

**Assessment of Soybean Leaf Area for Redefining Management
Strategies for Leaf-Feeding Insects**

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(ABSTRACT)

Commercially available leaf area index (LAI) meters are tools that can be used in making insect management decisions. However, proper technique must be determined for LAI estimation, and accuracy must be validated for the meters. Full-season soybean require LAI values of at least 3.5 to 4.0 by early to mid-reproductive developmental stages to achieve maximum yield potential, but the relationship between double-crop soybean LAI and yield is unknown. This research (1) evaluated minimum plot size requirements for mechanically defoliated soybean experiments using the LAI-2000 Plant Canopy Analyzer, (2) compared LAI estimates among LAI-2000 detector types which respond to different wavelengths of light, (3) compared LAI-2000 estimates with directly determined LAI values for 0, 33, 66, and 100% mechanical defoliation levels, (4) used linear and non-linear models to describe the response of full-season and double-crop soybean yields to reductions in LAI through mechanical defoliation, and (5) evaluated the response of double-crop soybean yields to reductions in LAI through insect defoliation.

The minimum plot size for obtaining accurate LAI estimates of defoliated canopies in soybean with 91 cm row centers is four rows by 2 m, with an additional 1 m at the ends of the two middle rows also defoliated. The wide-blue detector, which is found in newer LAI-2000 units and responds to wavelengths of light from 360 to 460 nm, gave higher LAI estimates than the narrow-blue detector, which responds to light from 400 to 490 nm. The unit with the narrow-blue detector gave estimates equal to directly determined LAI in two of three years for 0, 33, and 66% defoliation levels, while the units with the wide-blue detectors gave estimates higher than directly determined LAI in the two years that they were studied, except for a few accurate 33% defoliation estimates. Therefore, the LAI-2000 usually provides reasonable estimates of LAI. Yield decreased linearly with LAI when LAI values were below 3.5 to 4.0 by developmental stages R4 to

R5 in both full-season and double-crop soybean. Usually, there was no relationship between yield and LAI at LAI values greater than 4.0. There was an average yield reduction of $820 \pm 262 \text{ kg ha}^{-1}$ for each unit decrease in LAI below the critical 3.5 to 4.0 level; maximum yields ranged from 1909 to 3797 kg ha^{-1} . Insect defoliators did not defoliate double-crop soybean plots to LAI levels less than 4.0, and there was no yield difference between insect-defoliated and control plots. Therefore, double-crop soybean that maintains LAI values above the 3.5 to 4.0 critical level during mid-reproductive developmental stages is capable of tolerating defoliating pests.

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Chapter 1.

Introduction and Review of Literature

Soybean [*Glycine max* (L.) Merrill] is an important source of food, oil, and protein. Kogan and Turnipseed (1980) state that defoliation is the most visible and probably the most common type of soybean injury. Insect defoliators of soybean in the United States include the soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae), green cloverworm, *Plathypena scabra* (F.) (Lepidoptera: Noctuidae), velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), and saltmarsh caterpillar, *Estigmene acrea* (Drury) (Lepidoptera: Arctiidae). Soybean yield loss due to defoliation occurs through loss of leaf area and its subsequent effects on the plant. Ostlie (1984) described a linear relationship between defoliation and soybean leaf area. Reduced leaf area lowers light interception, resulting in reduced photosynthetic capacity of the plant, loss of leaf-stored material, or shortening of the seed-fill period; ultimately, these factors cause yield loss (Board et al., 1994). Nolting and Edwards (1989) and Grymes et al. (1999) discovered that soybean defoliation reduced yield linearly, while Browde et al. (1994) reported both linear and quadratic relationships between defoliation and yield.

Leaf area index (LAI), defined as the ratio of leaf area to land area, is strongly related to crop yield (Nolting and Edwards, 1989; Browde et al., 1994; Grymes et al., 1999). Research on full-season soybean indicates that LAI values in the critical region of 3.5 to 4.0 by developmental stage R2 to R4 (full bloom to full pod) are needed to approach maximum potential yield (Board and Harville, 1992; Higley, 1992; Westgate, 1999). Reductions in LAI below this critical level due to insect defoliation may reduce yield (Board et al., 1997). Mid-Atlantic double-crop soybean, which are planted later than full-season soybean, are subject to a shorter growing season, and may not have time to reach LAI values of 3.5 or higher. Therefore, maximum yield potential may not be

reached. To compensate for this, growers use narrow row spacing and high plant populations to create more uniform plant spacing and increase LAI. As a result, light interception increases (Board and Harville, 1992), and increased light interception during vegetative and early reproductive developmental stages increases yield (Board et al., 1992).

With the reduced leaf areas associated with double-crop soybean systems, parameters currently used for making insect management decisions in Mid-Atlantic states may be invalid. We hypothesized that defoliating insects reduce yield more in these systems than current thresholds indicate. There are two problems with current thresholds for Mid-Atlantic growers. First, they were developed primarily in the Midwest using full-season, not double-crop soybean systems (Todd and Morgan, 1972). Also, there are different pest complexes and defoliation periods between the Midwest and Mid-Atlantic regions. The green cloverworm is the primary defoliator of soybean in the Midwest (Hammond et al., 1991), where 90% of feeding is done in the final two instars (Hammond et al., 1979). In Mid-Atlantic states, defoliation by insects typically does not occur in a single event, but accumulates throughout the entire growing season. A complex of species that varies spatially and temporally contributes to defoliation. Second, the thresholds use percent defoliation to predict yield loss, which does not account for differences in canopy size. For example, the current threshold for defoliating insects used in many Mid-Atlantic states holds that approximately 15% defoliation can occur between the flowering and pod fill stages without significant yield loss (e.g., Virginia, North Carolina, Georgia, and Texas Cooperative Extension publications). Fifteen percent defoliation of a canopy with an LAI of 4.5 will reduce the LAI to 3.825; this may still allow for maximum potential yield. However, 15% defoliation of a canopy with an LAI of 3.5 will reduce the LAI to 2.975, which should reduce yield. Many researchers have found that leaf area remaining predicts yield better than percent defoliation (Fehr et al., 1977; Kogan and Turnipseed, 1980; Herbert et al., 1992; Higley, 1992; Klubertanz et al., 1996).

Response of soybean to defoliation depends on (1) the length and intensity of defoliation, (2) soybean developmental stage, (3) plant characteristics, and (4)

environmental and cultural factors. Regarding the first factors, Board et al. (1994) predicted that yield losses would decrease if severe (100%) defoliation occurred over time rather than all at once. Higley (1992) states that soybean is less able to tolerate a long defoliation period than a brief defoliation period.

The first factors, length and intensity of defoliation, are related to the second factor, developmental stage. Defoliation during vegetative developmental stages usually has no effect on yield (McAvoy, 1977), although Poston and Pedigo (1976) found that 50% defoliation at V3 (four nodes having leaves with unfolded leaflets) reduced yield by nearly 14%. Turnipseed (1972) discovered that 17% defoliation at any developmental stage did not reduce yields. Thomas et al. (1978) reported that heavy sequential defoliations beginning at R3 (beginning pod) through R6 (full seed) or R7 (beginning maturity) significantly reduced yield, whereas a single defoliation never reduced yield. Fehr et al. (1977) noted that highest yield losses occurred at R4 and R5 (beginning seed) defoliation. Thomas et al. (1974) found that the most sensitive developmental stages were R5 and R6, which could only tolerate 6% defoliation before yield loss occurred. In contrast, Talekar and Lee (1988) reported that soybean in Taiwan is most sensitive to defoliation at R3 and R4. Little or no yield loss is caused by defoliation after R7 (Thomas et al., 1974; Thomas, 1984; Herman, 1985).

The third factor that affects soybean response to defoliation is plant characteristics. It depends on leaf growth habits, delayed leaf senescence, and the ability to produce new leaves after defoliation. The size, shape, orientation, and spatial distribution of leaflets in the soybean canopy influence LAI. In a greenhouse study, Haile et al. (1998) observed that 'Clark' isolines had the same number of leaves per plant at developmental stage R2, but wide-leaflet isolines had greater LAI values than narrow-leaflet isolines. However, they noted that light interception was greater in the narrow-leaflet isoline, due to greater light penetration into lower portions of the canopy and its absorption by older leaflets. Small leaflets would also allow this, thus allowing more photosynthesis by lower leaflets. Haile et al. (1998) discovered that the narrow-leaflet isoline was better able to tolerate defoliation than wide-leaflet isolines because of the greater light penetration. Blad and Baker (1972) reported that mature soybean leaves tend

to be horizontally oriented, and newly expanding leaves are more vertically oriented. Vertical leaf arrangements allow greater light penetration to the lower canopy levels. Blad and Baker (1972) also stated that soybean have more leaf area distributed in the upper portion of the canopy. This is due to reduced light penetration into the lower portion of the canopy as the plants mature, and subsequent senescence and dropping of lower canopy leaves. Delayed leaf senescence may occur through improved light penetration into the lower canopy, allowing the lower (older) leaves to increase their photosynthesis (Turnipseed, 1972; Higley, 1992; Klubertanz et al., 1996). Whether the plant is determinate or indeterminate may affect its ability to produce new leaves after defoliation. Fehr et al. (1977) reported that determinate cultivars defoliated 100% from R2 to R7 were less able to compensate for defoliation than indeterminate cultivars. They stated that the simultaneous vegetative and reproductive development in indeterminate plants and the resulting different phases of flower, pod, and seed development give the plant less material to be stressed at any given stage. In contrast, Kogan and Turnipseed (1980) observed that there is no difference in defoliation tolerance between determinate and indeterminate varieties, except at very high defoliation levels. Klubertanz et al. (1996) noted that slightly defoliated, unstressed plants produced more leaves than non-defoliated plants by R6, showing that compensatory regrowth can occur after defoliation.

Soil fertility and moisture availability are important environmental considerations in plant response to defoliation, although some research has shown that drought stress does not affect the response (Klubertanz et al., 1996). Cultural practices affect LAI. LAI levels are higher when soybean is planted early (e.g., full-season) rather than late (e.g., double-crop), due to a longer developmental period (Board and Harville, 1992). Increasing plant populations reduces the amount of time that it takes to reach 95% light interception levels. Ninety-five percent light interception corresponds to LAI levels of 3.2 (Shibles and Weber, 1965) to 3.5 (Higley, 1992). Shibles and Weber (1965) showed that LAI is a function of population. High populations produce plants that are taller, have fewer lateral branches, longer petioles, smaller leaflets, longer vegetative stages, and greater leaf area production than lower populations (Shibles and Weber, 1966). Board et al. (1992) reported that narrowing rows from 100 to 50 cm increased light interception

and yield. Narrowing rows and increasing plant populations creates more equidistant plant spacing, reducing early-season competition within the row (Wells, 1991). Equidistant plant spacing increases LAI, consequently increasing light interception (Board and Harville, 1992), and increased light interception duration during vegetative and early reproductive developmental stages increases yield (Board et al., 1992).

Most research on the response of soybean to defoliation involves mechanical defoliation rather than insect defoliation. Some researchers suggest that the plant stresses produced by mechanical defoliation may not adequately represent actual insect defoliation (Mueller and Engroff, 1980; Ingram et al., 1981; Mesa and Fehr, 1984; Baldwin, 1990). Most insect defoliation patterns are gradual (Board et al., 1994), and typical insect consumption rates grow logarithmically by instar (Higley and Peterson, 1996) so that late instars eat the most plant tissue. In contrast, mechanical defoliation typically removes leaf area all at once. Insect consumption may vary depending on crop health. Carter et al. (1996) discovered that stressed soybean, which had a lower leaf nitrogen content than unstressed soybean, caused soybean looper larvae to nearly double their leaf consumption in an attempt to gain the required nitrogen for development. In support of the fidelity between mechanical and actual insect defoliation, Ostlie (1984) reported no differences in leaf transpiration rates or total water loss when these two methods were compared.

In order to develop an understanding of yield loss in insect-defoliated soybean, it is necessary to quantify the injury. It is difficult to quantify insect damage in the field (without destructive sampling) because insects consume the defoliated material. However, saving defoliated material after mechanical defoliation allows for quantification of leaf area removed with a leaf area meter. Removal of a known amount of leaf area (using hole-punchers) is also easily quantified. Quantification of insect damage is possible by collecting leaflets, photocopying them, cutting the “undefoliated” leaflet shape from the photocopy, and determining the difference in areas between the leaflets and the paper cutouts. Complications in research involving insect defoliation include cage effects and mortality factors, but these are avoided when leaves are mechanically defoliated. If the primary effect of insect defoliators is removal of leaf tissue, mechanical defoliation is justified (Haile et al., 1998).

Mechanical defoliation techniques include hand-picking entire leaflets, hole-punching, and cutting leaflets. Poston et al. (1976) noted that cutting the leaflet across the midrib increased photosynthesis. Talekar and Lee (1988) discovered that cutting along the midrib resulted in the lowest yield loss among the artificial defoliation techniques, while removing the entire leaflet resulted in highest yield losses. Hole-punching resulted in significantly greater water loss than removing entire leaflets, and more closely resembled water loss due to actual insect feeding (Hammond and Pedigo, 1981). However, Ostlie (1984) reported that water loss differences between hole-punching, entire leaflet, and actual insect defoliation occurred only during the first sixteen hours after defoliation. Whether or not the stem or petiole is removed may be important; however, little or no research has been done on this subject. Hand-defoliation of entire leaflets is the most commonly used mechanical defoliation method (Hutchins et al., 1988), and was shown to be an accurate estimate of the effect of caterpillar defoliation on yield by Thomas (1984). Thomas used the average leaf area consumed throughout the larval development of the cabbage looper (119.4 cm^2) to determine the number of larvae per row meter that would cause 6% defoliation; yields from 6% mechanical defoliation and insect defoliation were the same.

Some researchers use patterns when mechanically defoliating soybean leaves. Hintz et al. (1991) removed terminal leaflets from each trifoliolate to achieve 33% defoliation, and the terminal and one lateral leaflet to achieve 66% defoliation. In contrast, Grymes et al. (1999) had no preference for removal of lateral or terminal leaflets in their defoliation technique. Mesa and Fehr (1984) found that 100% removal of leaflets from every one of three (or two of three) plants, with the other plants undefoliated, gave greater yield reductions than 33 or 66% defoliation of every plant, possibly due to plant competition. Insect defoliators typically have a preference for location within a soybean canopy. Haile et al. (1998) report that typical lepidopterous defoliation proceeds from the top to the bottom of the soybean canopy. Klubertanz et al. (1996) and Haile et al. (1998) hand-defoliated leaflets from the upper two-thirds of the soybean canopy to simulate larval lepidopteran feeding patterns. Therefore, mechanical defoliation should proceed in a fashion similar to that of the pests.

Determining the relationship of LAI to yield in Mid-Atlantic double-crop and full-season soybean should allow researchers to redefine management strategies for defoliating insects. Instead of basing thresholds on numbers of pests, management decisions could focus on the damage done or that is in progress (i.e., whether or not defoliating insects are present in the field) and the LAI needed to achieve maximum potential yield. Therefore, research is needed to gain a more complete understanding of what canopy levels are critical for maximum yields, and at what point controls should be implemented to stop further leaf area loss by insect defoliators. The rapid, accurate determination of LAI is essential to the development of new pest management strategies.

Modern leaf area meters such as the LI-3000A and LI-3100 (LI-COR, Inc., Lincoln, NE) are calibrated with metal discs of known area, and their estimate of leaf area is usually considered absolute. LAI is determined by dividing the leaf area (determined by the leaf area meter) by ground area. A drawback to absolute determination of LAI is that it is time consuming; Welles and Norman (1991) reported that 70 person-hours were required to determine the absolute LAI of a single soybean plot with a radius of 4 m. Also, when used to determine percent insect defoliation, leaf area meters can overestimate the value, for they cannot discern between live and necrotic tissue (Bowers et al., 1999). Rapid, indirect measurements of LAI are possible using LAI meters such as the Delta-T SunScan (Delta-T Devices, Cambridge, UK), Decagon AccuPAR (Decagon Devices, Inc., Pullman, WA), and the LAI-2000 (LI-COR, Inc., Lincoln, NE). To estimate LAI, these meters measure the amount of available light above the canopy and the amount that penetrates through the canopy. However, this equipment must be validated and protocols for their use must be determined for individual crops.

Wilhelm et al. (2000) discovered that in corn (*Zea mays* L.), the three LAI meters listed above underestimated LAI when compared to absolute LAI (determined using the LI-3000A). Hunt et al. (1999) found that the LAI-2000 overestimated LAI in Nebraska full-season soybean defoliated by grasshoppers and *C. trifurcata*. Wilhelm et al. (2000) and Hunt et al. (1999) state that theoretically, the LAI meters should overestimate LAI since the meters cannot distinguish between leaf, stem, petiole, and reproductive (ear or pod) tissue. Therefore, LAI estimates should become less accurate as defoliation

increases, since more non-leaf plant tissue is exposed.

The minimum plot size for obtaining accurate LAI estimates must be determined so that the LAI meter does not detect plant material outside of the plot. Time, land, and labor constraints in small plot soybean defoliation research emphasize the importance of determining the smallest plot size that still allows accurate LAI estimates.

It is necessary for LAI meters of the same make to give similar estimates of LAI, so that meters may be used interchangeably. In the late 1990's, the spectral sensitivity range of the detector in the LAI-2000 was changed from 400-490 nm to 360-460 nm. Little or no research has been done outside of LI-COR, Inc. to validate the similarity between LAI estimates from these two detectors.

People have learned to accurately estimate percent defoliation. Bowers et al. (1999) reported that visual and quantitative estimates of insect-defoliated soybean were highly correlated, and that properly trained people can rapidly and accurately visually assess insect defoliation. However, basing thresholds on percent defoliation can be inappropriate. It is recommended that soybean be managed using LAI rather than percent defoliation (Herbert et al., 1992; Higley, 1992); therefore, training people to visually estimate soybean LAI would be in order. Images of soybean and their corresponding LAI values (from LAI-2000 estimates) are given in Appendix A.

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Chapter 2.

