 16 days after infestation with neonate soybean looper larvae (DPI). Percent defoliation of defoliated treatment relative to control at each of these sample dates was 13, 37, 43, 62, and 73%, respectively.

<table>
<thead>
<tr>
<th>Nodule:Root Ratio</th>
<th>Leaf Area (umol h⁻¹)</th>
<th>Nodule:Root Ratio</th>
<th>Leaf Area (umol h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₂H₄ 1000-cm⁻²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># Nodes</td>
<td>Ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(plant⁻¹)</td>
<td>(dry weight)</td>
<td></td>
<td>(dry weight)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>7 DPI</th>
<th>10 DPI</th>
<th>12 DPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.6 ± 0.2</td>
<td>0.77 ± 0.05</td>
<td>0.76 ± 0.04</td>
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<tr>
<td>Defol.</td>
<td>8.6 ± 0.2</td>
<td>0.80 ± 0.05</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.6* ± 0.2</td>
<td>0.79 ± 0.04</td>
<td>14.4 ± 1.2</td>
</tr>
<tr>
<td>Defol.</td>
<td>9.1 ± 0.1</td>
<td>0.70 ± 0.03</td>
<td>20.3* ± 1.4</td>
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(Continued next page)
(Table 1 continued)

<table>
<thead>
<tr>
<th></th>
<th>14 DPI</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.6 ± 0.2</td>
<td>0.71 ± 0.03</td>
<td>16.3 ± 0.7</td>
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<tr>
<td>Defol.</td>
<td>10.4 ± 0.2</td>
<td>0.77 ± 0.03</td>
<td>23.1* ± 1.9</td>
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</table>

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<th>16 DPI</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.3a± 0.2</td>
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<td>15.4 ± 1.5</td>
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<tr>
<td>Defol.</td>
<td>10.6 ± 0.3</td>
<td>0.71 ± 0.02</td>
<td>18.5 ± 1.1</td>
</tr>
</tbody>
</table>

Mean ± standard error of the mean: * indicates a significant difference (P < 0.05) between means of a common pair, based on PROC TTEST (SAS Institute 1985).

a P = 0.0525
b P = 0.0504
defoliation), control plants had significantly more nodes than defoliated plants and at 16 DPI (73% defoliation), control plants had 0.7 nodes more, although this difference was nonsignificant ($P = 0.0525$). This is similar to results from previous experiments in which defoliated plants averaged from 0.5 to 1 nodes fewer than control plants by the time they had sustained ca. 70% defoliation (Layton & Boethel 1988). Plants had begun to enter the reproductive stage by 16 DPI, because 50% of the plants harvested on that date were in the R2 stage of development (Fehr et al. 1971).

Nodule Development. The impact of defoliation on number of nodules (Fig 2A) was not manifested as quickly as the effects on stem and root dry weight. Although the defoliated plants had fewer nodules at 16 DPI (73% defoliation), this difference was not significant ($P = 0.0699$). Considering that 10 to 14 days are required for the development of new nodules (Carlson 1973), it was not surprising that number of nodules was not affected significantly, by 16 DPI. In previous experiments, it was found that although number of nodules was not significantly affected immediately following ca. 70% defoliation, there was a delayed effect of defoliation on number of nodules, resulting in large differences in number of nodules 1 to 2 weeks post-defoliation (Layton & Boethel 1988). Thus it is clear that defoliation does affect development of new nodules, but manifestation of this effect is often delayed.
Fig. 2. Number of nodules (A) and total dry weight (B) per two plants and mean dry weight of individual nodules (C) in 'Davis' soybean due to progressive insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
Average weight per nodule (Fig 2B) also was not affected on any sample date. However, defoliation caused a significant reduction in total nodule dry weight (Fig 2C) at 14 and 16 DPI, which indicates that defoliation had begun to affect nodule development by 14 DPI (62% defoliation). Because $N_2$-fixing ability of soybeans is largely dependent on total nodule mass (Weber 1966), one would expect this decrease in nodule dry weight to be manifested as a decrease in $N_2$-fixing ability.

At first, it may not seem logical that there was a significant difference in total nodule dry weight at 14 and 16 DPI when there was no difference in average nodule size and no significant difference in total number of nodules. However, nodulation characteristics of plants from a common treatment may differ considerably. Two plants may have similar total nodule weights, but one may have many small nodules while the other has fewer, but larger, nodules. Thus there is less inherent variation associated with the variable nodule dry weight than with either of the other two variables. The mean coefficient of variation across all sample dates for control plants was 15.2%, 31.7%, and 24.6% for total nodule dry weight, number of nodules, and mean dry weight per nodule, respectively. Therefore, total nodule dry weight is the best variable to use as an indication of nodule development, because its smaller variance relative to the other two variables increases the power of statistical tests for treatment differences.
The nodule:root ratio (Table 1) provides an index of nodule development relative to root development. The largest difference in nodule:root ratio occurred at 12 DPI, but was nonsignificant (P = 0.0504), and nodule:root ratio was generally unaffected during the period of progressive defoliation. Data from two previous experiments agree with this result (Layton & Boethel 1988), whereas data from a third experiment showed a significant decrease in this ratio in response to defoliation of 47% or greater (Layton & Boethel 1987).

