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Interactions of the Soybean Looper, Pseudoplusia Includens (Walker), and Soybean, Glycine Max (L.) Merrill, Involving Nitrogen: Effects on Host Symbiotic Nitrogen Fixation and Larval Development and Survival.

Alan Thomas Wier
Louisiana State University and Agricultural & Mechanical College

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INTERACTIONS OF THE SOYBEAN LOOPER, *PSEUDOPLUSIA INCLUDENS* (WALKER), AND SOYBEAN, *GLYCINE MAX* (L.) MERRILL, INVOLVING NITROGEN: EFFECTS ON HOST SYMBIOTIC NITROGEN FIXATION AND LARVAL DEVELOPMENT AND SURVIVAL

A Dissertation

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

by Alan T. Wier
B.S., Nicholls State University, 1983
M.S., Louisiana State University, 1987
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Acetylene reduction and nitrogen difference assays were used to evaluate effects of defoliation by soybean looper, *Pseudoplusia includens* (Walker), during reproductive growth stages on symbiotic nitrogen fixation by soybean. Defoliation (50-55%) of group VI cultivar Lee from full bloom into pod development stages caused up to 85% reduction of nitrogenase activity, whereas lower defoliation levels (32%) during seed development did not affect nitrogenase activity. Defoliation (74-94%) of group IV cultivar Clark caused 80-100% reduction of nitrogenase activity, regardless of whether defoliation occurred during bloom and pod development or during seed development. The nitrogen difference assay identified a reduction in the amount of nitrogen acquired from symbiotically-fixed sources in response to defoliation, but this occurred in only one experiment (Lee, 55% defoliation, full bloom into early seed development). Yield of Lee was reduced by 1492 kg/ha (50% defoliation, full bloom through pod development) or by 971 kg/ha (32% defoliation, early seed development). Yield of Clark was reduced by 800-1359 kg/ha (74-94% defoliation, full bloom through pod development) or by 750 kg/ha (94% defoliation, early seed development).

Chlorosis of non-nodulating isolines occurred during seed development and was associated with reduced feeding and poor establishment of soybean looper in field plots. As a
result, anticipated reductions in leaf area of non-nodulating isolines were not achieved during seed development, and three feeding bioassays were initiated to examine how chlorotic plants influenced soybean looper development. These bioassays used foliage from nodulating and non-nodulating isolines collected from field plots beginning at bloom and early seed development, and also examined foliage from greenhouse-grown non-nodulating soybeans supplied with six rates of nitrogen fertilizer (0-168 kg N/ha as NH₄NO₃). Responses by soybean looper to reduced nitrogen included development of greater number of stadia, lengthened duration of larval development, and reduced survival. Extended development resulted in greater foliage consumption and maximum larval weights of survivors that equalled those fed foliage with higher nitrogen concentrations. These responses to fluctuations in host nutrition may be mechanisms that allowed soybean looper to evolve in association with such a wide range of hosts (73 species in 29 families).
INTRODUCTION

Soybean, *Glycine max* (L.) Merrill, is attacked by a number of insect pests from orders including Coleoptera, Diptera, Hemiptera, Homoptera, Lepidoptera, Orthoptera, and Thysanoptera (Higley and Boethel 1994). The most important lepidopteran defoliators of soybean grown in the southern United States along the gulf coast region belong to the family Noctuidae and include velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, and soybean looper, *Pseudoplusia includens* (Walker) (Turnipseed and Kogan 1987). Although soybean looper develops on a number of hosts (73 plant species in 29 families, Herzog 1980), this insect occurs on soybean annually in the southeastern United States. Peak infestations that have the potential to limit yield usually occur in late August and September in this region.

Soybean yield responses to defoliation have been thoroughly reviewed and can depend on several factors including the plant growth stage, amount of defoliation, growth habit, and light interception by the remaining foliage (Turnipseed and Kogan 1987, and Hunt et al. 1994). Yield reductions of soybean are not expected to occur in response to <20 percent defoliation regardless of the stage of development (Turnipseed and Kogan 1987). Sometimes yield can be affected by high levels of defoliation that occur in early growth stages (Hunt et al. 1994); however, ≈30% defoliation generally does not reduce yield if it occurs before the end
of bloom or after full seed (Turnipseed and Kogan 1987). This compensation for 30% defoliation before the end of bloom was demonstrated even when adverse conditions including drought or late planting had stressed plant growth (Turnipseed 1972, and Caviness and Thomas 1980). The reproductive growth stages of soybean that involve pod and seed development are most sensitive to defoliation. After bloom, soybean generally can tolerate only 15-20% defoliation without yield reductions (Turnipseed 1972). Yield losses have occurred in response to 50-70% defoliation even during late seed development stages (Turnipseed 1972, and Board et al. 1994). Reproductive development is more synchronized in determinate varieties, and these varieties have demonstrated greater yield reductions in response to defoliation than indeterminate varieties during reproductive growth stages (Fehr et al. 1977). Most of the research on soybean yield responses to defoliation has involved mechanical defoliation. However, literature exists concerning effects of defoliation by soybean looper. Yield reductions >10% have occurred in soybean in response to defoliation by soybean looper (Bergman et al. 1985). Greater yield reductions (>30%) have occurred in northern Louisiana in response to heavy defoliation (80-90%) by natural infestations (107 larvae/25 sweeps, 38 cm diameter sweep net) of soybean looper in late August during late seed fill (Leonard et al. 1994).
The relationships among defoliation, symbiotic N fixation, and seed yield involve associations among plant sources and sinks where energy (carbohydrate) is produced or utilized. Defoliation affects the energy reserves of soybean at plant sources by reducing photosynthesis and removing starch reserves in foliage. This in turn suppresses energy 'sinks' that are dependent on photosynthate and starch reserves including the bacteria in root nodules that are responsible for symbiotic N fixation (Hardy and Havelka 1976, Mederski and Streeter 1977, Brun 1978, Gutschick 1978, Streeter et al. 1979, Schweitzer and Harper 1980, Williams et al. 1982, Denison and Sinclair 1985, and Millhollon and Williams 1986). Other factors that also affect symbiotic N fixation include soil temperature and water.

Symbiotic N fixation is an aerobic process that involves the reduction of N₂ to NH₄⁺ by bacteria, *Bradyrhizobium* spp., that reside in nodules on root systems of plants (mainly the Fabaceae) (Salisbury and Ross 1985). The reactions that result in N₂ being converted into NH₄⁺ and then into organic forms of nitrogen utilized by the plant are outlined as follows. Oxygen is transported in nodules by the protein leghemoglobin. The bacteria also require an energy source for reduction of N₂, which is supplied by the legume host as photosynthate (Hardy and Havelka 1976, Brun 1978, Gutschick 1978, and Streeter et al. 1979). Starch reserves can maintain energy requirements of the bacteria when
photosynthesis is suppressed such as in cloudy weather, shade, or darkness (Mederski and Streeter 1977, Schweitzer and Harper 1980, Williams et al. 1982, Denison and Sinclair 1985, and Millhollon and Williams 1986).

The chemical reaction for symbiotic N fixation is expressed as:

\[ \text{N}_2 + 6 \text{ electrons} + 8\text{H}^+ + \approx15\text{ ATP} \rightarrow 2\text{NH}_4^+ + \approx15\text{ ADP} + \approx15\text{ Pi}. \]

This equation denotes a proton, an electron, and an ATP requirement (Salisbury and Ross 1985). The source of protons and electrons is mainly sucrose that is translocated from foliar sources. Sources of sucrose include products of photosynthesis and starch degradation. The specific chemical reactions that result in production of \text{NH}_4^+ begin with reduction of these carbon sources and ferredoxin by NADH or NADPH. Ferredoxin is highly effective at reducing \text{N}_2 to \text{NH}_4^+. Nitrogenase, the enzyme catalyst of \text{N}_2 fixation, accepts electrons from reduced ferredoxin as it catalyses \text{N}_2 fixation. Nitrogenase consists of two components: Component I is an Fe-Mo protein (28 Fe atoms and 2 Mo atoms per molecule); and Component II is an Fe-protein (4 Fe atoms per molecule). Iron and Mo are reduced and then oxidized as nitrogenase accepts electrons from ferredoxin and transfers them to \text{N}_2 for formation of \text{NH}_4^+. Then ATP binds to Component II and increases this protein's strength as a reducing agent. Component II transfers electrons to Component I and ATP is
hydrolyzed to ADP. Component I completes the transfer of electrons to \(N_2\), and when 6 electrons and 8 protons (\(H^+\)) are accepted, 2 \(NH_4^+\) products are released from the enzyme. The \(NH_4^+\) is converted to organic N-rich compounds including glutamine, glutamic acid, asparagine, and ureides (allantoin and allantoic acid) in the cytosol. Three major forms of N are transported from nodules throughout the plant. These are asparagine, allantoin, and allantoic acid. Asparagine predominates in legumes of temperate origin, while ureides (allantoin and allantoic acid) predominate in legumes of tropical origin (Salisbury and Ross 1985).

Symbiotic nitrogen fixation can be measured in a number of ways including the acetylene reduction assay (Hardy et al. 1968) and the nitrogen difference method (Weaver 1986). The acetylene reduction assay is an indirect measurement of symbiotic N fixation. It measures nitrogenase activity and is dependent on the reduction of \(C_2H_2\) to \(C_2H_4\) by the bacteria in root nodules. This can be expressed as designated below-

\[
C_2H_2 + H_2 \xrightarrow{\text{nitrogenase}} C_2H_4.
\]

This method basically involves incubation of excavated root systems and nodules in a sealed container with 5\% \(C_2H_2\) and measurement of the amount of \(C_2H_4\) produced. This assay is a sensitive test that accurately measures the activity of the nitrogenase enzyme or how rapidly \(C_2H_2\) is being 'fixed' to \(C_2H_4\) by one plant relative to another. The acetylene
reduction assay should not be used to estimate the amount of N that was fixed over a growing season. Accuracy of this assay is dependent on complete and uniform removal of root systems and nodules (Weaver 1986). Sandy or loam soil types would aid root and nodule excavation for this assay. Nodules on lateral roots are relatively more important as plant maturity progresses, and incomplete removal of lateral roots late in the growing season can result in serious errors with this assay (Sloger et al. 1975).

Calculation of a N difference value or difference in soil-derived N from total plant N (soil-derived and atmospherically-derived or fixed N) also can be used to measure the amount of plant N that was acquired from symbiotically-fixed sources (LaRue and Patterson 1981). The N difference value is calculated by subtracting the N content of a non-fixing control plant (soil-derived N) from the N content of a nodulated plant (soil-derived plus fixed N). This method assumes that the two plant types accumulate similar amounts of soil-derived N in the above-ground portions (shoots), that they have similar ability to extract soil N, and that they accumulate soil N over the same period of time (Weaver 1986). However, some of these assumptions may be invalid because soil N concentration can affect the proportion of N acquired from soil compared to fixed sources in nodulated plants, and therefore, the amounts of N accumulated from soil sources may differ for fixing and non-
fixing plants. Non-nodulating isolines of soybean are optimal non-fixing control plants for evaluating symbiotic N fixation of soybean using this method. Similar rooting characteristics of nodulating and non-nodulating soybeans have been demonstrated (Mitchell and Russell 1971, and Nelson and Weaver 1980).

The acetylene reduction assay has been used extensively to measure differences in nitrogenase activity of soybean resulting from plant stress such as insect injury. Hutchinson (1979) reported that nitrogenase activity of soybean was reduced following soybean looper defoliation levels of approximately 33% that occurred over a 10-14 day period beginning at an early vegetative growth stage (V5, Fehr et al. 1971). He also found that mechanical defoliation, feeding and girdling by the three-cornered alfalfa hopper, Spissistilus festinus (Say), and feeding by the southern green stink bug, Nezara viridula (L.), reduced nitrogenase activity of soybean. All of these studies were carried out on greenhouse-propagated soybean during early vegetative growth stages. Lodging of field-grown soybean resulting from stem feeding by three-cornered alfalfa hopper also has reduced nitrogenase activity of soybean (Hutchinson 1979). Hicks (1978) and Hicks et al. (1984) found that higher 14C isotope activity occurred above petiole girdles caused by three-cornered alfalfa hopper feeding relative to below girdles. Their results indicated that girdling blocks
the flow of photosynthate from leaf sources to nodule sinks. Nodule feeding by insect larvae including bean leaf beetle, *Ceratoma trifurcata* (Forster), banded cucumber beetle, *Diabrotica balteata* LeConte, and soybean nodule fly, *Rivellia quadrifasciata* (Macquart), also has suppressed nitrogenase activity of soybean (Newsom et al. 1978, Hutchinson 1979, Layton 1983, and Newsom and Boethel 1985). Hutchinson (1979) and Lambert (1981) reported that soybean can compensate for nodule damage by producing nodules that are smaller and more numerous.

Layton and Boethel (1987, 1988, and 1989) performed three experiments examining the effects of defoliation on symbiotic N fixation of soybean. These experiments involved vegetative growth stage soybean propagated under greenhouse conditions. Layton and Boethel (1987) demonstrated that nitrogenase activity decreased linearly in response to increased levels of defoliation, and that 77% defoliation caused a 5-fold reduction in nitrogenase activity. Reduced nodule number, weight, and specific activity (*C*₂*H*₄ produced per unit nodule dry weight) were identified as factors that caused the reductions in nitrogenase activity. Layton and Boethel (1988) reported that recovery of nitrogenase activity occurred within 4 weeks following 70% defoliation. Factors that resulted in recovery of nitrogenase activity included partial recovery of total nodule weight per plant and complete recovery of nodule specific activity. Layton and
Boethel (1989) allowed soybean looper larvae to feed for 7, 10, 12, 14, and 16 days and measured defoliation levels of 13, 37, 43, 62, and 73%, respectively. They reported that significant reduction in nitrogenase activity began 10 days after infestation, that nitrogenase activity declined as defoliation progressed, and that nitrogenase activity was lowest at 16 days after infestation (73% defoliation caused 67% reduction in C₂H₄ production per plant). Russin et al. (1990) used the acetylene reduction assay to assess the impact of pest complexes on nitrogenase activity of soybean. These experiments also involved greenhouse-propagated, vegetative growth stage soybeans. Although defoliation levels up to 64% or establishment of soybean cyst nematode, *Heterodera glycines* Ichinohe (race 3), did not reduce nodule specific activity, stem canker fungus, *Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *caulivora* Athow and Caldwell, did reduce this measurement. Defoliation or stem canker fungus disease reduced nodule development and nitrogenase activity. Soybean cyst nematode did not reduce either of these factors. Interactions among these stresses were additive.