Techniques to Evaluate the LAI-2000 Plant Canopy Analyzer's Estimate of Leaf Area in Mechanically Defoliated Soybean, and Comparison of Two LAI-2000 Detector Types

ABSTRACT

Leaf area index (LAI) of soybean [*Glycine max* (L.) Merrill] is closely related to yield and has implications for improving pest and crop management. Instruments that provide a rapid estimate of LAI are commercially available, but their accuracy and utility for estimating double-crop soybean defoliation is unknown. This study evaluated minimum plot size requirements for mechanically defoliated soybean experiments using the LAI-2000 Plant Canopy Analyzer, compared LAI-2000 estimates with directly determined LAI values for 0, 33, 66, and 100% mechanical defoliation levels, and compared LAI estimates among LAI-2000 detector types which respond to different wavelengths of light. The minimum plot size for obtaining accurate LAI estimates of defoliated canopies with 91 cm row centers is four rows by 2 m, with an additional 1 m at the ends of the two middle rows also defoliated. Larger homogeneous plots should provide more accurate LAI estimates, but time, land, and labor constraints make this less feasible. A difference between the two detector types existed. The wide-blue detector, found in newer units, gave higher LAI estimates than the older narrow-blue detector due to differences in spectral sensitivity ranges. The accuracy of the instrument varied by year and detector type. The instrument with the narrow-blue detector gave estimates the same as directly determined LAI in two of three years that they were used for 0, 33, and 66% defoliation levels, while the instruments with the wide-blue detectors gave estimates higher than directly determined LAI in the two years that they were studied, except for a few accurate 33% defoliation estimates.

INTRODUCTION

Leaf area index (LAI) is the ratio of unit leaf area of the crop to unit ground area (e.g., a LAI of 3.0 means that there are 3 m² of leaves distributed above 1 m² of ground). LAI is closely related to soybean yield, as stated in Higley's (1992) defoliation/light

interception hypothesis: yield depends on photosynthesis during early soybean reproductive stages, and photosynthesis depends on canopy light interception, which can be described by LAI. An LAI value of 3.5 to 4.0 is correlated with 95% light interception, the value at which the crop should theoretically achieve canopy closure, maximum canopy photosynthesis for the development stage, and maximum yield for the environmental conditions (Board and Harville, 1992; Westgate, 1999). Reductions in LAI below this critical value due to insect defoliators may reduce yield (Board et al., 1997). Therefore, soybean LAI becomes a critical factor in defoliating insect pest management.

Direct measurement of LAI is labor intensive, involving removal of all leaflets in a given ground area, determining the area of the leaflets using a leaf area meter, and dividing the total leaf area removed by the ground area. Indirect measurement of LAI is possible with plant canopy analyzers such as the LAI-2000 (LI-COR, Inc., Lincoln, NE), which estimate LAI in under a minute through measurements of the gap frequency, the probability that a light ray will not contact vegetation as it passes through the canopy to the ground (Lang, 1991; Welles and Norman, 1991). Estimates of LAI should be within 15% of direct LAI for most research and crop management purposes (Buntin, 1994; Hunt et al., 1999). It is unknown if the LAI-2000 accurately estimates direct LAI measurements of double-crop soybean, which are typically planted following small grain, have narrower row spacing, higher plant populations, and are subject to a shorter growing season than full-season soybean. Furthermore, with the exception of Hunt et al. (1999), research is limited in estimating LAI under defoliated conditions.

The LAI-2000 uses a fish-eye lens and optical filters to allow wavelengths of light up to 490 nm to reach five silicon detectors arranged in concentric rings, which measure sky brightness at different zenith angles to estimate LAI (Welles and Norman, 1991; Hicks and Lascano, 1995). The manufacturer changed the sensitivity of the detector from 400-490 nm to 360-460 nm in the late 1990's to improve resolution. The two detector types must produce similar LAI estimates if plant canopy analyzers are used interchangeably, and so that all documented measurements involving plant canopy analyzers are similar.

The plant canopy analyzer operating manual (LI-COR, Inc., 1992a) lists the following assumptions about foliage for LAI estimates: (1) there is minimal radiation reflectance and transmittance; (2) foliage is randomly distributed and oriented; and (3) a minimum distance is maintained between the foliage and the fish-eye lens. The manual recommends not taking readings in direct sunlight, although it may be acceptable if opaque masks are used and the portion of the canopy that is visible to the fish-eye lens is shaded. Readings taken in direct sunlight give decreased LAI estimates because of leaf reflectance and light transmittance (Hicks and Lascano, 1995). Welles and Norman (1991) found that LAI estimates in soybean plots exposed to direct sunlight were 11% lower than in shaded plots. Hicks and Lascano (1995) found that in high-plains Texas cotton, the best LAI estimates were in plots shaded with a patio umbrella, recorded at solar noon; good estimates were also possible when sky conditions were overcast, and plots were not artificially shaded. Therefore, foliage radiation reflectance or transmittance can be minimized by taking LAI estimates on cloudy days, or by shading the plot canopy. The assumption that foliage is randomly distributed and oriented is difficult to justify in row crops, but sampling techniques such as using opaque masks to restrict the view of the fish-eye lens, taking multiple below-canopy readings, and measuring along diagonal transects across rows can counteract this problem (LI-COR, Inc., 1992a). The assumption that foliage should not be too close to the fish-eye lens can also be achieved by placing the plant canopy analyzer in a location at least four times the leaf width away from the nearest leaf. Research plots should also be large enough so that the fish-eye lens does not detect objects outside of the plot, but should not be so large that they require too much maintenance or become too labor-intensive.

In a soybean thinning experiment, Welles and Norman (1991) found that the LAI-2000 estimate of LAI was within 2% of directly determined LAI (3.25 and 3.18, respectively). Over several cropping system experiments, they discovered that the LAI-2000 generally gave estimates within 15% of directly determined LAI. Hunt et al. (1999) reported that LAI-2000 estimates were statistically the same as direct LAI values ranging from about 2.5 to 5.0 in full-season soybean with 76 cm row spacing. However, the plant canopy analyzer overestimated LAI. This may be partly explained by the plant canopy

analyzer's inability to differentiate between leaves and other plant material such as stems, pods, and petioles. When direct measurements are taken, only the leaves are counted, whereas the plant canopy analyzer estimate counts all plant material as "leaf area." Welles and Norman (1991) suggested that the term "foliage area index," rather than LAI, might be a better description of what is measured with the plant canopy analyzer. In contrast to Hunt et al. (1999), Wilhelm et al. (2000) found that three different LAI meters underestimated LAI values for corn, even though stem and ear tissue was present among leaf tissue.

The objectives of these experiments were: (1) to determine the minimum plot size for LAI estimates in soybean defoliation experiments using the LAI-2000, (2) to determine the accuracy of LAI-2000 estimates by comparison to direct measurements of LAI in full-season and double-crop soybean, and (3) to compare LAI estimates among LAI-2000 detector types.

MATERIALS AND METHODS

Minimum plot size determination

The LAI-2000 may detect objects outside of small plots, which would affect the LAI estimate. This experiment evaluated the effect of undefoliated border plants on LAI estimates of completely defoliated soybean plots. Knowing the area that the plant canopy analyzer detects allows minimum plot size for accurate LAI estimates to be determined in defoliation experiments.

In 1998, four plots (six rows with 91 cm row centers by 6 m) were established in maturity group V soybean on 22 and 23 September at the Virginia Tech Tidewater Agricultural Research and Extension Center in Suffolk, Virginia. The center two rows by 2 m were marked with string placed on the ground. A 4 m by 2 m blue tarpaulin stretched onto a polyvinyl chloride rectangular frame was held at an angle to shade the plots from the sun when LAI estimates were taken. For accurate LAI estimates, it is important that the tarpaulin is held in a location where it is not seen by the fish-eye lens, but still shades the entire plot. LAI readings were taken according to the instruction manual for row crops (LI-COR, Inc., 1992a) between late morning and early afternoon. An opaque mask with a 45-degree opening was used to restrict the viewing area of the

fish-eye lens and blocked out the view of the operator. One above-canopy reading was taken immediately before and in the same direction as a set of four below-canopy readings along a diagonal transect at 0, 25, 50, and 75% of the distance across the row, with each reading moving about 10 cm forward (Fig. 2.1). LAI readings were taken from all four sides of the plot, for a total of sixteen below-canopy readings per plot. This is twice the number of readings recommended by Hicks and Lascano (1995) for a crop with a heterogeneous canopy, and four times the number recommended for a crop with a homogenous canopy. To determine that the tarpaulin was not distorting the readings, a 2000-90 software package (LI-COR, Inc., 1992b) was used to delete readings from selected plots where the tarpaulin could have been detected by the plant canopy analyzer (e.g., those readings where the plant canopy analyzer was directed towards the tarpaulin). LAI values were re-computed, and standard errors showed that these LAI estimates were not different from the original LAI estimates where readings from all directions were combined (data not shown). To accomplish this, data were downloaded from the LAI-2000 in the “standard” rather than the “spreadsheet” format.

The following plot areas were sequentially 100% hand-defoliated, beginning with the smallest area in the center and working outwards until the largest area was completed: two rows by 2 m, four rows by 2 m, four rows by 2 m plus an additional 1 m at the ends of the two middle rows, four rows by 4 m, six rows by 4 m, and six rows by 6 m (Fig. 2.1 A-F). LAI estimates in these completely defoliated plots were taken before the plots were expanded to the next larger size. The point at which LAI estimates became similar for the expanding plot sizes was determined to be the minimum plot size necessary to achieve an adequate representation of LAI. The effect of defoliated plot size on LAI was analyzed using PROC GLM (SAS Institute, 1992). Means were separated using the Ryan-Einot-Gabriel-Welsch multiple comparison test (REGWQ) (SAS Institute, 1992).

Plant canopy analyzer validation

Field experiments at the Virginia Tech Tidewater Agricultural Research and Extension Center were done from 1998 to 2000 to determine the accuracy of the LAI-2000 in estimating soybean LAI compared to direct LAI estimates. In September 1998, four plots were established in double-crop, maturity group IV soybean (developmental

stage = late R6 [full seed]). Row spacing was 46 cm, and plot size was four rows by 2 m. A series of one above and four below-canopy LAI readings were taken from each side of the plot with the plant canopy analyzer (Serial number PCH 1186) for a total of sixteen below-canopy readings per plot. Below canopy readings followed diagonal transects spaced 0, 25, 50, and 75% of the distance across the row, with each reading moving about 10 cm forward (Fig. 2.2 A). An opaque mask with a 45-degree opening was used to restrict the view of the fish-eye lens. LAI readings were taken in the late morning or early afternoon, using a blue tarpaulin to shade the plots from direct sunlight. After the LAI was estimated, all leaflets were 100% defoliated by hand. Leaflets were placed in large brown paper bags and transported to the laboratory for direct leaf area determination with a leaf area meter (Model LI-3000A, LI-COR, Inc., Lincoln, NE). Direct LAI values were determined by dividing total leaf area measured by ground area.

In 1999, methods were the same as in 1998, except that full-season soybean plots at developmental stage R5 (beginning seed) were sequentially defoliated at levels of 0, 33, 66, and 100% by removing zero, one, two, or three leaflets of each trifoliolate, with LAI estimates taken by two researchers (each using separate plant canopy analyzers) after each defoliation. The blue tarpaulin was again used to shade the plots. LAI readings were taken from 0630 to 0830 h EST on 10 August. Leaflets were placed in separate bags for each defoliation level. LI-COR, Inc. recently introduced a new detector for the LAI-2000 that responds to a wider range of blue wavelength light than the previous detector. Therefore, LAI estimates were determined with the “older” plant canopy analyzer used in 1998 (PCH 1186), which will now be referred to as the “narrow-blue detector,” having spectral sensitivity from 400 to 490 nm, and also with the new “wide-blue detector” plant canopy analyzer (PCH 1326), having spectral sensitivity from 360 to 460 nm (Fig. 2.3). To make plots larger and less likely for the plant canopy analyzer to detect undefoliated plants exterior to the plots, border rows and 50 cm at the ends of the plots were defoliated to levels visually similar to corresponding plots, but these leaflets were not included in direct LAI determination. Leaf area was determined using a calibrated leaf area meter (Model LI-3100, LI-COR, Inc., Lincoln, NE).

In 2000, seven plots were established in maturity group III soybean using planting

dates that represented both full-season and double-crop systems (three plots represented full-season and four plots represented double-crop systems). Plots were sequentially defoliated at levels of 0, 33, 66, and 100% over four days. The LAI estimates were taken at the same time each evening to ensure that the sun was in the same position for each measurement. All measurements with the plant canopy analyzer were taken by the same individual. The placement of the fish-eye lens for below canopy readings changed since plots in 2000 consisted of five 38 cm rows instead of four 46 cm rows (Fig. 2.2 B). The soybean fields were approximately 50 m away from a hardwood forest, and the trees provided shade for LAI estimates. Therefore, the blue tarpaulin was not used in the 2000 experiments. Direct leaf area was determined as in 1999. LAI measurements in the full-season soybean test began on 7 August (developmental stage = late R3 [beginning pod]). LAI estimates were taken in the evening after the sun had dropped below the tree line; light scattering is reduced when measured and adjacent plots are completely shaded. On 8 August, one leaflet from each trifoliolate was removed by hand; leaflets were saved, and their area was determined using a calibrated LI-3100 leaf area meter. On that evening, LAI estimates were taken once the sun had dropped below the tree line. This process was repeated on 9 August for removal and determination of leaf area for the second of the trifoliolate leaflets, and again on 10 August for the third of the trifoliolate leaflets. Thus, plants were 100% defoliated by 10 August (only stems, small pods, and petioles remained). Treatment establishment of the 2000 season double-crop test began on 15 August (developmental stage = R3), and methods were the same as in the 2000 full-season test. An undefoliated check plot showed that LAI values obtained from the LAI-2000 (PCH 1326) did not change during the course of this experiment (LAI = 5.26 and 5.24 at the beginning and end of the experiment, respectively).

LAI-2000 estimates and direct measurements of LAI were compared using paired t-tests ($\alpha = 0.05$) since samples (plots) were not independent. Paired t-tests calculate the difference between the two methods of LAI determination for each plot and hence cancel out the plot-to-plot variability.

Comparison of plant canopy analyzers

Field experiments at the Virginia Tech Tidewater Agricultural Research and

Extension Center were done in 1999 and 2000 to determine whether narrow and wide-blue detectors in the LAI-2000 gave similar estimates of LAI. LI-COR, Inc., provided information on the spectral responses of the two detector types (Fig. 2.3). In 1999, the narrow and wide-blue detector plant canopy analyzers were compared by taking consecutive readings in 28 soybean plots with 46 cm row spacing. Four above and sixteen below canopy readings were taken per plot. An opaque mask with a 45-degree opening was used to restrict the view of the fish-eye lens. Care was taken to place the fish-eye lens in the same locations for each plot. Readings were taken on an overcast day (9 August, 0800-1330 h EST) by the same operator, and no tarpaulin was used.

Between the 1999 and 2000 seasons, the plant canopy analyzer with the wide-blue detector was returned to the manufacturer and its wide-blue detector was replaced with a narrow-blue detector, giving the researchers two narrow-blue detector plant canopy analyzers. A third narrow-blue detector plant canopy analyzer was borrowed from the University of Nebraska's Department of Entomology. LI-COR, Inc. provided the researchers a wide-blue detector LAI-2000 for use in the meter comparison study in 2000.

In 2000, LAI estimates were taken (as described above) with a narrow-blue and a wide-blue detector plant canopy analyzer on 6 September in ten plots of soybean with 38 cm row spacing. Additionally, LAI estimates were taken with three narrow-blue and one wide-blue detector plant canopy analyzers on 26 September in ten plots. One operator took all readings on completely overcast days. No tarpaulin was used in these experiments. The researcher tried to place the fish-eye lens in the same location for each below-canopy reading. In both years, measurements from different plant canopy analyzers were compared using paired t-tests.

RESULTS AND DISCUSSION

Minimum plot size determination

The manufacturer of the LAI-2000 recommends that its fish-eye lens should be at least three times the crop height away from any edge for below-canopy readings (LI-COR, Inc., 1992a). The mean height of the soybean plots was 81 cm \pm 1.5, so according to the manufacturer's recommendations, this would require the placement of the fish-eye lens a distance of 2.43 m from any edge. This condition was not met by the smaller plots

defoliated in this experiment (two rows by 2 m, four rows by 2 m), and the fish-eye lens detected more plant material outside of these plots than any other plots (Table 2.1). Assuming that the largest plot size (six rows by 6 m) had the most accurate LAI estimate, the smallest plot size statistically equal to the largest plot is the four rows by 2 m with an additional 1 m at the ends of the two middle rows also defoliated. This plot had a shape resembling a circle rather than a rectangle (Fig. 2.1 C), which is more likely the shape perceived by the fish-eye lens; this size provides a good balance between accuracy of the LAI estimate and time and labor constraints of small plot defoliation research. The larger plots may provide a slightly more accurate LAI estimate than smaller plots, but they may be too labor-intensive for most experiments. Although only soybean with 91 cm row centers were tested, Hunt et al. (1999) mentioned that row width or plant population should not affect plant canopy analyzer accuracy, due to the physics of the LAI measurement.

Plant canopy analyzer validation

1998 Experiment

In the four plots studied in 1998, there was no difference between the narrow-blue detector plant canopy analyzer estimate (2.70 ± 0.42) and direct LAI (2.50 ± 0.48) ($P = 0.24$).

1999 Experiment

One of the four soybean plots had to be dropped from the analysis after the initial LAI estimate because of changing sky conditions as that plot was being defoliated; the sun became too high to allow the tarpaulin to remain in the same position for subsequent LAI estimates. There was no significant difference between the narrow-blue detector plant canopy analyzer estimate and directly determined LAI at defoliation levels of 0 and 33%. Direct LAI values ranged from 3.0 to 5.4 for these levels. The narrow-blue detector plant canopy analyzer LAI estimate was marginally significantly higher than the directly determined LAI at 66% defoliation ($P = 0.075$), and was significantly higher at the 100% defoliation level ($P = 0.0004$) (Table 2.2). This overestimate of LAI is due to the plant canopy analyzer detecting more pod, petiole, and stem tissue at higher defoliation levels. These results were similar to those of Hunt et al. (1999). The wide-

blue detector plant canopy analyzer LAI estimates were significantly higher than directly determined LAI at 0, 66, and 100% defoliation levels (Table 2.2). With the wide-blue detector plant canopy analyzer, the LAI estimates at the 33% defoliation level were the same as directly determined LAI values ($P = 0.178$) (Table 2.2). Although the two researchers attempted to use the same technique in taking LAI estimates, some experimental error may have partly contributed to the difference between plant canopy analyzers this year.