\( \text{N}_2 \text{ Fixation.} \) Defoliation caused a decrease in \( \text{N}_2 \)-fixing ability (Fig 3A), as measured by total \( \text{C}_2\text{H}_4 \) production, by 10 DPI (37% defoliation). Because there was no reduction in total nodule weight of defoliated plants at 10 DPI, this decrease in \( \text{N}_2 \)-fixing ability is attributed to the significant decrease in NSA (a measure of \( \text{N}_2 \)-fixing ability per g of nodule dry weight) that occurred at 10 DPI (Fig 3B). At 16 DPI (73% defoliation), \( \text{N}_2 \)-fixing ability of control plants was 3.2-fold that of defoliated plants, while NSA of control plants was 2.3-fold greater than that of defoliated plants. Thus at 16 DPI, the reduction in \( \text{N}_2 \)-fixing ability was due largely to the decrease in NSA which was exacerbated by the reduction in total nodule weight. Because there was no effect on dry weight of individual nodules at any sample date (Fig 2B), the
Fig. 3: Total $\text{C}_2\text{H}_4$ production ($\text{N}_2$-fixing ability) per two plants per h (A), nodule specific activity (B), and $\text{C}_2\text{H}_4$ production per nodule (C) in 'Davis' soybean due to progressive insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
significantly lower \( C_2H_4 \) production per nodule of defoliated plants at 10, 14, and 16 DPI (Fig 3C) simply reflects the decreased NSA on those dates.

In previous experiments (Layton & Boethel 1988), \( C_2H_4 \) production per 1000-cm\(^2\) leaf area h\(^{-1}\) was reduced significantly in response to ca. 70% defoliation but rapidly increased to a level greater than that of controls as the plants recovered. Results of another experiment (Layton & Boethel 1987) indicated that relatively high levels of defoliation are required to cause a decrease in this variable, because defoliation of 66% did not cause a reduction, while 77% defoliation did cause a reduction. In the present study, defoliated plants reduced significantly more \( C_2H_2 \) to \( C_2H_4 \) per 1000 cm\(^2\) of leaf area than control plants at 12 and 14 DPI (43 & 62% defoliation), but by 16 DPI (73% defoliation), the difference between the two treatments was no longer significant (Table 1). Thus plants responded to the defoliation by initially becoming more efficient in \( N_2 \)-fixing ability per 1000 cm\(^2\) of remaining leaf area, but as defoliation progressed further, this effect declined.

Conclusions. Previous experiments have shown that insect-induced defoliation of ca. 70% can cause immediate multifold reductions in \( N_2 \)-fixing ability (Layton & Boethel 1987, 1988). The decrease in \( N_2 \)-fixing ability generally is due to a combination of reduced nodule weight and reduced NSA. In the present study, 32% defoliation at 10 DPI caused a significant decrease in NSA, resulting in decreased
$N_2$-fixing ability. As defoliation progressed, the effect on NSA became more pronounced, and total nodule dry weight per EU also was affected, resulting in an even greater reduction in $N_2$-fixing ability.
REFERENCES CITED


CHAPTER III

Recovery of $N_2$ Fixation
in Soybean
Following Insect-induced Defoliation

This chapter is written in the style of the Journal of Economic Entomology and has been accepted for publication in this journal.

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ABSTRACT

Recovery of $N_2$-fixing ability (measured by $C_2H_2-C_2H_4$ assay) of greenhouse-grown soybean following defoliation by soybean looper, *Pseudoplusia includens* (Walker) [Lepidoptera: Noctuidae], was examined in two experiments. Experiment 1 examined nodulation, $N_2$ fixation, and dry weight of plant parts of 'Forrest' soybean at 0, 2, and 4 weeks post-defoliation (WPD) (70% defoliation). Experiment 2 examined these same variables in 'Davis' soybean at 0, 1, 2, 3, 4, and 5 WPD (73% defoliation). In both experiments, leaf area, stem dry weight, and root dry weight of defoliated plants were greatly reduced at 0 WPD and never recovered to the level of control plants. Although $N_2$-fixing ability of defoliated plants was reduced 4.9-fold in Experiment 1 and 8.7-fold in Experiment 2 at 0 WPD, there was no significant difference in $N_2$-fixing ability of defoliated and control plants in either experiment at 4 WPD. Recovery of $N_2$-fixing ability was due to partial recovery of total nodule dry weight, combined with complete recovery of nodule specific activity.

INTRODUCTION

Insect-induced defoliation greatly reduces $N_2$-fixing ability of soybean (Hutchinson 1979, Layton & Boethel 1987).
The relationship between photosynthate supply to the nodules and \( N_2 \) fixation rate is well established (Hardy & Havelka 1976, Quebedeaux 1979, Streeter et al. 1979), and the decrease in \( N_2 \)-fixing ability in response to defoliation is undoubtedly caused by reduced photosynthate supply to roots and nodules. Photosynthate, primarily in the form of sucrose (Ogren & Rinne 1973), must be produced in the leaves and translocated to the nodules before \( N_2 \) fixation can occur. From 5 to 7 g of C must be supplied to the nodules of legumes for each 1 g of N that is fixed. This C is used 1) to support nodule development, 2) to supply the energy that drives the \( N_2 \) fixation process, and 3) to provide the molecular skeletons needed for assimilation of the fixed N (Pate 1985).