These reports established that insect defoliation of soybean and damage by other pests are associated with reductions in host nitrogenase activity. However, host N status also directly affects development of phytophagous insects. In order for a phytophagous insect species to develop and reproduce, host plants must provide the nutrients
(proteins, carbohydrates, lipids, vitamins, minerals, and salts) necessary for cell division, cell/tissue maintenance, and production of gametes (Simpson and Simpson 1990). Nitrogen is of particular importance in nutrition of heterotrophic organisms, including the Insecta, because this element is a major component of amino acids, nucleic acids, and protein. In comparison to carnivores, phytophagous insects convert food sources that are relatively low in N (10-50 mg N/g dry weight) into biomass that consists mainly of protein (70-140 mg N/g dry weight) (Scriber 1984a,b; and Simpson and Simpson 1990). Therefore, phytophagous insects are at a disadvantage because of the disproportionate amount of N (protein) in their bodies compared to that in their food sources, and N can be a limiting factor in the nutrition of insects.

Evidence that supports the concept that N is a limiting factor of insect nutrition has been reviewed (McNeil and Southwood 1978; Scriber 1984a,b; and Simpson and Simpson 1990). In 115 studies involving several insect orders including lepidopteran species, factors including insect growth rate, survival, and fecundity were suppressed when N concentration of host plants was reduced (Scriber 1984a,b). Exceptions to this relationship occurred in the literature and would be expected when considering how host plant suitability could affect how various insect species (i.e. predators, prey) interact within a given community.
Variability in insect population levels on fruit trees, ornamentals, and field crops often are associated with differences in nitrogen fertilization or soil fertility. Increased N fertilization has been associated with increased survival and increased pupal weight of leaf miners, *Liriomyza trifolii*, on tomato (Bethke et al. 1987). Increased adult population densities on, and increased ovipositional preference for chrysanthemum by the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), have been related to increased N fertilization and greater foliage N concentrations (Bentz and Larew 1992). Increased population levels of the spirea aphid, *Aphis spiraecola* Patch, have occurred on apple (Kaakeh et al. 1992) in association with increased N fertilization and increased foliage N concentration levels. Succulent, rapidly growing cotton terminals are preferred as ovipositional sites for cotton bollworm, *Helicoverpa zea* (Boddie), relative to older foliage (Gaines 1933). Higher larval densities of cotton bollworm also have occurred on cotton with increased N fertilization (Adkisson 1958).

Literature addressing variability of insect populations in association with changes in N concentration of soybean is limited. However, one study conducted by Todd et al. (1972) examined how N fertilization of non-nodulating soybeans affected insect populations. In their study, three rates of N fertilizer (78, 157, and 235 kg N/ha) and a non-N-
fertilized control were involved. Approximately two-fold increases in N concentration occurred in foliage from plants that received 235 kg N/ha relative to foliage from non-fertilized non-nodulating control plants. Populations of Mexican bean beetle, *Epilachna varivestis* Mulsant, were approximately five-fold higher for this same treatment comparison one month after infestation. Trends involving increased numbers of lepidopteran species including soybean looper, velvetbean caterpillar, and green cloverworm, *Plathypena scabra* (F.) associated with increased N fertilization rates also were reported; however, populations of these species were low, and these trends were non-significant.

Some insects compensate for low host N concentration through increased consumption (Scriber 1984 a,b; and Simpson and Simpson 1990). Compensatory responses of this nature are reported for lepidopteran larvae including the imported cabbageworm, *Pieris rapae* (L.), on crucifers (Slansky and Feeney 1977); the southern armyworm, *Spodoptera eridania* (Cramer), on legumes (Scriber 1979); and for larvae of the monarch butterfly, *Danaus plexippus* (L.), on milkweed (Schroeder 1976). This adaptation allows for survival during periods when host N and protein concentrations are sub-optimal. However, insects cannot compensate for extremely low dietary N by overeating *ad infinitum*, and minimum N requirements have been determined for several species.
(Mattson 1980). For example, the minimum N concentration required for survival and development of the southern armyworm is \( \approx 30 \text{ mg N/g dry weight (3\% N)} \) (Soo Hoo and Fraenkel 1966, and Dadd 1973). Many types of compensatory feeding have been reported (Simpson and Simpson 1994). Compensation for reduced host nutritional quality has resulted because of variation in duration of a meal, in meal size, or in the resting period between meals. These responses often result in greater consumption by specific stadia. Another type of compensation response involved increased consumption that resulted from a lengthening of the duration of a life stage (larva or nymph) (Al-Zubaidi and Capinera 1984, and Ohmart et al. 1985), and this response may not be characterized by increased consumption by specific stadia.

The literature illustrates that there is an association between N concentration in host plants and the development of phytophagous insects. However, it cannot be ruled out that effects of N are indirect (Simpson and Simpson 1990). Nitrogen is involved in many physiological processes in the plant. It is the basic constituent of chlorophyll and is required in synthesis of glutamate, of amino acids that function as enzymes in cellular metabolism, and of amino acids that link together to form protein (Tisdale and Nelson 1975, and Salisbury and Ross 1985). Nitrogen is a mobile element in plants, and when it is deficient, older foliage
becomes chlorotic as N moves out of these tissues and into younger growth. As leaves age, N and protein concentration levels decrease, and fiber, lignin, and tannin levels increase (Wittenbach et al. 1980, and Scriber 1984a,b). These plant components reduce digestibility of foliage, and can affect insect development (Scriber 1984a,b). Early instar soybean looper feed selectively on low fiber, highly-digestible soybean tissues (Kogan and Cope 1974), and age of soybean leaves affects development of soybean looper (Reynolds and Smith 1985) and cotton bollworm (McWilliams and Beland 1977). As mentioned previously, succulent, rapidly growing cotton terminals are preferred ovipositional sites for the cotton bollworm (Gaines 1933), and higher larval densities of this pest have been associated with increased N fertilization (Adkisson 1958). These insect/host plant interactions could have evolved as responses to tannins, lignin, and fiber of older, less succulent tissues and may not occur in response to N or protein concentration.

Total plant N may be less important to the nutrition of insects than the balance of certain amino acids. Increased population densities and fecundity of a species of whitefly, Aleurotrachelus jelinekii Frauneuf, have been associated with higher concentrations of certain essential amino acids in evergreen bush, Viburnum tinus, when differences in total N were not observed (McNeill and Southwood 1978). Amide (glutamine + asparagine) has been positively correlated with
population densities of certain aphid and leafhopper species, while other amino acids including phenylalanine, glycine, tyrosine, and proline were negatively correlated. Leafhopper species also are known to exploit hosts during periods of peak amide concentration. However, the relation of host amino acids and insect development may not be important in experiments where plant genotype does not vary. This is because the amount and balance of amino acids (protein quality) is regulated more by plant genotype than by the quantity of available N (Tisdale and Nelson 1975).

Plant nitrogen concentration also can influence allelochemical concentration, resulting in variable levels of host plant resistance. However, the relationship of N fertilization and concentration of secondary plant chemicals can be positively or negatively associated. Concentrations of N-based allelochemics including hydrocyanic acid have been positively correlated with N fertilization in Sudan grass, and the amount of increase in toxin concentration has varied depending on source of supplemental N (NH$_4^+$, NO$_3^-$, or urea). However, N deficiency also has been associated with increases in concentration of allelochemics including chlorogenic acids in sunflower and other plants (Scriber 1984b).

Foliage water content also can be correlated with greater digestibility by phytophagous insect consumers, and effects of water concentration on insect development can be difficult to distinguish from effects of N because these two
factors often are positively associated (Scriber 1984a, b; and Simpson and Simpson 1990). This literature links host N, insect nutrition, and insect development, but illustrates why caution should be used when evaluating general insect/plant interactions involving N. Changes in N can be related to changes in the concentration of a number of substances in the host including cellular enzymes that consist of non-essential and essential amino acids, insect-suppressive allelochemicals, and water. Therefore, although host N concentration and insect development are closely related, interactions of N and these other factors should be recognized.

Although the literature reviewed documents the involvement of N in insect/host plant interactions, research has not sufficiently addressed the effects of defoliation on symbiotic N fixation during reproductive growth stages, and relationships of defoliation level, nitrogenase activity, and yield need to be considered. Also, if non-nodulating soybean are used in experiments involving defoliation effects on symbiotic N fixation, the association of host N status and insect development should be evaluated.
LIST OF RESEARCH OBJECTIVES

1. To examine how defoliation by the soybean looper affects symbiotic N fixation and yield of soybean in field experiments during bloom and pod development or early seed development.

2. To evaluate feeding, growth, and survival of soybean looper larvae when fed nodulating or non-nodulating isoline soybean foliage collected during bloom and pod development or early seed development.

3. To evaluate feeding, growth, and survival of the soybean looper larvae when provided foliage from non-nodulating isoline soybean plants supplied with sequential applications of several rates of N fertilizer.
CHAPTER 1
SYMBIOTIC NITROGEN FIXATION AND YIELD OF SOYBEAN
CULTIVARS LEE AND CLARK FOLLOWING DEFOLIATION
BY THE SOYBEAN LOOPER (LEPIDOPTERA: NOCTUIDAE)
DURING POD OR SEED DEVELOPMENT

Introduction

Symbiotic nitrogen (N) fixation, or reduction of atmospheric N to the ammonium ion, requires oxygen and an energy source. In plants of the Fabaceae such as soybean, *Glycine max* (L.) Merrill, carbohydrates from photosynthesis are the main source of energy used by the N₂-fixing bacteria in root nodules to conduct this process (Salisbury and Ross 1985). Any plant stress that inhibits photosynthesis limits the supply of photosynthate to nutrient sinks including bacteria in root nodules and thereby has the potential to limit symbiotic N fixation (Hardy and Havelka 1976). However, carbohydrates stored as starch in plant shoots also can be used as a carbon source by bacteria in root nodules during periods when CO₂ assimilation (photosynthesis) is suppressed (cloudy or shady conditions or in darkness) (Mederski and Streeter 1977, Schweitzer and Harper 1980, Williams et al. 1982, Denison and Sinclair 1985, and Millhollon and Williams, 1986).

Insects that defoliate leguminous plants reduce energy reserves used by bacteria in root nodules for symbiotic nitrogen fixation by reducing the amount of photosynthate being produced and translocated to these bacteria and by removing energy reserves that are stored as starch in the
foliage. Hutchinson (1979), Layton and Boethel (1987, 1988, and 1989), and Russin et al. (1990) demonstrated that defoliation by soybean looper, *Pseudoplusia includens* (Walker), affected symbiotic N fixation of soybean. Up to 5-fold reductions in nitrogenase activity following 77% defoliation were reported (Layton and Boethel 1987).

Other types of insect damage can reduce symbiotic N fixation. Nodule feeding by coleopteran larvae [Coleoptera: Chrysomelidae, *Cerotoma trifurcata* (Forster)] (Newsom et al. 1978, Layton 1983, and Newsom and Boethel 1985), dipteran larvae [Diptera: Platystomatidae, *Rivellia quadrifasciata* (Macquart)] (Newsom et al. 1978), and stem girdling by homopteran nymphs and adults [Homoptera: Membracidae, *Spissistilus festinus* (Say)] (Hutchinson 1979, and Hicks et al. 1984) have been shown to reduce nitrogenase activity of soybean. Systemic, soil-applied insecticides can promote symbiotic N fixation indirectly by protecting soybean foliage and nodules from insect injury. Newson and Boethel (1985) demonstrated that treatment with carbofuran or aldicarb protected soybean from injury by a wide spectrum of insect pests and resulted in greater nodule dry weight per plant, greater nodule specific activity, and greater yield in treated plots.

Several experiments have examined the effect of soybean looper defoliation on symbiotic N fixation (Hutchinson 1979; Layton and Boethel 1987, 1988, and 1989; and Russin et al.
20

1990). These studies were conducted under greenhouse conditions, concentrated on effects of defoliation during vegetative growth stages, and did not evaluate yield. However, soybean looper infestations occur from early Aug through mid-Sep (Baldwin et al. 1994), coinciding with reproductive growth stages of soybean cultivars adapted to the southern United States. Therefore, experiments were initiated examining the effect of defoliation of soybean by soybean looper during pod and seed development stages on symbiotic N fixation and yield.

**Materials and Methods**

Experiments were conducted that involved the factorial arrangement of all combinations of nodulating or non-nodulating isolines of soybean and soybean looper-defoliated or non-defoliated plants. Each treatment combination was replicated four times. Plots were arranged in a randomized complete block design and planted on Commerce silty clay loam soil (fine-silty, mixed, nonacid, thermic Aeric Fluvaquent). Separate experiments utilizing this design were conducted for cultivars Lee and Clark soybean and for defoliation initiated R2 or R5 plant growth stages (Fehr et al. 1971). Each cultivar and stage of infestation was examined for two years.

A non-fixing control was required for indirect measurements of symbiotic N fixation using the N-difference method (LaRue and Patterson 1981). Cultivars Clark and Lee
soybean were chosen for these experiments because isogenic lines of each were available that do not nodulate. The cultivar Clark has an indeterminate growth habit and is in maturity group IV whereas Lee has a determinate growth habit and is in maturity group VI. These non-nodulating isolines were developed through crosses involving T201 (source of the single recessive rjl gene coding for the non-nodulating phenotype) and subsequent backcrosses and selection for the non-nodulating phenotype (Bernard et al. 1991, and Hartwig 1994). Therefore, these non-nodulating phenotypes differed from the recurrent parents mainly in ability to nodulate and fix N symbiotically. This choice of cultivars permitted examination of the effects of defoliation on symbiotic N fixation of soybeans in two different maturity groups with different growth habits (indeterminate compared with determinate).

Planting dates, infestation dates, and duration of the infestation periods are summarized in Table 1.1. Before planting, seed of each cultivar were inoculated with a commercial peat-based soybean inoculum, *Bradyrhizobium japonicum* (Kirchner) Buchanan (Nitragin brand, 'S' Culture, LiphaTech Inc., Milwaukee, WI). Ammonium salt of imazaquin (Scepter, 0.14 kg [AI]/ha, American Cyanamid, Wayne, New Jersey) was applied before seedling emergence in each experiment for weed control. Plots consisted of four 0.92 m rows, 1.5 m in length with 1.5 m borders at front and rear.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Year</th>
<th>Date Planted</th>
<th>Dates of Infestation</th>
<th>Duration of Infestation</th>
<th>Dates of Infestation</th>
<th>Duration of Infestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee</td>
<td>1990</td>
<td>10 May</td>
<td>13 Jul-31 Jul</td>
<td>18 d</td>
<td>17 Aug-6 Sep</td>
<td>20 d</td>
</tr>
<tr>
<td>Lee</td>
<td>1991</td>
<td>2 May</td>
<td>2 Aug-12 Sep</td>
<td>41 d&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 Sep-8 Oct</td>
<td>37 d&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clark</td>
<td>1991</td>
<td>2 May</td>
<td>12 Jul-30 Jul</td>
<td>18 d</td>
<td>9 Aug-23 Aug</td>
<td>14 d</td>
</tr>
<tr>
<td>Clark</td>
<td>1992</td>
<td>29 Apr</td>
<td>4 Jul-20 Jul</td>
<td>16 d</td>
<td>30 Jul-14 Aug</td>
<td>15 d</td>
</tr>
</tbody>
</table>

<sup>a</sup> These infestations occurred from full bloom (R2) through the 5-19 mm pod stage (R3-4).