2000 Experiment

The sun angle changed between LAI estimates in 1999 due to the time involved in defoliating the plots. Sun angle affects the amount of sunlit foliage detected by the plant canopy analyzer (LI-COR, Inc., 1992a). In 2000, techniques were changed so that the sun would be at approximately the same angle on each day when LAI estimates were taken. A disadvantage of this technique is that different sky conditions exist for each day, thus reducing uniformity between readings. However, this method gives researchers an entire day to defoliate the plots, while previously the plots had to be defoliated as rapidly as possible to minimize the effect of changing sun angle. At a significance level of $\alpha = 0.05$, all plant canopy analyzer estimates in both the full-season and double-crop tests in 2000 were significantly higher than direct LAI values, at all levels of defoliation, except for the 33% defoliation level in the double-crop test (Tables 2.3 and 2.4).

In a defoliated crop, the LAI estimate is expected to be slightly greater than the direct LAI, because of the increased proportion of pods, stems, and petioles seen by the fish-eye lens (Hunt et al., 1999). This non-leaf plant tissue intercepts a significant amount of radiation (Lang, 1991). A phenomenon that occurred in the full-season test in 2000 was that subtraction of the estimated remaining pod, stem, and petiole area (about 0.80 to 0.82) from the estimated LAI at 0, 33, and 66% defoliation resulted in estimates much closer to the directly determined LAI values in Table 2.3. Similar results occurred in the double-crop test in 2000 when 0.96 to 0.98 was subtracted from the LAI estimate for the 0% defoliation level (Table 2.4). An attempt was made to determine stem and pod area in the 100% defoliated plots using a LI-3100 leaf area meter, but this was unsuccessful as the instrument was designed to determine areas of two-dimensional, i.e.,

flat objects. While such manipulation of the 2000 data gives more accurate LAI estimates, similar treatment of the 1998 and 1999 data results in an underestimate of LAI. Therefore, it should not be assumed that the LAI-2000 does not adjust for stems and petioles.

After observing LAI estimate differences between 1998-1999 and the full-season 2000 experiments, the effect of shading technique on LAI estimates was examined. At 0800 h EST on 16 August 2000, with no clouds in the sky, LAI estimates were taken with the narrow-blue detector plant canopy analyzer in the four double-crop soybean plots with a blue tarpaulin shading the plots, to determine if tarpaulin-shaded LAI estimates were the same as tree-shaded LAI estimates. The LAI estimates were compared using paired t-tests. In these comparisons, light scattering from sunlit areas bordering the shaded plot should be greater when using the tarpaulin, therefore LAI should decrease. However, the tarpaulin-shaded LAI estimates were greater than the tree-shaded LAI estimates taken the previous evening ($P = 0.011$). Tarpaulin-shaded plots had a mean LAI of 5.11 ± 0.125 , while tree-shaded plots had a mean LAI of 4.82 ± 0.145 . Therefore, the tarpaulin-shaded LAI estimates were even further from the direct LAI values (3.96 ± 0.291) than the tree-shaded estimates. This is in contrast to Welles and Norman (1991), who observed decreased LAI measurements under direct sunlight where light scattering is greatest. It is possible that different sky conditions between morning and evening readings caused the discrepancy. Regardless, it was assumed that the difference between performances of the plant canopy analyzers between 1998-1999 and 2000 was not due to the elimination of the tarpaulin.

Comparison of plant canopy analyzers

1999 Experiment

The wide-blue detector plant canopy analyzer gave significantly higher LAI readings than the narrow-blue detector plant canopy analyzer (paired t-tests, $P < 0.001$) (Fig. 2.4). Inspection of the data indicated that the estimates were consistently different at LAI levels below 5.0, but they became more erratic above this level. This creates a problem if the narrow and wide-blue detector plant canopy analyzers are used interchangeably.

2000 Experiment

On 6 September, a marginally significant difference ($P = 0.054$) between the narrow-blue (PCH 1326) and wide-blue (PCH 0001) detector plant canopy analyzers was observed (Fig. 2.5); the wide-blue detector plant canopy analyzer gave higher LAI estimates. On 26 September, there were no differences ($\alpha = 0.05$) between the narrow-blue detector plant canopy analyzers (PCH 0095, PCH 1186, and PCH 1326). Two of the three narrow-blue detector plant canopy analyzers (PCH 1186 and PCH 1326) had significantly lower estimates of LAI than the wide-blue detector plant canopy analyzer (PCH 0001); the other narrow-blue detector plant canopy analyzer (PCH 0095) had LAI estimates marginally significantly lower than the wide-blue detector plant canopy analyzer (Table 2.5 and Fig. 2.6).

Although differences between the narrow and wide-blue detector plant canopy analyzers are statistically significant, these differences may or may not be biologically significant, depending on the accuracy required by the user. From Figs. 2.5 and 2.6, all plant canopy analyzers had LAI estimates at values from approximately 2.0 to 6.0 in 2000, and the wide-blue detector plant canopy analyzer LAI estimates were generally only 2% higher than the estimates from the narrow-blue detector plant canopy analyzers this year. This represents an average overestimation of less than 0.1 LAI, a difference that is biologically meaningless. But in 1999, the average wide-blue detector plant canopy analyzer estimate was 9% greater than the narrow-blue detector plant canopy analyzer estimate from 2.5 to 6.0 LAI, representing an average difference of 0.4 LAI. These data show that plant canopy analyzers with either detector type may be used to track LAI progression during a season. However, the difference may cause problems if more accurate LAI measurements are required.

The reason the two detector types performed differently may be partly explained by the wavelength of light they respond to and the scattering of light within the canopy. Chlorophyll reflects green light, thus leaflets absorb little green light. Where chlorophyll levels are high, as in a dense canopy, there is more scattering of green light. LI-COR, Inc. described the differences between the narrow and wide-blue detector plant canopy analyzers (Jon Welles, LI-COR, Inc., personal communication, 1999). The narrow-blue

detector spectral sensitivity is 400 to 490 nm, with a sharp peak at about 480 nm (Fig. 2.3). There is slightly less absorption of light in the long blue than in the short blue light spectrum (long blue light is closer to green light than short blue light), thus more light scattering. More light scattering means that the below-canopy reading is increased (the plant canopy analyzer senses that more light is coming through the canopy), resulting in a reduced LAI estimate. The wide-blue detector's spectral sensitivity is about 360 to 460 nm, with a wide peak range, the maximum occurring at 430 nm (Fig. 2.3). There is slightly more light absorption in this short blue light, thus less light scattering, and higher LAI estimates. Advantages of the new wide-blue detector plant canopy analyzer include greater resolution and the ability to determine LAI values up to 10 (the narrow-blue detector plant canopy analyzer could determine LAI values up to about 7) (Jon Welles, LI-COR, Inc., personal communication, 1999). The first LAI-2000 serial number to use the wide-blue detector is PCH-1194 (Jon Welles, LI-COR, Inc., personal communication, 2000); however, this should only be considered a guide, as replacement with a wide-blue detector could occur if a narrow-blue detector requires repair.

CONCLUSION

Soybean LAI estimates for defoliation experiments should be taken in plots no smaller than 4 rows by 2 m with an additional 1 m at the ends of the two middle rows also defoliated, regardless of row spacing. In plant canopy analyzer validation experiments, results varied by year and by detector type of the plant canopy analyzer (narrow vs. wide-blue detector). Narrow-blue detector plant canopy analyzer estimates of LAI were statistically the same (but numerically higher) as direct values from approximately 2.0 to 5.0 LAI in 1998 and 1999. Accurate estimates of LAI were unachievable in 2000 with either detector type, with the exception of the 33% defoliation level estimates in the double-crop test. Soybean defoliated below an LAI of 2.0 was likely skewed by a greater proportion of pods, stems, and petioles, causing significantly higher LAI estimates than direct LAI values. Caution must be taken if narrow and wide-blue detector plant canopy analyzers are used interchangeably in research, due to the tendency of the wide-blue detector plant canopy analyzer to read significantly higher than the narrow-blue detector plant canopy analyzer. But, these differences were small and probably biologically

insignificant.

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Table 2.1. Mean defoliated LAI estimates using a narrow-blue detector plant canopy analyzer (PCH 1186) in four soybean plots expanding in size from two rows (91 cm row centers) by 2 m to six rows by 6 m on 22-23 September 1998. Pods, stems, and petioles remained after each plot was 100% defoliated.

Plot size defoliated	Estimated mean LAI \pm SD
2 rows x 2 m	0.94 a [†] \pm 0.17
4 rows x 2 m	0.74 b \pm 0.13
4 rows x 2 m plus 1 m at ends of middle rows	0.67 bc \pm 0.14
4 rows x 4 m	0.65 bc \pm 0.15
6 rows x 4 m	0.62 c \pm 0.14
6 rows x 6 m	0.60 c \pm 0.11

[†] Means within a column followed by the same letter are not significantly different (Ryan-Einot-Gabriel-Welsch Multiple Comparison Test, $P > 0.05$).

Table 2.2. 1999 mean estimates of soybean LAI (at 0, 33, 66, and 100% plant defoliation levels) using the narrow-blue (PCH 1186) and wide-blue (PCH 1326) detector plant canopy analyzers, compared to direct mean LAI values using paired t-tests.

% defol	N	Narrow-blue (PCH 1186)		Wide-blue (PCH 1326)		Direct Mean LAI \pm SD
		Estimated mean LAI \pm SD	P [†]	Estimated mean LAI \pm SD	P [§]	
0	4	4.89 \pm 0.53	0.450	6.09 \pm 0.69	0.004	4.82 \pm 0.42
33	3	3.28 \pm 0.38	1.000	3.15 \pm 0.26	0.178	3.28 \pm 0.23
66	3	2.33 \pm 0.29	0.075	2.31 \pm 0.32	0.043	2.00 \pm 0.25
100	3	0.89 \pm 0.03	0.0004	1.02 \pm 0.04	0.0006	0.00 \pm 0.00

[†] P-value is from comparison of narrow-blue estimate of LAI and direct LAI values.

[§] P-value is from comparison of wide-blue estimate of LAI and direct LAI values.

Table 2.3. 2000 mean estimates of LAI in full-season soybean (at 0, 33, 66, and 100% plant defoliation levels) using two narrow-blue detector plant canopy analyzers (PCH 1326 and PCH 1186), and one wide-blue detector plant canopy analyzer (PCH 0001), compared to direct mean LAI values using paired t-tests.

% defol	N	Narrow-blue (PCH 1186)		Narrow-blue (PCH 1326)		Wide-blue (PCH 0001)		Direct Mean LAI \pm SD
		Estimated mean LAI \pm SD	P [†]	Estimated mean LAI \pm SD	P [§]	Estimated mean LAI \pm SD	P [‡]	
0	3	3.84 \pm 0.295	0.011	4.20 \pm 0.439	0.002	4.24 \pm 0.471	0.002	3.00 \pm 0.405
33	3	2.82 \pm 0.348	0.004	2.98 \pm 0.430	0.003	2.95 \pm 0.356	0.002	2.13 \pm 0.353
66	3	1.91 \pm 0.292	0.003	1.85 \pm 0.318	0.007	1.92 \pm 0.318	0.005	1.10 \pm 0.217
100	3	0.82 \pm 0.098	0.005	0.80 \pm 0.065	0.002	0.80 \pm 0.076	0.003	0.00 \pm 0.000

[†] P-value is from comparison of narrow-blue (PCH 1186) estimate of LAI and direct LAI values.

[§] P-value is from comparison of narrow-blue (PCH 1326) estimate of LAI and direct LAI values.

[‡] P-value is from comparison of wide-blue (PCH 0001) estimate of LAI and direct LAI values.

Table 2.4. 2000 mean estimates of LAI in double-crop soybean (at 0, 33, 66, and 100% plant defoliation levels) using one narrow-blue detector plant canopy analyzer (PCH 1326), and one wide-blue detector plant canopy analyzer (PCH 0001), compared to direct mean LAI values using paired t-tests.

% defol	N	Narrow-blue (PCH 1326)		Wide-blue (PCH 0001)		Direct Mean LAI \pm SD
		Estimated mean LAI \pm SD	P†	Estimated mean LAI \pm SD	P§	
0	4	4.82 \pm 0.145	0.006	4.91 \pm 0.096	0.009	3.96 \pm 0.291
33	4	3.13 \pm 0.221	0.085	3.06 \pm 0.209	0.144	2.86 \pm 0.305
66	4	2.16 \pm 0.197	0.049	2.16 \pm 0.176	0.039	1.56 \pm 0.226
100	4	0.96 \pm 0.074	0.0001	0.98 \pm 0.076	0.0001	0.00 \pm 0.000

† P-value is from comparison of narrow-blue estimate of LAI and direct LAI values.

§ P-value is from comparison of wide-blue estimate of LAI and direct LAI values.

Table 2.5. Comparison of LAI estimates from four plant canopy analyzers on 26 September 2000 in ten soybean plots.

Plant canopy analyzers compared†	P§
PCH 1326 and PCH 1186	0.915
PCH 1326 and PCH 0095	0.375
PCH 1326 and PCH 0001	0.016
PCH 1186 and PCH 0095	0.519
PCH 1186 and PCH 0001	0.009
PCH 0095 and PCH 0001	0.075

† Narrow-blue detector plant canopy analyzers include PCH 1326, PCH 1186, and PCH 0095; the wide blue-detector plant canopy analyzer was PCH 0001.

§ Paired t-tests were used to determine differences between pairs.

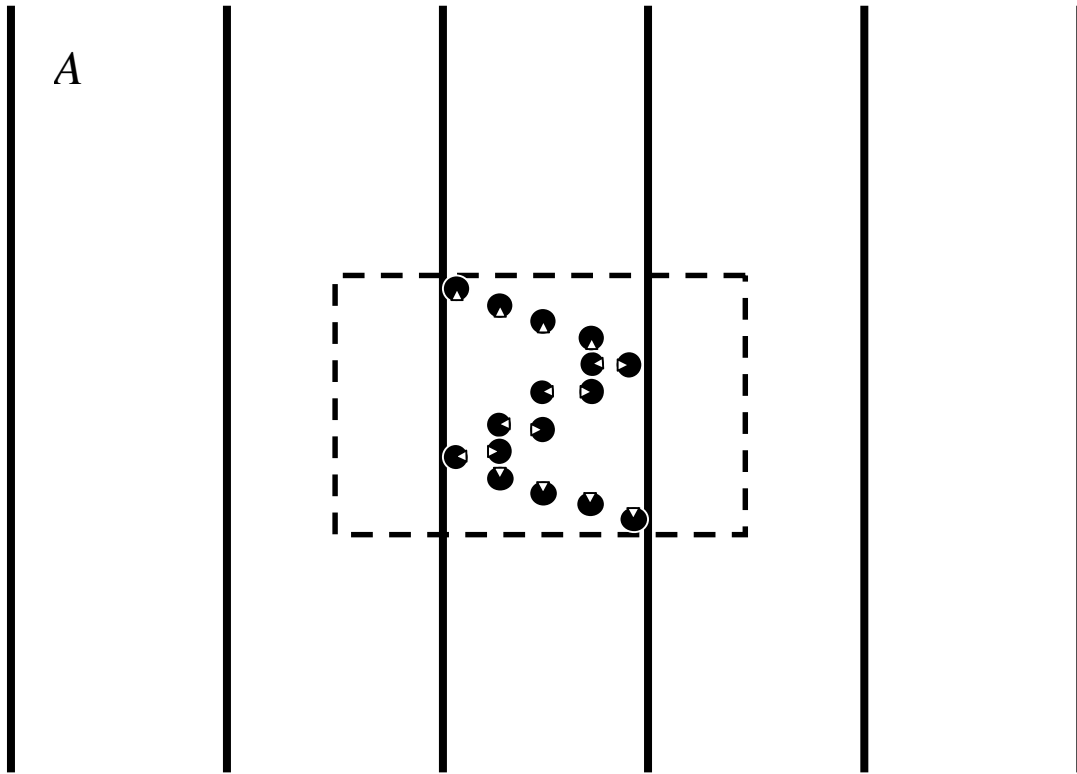


Fig. 2.1-A. Placement of LAI-2000 fish-eye lens (with 315-degree restricting viewcap) for below-canopy measurements in soybean plots with 100% defoliation of two rows by 2 m. All plants within the dotted lines were 100% defoliated; solid vertical lines represent 91 cm row centers.

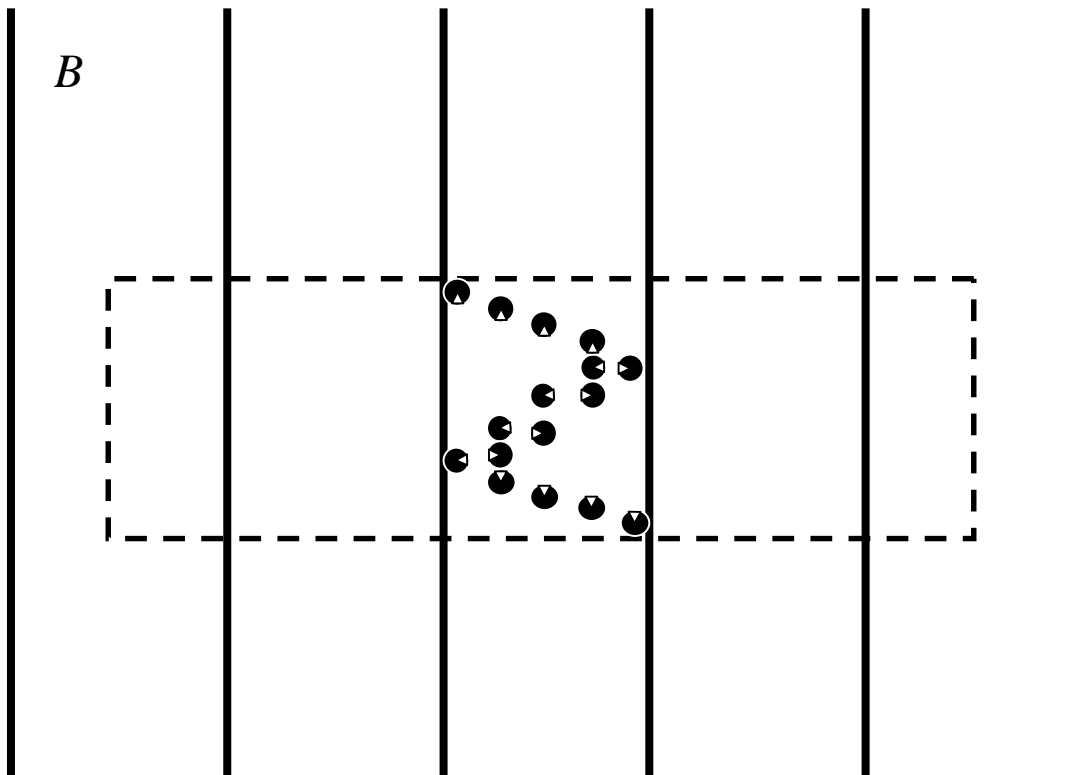


Fig. 2.1-B. Placement of LAI-2000 fish-eye lens (with 315-degree restricting viewcap) for below-canopy measurements in soybean plots with 100% defoliation of four rows by 2 m. All plants within the dotted lines were 100% defoliated; solid vertical lines represent 91 cm row centers.