Hardy & Havelka (1974) reported a 5-fold increase in rate of \( N_2 \) fixation as measured by \( \text{C}_2\text{H}_2-\text{C}_2\text{H}_4 \) assay \([\text{N}_2(\text{C}_2\text{H}_2)]\) in soybean in response to enhanced photosynthetic rate due to atmospheric \( \text{CO}_2 \) enrichment. Similarly, Lawn & Brun (1974) found that \( \text{N}_2(\text{C}_2\text{H}_2) \) fixation increased with increasing light intensity and decreased due to 50% shading or 60% artificial defoliation. It is clear that major increases in rate of \( N_2 \) fixation cannot occur without corresponding increases in photosynthetic rate (Hardy & Havelka 1976). Conversely, factors such as insect-induced defoliation that reduce overall photosynthetic rate will retard \( N_2 \) fixation.
Hutchinson (1979) reported large decreases in $N_2$ fixation immediately following insect-induced defoliation. Layton & Boethel (1987) further examined the immediate effects of insect-induced defoliation and found a linear reduction in $N_2(C_2H_2)$ fixation with increasing degree of defoliation. Furthermore, defoliation levels of ca. 47% or greater also caused reductions in nodule specific activity (NSA), a measure of $N_2$-fixing efficiency per g of dry nodule weight. Riggle et al. (1984) also reported decreased NSA in response to artificial defoliation.

Studies such as these have demonstrated that $N_2$ fixation is reduced following defoliation, but there is little information on subsequent recovery of $N_2$ fixation. Soybean in the vegetative and early reproductive stages can tolerate high levels of defoliation without sustaining significant losses in seed yield (Turnipseed 1972). This indication that soybean is often able to compensate for defoliation prompted us to examine the recovery response of $N_2$ fixation in soybean following insect-induced defoliation.

MATERIALS AND METHODS

Experiment 1. 'Forrest' soybean (maturity group V) was planted on 27 February in 42, 7.6-liter pots that contained 6 kg of sandy loam soil (organic matter = 0.24%). The seeds were inoculated with a commercial preparation containing
several strains of N₂-fixing bacteria, *Bradyrhizobium japonicum* (Kirchner) Buchanan, and planted at the rate of five per pot. Plants were thinned to two per pot shortly after emergence; thus, an experimental unit consisted of a single pot containing two plants. All pots were fertilized with 20 mg kg⁻¹ of K (potassium chloride) and 11 mg kg⁻¹ P (monocalcium phosphate) at 20 and 40 days after planting (DAP). From 0 to 71 DAP, the plants received supplemental lighting (photoperiod of 16:8 [L:D]) supplied by 40-W fluorescent lamps (Gro-Lux®) suspended ca. 70 cm above the tops of plants.

At 35 DAP, pots were randomly assigned to the treatments (control or defoliated); those assigned to the defoliated treatment were infested with 24 neonate larvae of the soybean looper, *Pseudoplusia includens* (Walker), per pot. Plants were in the V6 development stage (Fehr et al. 1971) at this time. Movement of larvae from infested pots to control pots was restricted by spacing pots approximately 50 cm apart so that leaves of plants in adjacent pots did not touch. Spacing of the pots in this manner also minimized shading by plants in adjacent pots. Larvae perished during the experiment due to various unknown factors, but this was expected and accounted for by the high initial infestation level. Pots were examined daily and larvae were often moved from one pot to another in order to maintain equal infestation of all pots and assure uniform defoliation. The larvae were allowed to feed for 21 days,
at which time many had pupated. Because maximum defoliation had occurred at this time (V11), this was designated 0 weeks post-defoliation (WPD). Remaining insects were removed from all plants, and seven randomly-chosen pots were harvested from each treatment. An additional seven pots were harvested from each treatment at 2 and 4 WPD (growth stages V15 and V18).

Experiment 2. The second experiment was conducted in a manner similar to the first, except for a few important differences. On 17 Dec, 104 pots were planted with 'Davis' soybean (maturity group VI) as described for Experiment 1. At 32 DAP (V4), the 52 pots that had been randomly assigned to the defoliated treatment were infested with 24 neonate soybean looper larvae. After 17 days, many of the surviving larvae had pupated, and plants had developed to the V8 stage. This was designated 0 WPD and eight randomly-chosen pots were harvested from each treatment. An additional eight randomly-chosen pots were harvested from each treatment at 1, 2, 3, and 4 WPD (growth stages V10, V11, V12, and R2, respectively). The remaining 12 pots per treatment were harvested at 5 WPD (growth stage R3). Fertilizer was applied at 20, 50, and 70 DAP, and supplemental lighting was supplied from 0 to 32 DAP.

Sampling Methods. Plants were harvested at the indicated times and subjected to the acetylene-ethylene \((C_2H_2-C_2H_4)\) reduction assay of Hardy et al. (1968), which provides an indirect measure of relative \(N_2\)-fixing ability.
Parameters determined for each experimental unit were: leaf area (measured using an area meter [LI-COR \textsuperscript{\textregistered} LI-3100]); number of nodes; stem dry weight; root dry weight; number of nodules; total nodule dry weight; mean individual nodule dry weight; \( \text{C}_2\text{H}_4 \text{ two-plants}^{-1} \text{ h}^{-1} \); \( \text{C}_2\text{H}_4 \text{ nodule}^{-1} \text{ h}^{-1} \); NSA (\( \text{C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule dry weight h}^{-1} \)); \( \text{C}_2\text{H}_4 \text{ 1000-cm}^{-2} \text{ leaf area h}^{-1} \); and nodule:root ratio (nodule dry weight/root dry weight).