<sup>b</sup> Extended into early seed development (R5).

<sup>c</sup> These infestation periods occurred during early seed development (R5).

<sup>d</sup> Extended into full seed stage (R6).
The two center rows of each plot were caged with 1.83 x 1.83 x 1.83 m cages throughout each defoliation period.

Soybean loopers used in all experiments were obtained as eggs from the USDA-ARS-SIML insectory at Stoneville, MS. In 1990, eggs were allowed to hatch and feed on foliage under controlled conditions (≈27°C, 60% humidity, 14 h light:10 h dark) in a laboratory before infestation of plots with third instars. Number of larvae used at each infestation of Lee in 1990 were as follows. Lee was infested with ≈325, 280, and 250 third instars on day 1, 11, and 12 of the defoliation period initiated at R2 in 1990, respectively, and with ≈200, 100, 100, 176, and 820 third instars on day 1, 4, 5, 8, and 12 of the defoliation period initiated at R5, respectively. All further infestations were conducted using an estimated number of eggs that were distributed throughout the canopy attached to paper towels as received from the USDA-ARS. The approximate number of eggs added to each plot were as follows. In 1991, Lee was infested with ≈200, 125, 250, 300, and 300 eggs on day 1, 8, 15, 19, and 20 of the defoliation period initiated at R2, respectively, and with ≈600, 500, 500, 250, 400 eggs on day 1, 8, 15, 26, and 30 of the defoliation period initiated at R5, respectively. Clark was infested with ≈250 and 350 eggs on day 1 and 6 of the defoliation period initiated at R2, respectively, and with ≈125, 250, and 150 eggs on day 1, 10, and 13 of the defoliation period initiated at R5, respectively. In 1992,
Clark was infested with ≈500 and 1000 eggs on day 1 and 8 of the defoliation period initiated at R2, respectively, and with ≈500 and 1000 eggs on day 1 and 9 of the defoliation period initiated at R5, respectively. Following each initial infestation, number of larvae or eggs added to plots were dependant on visual evaluations of defoliation. This helped insure that similar amounts of defoliation were achieved among plots within each infestation period.

Insecticides were used throughout these experiments as detailed below. Methyl parathion 4E (0.56 kg [AI]/ha, Red Panther Chemical Co., Clarksdale, MS) was applied to foliage for predator suppression 48 h before infestation of plants with soybean loopers. Chlorpyrifos (0.003 kg [AI]/liter, Lorsban 4E, DowElanco, Indianapolis, IN) also was applied to the soil around the perimeter of cages 48 h before each infestation to suppress predation by red imported fire ants (Hymenoptera: Formicidae, Solenopsis invicta Buren). Permethrin (0.11 kg [AI]/ha, Ambush 2EC, Zeneca Agricultural Products, Wilmington, DE) or thiodicarb (0.67 kg [AI]/ha, Larvin 3.2F, Rhone-Poulenc Agricultural Co., Research Triangle Park, NC) was applied to foliage to control soybean loopers at the termination of each defoliation period and when natural infestations of noctuid [Lepidoptera: Noctuidae, Anticarsia gemmatalis Hubner and/or Pseudoplusia includens (Walker)] pests occurred. Tralomethrin (0.027 kg [AI]/ha, Scout X-tra 0.9EC, Hoechst-Roussel Agri-Vet Co., Somerville,
NJ) was applied to control infestations by pentatomid [Heteroptera: Pentatomidae, Nezara viridula (L.) and/or Euschistus sp.] pests when required. Insecticides were applied to defoliated and non-defoliated plots each time to avoid confounding effects of defoliation with application of insecticides.

When each defoliation period ended, one row of the caged plants was carefully uprooted. This was accomplished by digging ≈20 cm into the ground in each furrow (≈45 cm away from main stems) along 1-1.5 m of row. Shovels were used as levers to lift soil on each side of the plants. The soil around the root systems was loosened using ≈25.4 cm long screwdrivers and shaken away from root systems. This process was done with care to avoid excessive loss of nodules and was never done under muddy conditions. Then six plants were selected at random, and the acetylene reduction assay was performed on this bulked root sample of six plants from each plot. This assay involved placement of the six soybean root systems and nodules in one 0.95 liter mason jar, replacing 50 ml of air with acetylene (5% by volume of the empty vessel), sampling the vessel at 60 minutes, and determining the amount of ethylene produced using a flame ionization detector gas chromatograph (model GC-14A, Shimadzu, Tokyo, Japan). The gas chromatograph was equipped with a 1.83 m x 3.2 mm stainless steel column containing 80-100 mesh HayeSep T porous polymer (Alltech, Deerfield, IL). Ethylene (100 ppm,
Alltech, Deerfield, IL) was used as a standard to identify ethylene peaks initially and to confirm accuracy following every 16 samples.

Plants were separated into components (leaves, stems, pods, roots and nodules) and leaf area per plant was determined (leaf area meter, model 3100, Li-Cor, Lincoln, NE). Nodule number per plant was determined. Plant components were dried in an oven at 80°C and weighed daily. When measurable reductions in weight (moisture loss) ceased, dry weights of plant components were measured. Immature seed were not separated from pods for any measurements. Above-ground plant portions (stems, leaves, and pods) were finely-ground, and N content of each component was determined using an elemental (C/H/N) analyzer (model 240C, Perkin Elmer, Norwalk, CT). Acetanilide (10.36% N, Fisher Scientific, Houston TX) was used as a N standard for this instrument, and standards were run initially and following every 10 samples to confirm accuracy. At growth stage R8 (full maturity), yield of 1 meter of row was determined. Yield samples were acquired from plants along the remaining, previously-caged portion of each plot that had not been disturbed when roots were dug.

Several additional parameters were calculated from these observations. These were percent defoliation [(mean leaf area of non-defoliated control - leaf area of defoliated experimental unit)/mean leaf area of non-defoliated
control*100]; dry weight per nodule; nodule to root ratio (dry weight/dry weight); N content of plant components and of total above-ground biomass; N difference in above-ground biomass (mg N of nodulating isoline minus mg N of non-nodulating isoline for each respective treatment); ethylene production per nodule, nodule dry weight (nodule specific activity), and per unit of leaf area; and percent N from symbiotically-fixed sources for non-defoliated control plants [N difference value (mg)/N in above-ground biomass (mg) x 100].

Data were analyzed separately by cultivar, year, and plant growth stage at infestation, using general linear model (GLM) procedure (sources- block, isoline, defoliation, isoline by defoliation) followed by preplanned, single degree of freedom contrasts to distinguish defoliation effects on specific isolines (SAS Institute 1990). Probability values for contrasts were reported only if appropriate GLM statistics were significant (P≤0.05). Contrasts were not required when the non-nodulating isoline was not involved (i.e. nodule parameters and ethylene production); therefore, GLM probability statistics were reported in these cases. The GLM or contrast probabilities were included throughout the text, and significant differences between defoliated and non-defoliated treatments (P≤0.05) were indicated with an * in figures.
**Results**

*Cultivar Lee.* Defoliation of Lee initiated at R2 in 1990 significantly reduced leaf area of nodulating (P>F ≤0.01) and non-nodulating (P>F ≤0.01) isolines, and this corresponded to 50 and 40% defoliation, respectively (Fig. 1.1). Defoliation of Lee initiated at R5 in 1990 significantly reduced leaf area of the nodulating isoline (P>F ≤0.05) which corresponded to 32% defoliation; however, leaf area of the non-nodulating isoline was not significantly affected. Defoliation of Lee initiated at R2 in 1991 significantly reduced leaf area of nodulating (P>F ≤0.001) and non-nodulating (P>F ≤0.06) isolines, and this corresponded to 55 and 31% defoliation, respectively. Defoliation of Lee initiated at R5 in 1991 did not significantly affect leaf area of either isoline; and consequently, significant differences were not observed for any of the other parameters because of defoliation initiated at this growth stage.

Defoliation of Lee initiated at R2 in 1990 significantly reduced leaf dry weight (P>F ≤0.05) and stem dry weight (P>F ≤0.05) of the nodulating isoline; however, defoliation did not affect root and pod dry weight of this isoline (Table 1.2). Defoliation initiated at R2 in 1990 did not significantly affect dry weights of components of the non-nodulating isoline. Defoliation of Lee initiated at R5 in 1990 significantly reduced leaf dry weight (P>F ≤0.05) of the nodulating isoline. Stem, root, and pod dry weights of
Figure 1.1. Leaf area per plant following defoliation of nodulating and non-nodulating isolines of cv. Lee soybean initiated at two growth stages.
Table 1.2. Dry weight (g) of leaf, stem, root, and pod components following defoliation of nodulating and non-nodulating isolines of cv. Lee soybean initiated at two growth stages.

<table>
<thead>
<tr>
<th>Component and Defoliation Treatment</th>
<th>Dry weight (g) Lee 1990</th>
<th>Dry weight (g) Lee 1991</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2</td>
<td>R5</td>
</tr>
<tr>
<td>Leaf Non-def.</td>
<td>17.4 15.1</td>
<td>17.4 12.0</td>
</tr>
<tr>
<td></td>
<td>12.2* 12.1</td>
<td>11.5* 10.4</td>
</tr>
<tr>
<td>Def.</td>
<td>12.2* 12.1</td>
<td>11.5* 10.4</td>
</tr>
<tr>
<td>Stem Non-def.</td>
<td>27.5 23.7</td>
<td>28.9 44.6</td>
</tr>
<tr>
<td></td>
<td>20.7* 20.5</td>
<td>23.8 25.1</td>
</tr>
<tr>
<td>Def.</td>
<td>20.7* 20.5</td>
<td>23.8 25.1</td>
</tr>
<tr>
<td>Pod Non-def.</td>
<td>0.8 0.4</td>
<td>29.1 15.4</td>
</tr>
<tr>
<td></td>
<td>1.0 0.6</td>
<td>19.5 13.6</td>
</tr>
<tr>
<td>Def.</td>
<td>1.0 0.6</td>
<td>19.5 13.6</td>
</tr>
<tr>
<td>Root Non-def.</td>
<td>6.5 5.1</td>
<td>6.8 5.3</td>
</tr>
<tr>
<td>Def.</td>
<td>4.7 5.2</td>
<td>5.5 5.8</td>
</tr>
</tbody>
</table>

*An * indicates significant differences (P ≤ 0.05) between defoliated and non-defoliated treatments.
*Defoliation did not significantly reduce leaf area when initiated at R5 in 1991.
this isoline were not significantly affected by defoliation (Table 1.2). Defoliation initiated at R5 in 1990 did not significantly affect dry weights of components of the non-nodulating Lee isoline. Defoliation of Lee initiated at R2 in 1991 significantly reduced leaf (P>F ≤0.001), root (P>F ≤0.01), and pod (P>F ≤0.01) dry weights of the nodulating isoline. Stem dry weight of this isoline was not significantly affected by defoliation during this period (Table 1.2). Defoliation of Lee initiated at R2 in 1991 significantly reduced leaf dry weight (P>F ≤0.05) of the non-nodulating isoline; however, stem, root, and pod dry weights of this isoline were not significantly affected. Dry weights of the components were not significantly affected by defoliation initiated at R5 in 1991.

Nodule dry weight of Lee expressed on a root dry weight basis was significantly greater (P=0.06) in response to defoliation initiated at R2 in 1991 (Table 1.3). Otherwise, nodule parameters of Lee were not significantly affected by defoliation initiated at either growth stage in either year. However, a trend that involved reduction of several nodule parameters of defoliated plants was observed for Lee when defoliation was initiated at R2 in 1990 and R5 in 1991.

Yield of the nodulating isoline was significantly reduced by 1492 or 971 kg/ha in response to defoliation when initiated at R2 (P>F ≤0.01) or R5 (P>F ≤0.05) in 1990, respectively (Fig. 1.2). Yields of the non-defoliated, non-
Table 1.3. Nodule parameters measured following defoliation of cv. Lee and Clark soybean initiated at two growth stages.

| Nodule Parameter | Defoliation Treatment | Cultivar Lee\(^a\) | | | Cultivar Clark\(^a\) | | |
|------------------|-----------------------|--------------------|-----------------|-----------------|-----------------|----------------|
| No. per Plant    | Non-defol.            | 143.7  | 98.4    | 99.3    | 36.3            | 65.0    | 80.1    | 50.8            | 43.1 |
|                  | Defoliated            | 90.9   | 113.7   | 109.9   | 20.7            | 61.3    | 76.7    | 42.3            | 32.8 |
| Dry Wt. (mg) per Plant | Non-defol.            | 166.5  | 329.2   | 314.3   | 237.5           | 431.3   | 587.6   | 388.3           | 346.3 |
|                  | Defoliated            | 83.5   | 350.0   | 333.8   | 125.0           | 317.3   | 330.8   | 257.7*           | 275.3 |
| Dry Wt. (mg) per Nodule | Non-defol.            | 1.3    | 3.5     | 2.9     | 7.0             | 6.7     | 7.5     | 7.7             | 8.0 |
|                  | Defoliated            | 0.9    | 3.2     | 2.9     | 6.1             | 5.2     | 4.3*    | 6.1\(^+\)        | 7.8 |
| Root Dry Wt. (mg/g) | Non-defol.            | 25.1   | 111.4   | 50.3    | 89.8            | 226.7   | 183.6   | 164.0           | 71.7 |
|                  | Defoliated            | 18.8   | 172.5\(^\dagger\) | 60.9    | 42.2            | 250.9   | 85.2    | 153.5           | 51.0 |

\(^{\dagger}\) and \(^*\) indicate probability levels of \(<0.06\) and \(<0.05\), respectively.

\(^b\)Defoliation did not significantly reduce leaf area of Lee or Clark when initiated at R5 in 1991.
Figure 1.2. Yield (kg/ha at 13% moisture) at maturity of nodulating and non-nodulating isolines of cv. Lee soybean following defoliation initiated at two growth stages.
nodulating isoline were 35-77% lower (P>F Isoline ≤0.01) than those of the respective nodulating isoline, and yields of the non-nodulating isoline were not affected by defoliation initiated at either growth stage in 1990. In 1991, yield of neither isoline of Lee was affected by defoliation, regardless of the period when defoliated.