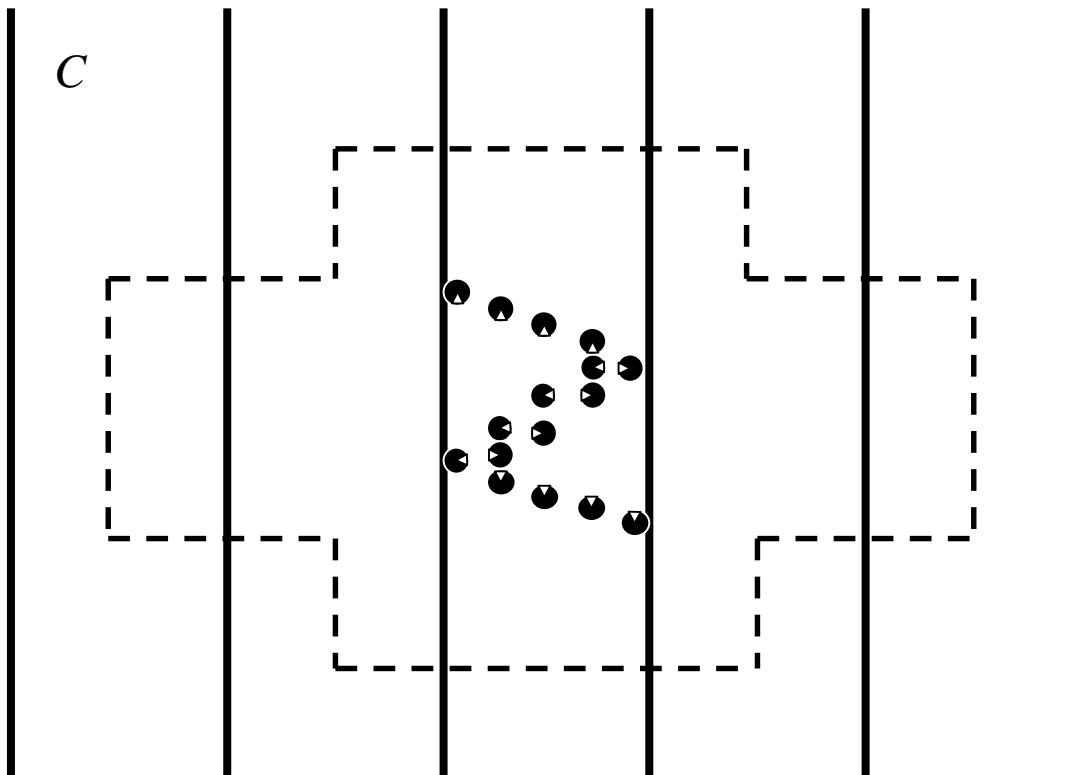


Fig. 2.1-C. Placement of LAI-2000 fish-eye lens (with 315-degree restricting viewcap) for below-canopy measurements in soybean plots with 100% defoliation of four rows by 2 m plus an additional 1 m at the ends of the two middle rows. All plants within the dotted lines were 100% defoliated; solid vertical lines represent 91 cm row centers.

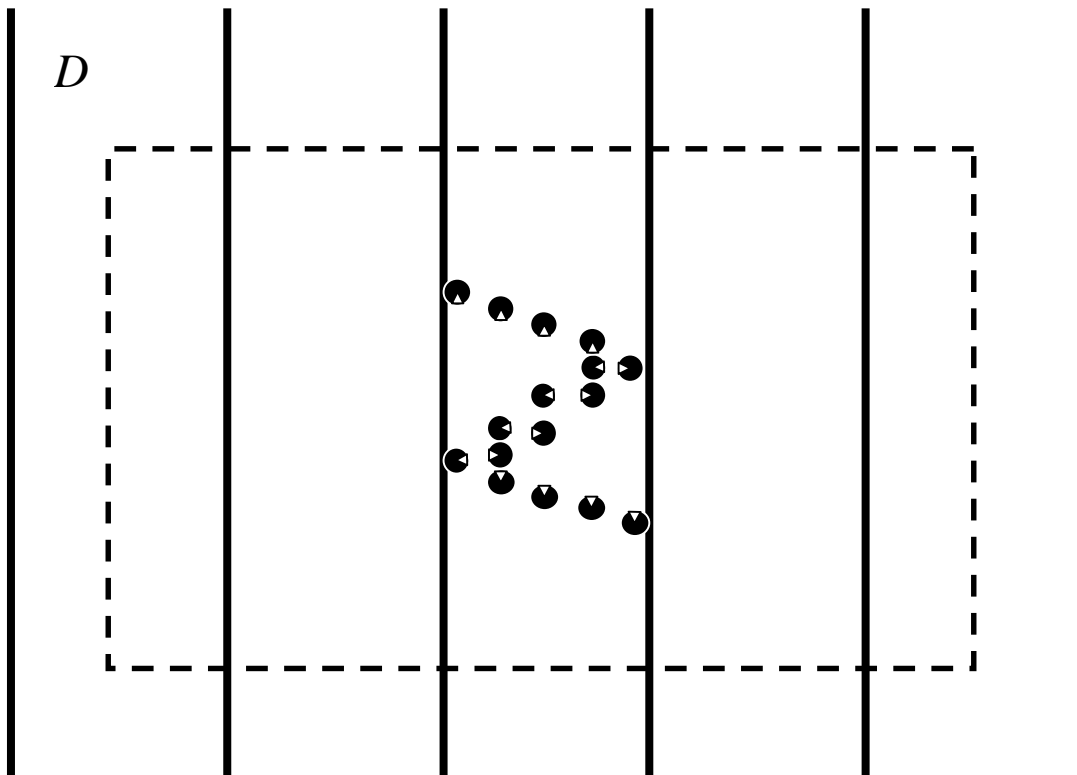


Fig. 2.1-D. Placement of LAI-2000 fish-eye lens (with 315-degree restricting viewcap) for below-canopy measurements in soybean plots with 100% defoliation of four rows by 4 m. All plants within the dotted lines were 100% defoliated; solid vertical lines represent 91 cm row centers.

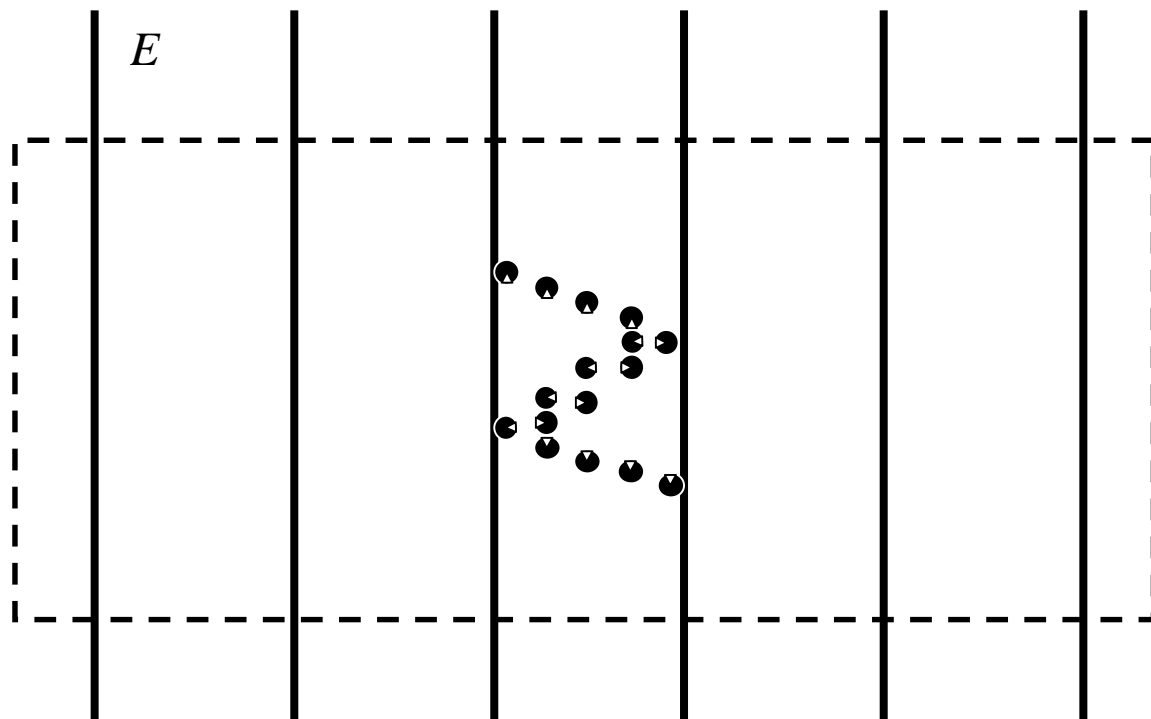


Fig. 2.1-E. Placement of LAI-2000 fish-eye lens (with 315-degree restricting viewcap) for below-canopy measurements in soybean plots with 100% defoliation of six rows by 4 m. All plants within the dotted lines were 100% defoliated; solid vertical lines represent 91 cm row centers.

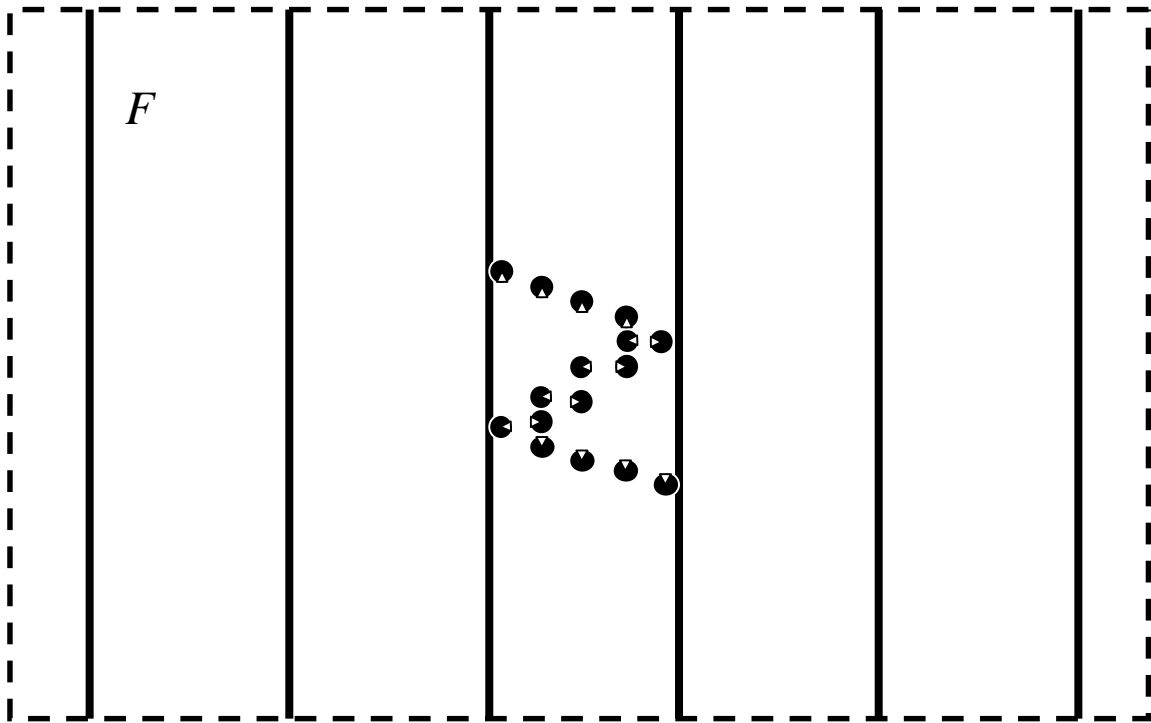


Fig. 2.1-F. Placement of LAI-2000 fish-eye lens (with 315-degree restricting viewcap) for below-canopy measurements in soybean plots with 100% defoliation of six rows by 6 m. All plants within the dotted lines were 100% defoliated; solid vertical lines represent 91 cm row centers.

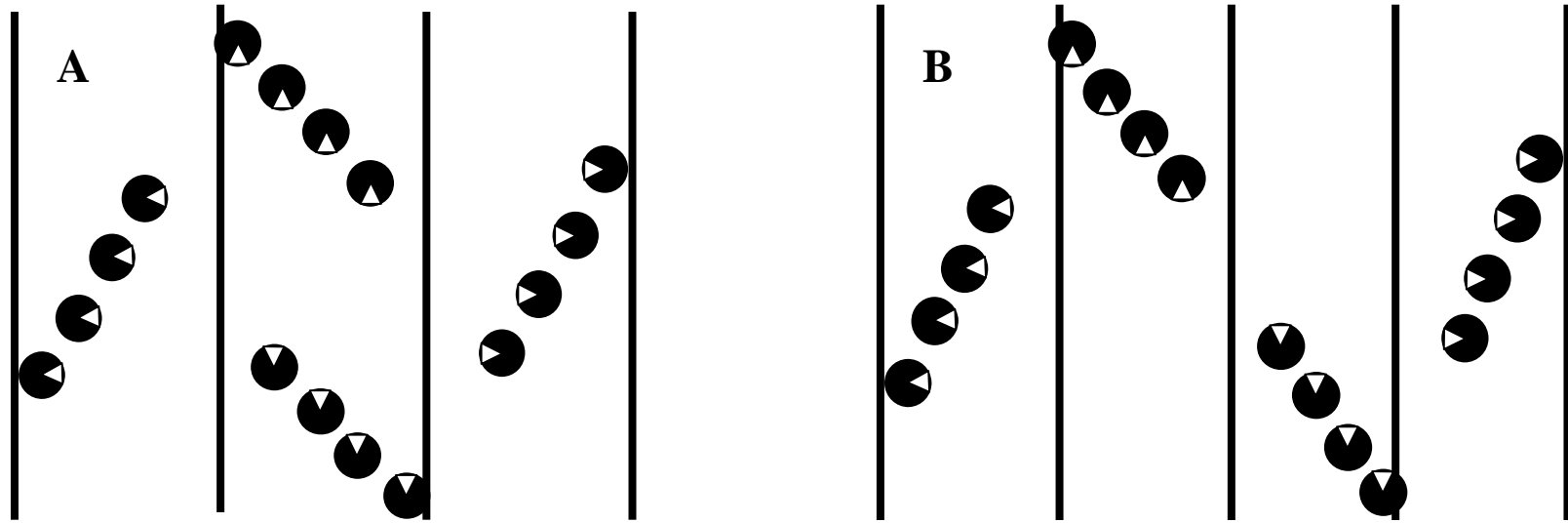


Fig. 2.2. Placement of LAI-2000 fish-eye lens (with 315-degree restricting viewcap, indicated by the black portion of the circle) for below-canopy measurements in soybean plots with (A) four rows with 46 cm row centers, and (B) five rows with 38 cm row centers. Vertical lines represent row centers.

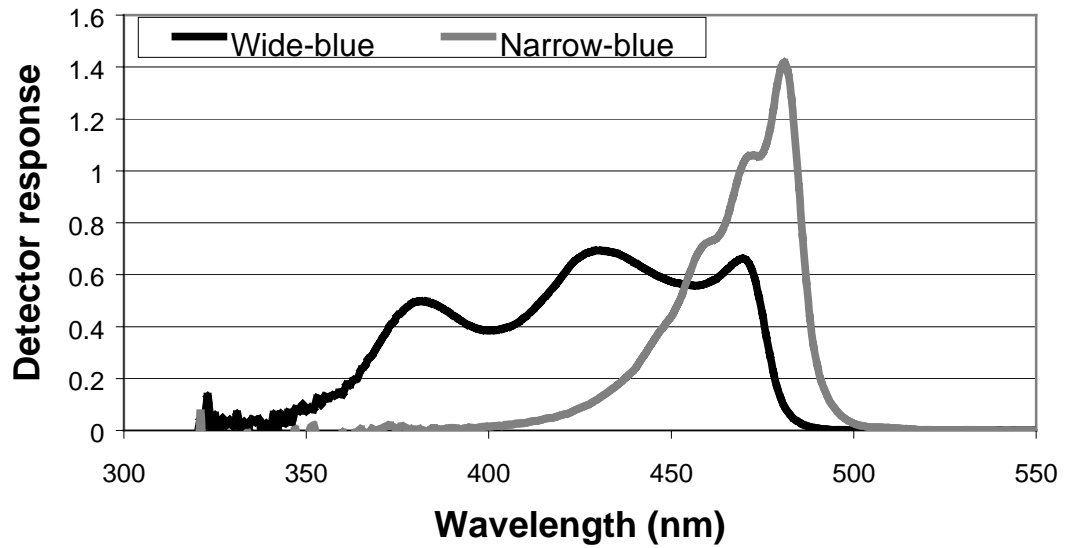


Fig. 2.3. Spectral sensitivities of the LAI-2000 narrow and wide-blue detectors (Source: LI-COR, Inc., Lincoln, NE).