The methods used in harvesting the plants and performing the \( \text{C}_2\text{H}_2+\text{C}_2\text{H}_4 \) reduction assay are described by Layton & Boethel (1987). All measurements were made on the two plant experimental units.

Statistical Analyses. Data from individual sample dates of each experiment were analyzed using PROC TTEST of SAS (SAS Institute 1985). Treatment means for a given sample date were declared significantly different when the following criteria were met: Experiment 1, \(|t| > 2.179, \text{ df} = 12, \text{ } P < 0.05\); Experiment 2, 0-4 WPD, \(|t| > 2.145, \text{ df} = 14, \text{ } P < 0.05\); Experiment 2, 5 WPD, \(|t| > 2.074, \text{ df} = 22, \text{ } P < 0.05\). Actual \( P \) values are noted in the text only for cases where \( 0.05 < P \leq 0.10 \).

RESULTS AND DISCUSSION

Experiment 1. At 0 WPD, defoliated plants suffered 70% loss of leaf area relative to controls [(\( \bar{X} \) leaf area of control - \( \bar{X} \) leaf area of defoliated)/\( \bar{X} \) leaf area of control]}
(Fig. 1A) and defoliation appeared uniform from top to bottom of the plants. Because the pots that were harvested at 0 WPD were chosen randomly from all pots in the experiment, this indicates that, at 0 WPD, the remaining plants also were defoliated an average of 70%. The absolute difference in mean leaf area per two plants was 1638 cm$^2$ at 0 WPD. By 4 WPD, defoliated plants had 38% less leaf area than controls, which indicated a relative degree of recovery due to the development of new foliage that was not subjected to defoliation. However, the absolute difference in leaf area had increased to 4788 cm$^2$.

These results imply that defoliation decreased the rate of new leaf development due to reduced photosynthate supply. This may seem paradoxical because leaves are the site of photosynthesis; however, the development of a new legume leaf requires an initial investment of photosynthate that is not returned until the leaf reaches ca. 50% of its full size (Ogren & Rinne 1973, Pate 1985). Turnipseed (1972) reported that a lower level of artificial defoliation (33%) did not affect the rate of development of new leaf tissue in field-grown soybean.

Supplemental lighting prevented the onset of reproductive development and artificially prolonged the vegetative development period, which allowed us to determine if defoliation affected development of new leaf area by reducing development of new nodes. Control plants had significantly more nodes than defoliated plants at 0 WPD
Fig. 1. Leaf area (A), stem dry weight (B), and root dry weight (C) per two plants in 'Forrest' soybean following 70% insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
(Table 1). However, there was no difference in number of nodes at 2 WPD when supplemental lighting was terminated. At 4 WPD, defoliated plants had significantly more nodes than control plants, and a few plants in both treatments had begun to bloom.

Therefore, defoliation did not retard development of new nodes, and the continued divergence in absolute leaf areas following removal of the soybean loopers was not due to reduced node development. Increased branch development and increased leaf size remain as possible explanations. While neither of these parameters was quantified, control plants did exhibit increased branching in the weeks following defoliation.

Defoliation also resulted in significant reductions in dry stem (Fig. 1B) and root (Fig. 1C) weight of defoliated plants on all sample dates. At 4 WPD, control plants had 1.9-fold greater stem weight and 2.2-fold greater root weight than defoliated plants. The reduction in stem dry weight was not due to reduced development of new nodes but rather to observed reductions in stem diameter and internode length.

In addition to indicating reduced photosynthate supply to the root system, reduced root mass also means fewer sites for development of nodules that house the N₂-fixing bacteria. However, from 10 to 14 days are required for development of new nodules (Carlson 1973), yet most defoliation occurred during the 7 to 10 days immediately
Table 1. Recovery of selected parameters in 'Forrest' soybean at 0, 2, and 4 weeks following 70% insect-induced defoliation (WPD)

<table>
<thead>
<tr>
<th></th>
<th>Nodule:Root Ratio</th>
<th>Leaf Area (umol h⁻¹)</th>
<th>C₂H₄ 1000-cm⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(dry weight)</td>
<td></td>
<td></td>
</tr>
<tr>
<td># Nodes (plant⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.5 ± 0.15</td>
<td>0.57 ± 0.026</td>
<td>13.6 ± 1.14</td>
</tr>
<tr>
<td>Defol.</td>
<td>10.6 ± 0.17</td>
<td>0.66 ± 0.035</td>
<td>9.1 ± 0.99</td>
</tr>
<tr>
<td>Control</td>
<td>14.9 ± 0.21</td>
<td>0.52 ± 0.015</td>
<td>7.1 ± 0.43</td>
</tr>
<tr>
<td>Defol.</td>
<td>14.8 ± 0.24</td>
<td>0.83 ± 0.042</td>
<td>8.5 ± 1.00</td>
</tr>
<tr>
<td>Control</td>
<td>18.0 ± 0.33</td>
<td>0.47 ± 0.022</td>
<td>4.8 ± 0.48</td>
</tr>
<tr>
<td>Defol.</td>
<td>18.9 ± 0.14</td>
<td>0.79 ± 0.047</td>
<td>6.3 ± 0.95</td>
</tr>
</tbody>
</table>

Mean ± standard error of the mean: * indicates a significant difference (P < 0.05) between means of a common pair, based on PROC TTEST (SAS Institute 1985).
before the 0 WPD sample date (soybean loopers consume ca. 90% of their total foliage intake during the 5th and 6th stadia [Boldt et al. 1975]). Therefore, there was no significant difference in number of nodules at 0 WPD (Fig. 2A), because insufficient time had elapsed since defoliation. By 2 WPD, control plants had developed twice as many nodules as they had at 0 WPD, while very few new nodules had developed on defoliated plants. This resulted in a significant difference in mean number of nodules at 2 WPD that persisted at 4 WPD.