Nitrogen content of leaves (P>F ≤0.01), stems (P>F ≤0.05), and total above-ground biomass (P>F ≤0.05) of the nodulating isoline was significantly reduced by defoliation initiated at R2 in 1990; however, pod N content of the nodulating isoline was not significantly reduced (Table 1.4). Nitrogen content of leaves (P>F ≤0.05), pods (P>F ≤0.05), and total above-ground biomass (P>F ≤0.05) of the nodulating isoline was significantly reduced because of defoliation initiated at R5 in 1990, whereas stem N content was not affected. Nitrogen content of leaves (P>F ≤0.01), pods (P>F ≤0.001), and total above-ground biomass (P>F ≤0.001) of the nodulating isoline was significantly reduced by defoliation initiated at R2 in 1991; however, N content of stems of this isoline also was not affected. Nitrogen content of components of the nodulating isoline were not significantly affected by defoliation initiated at R5 in 1991. Defoliation initiated at either growth stage in either year did not significantly affect N content of any plant component or of total above-ground biomass of the non-nodulating isoline (Table 1.4).
Table 1.4. Nitrogen content (mg per plant) in above-ground components of nodulating and non-nodulating isolines of cv. Lee soybean following defoliation initiated at two growth stages.

<table>
<thead>
<tr>
<th>Component and Defoliation Treatment</th>
<th>Nitrogen (mg/plant) Lee 1990</th>
<th>Nitrogen (mg/plant) Lee 1991</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf Non-def.</td>
<td>841</td>
<td>711</td>
</tr>
<tr>
<td>Def.</td>
<td>523*</td>
<td>537</td>
</tr>
<tr>
<td>Stem Non-def.</td>
<td>449</td>
<td>362</td>
</tr>
<tr>
<td>Def.</td>
<td>376*</td>
<td>335</td>
</tr>
<tr>
<td>Pod Non-def.</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Def.</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>Totalc Non-def.</td>
<td>1317</td>
<td>1086</td>
</tr>
<tr>
<td>Def.</td>
<td>931*</td>
<td>894</td>
</tr>
</tbody>
</table>

*An * indicates significant differences (P ≤ 0.05) between defoliated and non-defoliated treatments.

Defoliation did not significantly reduce leaf area when initiated at R5 in 1991.

Total above-ground biomass.
When assessed following R2 infestations of Lee, 18\% (1990) and 62\% (1991) of N in above-ground biomass of non-defoliated, nodulating control plants was acquired from fixed sources. When assessed following R5 infestations, 49\% (1990) or 68\% (1991) of N in above-ground biomass of non-defoliated, nodulating plants was acquired from fixed sources. The N difference values for above-ground biomass (P>F <0.01), in particular, the leaf component (P>F ≤0.05), were significantly reduced because of defoliation initiated at R5 in 1990 (Table 1.5). The N difference values for above-ground biomass (P>F ≤0.01) and its leaf (P>F ≤0.05) and pod (P>F ≤0.05) components were significantly reduced by defoliation initiated at R2 in 1991. The N difference values for above-ground biomass or any of its components were not significantly reduced by defoliation initiated at R2 in 1990 or R5 in 1991.

Defoliation of Lee initiated at R2 in 1990 significantly reduced ethylene production per plant by 85\% and reduced ethylene production per gram nodule dry weight (nodule specific activity) by 69\% (Table 1.6). Ethylene production per nodule and per unit leaf area was not significantly affected. Defoliation of Lee initiated at R2 in 1991 significantly reduced nodule specific activity by 53\%; however, other ethylene measurements were not significantly affected. Defoliation of Lee initiated at R5 in 1990 or 1991
Table 1.5. Nitrogen difference values (mg N per nodulating plant - mg N per non-nodulating plant) for above-ground components of cv. Lee and Clark soybean following defoliation initiated at two growth stages.

<table>
<thead>
<tr>
<th>Component and Defoliation</th>
<th>N Difference (mg/plant) Lee</th>
<th>N Difference (mg/plant) Clark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2</td>
<td>R5</td>
</tr>
<tr>
<td>Leaf</td>
<td>Non-def.</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>Def.</td>
<td>-14</td>
</tr>
<tr>
<td>Stem</td>
<td>Non-def.</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Def.</td>
<td>40</td>
</tr>
<tr>
<td>Pod</td>
<td>Non-def.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Def.</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>Non-def.</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>Def.</td>
<td>37</td>
</tr>
</tbody>
</table>

*An * indicates significant differences (P ≤ 0.05) between defoliated and non-defoliated treatments.

Significant defoliation of the non-nodulating isolate did not occur.

Defoliation did not significantly reduce leaf area when initiated at R5 in 1991.

Total above-ground biomass.
Table 1.6. Ethylene (µM) produced per plant, nodule, g nodule dry weight, and 1000 cm² leaf area at 60 minutes from the acetylene reduction assay for defoliated and non-defoliated cv. Lee soybean and defoliation initiated at two growth stages.

<table>
<thead>
<tr>
<th>Ethylene (µM) per</th>
<th>Defoliation Treatment</th>
<th>R2 Infestation*</th>
<th>R5 Infestation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td>Non-defol.</td>
<td>1372</td>
<td>6258</td>
</tr>
<tr>
<td></td>
<td>Defoliated</td>
<td>207*</td>
<td>3610</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2174</td>
<td>1383</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2327</td>
<td>592</td>
</tr>
<tr>
<td>Nodule</td>
<td>Non-defol.</td>
<td>10.7</td>
<td>64.2</td>
</tr>
<tr>
<td></td>
<td>Defoliated</td>
<td>3.6</td>
<td>32.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.4</td>
<td>39.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.9</td>
<td>28.5</td>
</tr>
<tr>
<td>Gram Nod.</td>
<td>Non-defol.</td>
<td>6992</td>
<td>19212</td>
</tr>
<tr>
<td>Dry Wt.</td>
<td>Defoliated</td>
<td>2195*</td>
<td>9070*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6768</td>
<td>5959</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5221</td>
<td>4237</td>
</tr>
<tr>
<td>Leaf Area</td>
<td>Non-defol.</td>
<td>314</td>
<td>3029</td>
</tr>
<tr>
<td></td>
<td>Defoliated</td>
<td>89</td>
<td>3097</td>
</tr>
<tr>
<td></td>
<td></td>
<td>677</td>
<td>1151</td>
</tr>
<tr>
<td></td>
<td></td>
<td>855</td>
<td>538</td>
</tr>
</tbody>
</table>

*An * indicates the significance level ≤0.05.

Defoliation did not significantly reduce leaf area of Lee when initiated at R5 in 1991.

Nodule specific activity.

Expressed x 1000 cm².
did not significantly affect any of the ethylene parameters measured.

**Cultivar Clark.** Defoliation of Clark initiated at R2 in 1991 significantly reduced leaf area of the nodulating \( (P>F < 0.01) \) and non-nodulating \( (P>F < 0.01) \) isolines, and this corresponded to 74 and 88% defoliation, respectively (Fig. 1.3). Defoliation initiated at R5 in 1991 did not significantly reduce leaf area of either isoline. Defoliation initiated at R2 in 1992 significantly reduced the leaf area of nodulating \( (P>F \leq 0.0001) \) and non-nodulating \( (P>F \leq 0.0001) \) isolines, and this corresponded to 94 and 93% defoliation (Fig. 1.3). Defoliation initiated at R5 in 1992 significantly reduced leaf area of the nodulating isoline \( (P>F \leq 0.001, 94\% \text{ defoliation}) \); however, leaf area of the non-nodulating isoline was not significantly affected.

Significant reductions of leaf \( (P>F \leq 0.0001) \), stem \( (P>F \leq 0.001) \), and pod \( (P>F \leq 0.05) \) dry weights of the nodulating isoline and leaf dry weight \( (P>F \leq 0.001) \) of the non-nodulating isoline occurred in response to defoliation initiated at R2 in 1991 (Table 1.7). Dry weights of plant components were not significantly affected by defoliation initiated at R5 in 1991. In 1992, leaf dry weight \( (P>F \leq 0.0001) \) of the nodulating isoline, and leaf \( (P>F \leq 0.0001) \), stem \( (P>F \leq 0.01) \), and root \( (P>F \leq 0.001) \) dry weights of the non-nodulating isoline were significantly reduced by defoliation initiated at R2. Defoliation initiated at R5 in
Figure 1.3. Leaf area per plant following defoliation of nodulating and non-nodulating isolines of cv. Clark soybean initiated at two growth stages.
Table 1.7. Dry weight (g) of leaf, stem, root, and pod components following defoliation of nodulating and non-nodulating isolines of cv. Clark soybean initiated at two growth stages.

<table>
<thead>
<tr>
<th>Component and Defoliation Treatment</th>
<th>Dry Weight (g)</th>
<th>Clark 1991</th>
<th>Dry Weight (g)</th>
<th>Clark 1992</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2</td>
<td>R5</td>
<td>R2</td>
<td>R5</td>
</tr>
<tr>
<td>Leaf Non-def.</td>
<td>6.1</td>
<td>4.7</td>
<td>5.5</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>1.8*</td>
<td>1.2*</td>
<td>3.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Leaf Def.</td>
<td>9.7</td>
<td>13.1</td>
<td>13.7</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>1.5*</td>
<td>1.4*</td>
<td>1.3*</td>
<td>4.2</td>
</tr>
<tr>
<td>Stem Non-def.</td>
<td>9.3</td>
<td>6.0</td>
<td>10.9</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>4.6*</td>
<td>5.0</td>
<td>10.5</td>
<td>9.9</td>
</tr>
<tr>
<td>Stem Def.</td>
<td>16.0</td>
<td>20.3</td>
<td>31.2</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>15.3</td>
<td>12.2*</td>
<td>28.8</td>
<td>24.8</td>
</tr>
<tr>
<td>Pod Non-def.</td>
<td>1.2</td>
<td>0.6</td>
<td>13.0</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>0.6*</td>
<td>0.4</td>
<td>12.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Pod Def.</td>
<td>3.3</td>
<td>1.6</td>
<td>25.7</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>0.8</td>
<td>21.5</td>
<td>13.7</td>
</tr>
<tr>
<td>Root Non-def.</td>
<td>1.9</td>
<td>2.0</td>
<td>2.5</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>1.6</td>
<td>1.7</td>
<td>2.9</td>
</tr>
<tr>
<td>Root Def.</td>
<td>3.4</td>
<td>5.1</td>
<td>5.0</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>3.5*</td>
<td>4.9</td>
<td>6.0</td>
</tr>
</tbody>
</table>

*An * indicates significant differences (P ≤ 0.05) between defoliated and non-defoliated treatments.

Defoliation did not significantly reduce leaf area when initiated at R5 in 1991.
1992 significantly reduced leaf dry weight (P>F ≤0.01) of the
nodulating isoline (Table 1.7). Otherwise, plant component
dry weights were not significantly affected by defoliation
initiated at R5 in 1992.

In general, nodule parameters of Clark decreased in
response to defoliation. However, significant reductions
occurred only for nodule dry weight per plant and for dry
weight per nodule because of defoliation initiated at R5 in
1991 or for dry weight per nodule in response to defoliation
initiated at R2 in 1992 (Table 1.3).

Yield of the nodulating isoline of Clark was
significantly reduced 800 kg/ha by defoliation initiated at
R2 in 1991 (P>F ≤0.05), 1359 kg/ha by defoliation initiated
at R2 in 1992 (P>F ≤0.0001), and 750 kg/ha by defoliation
initiated at R5 in 1992 (P>F ≤0.05) (Fig 1.4). Yields of the
non-defoliated, non-nodulating Clark isoline were 52–65% lower (P>F Isoline ≤0.02) than those of the respective
nodulating isoline, and yields of the non-nodulating Clark
isoline were not significantly affected by defoliation in
either year at either growth stage.

Nitrogen content of leaves (P>F ≤0.0001), stems (P>F
≤0.01), pods (P>F ≤0.01), and total above-ground biomass (P>F ≤0.001) of the nodulating isoline was significantly reduced
by defoliation initiated at R2 in 1991 (Table 1.8). Leaf N
content of the non-nodulating isoline was significantly
reduced (P>F ≤0.01) by defoliation initiated at R2 in 1991,
Figure 1.4. Yield (kg/ha at 13% moisture) at maturity of nodulating and non-nodulating isolines of cv. Clark soybean following defoliation initiated at two growth stages.
Table 1.8. Nitrogen content (mg per plant) in above-ground components of nodulating and non-nodulating isolines of cv. Clark soybean following defoliation initiated at two growth stages.

<table>
<thead>
<tr>
<th>Component and Defoliation Treatment</th>
<th>Nitrogen (mg/plant) Clark 1991</th>
<th></th>
<th>Nitrogen (mg/plant) Clark 1992</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2</td>
<td>R5</td>
<td>R2</td>
<td>R5</td>
</tr>
<tr>
<td>Leaf</td>
<td>Non-def. 280 159</td>
<td>219 157</td>
<td>452 507</td>
<td>541 193</td>
</tr>
<tr>
<td>Def.</td>
<td>77* 41*</td>
<td>148 97</td>
<td>62* 47*</td>
<td>40* 104</td>
</tr>
<tr>
<td>Stem</td>
<td>Non-def. 221 109</td>
<td>225 158</td>
<td>321 250</td>
<td>509 192</td>
</tr>
<tr>
<td>Def.</td>
<td>134* 112</td>
<td>277 172</td>
<td>275 187</td>
<td>544 229</td>
</tr>
<tr>
<td>Pod</td>
<td>Non-def. 54 22</td>
<td>604 345</td>
<td>129 44</td>
<td>964 380</td>
</tr>
<tr>
<td>Def.</td>
<td>21* 18</td>
<td>530 223</td>
<td>66* 25</td>
<td>777 408</td>
</tr>
<tr>
<td>Totalc</td>
<td>Non-def. 555 289</td>
<td>1048 660</td>
<td>902 801</td>
<td>2014 766</td>
</tr>
<tr>
<td>Def.</td>
<td>232* 171</td>
<td>955 491</td>
<td>403* 260*</td>
<td>1362 741</td>
</tr>
</tbody>
</table>

*An * indicates significant differences (P ≤ 0.05) between defoliated and non-defoliated treatments.

bDefoliation did not significantly reduce leaf area when initiated at R5 in 1991.

cTotal above-ground biomass.
whereas N content of other above-ground components and component totals for this isoline were not significantly affected. Nitrogen content of leaves (P>F ≤ 0.0001), pods (P>F ≤ 0.01), and total above-ground biomass (P>F ≤ 0.0001) of the nodulating isoline was significantly reduced by defoliation initiated at R2 in 1992 (Table 1.8). Nitrogen content of the leaf component (P>F ≤ 0.0001) and total above-ground biomass of the non-nodulating isoline was significantly reduced (P>F ≤ 0.001) by defoliation initiated at R2 in 1992. Defoliation initiated at R5 did not significantly affect N content of above-ground biomass or its components, except when a significant reduction of leaf N content of the nodulating isoline (P>F ≤ 0.001) occurred in 1992.