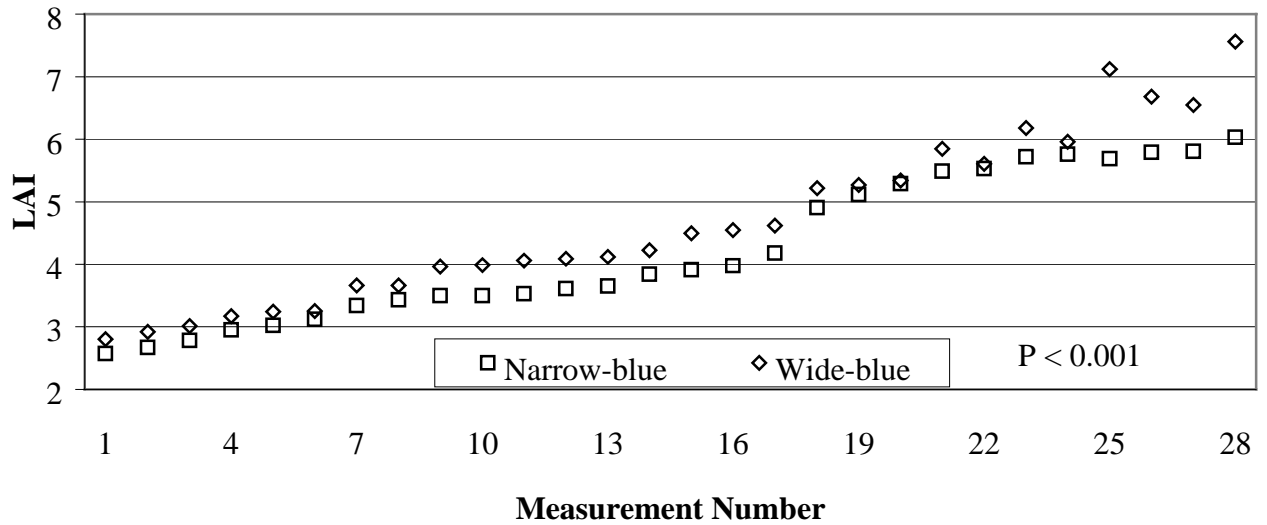


Fig. 2.4. Leaf area index (LAI) estimates using both narrow-blue detector (PCH 1186) and wide-blue detector (PCH 1326) plant canopy analyzers in 28 soybean plots in 1999. Estimates were compared using paired t-tests.

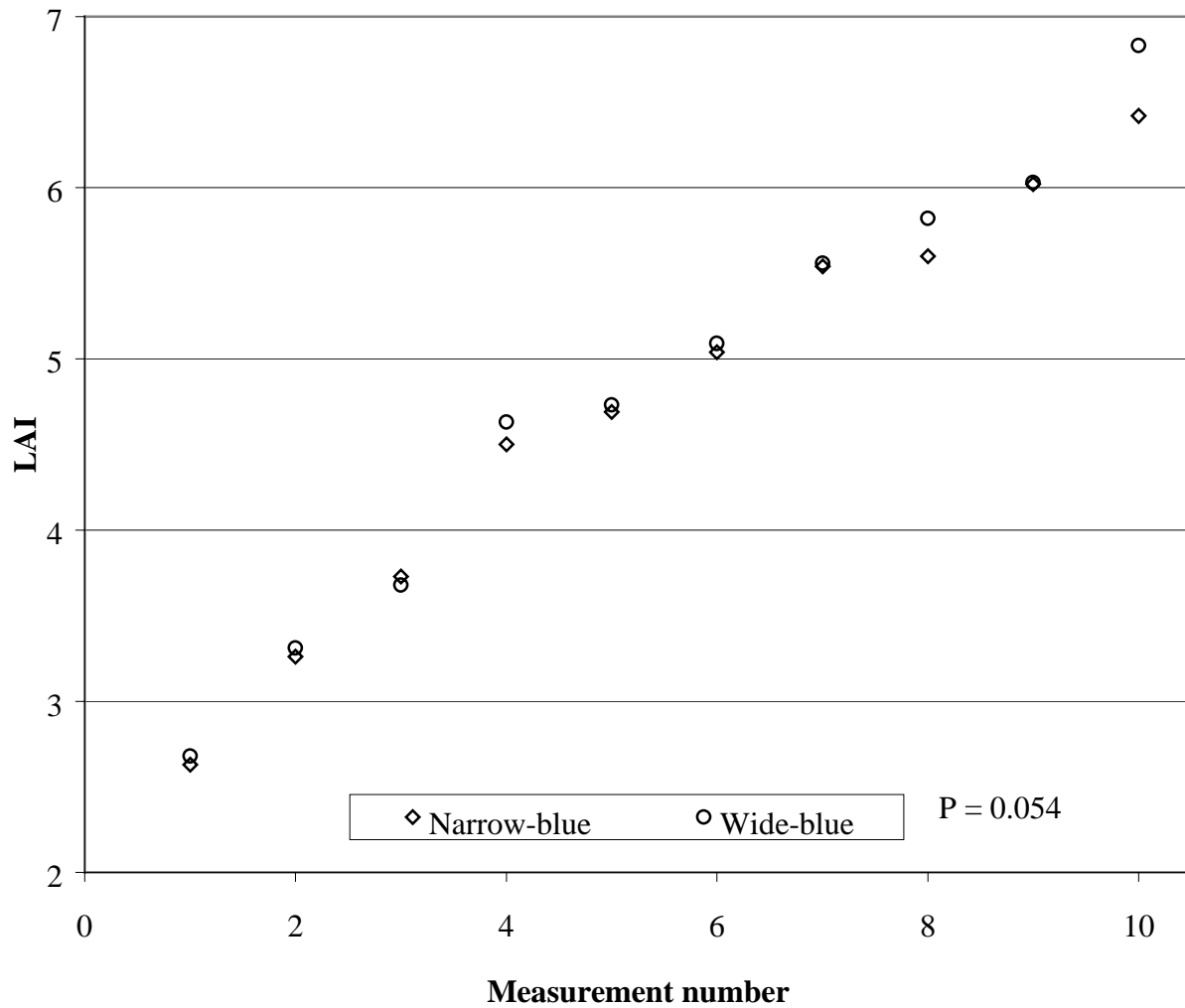


Fig. 2.5. Leaf area index (LAI) estimates using both narrow-blue detector (PCH 1326) and wide-blue detector (PCH 0001) plant canopy analyzers in 10 soybean plots on 6 September 2000. Estimates were compared using paired t-tests.

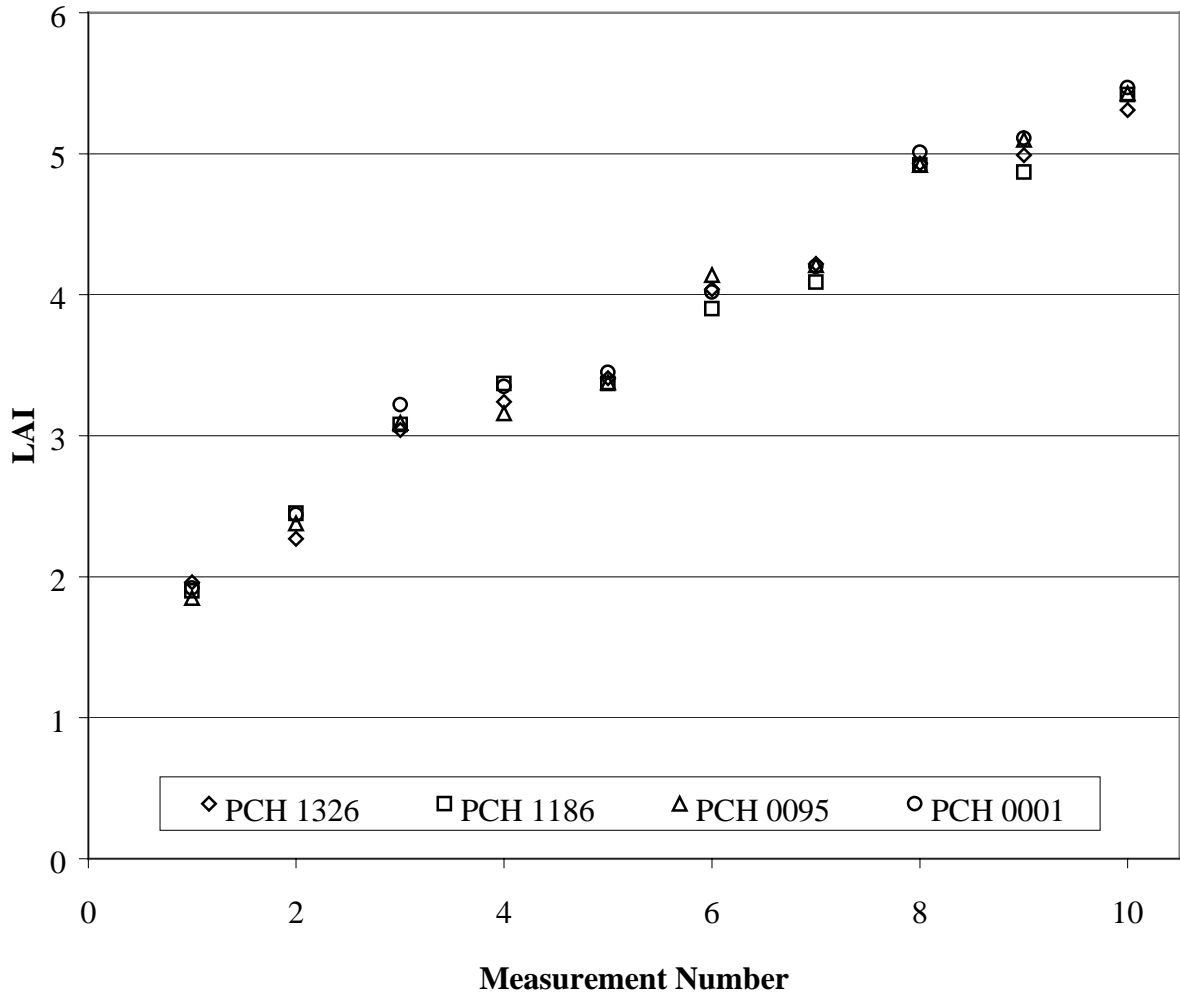


Fig. 2.6. Leaf area index (LAI) estimates using three narrow-blue detector plant canopy analyzers (PCH 1326, PCH 1186, and PCH 0095) and one wide-blue detector plant canopy analyzer (PCH 0001) in 10 soybean plots on 26 September 2000. Measurements were compared using paired t-tests; P-values for these comparisons are given in Table 2.5.

Chapter 3.

Effects of Mechanical and Insect Defoliation on Soybean Leaf Area Index and Yield

ABSTRACT

Previous research indicates a strong correlation between leaf area index (LAI) and yield of full-season soybean. LAI values of at least 3.5 to 4.0 in the reproductive stages are required for maximum potential yield. However, it is unknown how double-crop soybean yields respond to LAI. This study used linear and non-linear models to describe the response of full-season and double-crop soybean yields to reductions in LAI through mechanical defoliation, and evaluated the response of double-crop soybean yields to reductions in LAI through insect defoliation. Results of the mechanical defoliation experiment indicate that there is a significant linear decrease in yield in both full-season and double-crop soybean when LAI values are below 3.5 to 4.0 by developmental stages R4 to R5, while yields usually plateau at higher LAI levels. There was an average yield reduction of $846.7 \pm 282.2 \text{ kg ha}^{-1}$ (range = 538 to 1344 kg ha^{-1}) for each unit decrease in LAI below the critical 3.5 to 4.0 level. Insect defoliators did not defoliate double-crop soybean plots to levels that reduced yield; no plots had LAI levels below 4.0 by the developmental stage when LAI estimates were taken (R3 to R6). Therefore, double-crop soybean that maintains LAI values above the 3.5 to 4.0 critical level by mid-reproductive developmental stages can tolerate defoliating pests.

INTRODUCTION

Defoliation is the most visible and probably the most common type of injury to soybean [*Glycine max* (L.) Merrill] (Kogan and Turnipseed, 1980). The complex of insect defoliators of soybean includes lepidopterans such as the green cloverworm, *Plathypena scabra* (F.) (Lepidoptera: Noctuidae), and coleopterans such as the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae). Defoliation linearly reduces soybean leaf area (Ostlie, 1984), which is required for plants to capture and convert sunlight into seed.

Soybean in vegetative developmental stages and early reproductive stages can

tolerate defoliation due to production of new leaf area (Fehr et al., 1977). Additionally, delayed leaf senescence may prevent yield losses from defoliation (Higley, 1992). Soybean at developmental stage R7 (beginning maturity) or later can withstand defoliation since the seeds are beginning to mature and are no longer accumulating photosynthate or nutrients. Fehr et al. (1977) found that indeterminate soybean lost yield when defoliated between developmental stages R2 (full bloom) to R5 (beginning seed), and Board et al. (1994) report that near R5 is the most sensitive developmental stage. Defoliation is probably less severe when it occurs gradually, which is typical of most insect defoliation patterns, rather than all at once (Board et al., 1994).

Leaf area index (LAI) is the ratio of unit leaf area of the crop to unit ground area (e.g., a LAI of 3.0 means that there are 3 m² of leaves distributed above 1 m² of ground). Research on full-season soybean indicates that LAI values in the critical region of 3.5 to 4.0 by developmental stage R2 to R4 (full pod) are needed to maximize photosynthetic potential (Board and Harville, 1992; Higley, 1992; Westgate, 1999). Reductions in LAI below this critical region due to insect defoliators may reduce yield (Board et al., 1997). Browde et al. (1994) reported both linear and quadratic relationships between soybean defoliation and LAI, and between defoliation and yield. Therefore, soybean LAI becomes an important factor in making management decisions regarding defoliating insects.

It is unknown how LAI of double-crop soybean responds to insect defoliation. Double-crop soybean are typically planted following small grain, have narrower row spacing, higher plant populations, and are subject to a shorter growing season than full-season soybean. We hypothesized that double-crop soybean would be more sensitive to defoliation than full-season soybean, and that 15% defoliation thresholds (from current Virginia, North Carolina, Georgia, and Texas Cooperative Extension publications) from full-season soybean research may be too high for double-crop soybean. Additionally, there are problems with basing thresholds on percent defoliation.

Researchers have stated that descriptions of yield loss by insect defoliators should consider leaf area remaining (as determined by LAI), not leaf area removed (Fehr et al., 1977; Herbert et al., 1992; Higley, 1992). Leaf area removed is typically expressed as percent defoliation. A percent defoliation threshold has different meaning for plants with

different canopy sizes. For example, a plant with an initial LAI of 5.0 that is defoliated 15% results in an LAI of 4.25, while a plant with an initial LAI of 4.0 that is defoliated 15% results in an LAI of 3.4. The first plant still exceeds the critical 3.5 to 4.0 LAI requirement for maximum yield potential, while the second plant does not. Therefore, insect defoliation thresholds that are based on LAI, rather than percent defoliation, are more meaningful.

Plants can be defoliated either by insects or mechanically. In support of the fidelity between mechanical and actual insect defoliation, Ostlie (1984) reported no differences in leaf transpiration rates or total water loss when these two methods were compared. Additionally, Thomas (1984) discovered that hand-defoliation of soybean leaflets was an accurate estimate of the effect of caterpillar defoliation on yield. Advantages of using mechanical defoliation rather than insect defoliation include the ability to quantify the amount of defoliation and the elimination of cage effect (Baldwin, 1990). Of the mechanical defoliation techniques (hand-picking, hole-punching, or cutting leaflets), hand-picking is the method most often used (Hutchins et al., 1988).

The objectives of these experiments were: (1) to determine the relationship between LAI and yield in mechanically defoliated double-crop and full-season soybean, and (2) to determine the relationship between LAI and yield in insect defoliated double-crop soybean.

MATERIALS AND METHODS

Mechanical defoliation experiment

Research was done from 1998 to 2000 at the Virginia Tech Tidewater Agricultural Research and Extension Center in Suffolk, Virginia. Soybean cultivars representing indeterminate maturity groups III and IV, and determinate maturity group V were planted to represent full-season and double-crop systems. Experimental design was a randomized complete block with four replicates. There were slight changes in the experiment between years, such as cultivar selection, soil type, planting dates, target LAI values, defoliation dates, and harvest dates (Table 3.1). In 1998 and 1999, plot size was four (46 cm) rows by 2 m; in 2000 plot size was five (38 cm) rows by 2 m. Leaflets were hand-defoliated on 14 dates over a one-month period to reduce LAI values from a

maximum predicted value to target LAI values (Table 3.1). For example, we predicted that 1998 full-season undefoliated (control) soybean would reach a maximum LAI of 4.25, and set target LAI values of 2.50 and 1.50 for the low and high defoliation treatments, respectively. Target LAI values were selected to provide a range of LAI values for regression to yield. Insecticides were applied so that actual insect feeding would not confound the hand-defoliation of the plots. In 1998, lambda-cyhalothrin (Karate Z[®] [Syngenta, Greensboro, NC], 29.9 ml ha⁻¹) was broadcast once on full-season plots and twice on double-crop plots; in 1999, esfenvalerate (Asana XL[®] [E.I. DuPont De Nemours and Co., Wilmington, DE], 95.7 ml ha⁻¹) was broadcast once on all plots, and was broadcast twice in 2000.

The computer model DEFOL (developed by Leon G. Higley at the University of Nebraska, personal communication, 1998) was used to reach target defoliation levels. The DEFOL program models insect defoliation and calculates the number of leaflets to remove from each plot based on the previous days' number of leaflets picked and the total leaf area removed. To simulate the development of larval lepidopteran pests, the DEFOL program assumed that insect feeding was light at the onset of defoliation (representing early instars), increased in the middle (representing later instars), and declined towards the end of the study (representing instars that are ready to pupate). The original DEFOL program was expanded from two weeks to one month since defoliator complexes in Virginia often occur (or overlap) for several weeks or months. Hand-defoliation began at R2, working from the top towards the bottom of the canopy. No preference was given to removal of terminal or lateral leaflets. Control plots were walked through and plants were handled to simulate activities in other plots. Defoliated leaflets were collected from each plot and leaf area was determined using a leaf area meter (Model 3000A, LI-COR, Inc., Lincoln, NE).

A plant canopy analyzer (Model LAI-2000, LI-COR, Inc., Lincoln, NE) was used to estimate LAI. LAI readings were taken according to the instruction manual for row crops (LI-COR, Inc., 1992). An opaque mask with a 45-degree opening was used to restrict the viewing space of the fish-eye lens of the plant canopy analyzer, and blocked the instrument's view of the operator. Readings were taken on overcast days. One

above-canopy reading was taken immediately before and in the same direction as a set of four below-canopy readings along a diagonal transect at 0, 25, 50, and 75% of the distance across the row, with each reading moving about 10 cm forward (Fig. 3.1 A and B). LAI readings were taken from all four sides of the plot, for a total of sixteen below-canopy readings per plot.