Although there was no difference in number of nodules at 0 WPD, defoliated plants had significantly smaller nodules on that date (Fig. 2C). Individual nodules of control plants were 1.6-fold larger than those of defoliated plants. By 2 and 4 WPD, nodules of defoliated plants were roughly equal in size to those of control plants.
Apparenty, defoliated plants completed development of nodules already present, before growing new nodules.

Control plants had significantly greater total nodule dry weight than defoliated plants on all sample dates (Fig. 2B). At 0 WPD, this was due to reduced size of individual nodules on defoliated plants (numbers were equal). But, at 2 and 4 WPD, this was due to reduced number of nodules in the defoliated treatment (size was equal). Total nodule weight is an important factor that affects rate of $N_2$ fixation (Weber 1966).
Fig. 2. Number of nodules (A) and total nodule dry weight (B) per two plants and mean dry weight of individual nodules (C) in 'Forrest' soybean following 70% insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
No significant difference ($P = 0.0684$) was found in nodule:root weight ratio at 0 WPD (Table 1). At 2 and 4 WPD, however, the nodule:root ratio of defoliated plants was significantly greater than that of controls, indicating increased development of nodules per unit of root mass in defoliated plants.

$N_2$-fixing ability was significantly reduced in defoliated plants at 0 WPD, as indicated by the reduced rate of $C_2H_4$ production (Fig. 3A); control plants produced 4.9-fold more $C_2H_4$. At 2 WPD, control plants still exhibited significantly greater $N_2$-fixing ability, but the difference had decreased to 1.8-fold. By 4 WPD, there was a non-significant, 1.2-fold difference between treatments.

These results illustrated recovery of $N_2$-fixing ability in defoliated plants. This can be partly explained by partial recovery of total nodule dry weight of defoliated plants. Nodule weight of control plants was 1.8-fold that of defoliated plants at 2 WPD but only 1.3-fold at 4 WPD (Fig. 2B). Thus, from 2 to 4 WPD, defoliated plants developed more new nodule mass (1427 mg two-plants$^{-1}$) than controls (992 mg two-plants$^{-1}$).

NSA of control plants was 3-fold that of defoliated plants at 0 WPD, a significant difference, but there was no significant difference in NSA at 2 and 4 WPD (Fig. 3B). The reduction in NSA at 0 WPD was attributed to a reduced supply of photosynthate, which provides the energy that fuels the $N_2$ fixation process within the nodules (Pate 1985). Riggle
Fig. 3. Total $C_2H_4$ production ($N_2$-fixing ability) per two plants per h (A), nodule specific activity (B), and $C_2H_4$ production per nodule (C) in 'Forrest' soybean following 70% insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
et al. (1984) observed reduced NSA in response to artificial defoliation and partial shading, which they also attributed to reduced photosynthate supply.

The essentially complete recovery of NSA by 2 WPD suggests preferential partitioning of available photosynthate to nodules in order to sustain biological $N_2$ fixation. However, it must be noted that by 2 WPD, defoliated plants had less total nodule mass than controls (Fig. 2B), and therefore would have required less photosynthate in order to maintain an equivalent NSA.

At 0 WPD, control plants produced 4.8-fold more $C_2H_4$ per nodule than defoliated plants, but this significant difference disappeared by 2 and 4 WPD (Fig. 3C). The large difference at 0 WPD was again attributed to reduced photosynthate supply but was also magnified by the fact that nodules of defoliated plants were smaller than those of control plants at 0 WPD (Fig. 2C).

Control plants produced significantly more $C_2H_4$ 1000-cm$^{-2}$ leaf area h$^{-1}$ than defoliated plants at 0 WPD (Table 1). However, by 2 and 4 WPD, defoliated plants slightly exceeded control plants in this variable, though not significantly.

Experiment 2. Results of Experiment 2 provide a more complete view of the recovery response of soybean following insect-induced defoliation, because samples were taken at weekly intervals through 5 WPD. The absolute difference in mean leaf area was 801 cm$^2$ at 0 WPD (Fig. 4A), which
Fig. 4. Leaf area (A), stem dry weight (B), and root dry weight (C) per two plants in 'Davis' soybean following 73% insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
represented 73% defoliation. By 4 WPD, the absolute difference in mean leaf areas had increased to 1703 cm², in a response similar to that observed with 'Forrest'. In both experiments, the reduction in leaf area due to reduced leaf growth following defoliation was greater than the loss of leaf area due to consumption by the soybean loopers.

Defoliated plants trailed controls in development of new nodes on all sample dates (Table 2). The situation was similar to that observed with 'Forrest' in that reduced node development did not account fully for the reduced leaf development subsequent to defoliation. Defoliation did not appear to delay reproductive development, because by 5 WPD, both treatments had developed to the R3 stage.