When the non-defoliated, nodulating isoline of Clark was assessed following infestations initiated at R2, 48% (1991) or 11% (1992) of N in above-ground biomass had been acquired from fixed sources. When assessed following infestations initiated at R5, 37% (1991) or 62% (1992) of N in above-ground biomass of these control plants had been acquired from fixed sources. The N difference values for above-ground components of Clark were not significantly reduced by defoliation initiated at R2 or R5 in 1991, or R2 in 1992 (Table 1.5). The N difference value for the leaf component was significantly reduced (P>F ≤ 0.05) by defoliation initiated at R5 in 1992, whereas other above-ground plant
components and total above-ground biomass were not significantly affected.

Ethylene production by Clark per plant and per nodule, and nodule specific activity were significantly reduced by >80% in response to defoliation initiated at R2 in 1991 (Table 1.9). In addition, ethylene production per unit leaf area also was significantly reduced by defoliation initiated at R2 in 1992, and all four of these measurements were reduced by 99-100% in that experiment (Table 1.9). Defoliation of Clark initiated at R5 in 1991 did not significantly affect any of the ethylene parameters measured. However, in 1992 defoliation initiated at that growth stage reduced all ethylene measurements measured by 100% (Table 1.9).

Discussion

This research demonstrated that defoliation by soybean looper can affect symbiotic N fixation and influence yield of soybean under field conditions and during the growth stages that are normally attacked by this pest. When acetylene reduction was used to indicate effects of defoliation on symbiotic N fixation, up to 85% reduction of nitrogenase activity was demonstrated for the determinate cv. Lee when 50-55% defoliation occurred from full bloom (R2) into pod development stages (R3-4). However, when 32% defoliation of Lee occurred during early seed development (R5), reductions
Table 1.9. Ethylene (µM) produced per plant, nodule, g nodule dry weight, and 1000 cm² leaf area at 60 minutes from the acetylene reduction assay for defoliated and non-defoliated cv. Clark soybean and defoliation initiated at two growth stages.

<table>
<thead>
<tr>
<th>Ethylene (µM) per</th>
<th>Defoliation Treatment</th>
<th>R2 Infestation*</th>
<th>R5 Infestation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td>Non-defol.</td>
<td>13977</td>
<td>13047</td>
</tr>
<tr>
<td></td>
<td>Defoliated</td>
<td>2851**</td>
<td>21***</td>
</tr>
<tr>
<td>Nodule</td>
<td>Non-defol.</td>
<td>217.4</td>
<td>171.1</td>
</tr>
<tr>
<td></td>
<td>Defoliated</td>
<td>35.6**</td>
<td>0**</td>
</tr>
<tr>
<td>Gram Nod. Dry Wt</td>
<td>Non-defol.</td>
<td>32434</td>
<td>24327</td>
</tr>
<tr>
<td></td>
<td>Defoliated</td>
<td>6404**</td>
<td>86***</td>
</tr>
<tr>
<td>Leaf Area</td>
<td>Non-defol.</td>
<td>20307</td>
<td>4494</td>
</tr>
<tr>
<td></td>
<td>Defoliated</td>
<td>3877</td>
<td>57**</td>
</tr>
</tbody>
</table>

*An *, **, or *** indicate significance levels ≤0.05, ≤0.01, or ≤0.001, respectively.

bDefoliation did not significantly reduce leaf area of Clark when initiated at R5 in 1991.

cNodule specific activity.

dExpressed x 1000 cm².
in nitrogenase activity were not observed. This may have been because ≈66% of foliar energy sources (photosynthetically-active leaf area and foliar starch reserves) still remained and provided enough energy for symbiotic N fixation to continue at rates that were relatively unaffected. Defoliation of the indeterminate cv. Clark at levels of 74-94% caused 80-100% reduction of nitrogenase activity, regardless of whether defoliation occurred at full bloom (R2) and extended into pod development (R3-4), or if it occurred during early seed development (R5). Hutchinson (1979) and Layton and Boethel (1987) reported similar results concerning reduced nitrogenase activity following defoliation of early vegetative growth stage soybeans (V5-8). Hutchinson (1979) found that 39% defoliation caused a 35% reduction in nitrogenase activity of 'Bossier' soybeans (maturity group VIII). According to the linear equation (y = 28.7 - 0.3x, r² = 0.90) reported by Layton and Boethel (1987) for 'Davis' soybean (maturity group VII), nitrogenase activity (y) would approach 0 when defoliation levels (x) exceeded 90%; and 30, 50, or 70% defoliation would be expected to cause 34, 54, or 78% reduction of nitrogenase activity, respectively. However, the rates of nitrogenase activity were much greater in my study for non-defoliated plants. This is reasonable considering that root systems and nodulation of soybean plants in reproductive stages are extensive relative to those of young, early vegetative stage
(V5) plants (Howell 1963, Mitchell and Russell 1971, Sloger et al. 1975, and Hicks 1978). Therefore, defoliation influenced nitrogenase activity similarly, regardless of the age, size, or maturity of the N fixing system of these soybean plants. Direct reductions of N content of leaves resulted from defoliation of nodulating isoline plants in most experiments (Table 1.4 and 1.8). Nitrogen reserves in pod and seed sinks also were indirectly reduced by defoliation. This indicated that not only did soybean looper defoliation affect photosynthate supply to the bacteria in nodules, but defoliation also reduced non-foliar N reserves used for growth, maintenance, and seed production.

Forty-eight to 68% of the N in non-defoliated, nodulating plants was acquired from fixed sources in most of these experiments. However, at termination of R2 infestations of Lee in 1990 and of Clark in 1992, a large percentage of N in above-ground biomass (82 or 89%, respectively) was acquired from the soil source. Therefore, soil N had a greater influence than fixed N on plants at those two periods. This factor probably resulted in a negative N difference value for the leaf component of Clark following defoliation initiated at R2 in 1992 (Table 1.5). Hardy and Havelka (1976) reported that the average amount of N that is provided from symbiotically-fixed sources for soybean averaged 25% (ranged from 10-35%), but that greater amounts would be obtained from the symbiotically-fixed source
on N-deficient soils. Nitrogen deficiency symptoms (chlorosis) occurred by R5 in non-nodulating isoline plants in these experiments, while nodulating isolines remained dark green. This indicated that soil N was depleted by R5 and may be the reason that higher amounts of plant N were obtained from symbiotically-fixed sources by the end of the infestations initiated at R5 compared with those initiated at R2.

Use of the non-nodulating isoline and the N difference assay for evaluating effects of defoliation identified a reduction of symbiotic N fixation in only one experiment. This occurred when defoliation of the determinate cv. Lee began at full bloom (R2) and extended into early seed development (R5), resulting in 55% defoliation over a 41 d period. The N difference value also suggested that symbiotic N fixation was reduced by defoliation of Lee initiated at R5 in 1990 (Table 1.5). However, significant reductions of leaf area of the non-nodulating Lee isoline did not occur when defoliation was initiated at this growth stage in 1990 (Fig. 1.1). Therefore, the N difference value could not be used as a valid measurement of symbiotic N fixation of Lee following defoliation initiated at R5 in 1990.

Higher levels of defoliation were achieved in experiments with Clark (74-94%) than in experiments with Lee (31-55%). One explanation for this involves canopy size. The canopy of Lee often was more extensive than that of
Clark. Averaged across years, Lee had 23% greater leaf area than Clark at the end of the defoliation period initiated at R2, and in specific years Lee (1990) had 66% greater leaf area than Clark (1991). General differences in canopy size among cultivars were not as extreme following defoliation periods initiated at R5.

Defoliation (74-94%) of Clark initiated at R2 consistently decreased ethylene production by 80-100%, but did not affect the N difference value, even though leaf area of the non-nodulating isoline was significantly reduced. Defoliation (94%) of Clark initiated at R5 in 1992 also significantly decreased the ethylene measurements but did not affect the N difference value. However, in this case leaf area of the non-nodulating isoline was not significantly reduced. So with Clark, the N difference value did not indicate differences in N fixation, although large amounts of defoliation occurred and ethylene production was greatly reduced. Therefore, this technique for measurement of symbiotic N fixation provided variable results and appeared to have limited utility in experiments of this nature. The N difference technique may be more useful in experiments evaluating effects of defoliation after a recovery period, which would allow enough time for the defoliated plants to accumulate N in new growth from soil and fixed sources.

Soybean looper does not feed or develop normally on chlorotic non-nodulating soybean plants, and similar
defoliation levels were not achieved for nodulating compared to non-nodulating isolines in these experiments. Variable amounts of feeding among isolines were first observed at the R5 infestation of Lee in 1990, and bioassays were initiated later to evaluate this response using Clark foliage (See Chapter 2). In the feeding bioassays, 6.5-fold greater mortality occurred when soybean looper larvae consumed the non-nodulating isolate at R5 (23 mg N/g dry weight) relative to when larvae consumed the nodulating isolate (40 mg N/g dry weight) (Chapter 2, Wier and Boethel 1994). This factor probably was responsible for absence of reductions of leaf area of the non-nodulating isolate in these experiments with Lee at R5 (Fig. 1.1) in 1990 and Clark at R5 in 1992 (Fig. 1.3) and for negative N difference values for the leaf component of Clark in 1992 following defoliation initiated at R5.

Nodule parameters were useful in some cases for identifying effects of defoliation on symbiotic N fixation. When defoliation of Lee was initiated at R2 in 1991, a higher nodule weight per unit root weight was observed for defoliated plants (Table 1.3). This indicated that defoliation stimulated nodule formation and/or nodule enlargement. This was following the defoliation period of Lee that began at R2, lasted 41 days, and extended into R5. Other trends were observed that tend to support the stimulation of nodule development following this defoliation
period. These trends involved greater nodule number and nodule dry weight per plant for defoliated plants (Table 1.3). Ten to 14 days are required for soybean to begin to develop new nodules (Carlson 1973), and because defoliation occurred over 41 d, this compensatory response had time to occur.

Greater nodule weight per unit root weight was not observed in response to defoliation of early vegetative growth stage soybean in experiments by Layton and Boethel (1987, 1988, and 1989). When defoliation of Clark was initiated at R2 in 1992, the average dry weight per nodule was significantly reduced, or stated differently, the average weight per nodule of non-defoliated plants was significantly greater. This indicated that defoliation retarded nodule development. Reduced weight per nodule has been reported by Hutchinson (1979) following 39% defoliation of early vegetative growth stage soybean and by Layton and Boethel (1987, and 1988) following 66-88% defoliation during early vegetative growth stages. Although nodule weight per plant and weight per nodule of Clark were significantly reduced by defoliation initiated at R5 in 1991 (Table 1.3), defoliation did not significantly reduce leaf area of that cultivar following that defoliation period. Therefore in this case, nodule parameters could not be linked to effects of defoliation.
Defoliation of Lee initiated at R2 significantly reduced nodule specific activity both years (Table 1.6). Therefore, on a weight basis, nodules on roots of defoliated plants of this cultivar were less efficient at fixing N, and compensation for defoliation through increased nitrogenase activity did not occur. Defoliation of Clark initiated at R2 significantly reduced all ethylene measurements, with exception of ethylene per unit leaf area following defoliation initiated at R2 in one year (1991) (Table 1.9). Therefore, defoliation of Clark affected symbiotic N fixation in several ways when initiated at R2. Nitrogen fixation was affected through reduction of the overall efficiency of nodules of defoliated plants when compared relative to the number of nodules (ethylene/nodule), mass of nodules (nodule specific activity), or remaining leaf area. These same findings concerning ethylene data apply to Clark when defoliation was initiated at R5, although only 1992 data are relevant because significant reductions in leaf area of Clark did not occur at R5 in 1991. Thus, it appears that Clark did not compensate for defoliation by producing greater amounts of symbiotically-fixed N.

Layton and Boethel (1987) attributed reductions in symbiotic N fixing ability following >47% defoliation of 'Davis' soybean at early vegetative growth stages to reduced nodule number, weight, and nodule specific activity. They later found that even lower defoliation levels (37%) reduced
nodule specific activity, while 62 and 73% defoliation reduced both nodule specific activity and nodule weight (Layton and Boethel 1989). Vegetative soybeans did not recover symbiotic N fixing ability until 4 weeks after defoliation was terminated (Layton and Boethel 1988). Research by Lawn and Brun (1974) and Riggle et al. (1984) demonstrated that mechanical defoliation (60%) of soybean imposed at R3-4 or R5 growth stages resulted in reductions in nodule specific activity of 20-60%. However, reductions in nitrogenase activity are not always associated with reduced nodule specific activity. In experiments by Russin et al. (1990) involving defoliation of soybean from V6 to R1 growth stages, reductions in nodule specific activity were not observed following 22-64% defoliation, although ethylene produced per plant and nodule number, nodule dry weight per plant, and dry weight per nodule were significantly reduced. My research indicated that reductions in nitrogenase activity resulted from reduced nodule specific activity of Lee when 50-55% defoliation occurred from full bloom (R2) and continued into pod development stages (R3-4) or into early seed development (R5). Reductions of nodule specific activity of Clark occurred regardless of when defoliation occurred and were related to reductions of nodule dry weight per plant and dry weight per nodule.

Yield reductions for Lee of 35% (1492 kg/ha) occurred in response to 50% defoliation at full bloom through pod
development, whereas 32% defoliation during early seed development caused yield reductions of 22% (971 kg/ha). Yield reductions for Clark of 48% (800 kg/ha) and 95% (1359 kg/ha) occurred in response to 74 and 94% defoliation from full bloom into pod development stages, whereas 94% defoliation during early seed development caused yield reductions of 41% (750 kg/ha). Yield reductions of similar magnitude have been demonstrated for soybean in response to defoliation during reproductive growth stages (McAlister and Krober 1958, Teigen and Vorst 1975, Caviness and Thomas 1980, Fehr et al. 1981, Pickle and Caviness 1984, Goli and Weaver 1986, and Turnipseed and Kogan 1987). Turnipseed and Kogan (1987) reviewed data concerning effects of insect defoliation on soybean yield and concluded that yield reductions are not expected to occur in response to < 20% defoliation at any growth stage or to < 30% defoliation before R2 or after R6 growth stages. They concluded that defoliation levels > 30% caused yield decreases when imposed at stages from full bloom (R2) to full seed (R6). In my experiments, defoliation levels above this critical level of 30% identified by Turnipseed and Kogan (1987) were achieved, and infestations occurred at growth stages when yield is influenced. However, their research used mechanical defoliation (cutting leaves off at the tip of the petiole) over a relatively short period of time during specific reproductive stages, and therefore, may not be comparable to my experiments where effects of
insect defoliation occurred over an extended period (14-41 days).