At the end of the defoliation period, rows adjacent to each plot and 50 cm at the end of each plot were defoliated to levels visually similar to those of the plot. This made it less likely for the plant canopy analyzer to detect plants exterior to the plots, which would have different leaf areas. Soybean were hand-harvested in 1998 and 1999, and were threshed in a Wintersteiger plot combine. In 2000, plots were harvested and threshed by the combine. Harvested seed weights were adjusted to 130 g kg⁻¹ moisture.

In the 1999 season, full season maturity group V soybean plots were established and were initially defoliated on 23 July; however, due to severe lodging the defoliation treatments were stopped after one week, and these plots were dropped from the experiment.

Relationships between soybean yield and LAI at the end of the defoliation period were analyzed using simple linear regression (SLR) and non-linear regression models (linear-plateau [LP], linear-slope [LS], and linear-slope-plateau [LSP] models). When LAI did not have a significant effect on yield, a line with zero slope was reported. The SLR model was analyzed using PROC GLM, and the non-linear models used PROC NLIN (SAS Institute, 1992). The model with the best fit was used to describe each data set (each cultivar/planting date/year combination). Goodness-of-fit was determined by whether or not the residuals were normally distributed about a mean of zero, as determined by a Shapiro-Wilk W test (SAS Institute, 1992). SLR models have one segment with non-zero slope. LP models have a sloping linear segment followed by zero slope; the LS model joins two segments with non-zero slopes; and the LSP model has two line segments, each with a different slope, that connect to a segment with zero slope (Schabenberger and Pierce, 2001). In all but the SLR model, the point where changes between the functions occur is called the join-point(s). The following terms were used for the SLR, LP, LS, and LSP models:

$$\theta_1 = \beta_0 + \beta_1 * LAI$$

$$\theta_2 = \beta_0 + \beta_1 * \text{jointpoint}_1$$

$$\theta_3 = \beta_0 + \beta_1 * \text{jointpoint}_1 + \beta_2 * (LAI - \text{jointpoint}_1)$$

$$\theta_4 = \beta_0 + \beta_1 * \text{jointpoint}_1 + \beta_2 * (\text{jointpoint}_2 - \text{jointpoint}_1).$$

θ_1 is the linear slope of the first segment, θ_2 is the yield when the first segment reaches jointpoint_1 , θ_3 is the slope of the second segment, and θ_4 is the yield when the second segment reaches jointpoint_2 (Schabenberger and Pierce, 2001). The following mathematical expressions were used in the model statement (SAS Institute, 1992):

SLR: $\text{yield} = \theta_1$

LP: $\text{yield} = \theta_1$ (when $LAI \leq \text{jointpoint}_1$) + θ_2 (when $LAI > \text{jointpoint}_1$)

LS: $\text{yield} = \theta_1$ (when $LAI \leq \text{jointpoint}_1$) + θ_3 (when $LAI > \text{jointpoint}_1$)

LSP: $\text{yield} = \theta_1$ (when $LAI \leq \text{jointpoint}_1$) + θ_3 (when $\text{jointpoint}_1 < LAI < \text{jointpoint}_2$) + θ_4 (when $LAI > \text{jointpoint}_2$).

Non-linear regression models estimated the join-point(s). The 95% confidence limits for the estimate of the join-point in these models were required to completely contain the original LAI values (thus ensuring that the estimate of the join-point was within the range of the original data) to be accepted over SLR models. When 95% confidence limits contained the value zero, we rejected the model and assumed that the slope of that portion of the graph was zero. The r^2 values were determined for acceptable models.

Insect defoliation experiment

Research was done in 1999 and 2000 in cooperation with four Virginia Cooperative Extension Agriculture and Natural Resources agents from the counties of Essex, New Kent, Middlesex, and Prince George. Each agent established a test site in double-crop soybean in his county. Soybean was planted in early July at a row spacing of 19 cm. Plot size was 3.66 m wide by 18.3 m long. Treatments were insect defoliators present or absent in the plots. Insecticides were sprayed at the agents' discretion throughout July and August to establish the insect-free plots, while unsprayed plots encouraged the presence of defoliating insects. Experimental design was a randomized complete block with 6 or 8 replicates. In the 1999 Essex County test, an abundance of

corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), necessitated that untreated plots be split, so that half of the plot would receive a lambda-cyhalothrin spray (Karate Z[®], 12 ml ha⁻¹) to control this pod-feeder (applied 26 August); the other half was left untreated. The plots that had been sprayed the entire season in Essex County also received the lambda-cyhalothrin spray.

Insect defoliators were sampled in 1999 by taking 15 sweeps with a 38 cm diameter sweep net in the untreated plots. Sweep net samples were taken from the front of the plots so that LAI readings and harvest samples could be taken from the back of the plots where leaves were not touched or damaged by the sweep net. Occasionally sweep net samples were taken in treated plots to ensure that insect defoliators were being controlled by the spray schedule. Insects were not sampled in 2000.

In 1999, LAI readings were taken on an overcast day with a plant canopy analyzer using previously described techniques. Readings were taken at developmental stage R3 (beginning pod) in Essex, Middlesex, and Prince George Counties, and at R5 in New Kent County. In 2000, LAI readings were taken on an overcast day at developmental stage R6 (full seed) in Essex and New Kent Counties, and at R5 in Prince George and Middlesex Counties.

In early November 1999, four rows by 1 m were hand-harvested at the back of the plots and threshed in a Wintersteiger plot combine. Trash was removed from the samples, and seed weight and moisture were recorded. In early November 2000, four rows by 1 m were hand-harvested at the Prince George County location, but entire plots at Essex, Middlesex, and New Kent Counties were harvested by combine. Plot weights were adjusted to 130 g kg⁻¹ moisture. Yield differences were analyzed with PROC GLM (SAS Institute, 1992).

RESULTS AND DISCUSSION

Mechanical defoliation experiment

By the end of the defoliation period, the amount of leaf area removed was within 1 to 5% of the amount required by DEFOL, indicating that the computer model was closely followed. However, target LAI values were not always achieved. This was partly due to difficulties in predicting the maximum LAI for each cultivar/planting date/year at

the beginning of the experiment, with little knowledge of how environmental factors would affect the experiment. DEFOL bases defoliation levels on this maximum predicted LAI. However, the target LAI values were only a means to achieve a range of LAI values for the regression analyses, so all data were usable. Ideally, there would be an even distribution of data points from the lowest target LAI value through the control LAI values, so that regressions would not have gaps.

In Suffolk, Virginia, the yields that can be expected under a high level of management in the moderately well drained Eunola sandy loam and somewhat poorly drained Lynchburg fine sandy loam soils are 2016 to 2352 and 3024 kg ha⁻¹, respectively (USDA-SCS Soil Survey Division, 1981). The somewhat poorly drained soil has greater yield potential than the moderately well drained soil partly due to increased moisture availability. Maximum yield potential determines the percent yield loss when yield reductions occur. For example, a 1000 kg ha⁻¹ reduction in yield for every unit decrease in LAI in soybean with a maximum yield potential of 2000 kg ha⁻¹ results in a greater percent yield loss than in soybean with a 3000 kg ha⁻¹ potential.

Of the fifteen data sets, three had no significant relationship between LAI and yield, seven were described by SLR, and five fit the LP model (Table 3.2 and Figs. 3.2-3.16). The LS and LSP models did not describe any of the data sets.

The three data sets that had no significant relationship between LAI and yield all occurred in 1999 (Southern States brand cultivar RT-386 planted full-season and double-crop, and RT-446N planted full-season). These soybean had high LAI values; full-season LAI values were above 4.5 and most double-crop LAI values were above 4.0. The high LAI values were likely due to adequate rainfall that season, allowing plants to better compensate for leaf area loss through leaf regrowth. Haile et al. (1998) reported similar post-defoliation soybean leaf regrowth in seasons with ample rainfall. A relationship between LAI and yield was not expected in these data sets since leaf areas were so high; plateau levels (with zero slope) had been achieved.

Three of the seven data sets described by SLR occurred in 1998. In that year, yield of full-season Asgrow brand cultivar AG4702 was reduced 1374.5 kg ha⁻¹ for every unit decrease in LAI between values of 4.4 to 2.8 (Fig. 3.3). Double-crop AG3701 yield

was reduced 677.5 kg ha^{-1} for every unit decrease in LAI between values of 3.3 to 0.9 (Fig. 3.4), and double-crop AG4702 yield was reduced 746.1 kg ha^{-1} for every unit decrease in LAI between values of 4.4 and 1.8.

The 1999 RT-446N cultivar planted double-crop, and the 2000 RT-386 planted both full-season and double-crop in moderately well drained soil (Eunola sandy loam) had yield reductions described by SLR at LAI values greater than 5.0 (Figs. 3.9, 3.11, and 3.13). This indicates that there may be factors other than leaf area involved in determining soybean yield. Therefore, the 3.5-4.0 critical LAI level (established through previous research in full-season soybean) does not guarantee that maximum yield potential will be reached. Kumudini et al. (2001) discovered that longer leaf area duration improved soybean yield through increased dry matter accumulation during seed-fill. Soybean with high yield potential may be especially sensitive to leaf area duration, due to additional photosynthate requirements. For example, the 2000 full-season RT-386 soybean had a maximum yield potential of approximately 4300 kg ha^{-1} . The 2000 RT-446N planted double-crop in somewhat poorly drained soil (Lynchburg fine sandy loam) had linear decreases in yield from LAI values of 4.4 to 1.0 (Fig. 3.16).

The LP model described five of the data sets. Each of these had residuals normally distributed about a mean of zero (Shapiro Wilk W-test, $P > 0.05$). Thus, the LP model fit the data. In 1998, the full-season AG3701 soybean had linear yield reductions of $1371.1 \text{ kg ha}^{-1}$ for each unit decrease in LAI from values of 3.5 to 1.7; yields reached a plateau level when LAI values were above 3.5 (Fig. 3.2). The value 3.52 is the join-point in this model, the point where the sloping segment changes to the zero-slope segment. The join-point is the critical LAI value. In 1999, the double-crop RT-557N soybean had a join-point of 3.54 (Fig. 3.10). A LP model described the 2000 full-season RT-446N soybean, its join-point occurring at 3.69 (Fig. 3.12). The 2000 double-crop RT-386 in poorly drained soil had an ideal, wide range of data points (Fig. 3.15). This LP model had a join-point at an LAI value of 3.6. The join-point for 2000 double-crop RT-446N soybean in moderately well drained soil occurred at 4.30, but this number may be artificially high due to a gap in data between LAI values of 3.3 to 4.3 (Fig. 3.14); had there been more data points in this range, the plateau may have fallen somewhere

between these values.

Models with no significant relationship between LAI and yield had 88% of LAI values greater than 4.0. In only three of fifteen data sets were there significant linear relationships between LAI and yield when LAI values were above 4.4. Critical LAI values of full-season LP models were 3.52 and 3.69. This supports research by Board and Harville (1992), Higley (1992), and Westgate (1999), who determined that the critical LAI level is between 3.5 and 4.0. The LP models indicate similar critical LAI values for double-crop soybean (3.54 and 3.60, excluding the 2000 RT-446N planted double-crop in moderately well drained soil with the gap in LAI values from 3.3 to 4.3). Therefore, the critical LAI value for both double-crop and full-season soybean is approximately 3.5 to 4.0.

Insect defoliation experiment

Sweep net samples determined that *P. scabra* was the main defoliator pest in all four tests in 1999, and it remained in the plots for at least one month. Other defoliator pests and their numbers are given in Appendix B. In both years, there was not enough feeding by defoliating insects to drive LAI values below the critical 3.5 to 4.0 level in any of the tests. By the developmental stage when LAI readings were taken (R3 to R6), 75% (47 of 63) of the untreated plots had LAI values greater than 5.0. With sufficient leaf area untreated plots had the same yield as insecticide-treated plots, except for the 1999 Essex County test (Tables 3.3 and 3.4). Soybean plot yields and LAI values are provided in Appendix C.

In the 1999 Essex County test, there was a large *H. zea* population in late August through early September. An average of 21.3 larvae were caught per 15-sweep sample, well above the current Virginia threshold of 2.5 larvae in soybean with 7-inch row spacing (Herbert, 2001). This pest decimated the pods in the untreated plots, resulting in average yields of only $262 \text{ kg ha}^{-1} \pm 130$. McPherson and Moss (1989) reported that soybean could compensate for *H. zea* defoliation and depodding by producing new pods. They showed that soybean with nearly complete defoliation and depodding only lost 40% of maximum yield. We found that plots that had been untreated the entire season, but received one spray to control *H. zea* had the same yield as plots treated the entire season

(Table 3.3).

This research indicates that control of defoliating insects is not necessary when double-crop soybean LAI values of 5.0 are maintained from developmental stage R3 to R6. There was a similar response of soybean to insect-induced and mechanical defoliation, where no yield losses occurred when LAI values were above 4.0. Yields tended to plateau when LAI values were above the critical 4.0 level, indicating no significant relationship between LAI and yield. Soybean management decisions could therefore focus on the damage done or that is in progress, and LAI. The appearance of pod-feeding pests such as *H. zea* still warrants recommended scouting and control measures.

CONCLUSION

Double-crop soybean, similar to full-season soybean, requires a critical LAI of 3.5 to 4.0 by developmental stage R4 to R5 to achieve maximum yield potential. Measurement of LAI can provide an estimate of the health of a crop, and soybean management could be partially based on the presence of defoliating pests and whether soybean leaf area exceeds the critical LAI. Crops that do not meet the critical LAI requirement and have defoliating insects present are at the greatest risk to yield loss. Therefore, soybean that appears the “worst” may need the most protection from defoliators. Soybean that appears the “best” probably has adequate leaf area and may not need to be treated with insecticides for defoliating pests. It may seem counter-intuitive that soybean with large canopies, capable of greater potential yields than soybean with smaller canopies, may not need as much chemical protection, but these data indicate that large canopies can tolerate a large amount of defoliation without any significant yield loss. Soybean that maintains LAI values greater than the critical 3.5 to 4.0 level can be considered safe from yield loss due to insect defoliators. However, some cultivars with high yield potentials may require LAI values greater than 3.5-4.0, due to additional photosynthate demand by the seeds. If LAI values are below the critical level for reasons other than insect defoliation, practices that increase LAI, such as increasing plant population, narrowing row spacing, or earlier planting may need to be considered for future plantings. Currently, LAI estimates may be taken with plant canopy analyzers, and

in the future it may be possible to remotely sense LAI. Remote sensing would facilitate the management of large crop areas, but would still require ground-truthing of LAI either directly or by estimates taken with plant canopy analyzers.

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Table 3.1. Description of mechanical defoliation experiments.

Year	Cropping system†	Planting date	Soil type§	Cultivar‡	Target LAI levels	Defoliation dates	Harvest date
1998	FS	22 May	Lynchburg fine sandy loam	AG3701	4.25, 2.50, 1.50	8 Jul-10 Aug	21 Sep
	FS	22 May	Lynchburg fine sandy loam	AG4702	4.25, 2.50, 1.50	8 Jul-10 Aug	24 Sep
	DC	23 Jun	Lynchburg fine sandy loam	AG3701	3.00, 1.80, 1.05	5 Aug-3 Sep	19 Oct
	DC	23 Jun	Lynchburg fine sandy loam	AG4702	3.00, 1.80, 1.05	5 Aug-3 Sep	31 Oct
1999	FS	12 May	Lynchburg fine sandy loam	RT-386	4.50, 4.00, 3.50, 3.00, 2.00	7 Jul-5 Aug	27 Sep
	FS	12 May	Lynchburg fine sandy loam	RT-446N	5.00, 4.00, 3.50, 3.00, 2.00	7 Jul-5 Aug	15 Oct
	DC	29 May	Eunola loamy fine sand	RT-386	3.50, 3.00, 2.50, 2.00, 1.50	5 Aug-3 Sep	3 Nov
	DC	29 May	Eunola loamy fine sand	RT-446N	4.00, 3.50, 3.00, 2.50, 2.00	5 Aug-3 Sep	4 Nov
	DC	29 May	Eunola loamy fine sand	RT-557N	5.50, 4.00, 3.50, 3.00, 2.00	18 Aug-17 Sep	12 Nov
2000	FS	2 Jun	Eunola sandy loam	RT-386	4.00, 3.60, 3.10, 2.60, 2.10	12 Jul-11 Aug	16 Oct
	FS	2 Jun	Eunola sandy loam	RT-446N	4.50, 4.10, 3.60, 3.10, 2.10	12 Jul-11 Aug	16 Oct
	DC-1	19 Jun	Eunola sandy loam	RT-386	3.50, 3.25, 3.00, 2.50, 2.00	26 Jul-25 Aug	31 Oct

DC-1	19 Jun	Eunola sandy loam	RT-446N	4.00, 3.75, 3.25, 2.50, 2.00	26 Jul-25 Aug	31 Oct
DC-2	16 Jun	Lynchburg fine sandy loam	RT-386	3.50, 3.25, 3.00, 2.50, 2.00	26 Jul-25 Aug	30 Oct
DC-2	16 Jun	Lynchburg fine sandy loam	RT-446N	4.00, 3.75, 3.25, 2.50, 2.00	26 Jul-25 Aug	30 Oct

† FS, full season; DC, double crop

§ Higher taxonomic classes: Lynchburg (fine-loamy, siliceous, thermic Aeric Paleaquults); Eunola (fine-loamy, siliceous, thermic Auqic Hapludults)

‡ The “AG” prefix indicates an Asgrow brand cultivar; “RT” indicates a Southern States brand cultivar

Table 3.2. Results of fitting models to the mechanical defoliation data. The join points were estimated from the data.