Dry stem (Fig. 4B) and root (Fig. 4C) weight were significantly lower in the defoliated treatment on all sample dates, as also seen in Experiment 1. At 0 WPD, control plants had 1.7-fold more stem and root dry weight than defoliated plants. By 5 WPD, the differences were still large with the control having 1.6-fold more stem dry weight and 2-fold more root dry weight. Thus, the effects of defoliation in retarding development of these plant parts persisted for the duration of the experiment, and defoliated plants entered reproductive development in a stunted condition.

As with 'Forrest', there was no significant difference in number of nodules at 0 WPD, but from 1 to 4 WPD, control plants had significantly more nodules (Fig. 5A). Although
Table 2. Recovery of selected parameters in 'Davis' soybean at 0, 1, 2, 3, 4, and 5 weeks following 73% insect-induced defoliation (WPD)

<table>
<thead>
<tr>
<th></th>
<th># Nodes (plant⁻¹)</th>
<th>Nodule:Root Ratio (dry weight)</th>
<th>C₂H₄ 1000-cm⁻² (umol h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 WPD</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>8.1 ±0.18</td>
<td>0.55 ±0.043</td>
<td>7.7* ±0.72</td>
</tr>
<tr>
<td>Defol.</td>
<td>7.4 ±0.24</td>
<td>0.53 ±0.019</td>
<td>3.5 ±0.41</td>
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<tr>
<td><strong>1 WPD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.9* ±0.13</td>
<td>0.61* ±0.037</td>
<td>13.6 ±1.32</td>
</tr>
<tr>
<td>Defol.</td>
<td>9.3 ±0.16</td>
<td>0.47 ±0.041</td>
<td>13.5 ±0.80</td>
</tr>
<tr>
<td><strong>2 WPD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.8 ±0.27</td>
<td>0.61 ±0.029</td>
<td>7.9 ±1.06</td>
</tr>
<tr>
<td>Defol.</td>
<td>10.3 ±0.23</td>
<td>0.63 ±0.041</td>
<td>15.0* ±0.69</td>
</tr>
<tr>
<td><strong>3 WPD</strong></td>
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</tr>
<tr>
<td>Control</td>
<td>12.4* ±0.21</td>
<td>0.59 ±0.060</td>
<td>7.0 ±1.00</td>
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<tr>
<td>Defol.</td>
<td>11.7 ±0.19</td>
<td>0.76* ±0.146</td>
<td>14.9* ±1.51</td>
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(Table 2 continued)

<table>
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<th>5 WPD</th>
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<tr>
<td>Control</td>
<td>13.8 ±0.25</td>
<td>15.0* ±0.20</td>
</tr>
<tr>
<td></td>
<td>0.75 ±0.040</td>
<td>0.58 ±0.030</td>
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<tr>
<td></td>
<td>5.4 ±0.68</td>
<td>5.3 ±0.49</td>
</tr>
<tr>
<td>Defol.</td>
<td>13.1 ±0.22</td>
<td>14.2 ±0.26</td>
</tr>
<tr>
<td></td>
<td>0.91* ±0.041</td>
<td>0.83* ±0.023</td>
</tr>
<tr>
<td></td>
<td>13.5* ±1.79</td>
<td>11.7* ±0.64</td>
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</table>

Mean ± standard error of the mean: * indicates a significant difference (P < 0.05) between means of a common pair, based on PROC TTEST (SAS Institute 1985).
Fig. 5. Number of nodules (A) and total nodule dry weight (B) per two plants and mean dry weight of individual nodules (C) in 'Davis' soybean following 73% insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
the control had an average of 127 nodules two-plants\(^{-1}\) at 5 WPD vs 99 for the defoliated treatment, the difference was not significant (\(P = 0.0656\)).

Defoliated plants had significantly smaller nodules than controls from 0 to 3 WPD, but not at 4 and 5 WPD (Fig. 5C). Thus, in both experiments, defoliation caused an initial decrease in mean nodule size followed by subsequent recovery. Defoliation significantly reduced total nodule dry weight on all sample dates (Fig. 5B).

Examination of the nodule:root ratio for 'Davis' (Table 2) revealed an interesting pattern in development of nodules and roots as affected by defoliation. There was no significant difference in this ratio at 0 WPD. Control plants had a significantly greater nodule:root ratio at 1 WPD, but the difference disappeared at 2 WPD. From 3 to 5 WPD, there was again a significant difference in nodule:root ratio, but it was in favor of defoliated plants. Results of both experiments showed that defoliated plants responded to the photosynthate deficit caused by defoliation by restricting root development in favor of nodule development.

\(\text{C}_2\text{H}_4\) production in the control treatment was 8.2 \(\mu\text{mol two-plants}^{-1}\ \text{h}^{-1}\) at 0 WPD vs 0.9 for the defoliated treatment, a significant 8.7-fold difference (Fig. 6A). At 1 WPD, the difference decreased to 3.3-fold, which was still significant. At 2 WPD, \(\text{N}_2\)-fixing ability of control plants was 1.6-fold that of defoliated plants, but this did not represent a significant difference. \(\text{N}_2\)-fixing ability of
Fig. 6. Total \( C_2H_4 \) production (\( N_2 \)-fixing ability) per two plants per h (A), nodule specific activity (B), and \( C_2H_4 \) production per nodule (C) in 'Davis' soybean following 73% insect-induced defoliation. * indicates a significant difference between treatment means for a sample date.
the two treatments was essentially equal at 3 WPD, and, surprisingly, by 4 and 5 WPD, C\textsubscript{2}H\textsubscript{4} production was slightly, but not significantly, greater in the defoliated treatment. Total N\textsubscript{2}(C\textsubscript{2}H\textsubscript{2}) fixation for the 5-week period was lower in defoliated plants than in controls (Fig. 6A).