Yield reductions of determinate cultivars including Lee have been greater compared to indeterminate cultivars in response to 100% defoliation during reproductive growth stages (Fehr et al. 1977, and Fehr et al. 1981). In my experiments, general comparisons of determinate and indeterminate cultivars were questionable because of variable levels of defoliation among Lee and Clark. However, general comparisons of stage of infestation illustrated that 94% defoliation caused greater yield reductions of Clark when defoliation occurred from full bloom (R2) through pod development (R3-4) (95% yield reduction) compared to when defoliation occurred during early seed development (R5) (41% yield reduction). This contradicts findings by Fehr et al. (1977, and 1981) that maximum yield loss from 100% defoliation of indeterminate cultivars occurred at R5 or R5.5 (82% yield reduction) compared to earlier reproductive growth stages (R1-R4). However, that research also involved mechanical defoliation over a relatively short time period, and may not be comparable to my experiments where insect defoliation occurred over extended periods during bloom and pod development or early seed development.

Nitrogen content of non-defoliated, non-nodulating isolines was as much as 69% lower relative to that of respective non-defoliated, nodulating isolines following
experiments that involved defoliation periods initiated at R5 (Table 1.4 and 1.8). Therefore, the non-nodulating isolines were extremely stressed by N deficiency during the seed filling period, which probably suppressed effects of defoliation on yield. Yield of the non-nodulating isolines appeared to be more limited by N deficiency than by defoliation. Yields of these isolines were never reduced because of defoliation, even following defoliation at levels up to 93%. However, relative to non-defoliated, nodulating isolines, yields of the non-nodulating isolines were reduced extensively (35-77%). In summary, these experiments demonstrated that defoliation of soybean (32-94%) by soybean looper during pod (R2-4) or early seed development (R5) influenced symbiotic N fixation and thereby reduced yield.
CHAPTER 2

FEEDING ACTIVITY, GROWTH, AND SURVIVAL OF SOYBEAN LOOPER ON LOW NITROGEN NON-NODULATING SOYBEAN

Introduction

The soybean looper is reported to feed on 73 plant species in 29 families including soybean (Herzog 1980). Early-instar soybean loopers feed selectively on low fiber, highly-digestible soybean tissues (Kogan and Cope 1974). Age of soybean leaves also affects development of the soybean looper (Reynolds and Smith 1985) and the bollworm, Helicoverpa zea (Boddie) (McWilliams and Beland 1977). In these studies older leaves suppressed feeding and larval weight. As soybean leaves age, nitrogen and protein concentration levels decrease; and fiber, lignin, and tannin levels increase (Wittenbach et al. 1980, and Scriber 1984a). Therefore, effects of leaf age on growth and development of lepidopteran insects may be related to host nutritional quality.

Scriber (1984a,b) cited numerous studies in which reduced feeding and development of phytophagous insects, including several Lepidoptera, were associated with reduced foliage N concentration in host plants. The soybean looper is an economic pest of soybean (Newsom et al. 1980); however, its hosts include non-cultivated plants outside the Fabaceae family (Herzog 1980) that are N limited (generally exhibiting deficiency symptoms without supplemental N) because of their
inability to symbiotically-fix N. Therefore, soybean loopers probably are adapted to a wide range of host N concentrations levels. Feeding studies examining the effect of low foliage N on soybean looper larval feeding and development may help explain how low N, non-legume host plants influence population dynamics of this polyphagous insect.

While examining the effect of defoliation on symbiotic N fixation of soybean in greenhouse and field studies, I observed that reduced defoliation and larval development of soybean looper occurred on chlorotic, non-nodulating Clark soybean plants. Chlorotic foliar symptoms observed in those experiments were typical of N deficiency (Janick et al. 1974). The non-nodulating Clark phenotype differs from Clark only in ability to nodulate and fix N symbiotically (Bernard et al. 1991), and foliage from N deficient, non-nodulating soybean plants could be used to examine the effects of N deficiency on feeding and development of soybean looper.

In view of these observations, a laboratory study was designed to quantify foliage N concentration level and evaluate the effect of diets of nodulating and non-nodulating isolate foliage collected at two reproductive growth stages on feeding and development of the soybean looper.

Materials and Methods

Seed of nodulating Clark and non-nodulating (L63-1889) soybean isolines were inoculated with peat-based
Bradyrhizobium japonicum (Nitragin brand, 'S' Culture, LiphaTech Inc., Milwaukee, WI) and planted in four-row plots (0.92 m row spacing) 1.83 m in length on Commerce silty clay loam soil (fine-silty, mixed, nonacid, thermic Aeric Fluvaquent). Plots were arranged in a randomized complete block design with four blocks.

Fully expanded trifoliates were collected from the upper third of the canopy of field plots. Fifty larval feeding chambers consisting of a petri dish (100 by 15 mm) containing a leaflet and a moistened filter paper disk (90 mm) were prepared for each isoline. Soybean looper eggs were obtained from the USDA, ARS Insectary at Stoneville, Mississippi; and a single neonate soybean looper was introduced into each feeding chamber. Foliage and filter paper were replaced at 24- to 48-h intervals. The experiment was maintained in a completely randomized design at ≈25 °C with a photoperiod of 16 h of light to 8 h of darkness. Separate experiments were conducted for foliage collected from plants in full bloom (R2 plant growth stage, Fehr et al. 1971) and for the beginning seed stage (R5).

Developmental stage (stadium) of larvae was assessed at 0900 to 1200 h daily according to methods described by Shour and Sparks (1981). The criterion for determining if a molt occurred was presence of exuviae (head capsule and/or cast larval skin) following ecdysis. Larval weight (top-loading electronic balance Model TL110, American Scientific, McGraw
Park, IL) and leaf area (portable area meter Model LI-3000, Li-Cor, Lincoln, NE) were measured every other day beginning when larvae fed nodulating isoline foliage reached the fourth stadium. Nitrogen content (elemental C/H/N analyzer Model 240C, Perkin-Elmer, Norwalk, CT) also was determined (mg N per g leaf dry weight). Nitrogen samples consisted of all foliage from six plants from each of four replicated plots per isoline.

Data analysis consisted mainly of single degree of freedom $t$-tests among data for each isoline, with the Satterthwaite $t$-statistics being reported under unequal variance conditions ($t$test cochran procedure, SAS Institute 1990). However, frequency data were analyzed using Chi-square analysis.

**Results**

**R2 Growth Stage.** Foliage N content of the non-nodulating isoline (38.8 mg g$^{-1}$) did not significantly differ from that of the nodulating isoline (46.7 mg g$^{-1}$) at R2. The number of stadia was not significantly affected by isoline (5.7 vs 5.9 stadia for larvae fed nodulating vs. non-nodulating isolines). The duration of each stadium was not affected by isoline when the majority of the larvae were considered (96% required 6 stadia regardless of isoline, Fig. 2.1). However, differences in the duration of the seventh stadium were observed for the 4% of the larvae that required
Figure 2.1. Days required for development (means ± SE) of soybean looper larvae in each stadium when fed nodulating or non-nodulating isoline soybean foliage; collected at R2, full bloom, or at R5, beginning seed.
a seventh stadium. Larvae required significantly longer to develop on non-nodulating isoline foliage (15.0 vs. 15.6 days for larvae fed nodulating vs. non-nodulating isolines), and pupal development time was significantly shorter following development on non-nodulating isoline foliage (7.4 vs. 6.8 days for larvae fed nodulating vs. non-nodulating isolines). Significantly fewer larvae developed to the pupal stage following only five stadia when fed non-nodulating isoline foliage (30 vs. 10% of larvae fed nodulating vs. non-nodulating isolines). The number of larvae that required development through six or seven stadia before pupation was not affected by isoline. Percent larval mortality was not affected by isoline; however, percent of the individuals that developed to the adult stage (percent adult emergence) was reduced following larval development on non-nodulating isoline foliage (Fig. 2.2).

Foliage consumption by larvae within specific stadia was not affected by isoline (Fig. 2.3). Total foliage consumption (81.4 vs. 88.8 cm² for larvae fed nodulating vs. non-nodulating isoline foliage) was not significantly affected by isoline unless corrected for mortality. When only larvae that survived to the pupal stage were considered, significantly greater total amounts of foliage were consumed by larvae fed the non-nodulating isoline (83.4 vs. 98.9 cm² for larvae fed nodulating vs. non-nodulating isoline foliage). Larval weight was significantly reduced during
Figure 2.2. Percent larval mortality and adult emergence (means ± SE) for soybean loopers fed nodulating or non-nodulating isoline soybean foliage; collected at R2, full bloom, or at R5, beginning seed.
Figure 2.3. Foliage consumption (means ± SE) by soybean looper larvae in specific stadia when fed nodulating or non-nodulating isoline soybean foliage; collected at R2, full bloom, or at R5, beginning seed.
stadium 3, 4, or 5 when larvae consumed non-nodulating isoline foliage (Fig. 2.4). Larval weight was not affected by isoline when larvae were in stadium 6 or 7. Pupal weight (245.2 vs. 217.0 mg for larvae fed nodulating vs. non-nodulating isolines) was significantly lower for larvae fed non-nodulating isoline foliage.

**R5 Growth Stage.** Foliage N of the non-nodulating isoline (23 mg g\(^{-1}\)) was significantly reduced to approximately one-half that of the nodulating isoline (40.2 mg g\(^{-1}\)) at R5. Non-nodulating isoline foliage collected at R5 was more chlorotic relative to that collected at R2, and percent N of this isoline generally was lower at R5 (23 mg g\(^{-1}\)) than non-nodulating isoline foliage collected at R2 (38.8 mg g\(^{-1}\)). Foliage of the nodulating isoline remained dark green, and N concentration levels generally did not change from R2 (46.7 mg g\(^{-1}\)) to R5 (40.2 mg g\(^{-1}\)).

Larvae that consumed non-nodulating isoline foliage required a significantly greater number of stadia (5.5 vs. 6.5 stadia for larvae fed nodulating vs. non-nodulating isolines). Duration of the first, second, and fourth stadia was longer when larvae were fed non-nodulating isoline foliage (Fig. 2.1). Duration of the third, fifth, and sixth stadia was not significantly affected by isoline. Significantly fewer larvae pupated following development through only five stadia (44 vs. 0% for larvae fed nodulating
Figure 2.4. Weight of soybean looper larvae (means ± SE) in specific stadia when fed nodulating or non-nodulating isoline soybean foliage; collected at R2, full bloom, or at R5, beginning seed.
vs. non-nodulating isoline foliage) or six stadia (44 vs. 14% for larvae fed nodulating vs. non-nodulating isoline foliage) when fed the non-nodulating isoline. Only larvae that consumed non-nodulating isoline foliage required a seventh stadium (6% of larvae) or eighth stadium (2% of larvae). Larvae required significantly longer to develop when fed non-nodulating isoline foliage (15.4 vs. 18.8 days for larvae fed nodulating vs. non-nodulating isoline foliage). However, pupal development time was not significantly affected. Percent larval mortality was 6.5-fold greater, and percent adult emergence was reduced by 3.9-fold following development on non-nodulating isoline foliage (Fig. 2.2).

Foliage consumption was significantly less during stadium 4 or 5 when larvae were fed non-nodulating isoline foliage (Fig. 2.3). Foliage consumption was not affected by isoline when larvae were in stadium 3 or 6. Total foliage consumption by larvae fed non-nodulating isoline foliage (48.2 cm²) was reduced by 33% relative to those fed nodulating isoline foliage (72.6 cm²). However, when corrected for mortality, significantly greater total amounts of foliage were consumed when larvae were fed the non-nodulating isoline (73.4 vs. 109.5 cm² for larvae fed nodulating vs. non-nodulating isoline foliage). Larval weight was significantly reduced during stadium 3, 4, or 5 when larvae were fed non-nodulating isoline foliage (Fig. 2.4). Larval weight was not affected by isoline when larvae
were in stadium 6. Pupal weights were not significantly affected by isoline (199.8 vs. 179.5 mg for larvae fed nodulating vs. non-nodulating isolines).

Discussion

Nitrogen is an important component of an insect's diet because it is the basic constituent of amino acids, protein, and vitamins. Phytophagous lepidopteran insects convert a food source relatively low in N (10 to 50 mg g\(^{-1}\)) into biomass consisting mainly of protein (70 to 140 mg N/g dry weight) (Scriber 1984a,b). Therefore, phytophagous insects are at a disadvantage initially because of the disproportionate amount of N in their body and their natural food sources. Several Lepidoptera compensate for reduced protein and N concentration levels of certain diets by increasing food consumption. This compensatory feeding response was reported for the imported cabbageworm, *Pieris rapae* (L.), on crucifers with reduced N concentration levels (Slansky and Feeny 1977), for the southern armyworm, *Spodoptera eridania* (Cramer), when fed legumes with reduced N concentration levels (Scriber 1979), and for larvae of the monarch butterfly, *Danaus plexippus* (L.), on milkweed (Schroeder 1976). Compensatory feeding in response to low foliage N was observed in my experiments for a small portion of the soybean loopers that survived (22%) on low N foliage collected at R5. However, a large portion of this population of soybean loopers (78%)
could not survive on low N foliage by implementing this or some other survival mechanism. Mattson (1980) reported that insects cannot compensate for extremely low dietary N by unlimited overeating, and that minimum N requirements have been determined for a few organisms. The minimum N requirement for the southern armyworm is ≈30 mg g⁻¹ (Soo Hoo and Fraenkel 1966, and Dadd 1973). Even though limited compensatory feeding occurred, this experiment indicates that the N requirement for soybean looper is >23 mg g⁻¹ and that 40 mg g⁻¹ is sufficient for development from the neonate to the adult stage. Soybean commonly has foliage N concentration levels of 40 mg g⁻¹ in reproductive growth stages (deMooy et al. 1973).

Literature addressing the effect of reduced host N concentration levels on feeding and development of the soybean looper is limited. Todd et al. (1972) examined the relationship of N fertilization of non-nodulating soybeans and population levels of Mexican bean beetle, *Epilachna varivestis* Mulsant, and reported that levels of this pest were positively correlated with N fertilization rate and leaf protein concentration level of non-nodulating isoline soybean. They observed similar trends with soybean looper and other lepidopteran pests; however, population levels of these pests were considered too low for valid comparisons. Nutritional studies have indicated that decreased foliage N content is associated with reduced feeding and development of
lepidopteran larvae including the black swallowtail, *Papilio polyxenes asterius* Stoll. Scriber (1984a,b) reported a 1.6-fold reduction in relative growth rate (mg gain per day per mg mean biomass during a particular stadium) when N concentration level among various host species in the Apiaceae family differed from 40 to 20 mg g⁻¹. Although relative growth rate was not measured in my study, this reference confirms that growth of other lepidopteran larvae is reduced under host N regimes similar to those encountered in my study.

In summary, only slight effects on feeding and development were observed at R2 when foliage N concentration levels of nodulating and non-nodulating plants did not differ. However, chlorosis of the non-nodulating isolate was more evident, and a 43% reduction of foliage N of this isolate occurred at R5, resulting in limited compensatory feeding, extended larval development, and reduced survival. Foliage N concentrations of ≈40 mg g⁻¹ were sufficient for soybean looper development from the neonate to the adult stage, while concentrations of 23 mg g⁻¹ were inhibitory.