Year	Cropping system†	Cultivar	Model type§	P	r ²	Shapiro-Wilk W-test's P‡	Join point ± SE	Slope¥ ± SE	Intercept ± SE
1998	FS	AG3701	LP	0.0004	0.86	0.15	3.52 ± 0.25	1371.1 ± 248.6	-1465.1 ± 675.4
	FS	AG4702	SLR	0.0009	0.77	---	---	1374.5 ± 268.8	-2083.2 ± 952.6
	DC	AG3701	SLR	0.0001	0.88	---	---	677.5 ± 80.9	531.2 ± 196.9
	DC	AG4702	SLR	0.0001	0.88	---	---	746.1 ± 88.6	176.8 ± 287.2
1999	FS	RT-386	NS	0.6931	---	---	---	---	---
	FS	RT-446N	NS	0.1487	---	---	---	---	---
	DC	RT-386	NS	0.0550	---	---	---	---	---
	DC	RT-446N	SLR	0.0026	0.46	---	---	335.8 ± 93.2	728.6 ± 436.1
	DC	RT-557N	LP	0.0163	0.47	0.47	3.54 ± 0.23	830.9 ± 475.8	-1047.1 ± 1520.4
2000	FS	RT-386	SLR	0.0001	0.86	---	---	545.8 ± 53.3	1385.7 ± 216.5
	FS	RT-446N	LP	0.0001	0.88	0.94	3.69 ± 0.25	859.8 ± 100.7	580.6 ± 262.8
	DC-1	RT-386	SLR	0.0001	0.70	---	---	485.4 ± 74.9	639.2 ± 319.0
	DC-1	RT-446N	LP	0.0001	0.95	0.86	4.30 ± 0.20	654.2 ± 55.2	86.2 ± 149.3
	DC-2	RT-386	LP	0.0001	0.84	0.36	3.60 ± 0.19	778.0 ± 107.1	-110.2 ± 302.1
	DC-2	RT-446N	SLR	0.0001	0.88	---	---	573.4 ± 50.4	526.4 ± 167.9

† FS, full-season; DC, double-crop.

§ SLR, simple linear regression model; LP, linear plateau model; NS, not significant.

‡ In determining goodness-of-fit for LP models, non-significant Shapiro-Wilk W-test values ($P > 0.05$) indicate that the residuals were normally distributed about a mean of zero.

¥ Slopes are for non-zero portions of the model only. Plateau and non-significant portions of models have zero slope.

Table 3.3. Yield results of insect defoliation tests in four Virginia counties—1999.

Treatment	Yield† (kg ha ⁻¹)			
	Essex	Middlesex	New Kent	Prince George
Treated§	2123.5 a‡	2083.2 a	2573.8 a	2634.2 a
Untreated	262.1 b	2197.4 a	2446.1 a	2385.6 a
<i>H. zea</i> spray¥	2257.9 a	----	----	----

† Yield based on weight of soybean with a moisture content of 130 g kg⁻¹.

§ Esfenvalerate (Asana XL[®] [E.I. DuPont De Nemours and Co., Wilmington, DE) was applied as needed with a backpack sprayer to maintain insect-free plots.

‡ Means within a column followed by the same letter are not significantly different (LSD, P > 0.05).

¥ Lamda-cyhalothrin (Karate Z[®] [Syngenta, Greensboro, NC]) was applied on previously untreated plots at the Essex County location to control *H. zea* on 26 August 1999.

Table 3.4. Yield results of insect defoliation tests in four Virginia counties—2000.

Treatment	Yield† (kg ha ⁻¹)			
	Essex	Middlesex	New Kent	Prince George
Treated§	3124.8 a‡	2889.6 a	3265.9 a	3299.5 a
Untreated	3171.8 a	2782.1 a	3030.7 a	3131.5 a

† Yield based on weight of soybean with a moisture content of 130 g kg⁻¹.

§ Insecticide was applied as needed with a backpack sprayer to maintain insect-free plots.

‡ Means within a column followed by the same letter are not significantly different (PROC GLM, P > 0.05).

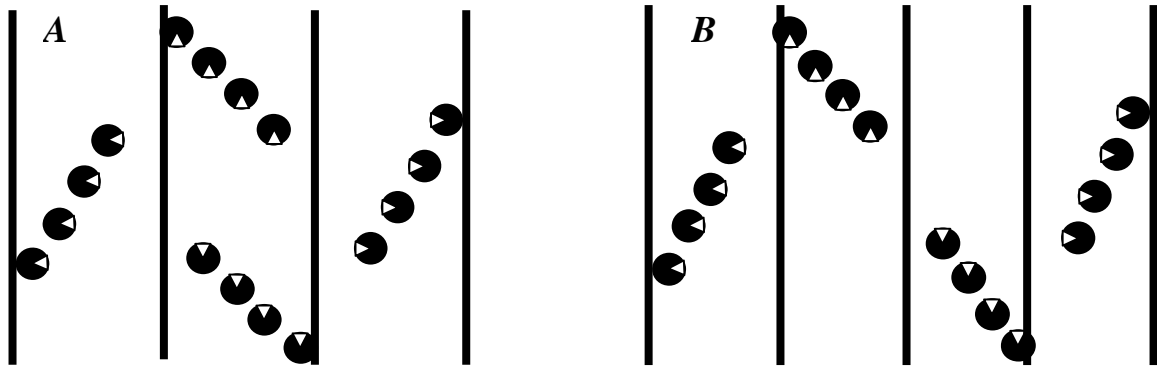


Fig. 3.1. Placement of LAI-2000 fish-eye lens (with 315-degree restricting viewcap, indicated by the black portion of the circle) for below-canopy measurements in soybean plots with (A) four rows with 46 cm row centers, and (B) five rows with 38 cm row centers. Vertical lines represent row centers.

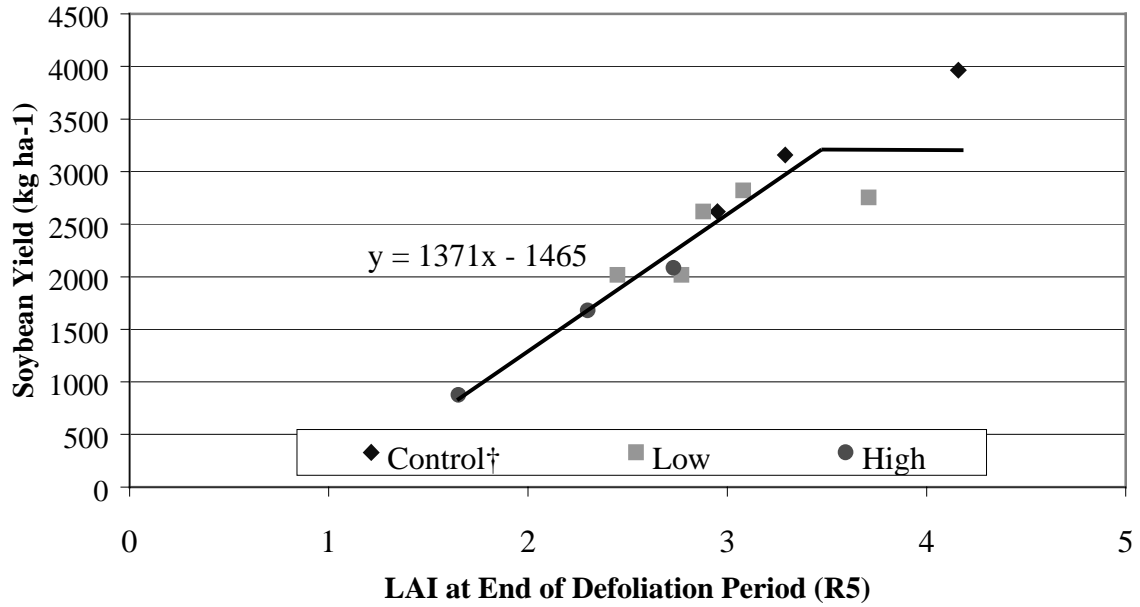


Fig. 3.2. Linear plateau regression of LAI and yield for 1998 Asgrow brand cultivar AG3701 soybean planted full-season. † The legend indicates level of mechanical defoliation.

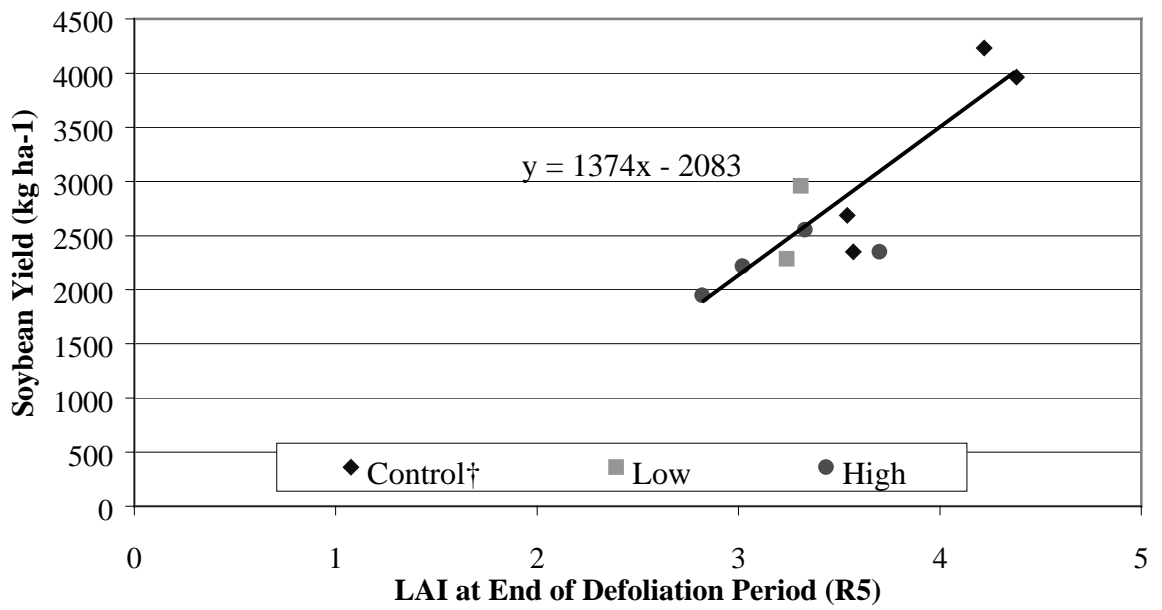


Fig. 3.3. Simple linear regression of LAI and yield for 1998 Asgrow brand cultivar AG4702 soybean planted full-season. † The legend indicates level of mechanical defoliation.

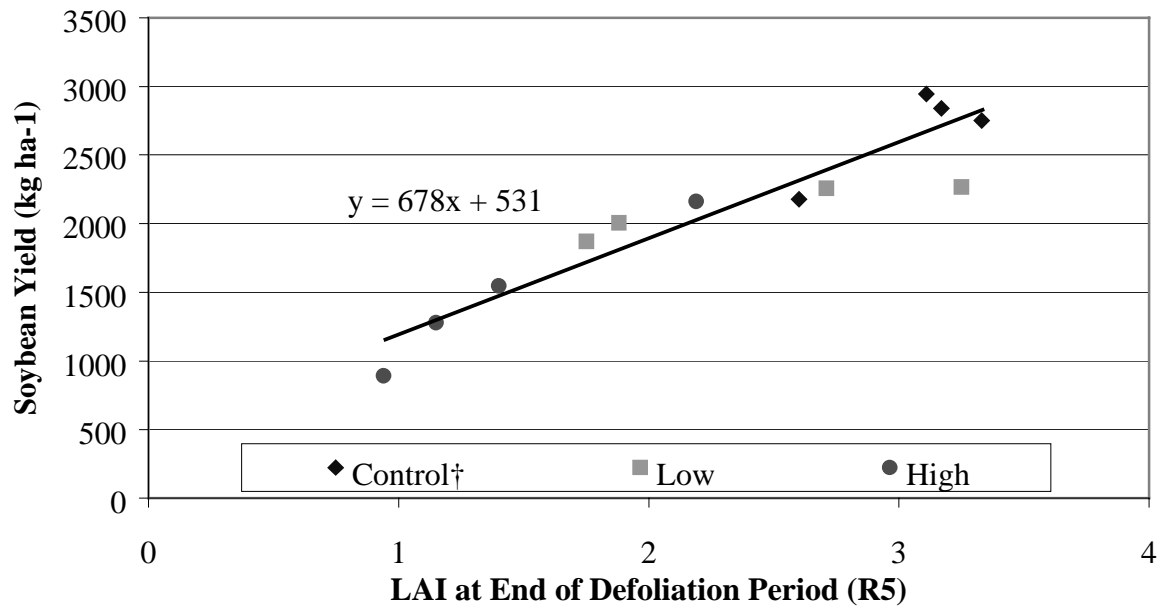


Fig. 3.4. Simple linear regression of LAI and yield for 1998 Asgrow brand cultivar AG3701 soybean planted double-crop. † The legend indicates level of mechanical defoliation.

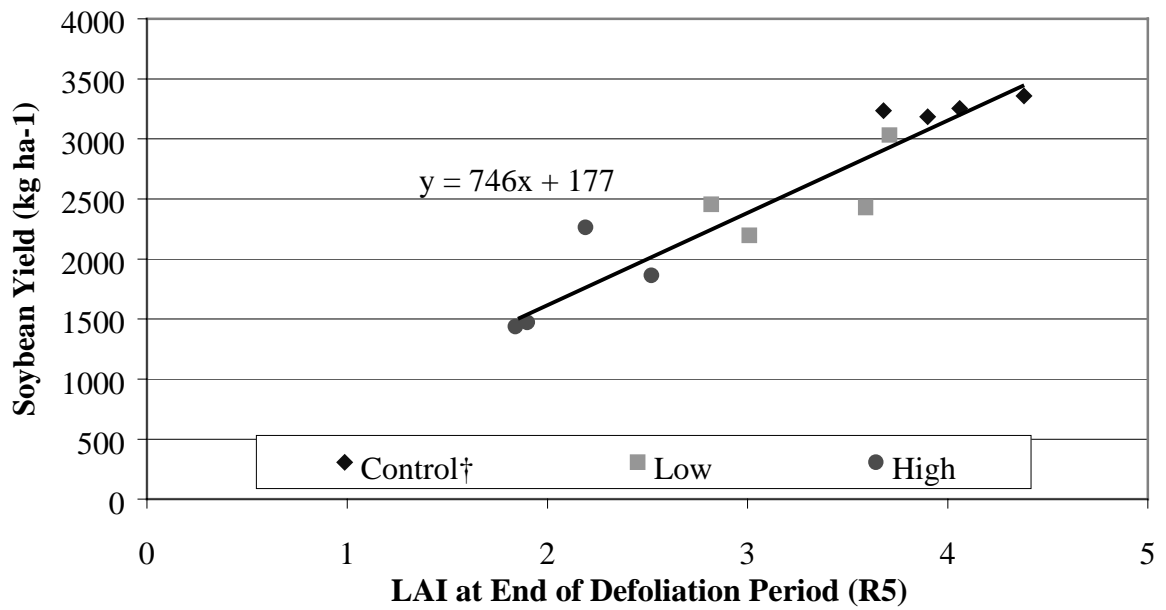


Fig. 3.5. Simple linear regression of LAI and yield for 1998 Asgrow brand cultivar AG4702 soybean planted double-crop. † The legend indicates level of mechanical defoliation.

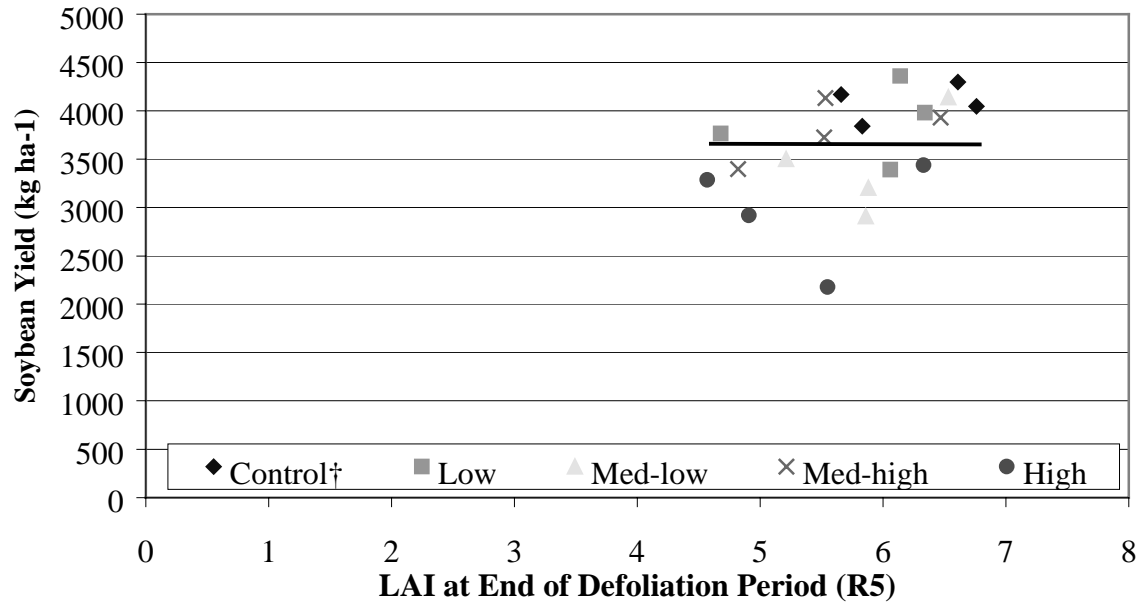


Fig. 3.6. Non-significant relationship between LAI and yield for 1999 Southern States brand cultivar RT-386 soybean planted full-season. † The legend indicates level of mechanical defoliation.