These data show full recovery of N\textsubscript{2}-fixing ability of defoliated plants by only 3 WPD. This is despite the fact that these plants had sustained 73% defoliation at 0 WPD, had only 46% as much leaf area as control plants at 3 WPD, and also had less stem, root, and nodule mass.

Data on NSA for Experiment 2 (Fig. 6B) show that recovery of N\textsubscript{2}-fixing ability was due largely to an increase in efficiency of N\textsubscript{2} fixation per g of nodule weight. NSA of controls was 5-fold greater than that of defoliated plants at 0 WPD, but by 1 WPD, there was no significant difference. By 2 WPD, NSA of defoliated plants had significantly exceeded that of controls, and it remained significantly greater from 3 to 5 WPD. These results agree with those for Experiment 1 in which NSA of defoliated plants had recovered to the level of controls by 2 WPD.

C\textsubscript{2}H\textsubscript{4} produced per nodule (Fig. 6C) followed a trend similar to that for NSA. Control plants produced significantly more C\textsubscript{2}H\textsubscript{4} per nodule at 0 and 1 WPD, but by 2 WPD, defoliated plants slightly exceeded control plants. Defoliated plants produced significantly more C\textsubscript{2}H\textsubscript{4} per nodule than controls from 3 to 5 WPD.
At 0 WPD, control plants produced 2.2-fold more $C_2H_4$ 1000-cm$^{-2}$ leaf area h$^{-1}$, a significant difference (Table 2). There was no difference between the treatments at 1 WPD, but from 2 to 5 WPD, defoliated plants produced roughly 2-fold as much $C_2H_4$ 1000-cm$^{-2}$ leaf area h$^{-1}$ as control plants. There are two possible explanations for this shift. Either defoliated plants compensated for defoliation by increased photosynthetic efficiency of remaining leaf tissue or defoliated plants allocated a greater proportion of their available photosynthate to the $N_2$-fixing process. Both Poston et al. (1976) and Ingram et al. (1981) found no lasting change in net photosynthetic rate of remaining soybean foliage following defoliation. However, Martens & Trumble (1987) cite several examples in other crops of increased photosynthetic efficiency of remaining foliage following defoliation, and Thorne and Koller (1974) reported increased net photosynthesis in single soybean leaves when all other leaves were severely shaded.

Because no measure of photosynthetic rate was taken in either of our experiments, it is impossible to determine whether photosynthetic compensation occurred. However, it is obvious that defoliated plants recovered $N_2$-fixing ability more rapidly than they recovered leaf area, stem dry weight, or root dry weight. This suggests that defoliated plants preferentially allocated a greater proportion of their available photosynthate to the $N_2$-fixing process; otherwise all parameters should have recovered similarly.
This conclusion is further supported by the increase in nodule:root ratio of defoliated plants and by Thrower's (1962) report that defoliation increased the proportion of photosynthate translocated to the root system from undamaged soybean leaves.

It must be noted that these results were obtained from greenhouse-grown soybean defoliated during vegetative development. Rate of $N_2$ fixation is greater during reproductive development (Hardy & Havelka 1976, Ryle et al. 1978) and developing seed compete with nodules for photosynthate (Lawn & Brun 1974). Also, ability of soybean to recover from defoliation without yield loss decreases as reproductive development proceeds (Weber & Caldwell 1966). Furthermore, the low organic matter content of the soil used in these experiments indicated low N content (Brady 1974). This soil was used in order to maximize the plants' dependence on biologically fixed N. Thus, the recovery responses of reproductive soybean or of plants less dependent on biological $N_2$ fixation may differ.

The recovery curves illustrated in Fig. 7 summarize results of both experiments. These curves were determined by calculating the defoliated:control ratio of the treatment means for total $C_2H_4$ production per experimental unit on each sample date. Thus, return of the ratio to a value of unity represents full recovery of $N_2$-fixing ability. Although defoliated plants never fully recovered in Experiment 1 (Fig. 7A), the trend toward recovery is
Fig. 7. Recovery of N₂-fixing ability in 'Forrest' (A) and 'Davis' (B) soybean following ca. 70% insect-induced defoliation.
evident, and indeed, the difference between treatments on the final sample date was not significant. The recovery curve for 'Davis' (Fig. 7B) shows full recovery of N₂-fixing ability by 3 WPD and greater N₂-fixing ability of defoliated plants at 4 and 5 WPD. Therefore, results of both experiments show a dramatic decrease in N₂-fixing ability immediately after insect-induced defoliation, followed by a period of rapid recovery.