Non-nodulating soybean provides an excellent mechanism for studying N nutrition of phytophagous insects. Production soybeans generally are not N limited and provide a stable, high N food source for the soybean looper. Vegetables, ornamentals, and several non-cultivated plants are overwintering hosts of the soybean looper (Newsom et al.)
1980, and Herzog 1980), and N status on these alternate hosts could impact the overall reservoir of soybean loopers that will ultimately infest soybean.
CHAPTER 3

FEEDING, GROWTH, AND SURVIVAL OF SOYBEAN LOOPER (LEPIDOPTERA: NOCTUIDAE) IN RESPONSE TO NITROGEN FERTILIZATION OF NON-NODULATING SOYBEAN

Introduction

The soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae), is a pest of soybean, *Glycine max* (L.) Merrill, in the southern United States (Newsom et al. 1980). As a legume host, soybean foliage is a high nitrogen (N) food source for insect pests (~40 mg N/g dry weight, deMooy et al. 1973). However, soybean loopers feed on non-legumes also. This insect has 73 known host plant species in 29 families including several non-cultivated hosts (Herzog 1980). Therefore, the soybean looper probably is adapted to a range of host plant N levels.

Nitrogen is a constituent of amino acids, protein, and chlorophyll and thus is a critical nutrient for plants. Dietary N also is critical for phytophagous insects. Scriber (1984a,b) cited numerous studies in which reduced feeding and development of insects, including several Lepidoptera, were attributed to reduced foliage N concentration of host plants. In field studies that involved non-nodulating (nn) soybean plants, I observed reduced feeding and growth of soybean looper larvae when these plants exhibited chlorotic foliar symptoms typical of N deficiency. Feeding bioassays were initiated, and a reduction in foliage N concentrations from ~40 mg/g dry weight (nodulating isoline) to 23 mg/g dry
weight (nn isoline) resulted in limited compensatory feeding, extended larval development, and reduced survival of soybean looper larvae (Chapter 2, Wier and Boethel 1994). Additional studies that examine how fertilizer N influences soybean looper larval feeding and development appeared necessary to define host N requirements of this pest and explain how N fertilization of non-legume host plants influences population dynamics of this phytophagous insect.

The objective of this experiment was to evaluate feeding, growth, and survival of the soybean looper when provided foliage from nn isoline soybean plants supplied with sequential applications of several rates of N fertilizer.

Materials and Methods

Plastic pots (25.4 cm in diameter) lined with plastic bags were filled with Convent silt loam soil (coarse-silty, mixed, non-acid, thermic Aeric Fluvaquent) and flooded for six weeks to encourage denitrification. The soil was dried at ambient greenhouse temperature for several weeks and then adjusted to a uniform weight of soil per pot. Oven dry weight of soil per pot was determined, and the amount of NH₄NO₃ required per pot was calculated for five fertilizer rates (5.25, 21, 42, 84, and 168 kg N/ha as NH₄NO₃). A non-fertilized, nn control also was examined in this study.

Non-nodulating (D68-0099) isolines of cv. Lee soybean were obtained from E. E. Hartwig (USDA-ARS SIML, Stoneville,
MS). The nn Lee phenotype differs from the nodulating Lee phenotype mainly in ability to nodulate and fix N symbiotically (Hartwig 1994). Seed were planted on 27 Sep, 1993. Pots were arranged in a completely randomized design with 25 pots per fertilizer treatment, and plants were thinned at emergence to 6 plants per pot. Plants were grown under supplemental lighting (1000 watt metal halide lamps ≈ 1 m above plants) with a photoperiod of 14:10 (L:D) h. Full rates of fertilizer were pipetted from stock solutions onto the soil surface at 9, 21, 35, and 55 days after planting (DAP) and lightly watered in. To help prevent leaching of fertilizer with regular watering, pots were watered from the bottom into saucers placed under each pot. Deionized water was used to prevent buildup of excessive salts in the soil. At 46 DAP, plants were in growth stage V8 (Fehr et al. 1971) and feeding bioassays using foliage from these plants were initiated. Plants were beginning to bloom when feeding bioassays were terminated.

At 51 and 60 DAP, respectively, 2 or 4 leaf samples were taken at random from the top 4 nodes having a fully expanded trifoliate, oven dried (80°C), and ground. Total N concentration (mg/g dry weight) was determined (elemental analyzer Model B-6450, Heraeus C/H/N/O Rapid Analyzer, UIC Scientific Distributors, Joliet, IL). Foliage nitrogen concentration was averaged across the 51 and 60 DAP samples for analysis. At 51 DAP, chlorophyll concentration was
determined colorimetrically (Mackinney 1941) at 652 nm (Spectronic 20, Milton Roy, Rochester, NY). Samples for chlorophyll analysis consisted of foliage from 5 plants selected at random from each fertilizer treatment. Leaves for this analysis also were sampled from the top 4 nodes having a fully expanded trifoliate.

Leaves for feeding bioassays were collected from the same location on the plants as N and chlorophyll samples. Twenty larval feeding chambers consisting of a petri dish (100 by 15 mm) that contained a leaflet on a moistened filter paper disk (90 mm) were prepared for each fertilizer treatment. Soybean looper eggs were obtained from the USDA, ARS Insectary at Stoneville, Mississippi, and a single neonate soybean looper was introduced into each feeding chamber. Foliage was replaced at 24 to 48 h intervals. The feeding chambers were arranged in a completely randomized experimental design and held at ≈23 °C with a photoperiod of 16:8 (L:D) h.

Development stage (stadium) of larvae was assessed at 0800 to 1400 h daily according to methods described by Shour and Sparks (1981). The criterion for determining if a molt had occurred was presence of exuviae (head capsule and/or cast larval skin) following ecdysis. Larval weights were measured to the nearest 0.1 mg (top-loading electronic balance Model A-30, Mettler, Hightstown, NJ) and leaf area consumed was determined (portable area meter Model LI-3000,
Li-Cor, Lincoln, NE) every other day beginning on day 7, when larvae fed foliage from plants given the two highest rates of N were in the fourth stadium. These measurements were initiated at that time because that was when leaf area consumed and larval weights became great enough to measure. The sum of foliage consumption for each specific stadium, and total consumption throughout the larval stage were averaged across the number of individuals tested. The maximum weight of larvae in each specific stadium, and when all stadia were considered also were averaged across the number of individuals tested. However, only individuals that survived through the specific stadium being examined (consumption per stadium or larval weight per stadium) or to pupation (total consumption or maximum larval weight) were included.

The relationships of foliage N and chlorophyll concentration to N fertilizer rates were analyzed using regression analysis (reg procedure, SAS Institute, 1990). Other non-frequency data were analyzed using analysis of variance, and means were separated using LSD (glm and means/lsd procedures, SAS Institute 1990). Frequency data were analyzed using Chi-square analysis (frequency/chi square procedure, SAS Institute 1990), and the criterion for significant mean separations of frequency data was non-overlap of 95% confidence intervals (Steel and Torrie 1980).
Results

Variable levels of chlorosis among plants fertilized with the different N fertilizer concentrations were observed. A positive linear relationship occurred for foliage N concentration in relation to N fertilizer concentration (Fig. 3.1). Average foliage N concentration was 11.4, 10.4, 12.3, 16.9, 22.4, and 33.4 mg/g dry weight for the 0, 5.25, 21, 42, 84, and 168 kg N/ha treatments, respectively. A positive cubic relationship was observed for chlorophyll concentration in relation to N fertilizer concentration (Fig. 3.1). Average chlorophyll concentration was 0.51, 0.47, 0.80, 1.15, 1.16, and 1.71 mg/g fresh weight for the 0, 5.25, 21, 42, 84, and 168 kg N/ha treatments, respectively.

In general, larval development time increased within each stadium as N fertilizer rate decreased (Table 3.1). However, larval development time was extended as larvae approached pupation, resulting in a similar or shorter duration of stadia 6, 7, 8, and 9 as N fertilizer rates decreased. The duration of stadium 7 was greater for larvae fed foliage from the 84 and 168 kg N/ha treatments compared to the 21 and 42 kg N/ha treatments. Differences in duration of stadia 6, 8, and 9 were not observed. Both numbers of stadia and duration of the larval stage were inversely related to N fertilizer rate (Table 3.2). Duration of the pupal stage was reduced only when larvae were fed foliage from the highest N treatment.
Figure 3.1. Relationship between fertilization of non-nodulating isoline cv. Lee soybean with six rates of NH₄NO₃ fertilizer and N concentration (P<0.01, y=0.143x + 10.312) and chlorophyll concentration (P<0.01, y=0.026x - 0.000297x² + 0.00000111x³ + 0.422) of foliage.
Table 3.1. Duration of each stadium in days and number of surviving insects (n) for soybean looper larvae fed non-nodulating isolate cv. Lee soybean foliage following sequential applications of six rates of NH$_4$NO$_3$ fertilizer.

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<th>N Fertilizer Rate (kg N/ha)</th>
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<td>5.0a (2)</td>
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<td>5.25</td>
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<td>21</td>
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<td>3.3a (4)</td>
<td>3.3a (3)</td>
<td>3.0ab (3)</td>
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<td>4.5a (2)</td>
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<tr>
<td>42</td>
<td>3.1bc (14)</td>
<td>2.8a (10)</td>
<td>2.7b (9)</td>
<td>3.3a (9)</td>
<td>2.7b (9)</td>
<td>3.6a (9)</td>
<td>3.6b (9)</td>
<td>5.2a (9)</td>
<td>6.4a (5)</td>
</tr>
<tr>
<td>84</td>
<td>3.0c (20)</td>
<td>2.1b (20)</td>
<td>2.5bc (20)</td>
<td>2.6bc (20)</td>
<td>3.0b (19)</td>
<td>3.3a (19)</td>
<td>5.7a (19)</td>
<td>5.0a (15)</td>
<td>---</td>
</tr>
<tr>
<td>168</td>
<td>2.8c (19)</td>
<td>2.0b (19)</td>
<td>2.2c (19)</td>
<td>2.3c (19)</td>
<td>3.1b (19)</td>
<td>3.8a (19)</td>
<td>6.3a (19)</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

P>F ANOVA 0.0001 0.0001 0.0008 0.0012 0.0108 0.5342 0.0001 0.8383 0.8457 ---

$^1$Column means followed by the same letter are not significantly different (P=0.05, LSD). Number of surviving individuals (n).
Table 3.2. Growth and development parameters for soybean looper larvae fed non-nodulating isoline cv. Lee soybean foliage following fertilization with several rates of NH$_4$NO$_3$.

<table>
<thead>
<tr>
<th>N Fertilizer Rate (kg N/ha)$^1$</th>
<th>No. of Stadia</th>
<th>Duration of Stages (days)</th>
<th>Total Foliage Consumed (cm$^2$)</th>
<th>Maximum Larval Wt. (mg)</th>
<th>Pupal Wt. (mg)</th>
<th>Mortality (%)$^2$</th>
<th>Emergence (%)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>8.6a</td>
<td>30.4a</td>
<td>146.9a</td>
<td>253.3a</td>
<td>147.9b</td>
<td>55±23.3b</td>
<td>35±22.3b</td>
</tr>
<tr>
<td>84</td>
<td>7.3b</td>
<td>23.3b</td>
<td>124.5b</td>
<td>269.9a</td>
<td>207.9a</td>
<td>5±10.2a</td>
<td>95±10.2a</td>
</tr>
<tr>
<td>168</td>
<td>6.8c</td>
<td>21.2c</td>
<td>100.7c</td>
<td>270.3a</td>
<td>202.8a</td>
<td>10±14.0a</td>
<td>90±14.0a</td>
</tr>
</tbody>
</table>

$^1$All larvae died before pupation when fed foliage from plants fertilized with 0, 5.25, or 21 kg N/ha. Row means followed by the same letter did not differ significantly (P>0.05, LSD).

$^2$Row means ± 95% confidence intervals followed by similar letters did not differ significantly. Criterion for differences among row means was non-overlap of 95% confidence intervals.

$^3$Indicates the percentage of larvae that died before pupation.

$^4$Indicates the percentage of larvae that survived to the adult stage.
Increased mortality occurred in response to decreased rate of N fertilizer (Table 3.2). All larvae died before pupation when larvae were fed foliage from the 0, 5.25, and 21 kg N/ha treatments. The majority of the larvae survived (>90%) when larvae were fed foliage from the 84 and 168 kg N/ha treatments, and all of these larvae pupated and emerged as adults. However, mortality of larvae fed foliage from the 42 kg N/ha treatment was moderate (55% mortality), and 10% mortality occurred in the pupal stage.

Within each stadium, less foliage was consumed as N rate decreased (Fig. 3.2). Foliage consumption was greater when larvae were fed foliage from the 168 kg N/ha treatment than for any other treatment for larvae in stadium 4 or 6. When larvae were in stadium 5 or 7, differences in foliage consumption were not observed for larvae fed foliage from the 21 and 42 kg N/ha or the 84 and 168 kg N/ha treatments. However, the 84 and 168 kg N/ha treatments resulted in greater foliage consumption than the 21 and 42 kg N/ha treatments when larvae were in these stadia. Larvae fed foliage from the 168, 84, and 42 kg N/ha treatments pupated following stadia 7, 8, and 9, respectively. Larvae in stadium 8 fed foliage from the 84 kg N/ha treatment consumed greater amounts of foliage than those fed foliage from the 42 and 21 kg N/ha treatments. Differences in foliage consumption were not observed for the 21 and 42 kg N/ha treatments for larvae in stadium 8. Larvae in stadium 9
Figure 3.2. Foliage consumption by soybean looper larvae in specific stadia when fed foliage from a non-nodulating isoline of soybean that received NH$_4$NO$_3$ at rates of 21, 42, 84, and 168 kg N/ha. Bars within each stadium category assigned the same letters are not significantly different (P>0.05, LSD).
consumed greater amounts when fed foliage from the 42 kg N/ha treatment relative to the 21 kg N/ha treatment. Regardless of the trends within each stadium, higher total amounts of foliage were consumed throughout the duration of larval development with each decrease in N fertilizer rate from 168, to 84, to 42 kg N/ha (Table 3.2).

With few exceptions, decreased N rate resulted in decreased larval weights within each stadium (Fig. 3.3). Decreased larval weight occurred with each decrease in N rate from 168, to 84, to 42 kg N/ha when larvae were in stadia 5, 6, and 7. Rates of 21 and 42 kg N/ha did not result in differences in larval weights for stadia 4, 5, 6, and 7; however, differences in larval weights for these two treatments occurred in stadia 8 and 9. Differences in larval weights among the 42 and 84 kg N/ha treatments occurred except when larvae were in stadium 4. Even though variation was observed in total quantity of foliage consumed, in larval weight within each stadium, and in the number of stadia when larvae were fed foliage from the 42, 84, or 168 kg N/ha treatments, larvae reached similar maximum weights before pupation regardless of these three N fertilizer rates (Table 3.2). Pupal weights were similar for larvae fed foliage from the 84 and 168 kg N/ha treatments, but higher than for larvae fed foliage from the 42 kg N/ha treatment.
Figure 3.3. Weight of soybean looper larvae during specific stadia when fed foliage from a non-nodulating isoline of soybean that received NH$_4$NO$_3$ at rates of 21, 42, 84, and 168 kg N/ha. Bars within each stadium category assigned the same letters are not significantly different (P>0.05, LSD).
Discussion

In a previous study (Chapter 2, Wier and Boethel 1994), I reported significant differences in foliage N concentrations of a nodulating isoline (≈40 mg/g dry weight) vs. the corresponding nn isoline (23 mg/g dry weight) when grown under field conditions. A diet of the N-deficient, nn isoline foliage was associated with limited compensatory feeding, extended larval development, and reduced survival of soybean looper larvae. Todd et al. (1972) examined the relationship of N fertilization of nn soybean on populations of Mexican bean beetle, *Epilachna varivestis* Mulsant, and soybean looper and reported that field population levels of Mexican bean beetle were positively correlated with both N fertilization rate and resultant leaf protein level of nn isoline soybean. Similar trends were observed with soybean looper in their study; however, population levels of this pest were too low for valid comparisons. Other studies also have documented reduced growth rates of lepidopteran (Papilionidae) larvae in response to foliage diets containing reduced N concentrations (20 mg N/g dry weight, Scriber 1984a,b).

This experiment further defined effects of small reductions in foliage N concentration on development of soybean looper larvae. I identified foliage N concentrations that caused 100% mortality (10 and 12 mg N/g dry weight), that caused moderate levels of mortality (17 mg N/g dry
weight), and that supported ≥90% survival to the adult stage (22-33 mg N/g dry weight). At 12 mg N/g dry weight, several larvae developed up to 4 supernumerary stadia before mortality occurred. This concentration (12 mg N/g dry weight) was the minimum concentration where this survival mechanism (increased number of stadia) was utilized by soybean looper larvae in my study. Foliage concentrations of 23 mg N/gram dry weight were associated with much higher levels of mortality (80%) in my earlier bioassay (Chapter 2, Wier and Boethel 1994). However, N samples for those bioassays consisted of foliage from the whole plant, while foliage for bioassays consisted of leaves from the upper 1/3 of the plant only. In the current study, foliage for N analysis and for bioassays came from the same location (upper 4 nodes with a fully expanded leaf); therefore, the actual N concentration of foliage consumed by soybean looper larvae was better defined.

Effects of reduced foliage N concentration on soybean looper development included greater number of stadia, extended duration of the larval development period, and greater total amounts of foliage being consumed. Number of stadia required by soybean looper larvae increased from that observed in my earlier study (5.5-5.9 stadia, 39-47 mg N/g dry weight, Wier and Boethel, 1994) to 6.8-8.6 stadia when foliage containing 17-33 mg N/g dry weight was consumed. Duration of larval development also increased from ≈15 days
when larvae consumed foliage containing 39-47 mg N/g dry weight (Chapter 2, Wier and Boethel 1994) to 21-30 days when larvae consumed foliage containing 17-33 mg N/g dry weight. Larvae were held at a slightly lower temperature in this study (23°C) compared to 25°C in the earlier study (Chapter 2, Wier and Boethel 1994). This reduction in temperature may have slowed larval development by ≈5 days (Mitchell 1967).

Similar maximum larval weights were achieved for larvae fed foliage containing the three greatest N levels. This was achieved through increased number of stadia, increased duration of the larval development period, and through compensatory feeding by soybean looper larvae, all of which were in response to reduced foliage N. Other lepidopteran insects also have compensated for reduced N levels of certain diets by increasing food consumption. This response is documented for the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Noctuidae), when fed crucifers with reduced N levels (Slansky and Feeny 1977) and the southern armyworm, *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae), when fed legumes with reduced N levels (Scriber 1979).

In the experiments with imported cabbageworm and southern armyworm, compensation for reduced N resulted because of increased rates of consumption and not through extended development. Increased rates of consumption generally result in greater consumption by specific stadia (Simpson and Simpson 1990). Although, the response of
soybean looper to reduced N involved lengthened duration of specific stadia (Table 3.1), increased consumption in specific stadia was not observed (Fig. 3.2), but only occurred because of the extended duration of the larval stage. This same type of compensatory response was associated with 23 mg N/g dry weight in my earlier study for the 20% of the soybean looper larvae that survived to pupation (Chapter 2, Wier and Boethel 1994). Larvae of other species including the beet armyworm, (Lepidoptera: Noctuidae) Spodoptera exigua (Hübner) (Al-Zubaidi and Capinera 1984), and a chrysomelid, Paropsis atomaria Oliver (Coleoptera: Chrysomelidae) (Ohmart et al. 1985), also have exhibited the type of compensation response to host N limitations that I observed with soybean looper.

Chlorosis is a typical symptom of N deficiency (Tisdale and Nelson 1975) and can be used to indicate the general nitrogen status of a plant. In this study, high mortality was associated with consumption of chlorotic plants that were low in chlorophyll and nitrogen, and high survival was associated with dark green plants that contained higher concentrations of chlorophyll and nitrogen. Therefore, chlorosis or chlorophyll concentration generally indicated the fitness of the plants, and these two factors could indicate when other hosts would support soybean looper larvae.
Even though consumption, development, and survival were closely related to host N concentration, interactions of N and other factors (water, allelochemics, lignin, tannins, and resins) could have affected host digestibility and nutrition and contributed to the results that were observed. However, these findings agreed with my previous conclusions that several mechanisms (lengthened larval development, increased number of stadia, and increased consumption) help soybean loopers survive on hosts with less than optimal foliage N levels (Chapter 2, Wier and Boethel 1994). In addition, I further defined how N limitations of host plants influence soybean looper development. These experiments suggest how fertilizer practices on low N non-legume hosts might influence development and survival of soybean loopers that ultimately infest soybean.
SUMMARY AND CONCLUSIONS

Through the course of these experiments, information concerning the influence of insect defoliation on symbiotic N fixation was compiled. This topic was addressed at plant growth stages (pod and early seed development) that soybean looper commonly infest, under field conditions that allowed measurement of yield. These experiments prompted further research to understand how host N status influences development and survival of the soybean looper.

CHAPTER 1. Symbiotic Nitrogen Fixation and Yield of Soybean Cultivars Lee and Clark Following Defoliation by the Soybean Looper (Lepidoptera: Noctuidae) During Pod or Seed Development.

a. In most experiments, 48-86% of plant N was provided from fixed sources, although soil N accounted for the majority of plant N (82-89%) in two experiments. The N difference assay identified a reduction of symbiotically-fixed N in only one of eight experiments (55% defoliation of Lee over 41 days from full bloom into early seed development).

b. Acetylene reduction assays indicated that defoliation affected symbiotic N fixation. Up to 85% reduction of nitrogenase activity was demonstrated for the determinate cultivar Lee when 50-55% defoliation occurred from full bloom (R2) into pod development stages (R3-4). However, when 32% defoliation of Lee occurred during early seed development
(R5), reductions in nitrogenase activity were not observed. Defoliation of the indeterminate cv. Clark at levels of 74-94% caused 80-100% reduction of nitrogenase activity, regardless of whether defoliation occurred at full bloom (R2) and extended into pod development (R3-4), or it occurred during early seed development (R5).

c. Yield reductions for Lee of 35% (1492 kg/ha) occurred in response to 50% defoliation at full bloom through pod development, whereas 32% defoliation during early seed development caused yield reductions of 22% (971 kg/ha). Yield reductions for Clark of 48% (800 kg/ha) and 95% (1359 kg/ha) occurred in response to 74 and 94% defoliation from full bloom into pod development stages, while 94% defoliation during early seed development caused yield reductions of 41% (750 kg/ha).

d. The non-nodulating isolines exhibited foliar symptoms typical of N deficiency that were accentuated at the beginning seed stage (R5).

e. When plants were infested at R5 growth stage, defoliation was suppressed, and significant reductions in leaf area of non-nodulating isolines were not achieved for either cultivar in either year.

f. Yield of the non-nodulating isolines appeared to be more limited by N deficiency than by defoliation. Yields of these isolines were never reduced because of defoliation, even following defoliation at levels up to 93%. However, relative
to non-defoliated, nodulating isolines, yields of the non-nodulating isolines were reduced extensively (35-77%).


a. During bloom and early pod development (R2-3), foliage N concentration of the non-nodulating isolate was not significantly lower than that of the nodulating isolate, and consequently, only slight effects on feeding and development were attributable to consumption of non-nodulating isolate foliage.

b. During early seed development (R5), chlorosis and a 43% reduction in the foliage N concentration of the non-nodulating isolate occurred, resulting in limited compensatory feeding, extended larval development, and reduced survival when soybean loopers consumed this isolate.

c. Foliage N concentrations of ≈40 mg N/g leaf dry weight were sufficient for soybean looper development from the neonate to the adult stage, whereas concentrations of 23 mg N/g leaf dry weight were inhibitory.

CHAPTER 3. Feeding, Growth, and Survival of Soybean Looper (Lepidoptera: Noctuidae) in Response to Nitrogen Fertilization of Non-nodulating Soybean.

a. The six N fertilizer treatments resulted in foliage N concentrations that ranged from 10 to 33 mg/g dry weight and
chlorophyll concentrations that ranged from 0.5 to 1.7 mg/g fresh weight.

b. Foliage from non-fertilized control plants and those fertilized with 5.25 and 21 kg N/ha were low in N (<12 mg/g) and caused 100% mortality when fed to soybean looper larvae.

c. Foliage from plants fertilized with 42 kg N/ha contained 17 mg N/g dry weight and resulted in moderate levels of mortality (55%), whereas the higher N rates (84 and 168 kg N/ha) resulted in foliage N concentrations of 22 and 33 mg/g dry weight and ≥90% survival.

d. Although the highest three foliage N concentrations were sufficient for survival of soybean looper larvae, effects on development of larvae were observed. Increases in number of stadia, duration of the larval development period, and foliage consumption were observed with each reduction in foliage N from 33.4, to 22.4, to 16.9 mg/g; but ultimately, similar maximum larval weights were achieved. These three factors appear to be survival mechanisms that allow the soybean looper to compensate for reduced N and may be the mechanisms that allow this species to maintain itself on such a wide range of hosts.

Results from the field experiments confirmed that soybean looper defoliation during reproductive growth stages impacts symbiotic N fixation and yield of soybean. The impact of defoliation on symbiotic N fixation probably is one
reason for the decreases in yield that were observed; however, defoliation affects other factors that can have an influence on yield that were not in the scope of my research. Other factors that could have been considered include effects on physiological factors such as seed filling rate, seed filling period, \( \text{CO}_2 \) assimilation, starch reserves, the priority of plant sinks for plant nitrogen and carbon.

Results from the feeding bioassays helped delineate the specific \( \text{N} \) requirements for soybean looper. Chlorosis was an obvious symptom of \( \text{N} \) deficiency in these experiments and can be used to indicate the general nitrogen status of other plants. High mortality was associated with consumption of chlorotic plants that were low in chlorophyll and nitrogen, and high survival was associated with dark green plants that contained higher concentrations of chlorophyll and nitrogen. Therefore, chlorosis or chlorophyll concentration generally indicated the fitness of plants in this experiment, and these two factors could indicate whether other hosts would support soybean looper larvae. Again caution must be used in making general conclusions concerning \( \text{N} \) concentration and insect development considering the many avenues where \( \text{N} \) is involved in plants. Effects of \( \text{N} \) on other factors (concentration of water, allelochemics, fiber, lignin, or tannin) also may have an impact on the biology of the soybean looper, and interactions of \( \text{N} \) with these materials should be examined.
Through further definition of the N limitations of soybean looper, I have answered some basic questions concerning the interactions between this pest and the soybean host and can make inferences concerning the effects of alternative hosts on the biology of the soybean looper. The same mechanisms (lengthened larval development, increased number of stadia, and increased consumption) that enabled soybean looper to survive on soybean with less than optimal foliage N concentration levels might influence development and survival of soybean loopers on alternative hosts that support populations that could ultimately infest soybean. Also, timing and rates of N fertilizer applied to non-legume hosts are considerations that may be relevant to pest management programs directed against this pest.
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29 August 1994

Alan T. Wier
Department of Entomology
402 Life Sciences Building
Louisiana State University
Baton Rouge, LA 70803

Dear Mr. Wier,

Per your recent request, as an author of the manuscript "Feeding activity, growth, and survival of soybean looper on low-nitrogen nonnodulating soybean" by Wier and Boethel, and published in *Agronomy Journal*, you retain the right to use material from the article at your discretion. As such, this letter serves as confirmation that you are indeed an author of the article and thus have the right to use any and all material from the article in your dissertation or other outlets of your choosing.

Sincerely,

David M. Kral
Associate Executive Vice President
North Carolina State University  
Department of Entomology  
College of Agriculture and Life Sciences  

Mr. Alan T. Wier  
Department of Entomology  
402 Life Sciences Building  
Louisiana State University  
Baton Rouge, Louisiana 70803  

Dear Mr. Wier:

Per your recent request, as an author of the manuscript, "Feeding, growth, and survival of soybean looper in response to nitrogen fertilization of non-nodulating soybean," by Wier and Boethel, and being published in Environmental Entomology, you retain the right to use material from the article at your discretion. As such, this letter serves as confirmation that you are indeed an author of the article and thus have the right to use any and all material from the article in your dissertation or other outlets of your choosing.

Sincerely,

R. E. Stinner  
Editor  
Environmental Entomology
VITA

Alan T. Wier was born on October 26, 1962, in Baton Rouge, Louisiana. He graduated from Broadmoor High School in Baton Rouge in 1980. Alan received a Bachelor of Science Degree in Plant Science from Nicholls State University in 1983, he received a Master of Science Degree in Agronomy from Louisiana State University in 1987, and he is currently a candidate for a Ph.D. in Entomology at Louisiana State University. Alan is married to Gina Cabrini Ghirardi Wier and has two children Diana Jeanne and Jacob Thomas.
Doctoral Examination and Dissertation Report

Candidate: Alan T. Wier

Major Field: Entomology

Title of Dissertation: Interactions of the Soybean Looper, Pseudoplusia includens (Walker), and Soybean, Glycine max (L.) Merrill, Involving Nitrogen: Effects on Host Symbiotic Nitrogen Fixation and Larval Development and Survival

Approved:

[Signature]
Major Professor and Chairman

Dean of the Graduate School

Examinig Committee:

[Signature]
[Name]

[Signature]
[Name]

[Signature]
[Name]

Date of Examination:

October 20, 1994