In Experiment 1, recovery of N₂-fixing ability was due to a combination of partial recovery of nodule mass and full recovery of NSA of defoliated plants. In Experiment 2, the recovery primarily was due to increased NSA of defoliated plants, which was also reflected as an increase in C₂H₄ production per individual nodule as well as in C₂H₄ production 1000-cm⁻² of leaf area. The response to defoliation as measured by these three somewhat analogous variables suggested that at 4 WPD, defoliated plants invested roughly as much photosynthate into N₂ fixation as control plants, although they had less leaf area with which to produce photosynthate. Thus, in both experiments, plants recovered from the adverse effects of defoliation on N₂ fixation within a few weeks, but the adverse effects on overall plant development persisted.
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SUMMARY

The more important findings of this research effort are enumerated below, by chapter:

CHAPTER I. Reduction in $N_2$ Fixation by Soybean in Response to Insect-induced Defoliation:

1) $N_2$-fixing ability decreased linearly as percent defoliation increased.

2) Nodule specific activity (NSA) was unaffected by defoliation of less than ca. 47%, but decreased sharply as defoliation exceeded 47%.

3) Stem, root, and nodule dry weight decreased linearly as percent defoliation increased.

4) The reduction in $N_2$-fixing ability was due solely to reductions in nodule dry weight at defoliation levels less than ca. 47%, and was due to a combination of reduced nodule dry weight and reduced NSA at defoliation levels exceeding ca. 47%.

CHAPTER II. Response of Soybean Growth and $N_2$-fixing Ability to Progressive Insect-induced Defoliation:

1) $N_2$-fixing ability was adversely affected as early as 10 days after plants were infested (DPI) with neonate soybean looper larvae, or by the time defoliation reached 37%.

2) This reduction in $N_2$-fixing ability at 10 DPI was the result of reduced NSA.
3) Significant decreases were observed in dry weight of stems, by 10 DPI (37% defoliation), and in dry weight of roots and nodules, by 14 DPI (62% defoliation).

4) Thus, the significant reductions in N₂-fixing ability at 14 and 16 DPI (62 & 73% defoliation) were the result of decreases in both nodule dry weight and NSA.

CHAPTER III. Recovery of N₂-fixing Ability in Soybean Following Insect-induced Defoliation:

1) In two experiments, defoliation of 70 and 73% caused 4.9 and 8.7-fold decreases in N₂-fixing ability at 0 weeks post-defoliation (WPD).

2) These reductions in N₂-fixing ability were due to decreases in both nodule dry weight and NSA.

3) In both experiments, defoliated plants recovered their N₂-fixing ability, relative to controls, by 4 WPD.

4) Recovery of N₂-fixing ability was due to a combination of full recovery of NSA and partial recovery of total nodule dry weight. In Experiment 2, defoliated plants had significantly greater NSA than controls at 2, 3, 4, and 5 WPD.

5) In both experiments, leaf area, and stem and root dry weight of defoliated plants were greatly reduced at 0 WPD and never recovered to the level of control plants. Thus, defoliated plants recovered their N₂-fixing ability despite prolonged effects on plant growth.
Many factors must be considered in assessing the long-term significance of the impact of insect-induced defoliation on soybean. There is currently a large research effort devoted toward developing ways to increase the rate of $N_2$ fixation in soybean and other legumes. Because of the dependence of $N_2$ fixation on photosynthate supply, it is obvious that, if this effort is successful, photosynthate requirements will likely increase along with the rate of fixation. If this occurs, the impact of defoliation on $N_2$ fixation will also increase.

Next, one must realize that most soil N is stored in the organic matter of the soil and slowly becomes available to plants as the organic matter decomposes. In many areas of the country, present cropping practices are causing a gradual decline in soil organic matter content, which consequently reduces the amount of N available to following crops. In such situations, biological fixation will become more important as a source of N, and the significance of any factor that limits $N_2$ fixation will increase.

Finally, one must also remember that the energy needed to produce synthetic N fertilizers is derived primarily from fossil fuels. The petroleum shortage of the 1970s resulted in sharp increases in the costs of N fertilizers. Unless alternative methods of producing N fertilizers can be developed and adopted, there is little doubt that future petroleum shortages will result in increased prices and decreased availability of synthetic N fertilizers.
Therefore, our dependence on biologically fixed N will likely increase in the future, and consequently, our concern with factors, such as insect-induced defoliation, that adversely affect \( \text{N}_2 \) fixation will also increase.

The results presented in this study show that insect-induced defoliation can greatly reduce the rate of \( \text{N}_2 \) fixation. However, the effects of this decreased \( \text{N}_2 \) fixation on seed yield remain to be assessed. As indicated in previous chapters, dependence on symbiotically fixed N increases as levels of N in the soil decrease. Therefore it seems reasonable to conclude that in soybeans that are largely dependent on symbiotically fixed N, decreased \( \text{N}_2 \)-fixing ability due to defoliation will adversely affect total N accumulation and subsequently, affect seed yield. This implies that the impact of a given degree of defoliation will be greater in soybeans that are heavily dependent on symbiotic fixation than in soybeans that are growing in N rich soils.

In the future, pest management decisions may have to consider both the effects of defoliation on \( \text{N}_2 \) fixation and the N fertility status of the soil in which the crop is growing, in addition to the other factors that traditionally enter into pest management decisions involving defoliating pests. Development of pest management programs with this degree of refinement remains a long term goal, requiring much additional research. By increasing our understanding of how defoliation affects growth and \( \text{N}_2 \)-fixing ability in
vegetative soybeans, the results of this research effort have moved us a few steps closer to the achievement of this goal.
VITA

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Major Field: Entomology

Title of Dissertation: Response of Soybean Growth and Dinitrogen-fixing Ability to Insect-induced Defoliation

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Date of Examination:

November 17, 1987