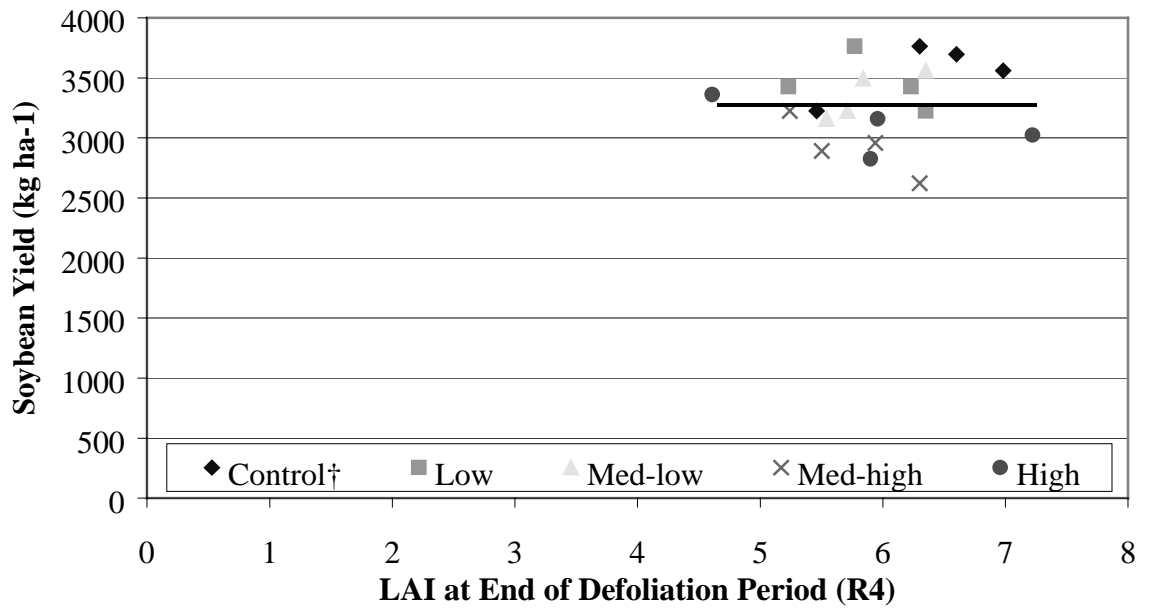


Fig. 3.7. Non-significant relationship between LAI and yield for 1999 Southern States brand cultivar RT-446N soybean planted full-season. † The legend indicates level of mechanical defoliation.

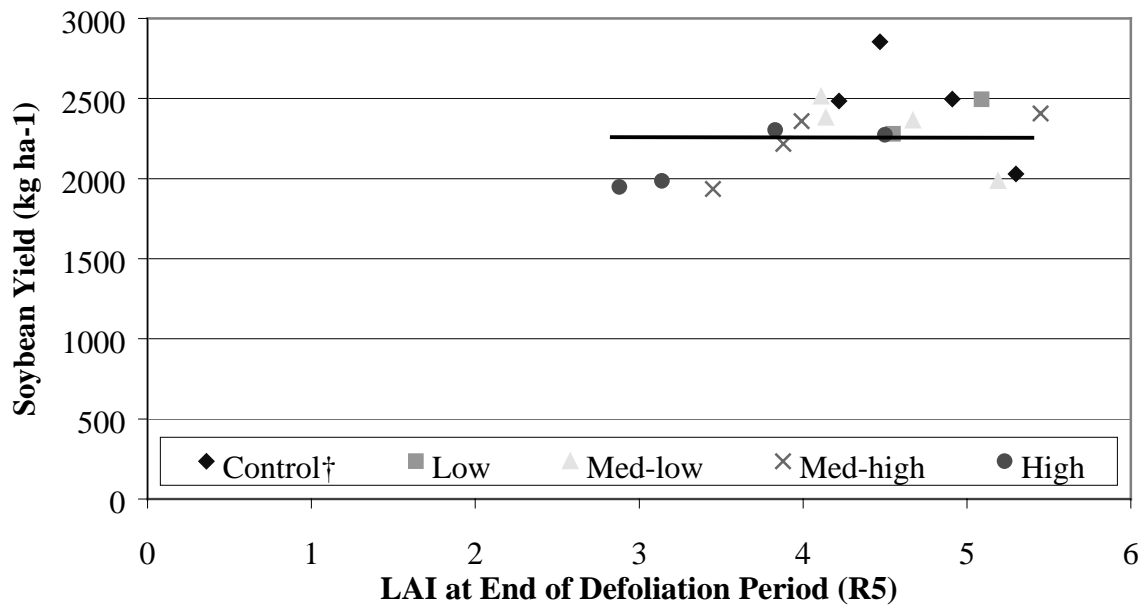


Fig. 3.8. Non-significant relationship between LAI and yield for 1999 Southern States brand cultivar RT-386 soybean planted double-crop. † The legend indicates level of mechanical defoliation.

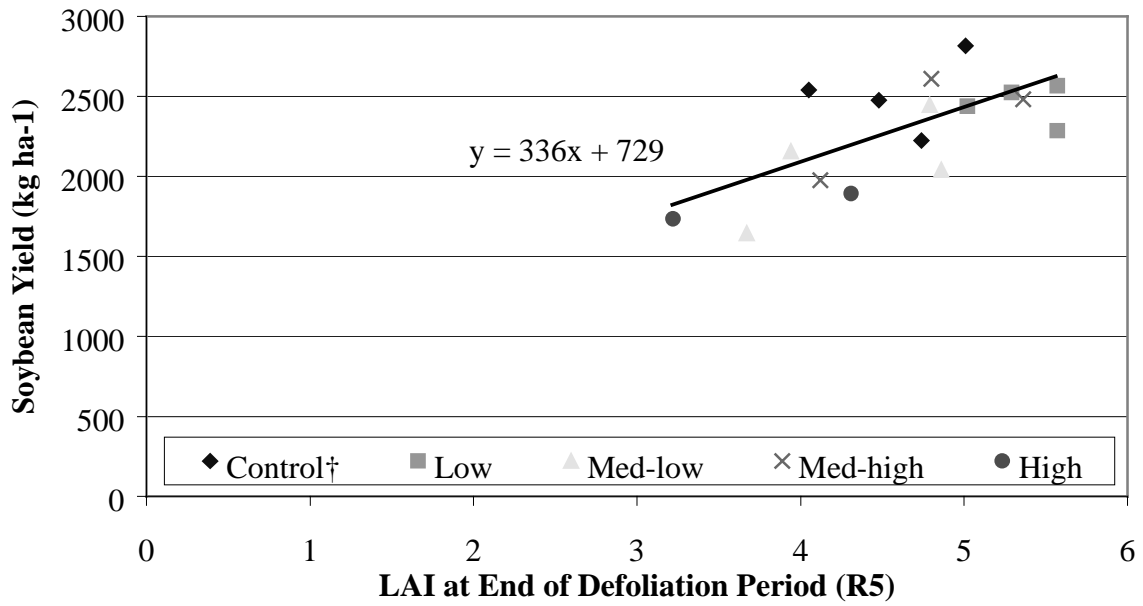


Fig. 3.9. Simple linear regression of LAI and yield for 1999 Southern States brand cultivar RT-446N soybean planted double-crop. † The legend indicates level of mechanical defoliation.

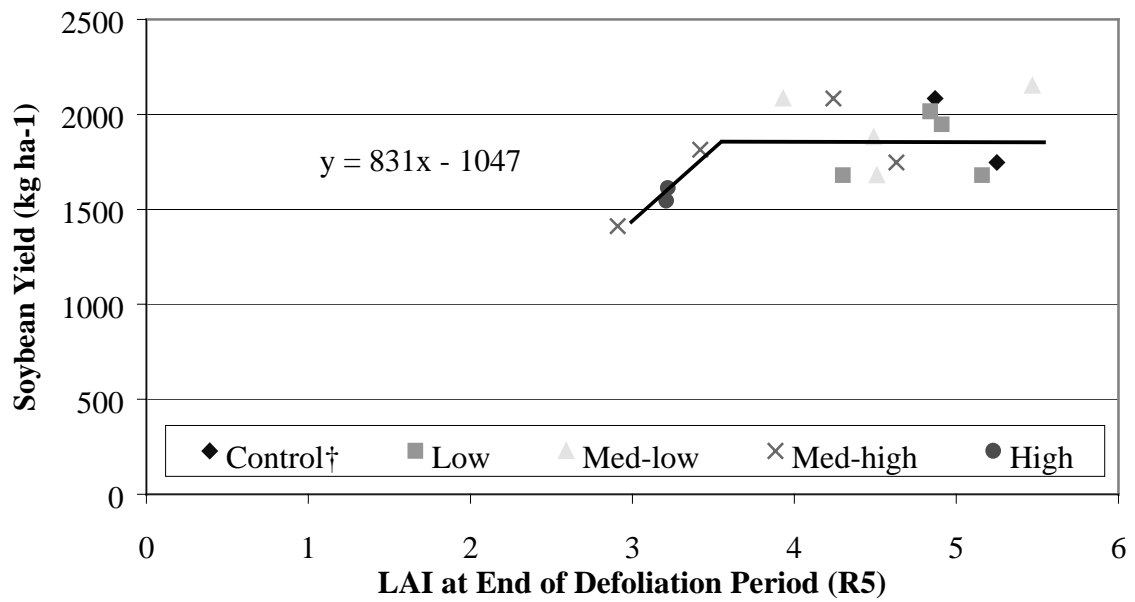


Fig. 3.10. Linear plateau regression of LAI and yield for 1999 Southern States brand cultivar RT-557N soybean planted double-crop. † The legend indicates level of mechanical defoliation.

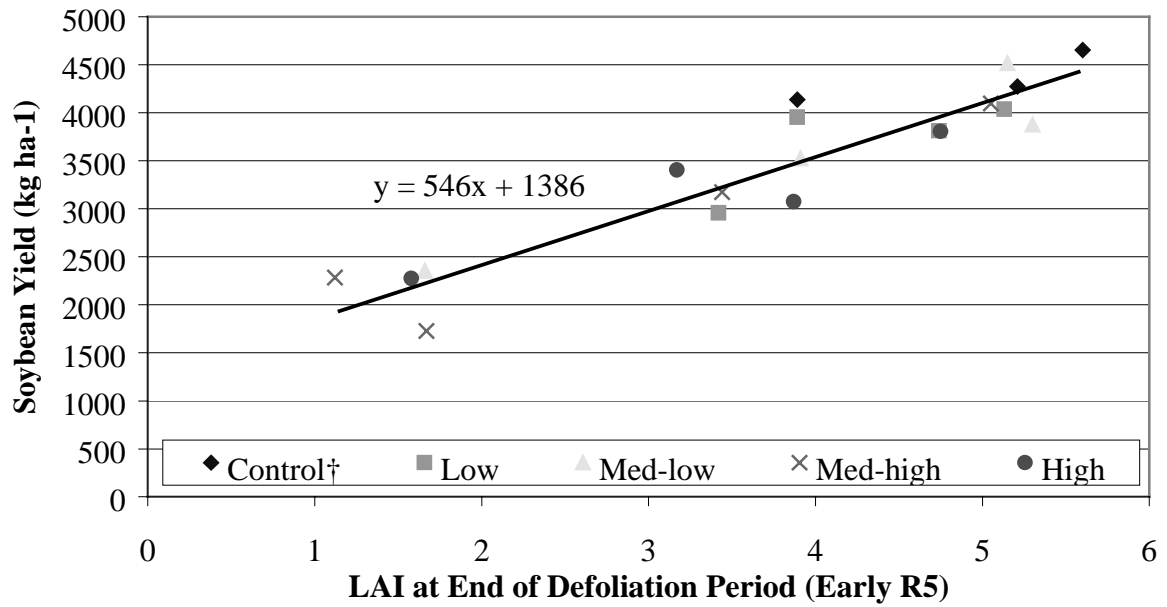


Fig. 3.11. Simple linear regression of LAI and yield for 2000 Southern States brand cultivar RT-386 soybean planted full-season. † The legend indicates level of mechanical defoliation.

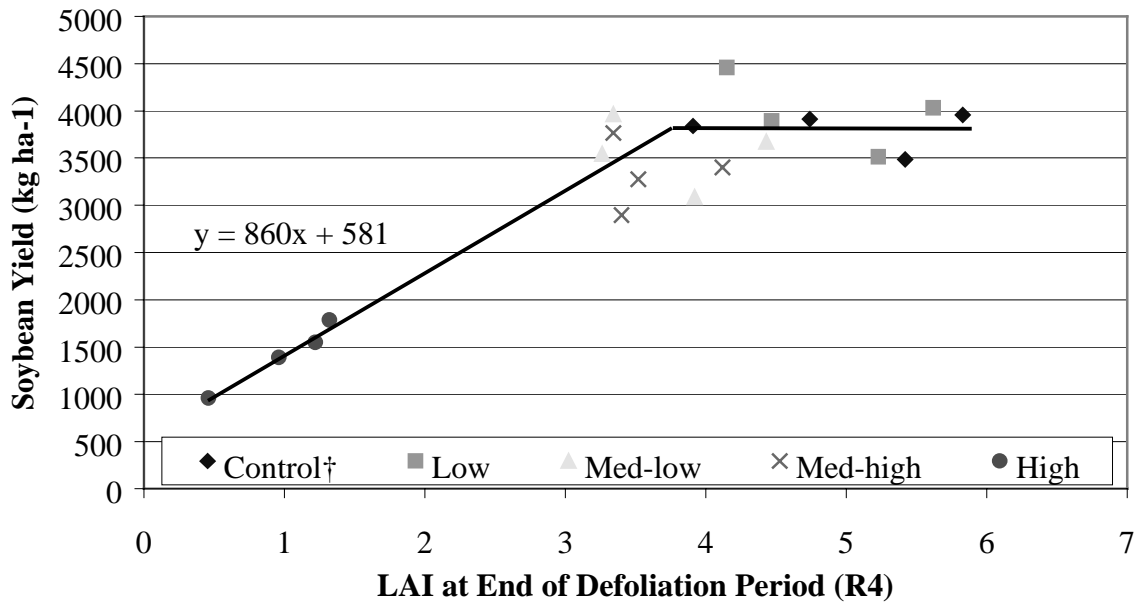


Fig. 3.12. Linear plateau regression of LAI and yield for 2000 Southern States brand cultivar RT-446N soybean planted full-season. † The legend indicates level of mechanical defoliation.

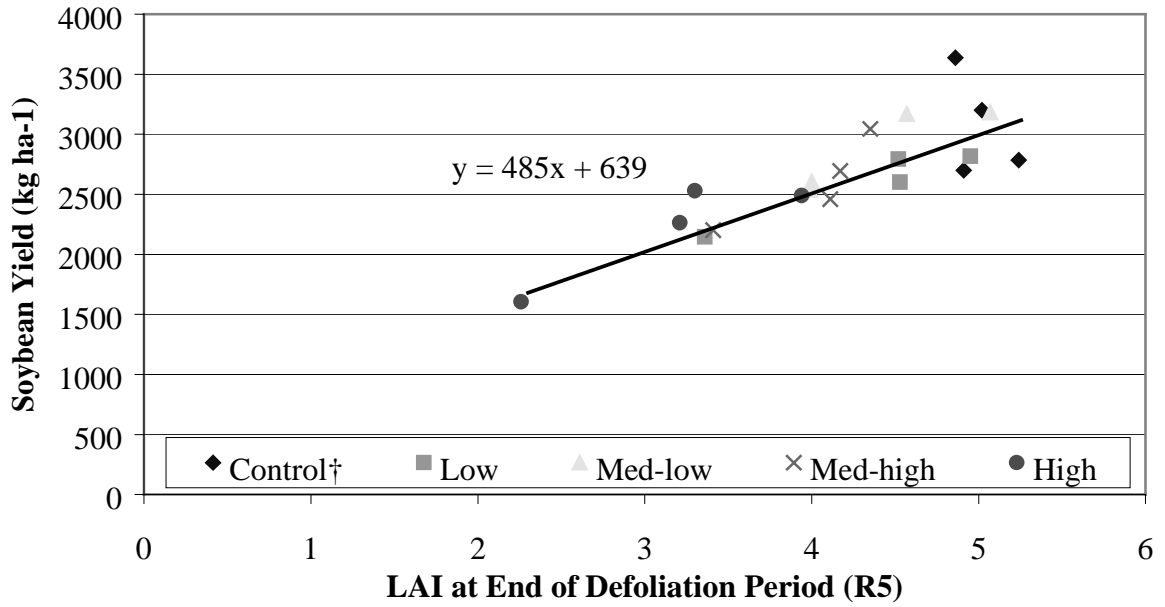


Fig. 3.13. Simple linear regression of LAI and yield for 2000 Southern States brand cultivar RT-386 soybean planted double-crop in a moderately well drained soil. † The legend indicates level of mechanical defoliation.

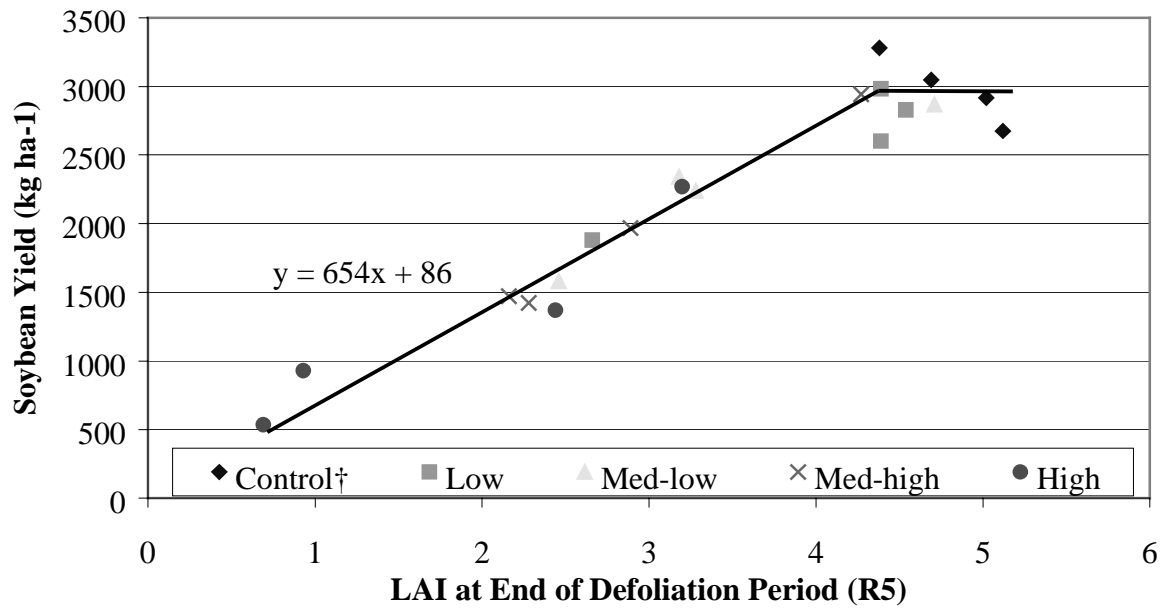


Fig. 3.14. Linear plateau regression of LAI and yield for 2000 Southern States brand cultivar RT-446N soybean planted double-crop in a moderately well drained soil. † The legend indicates level of mechanical defoliation.

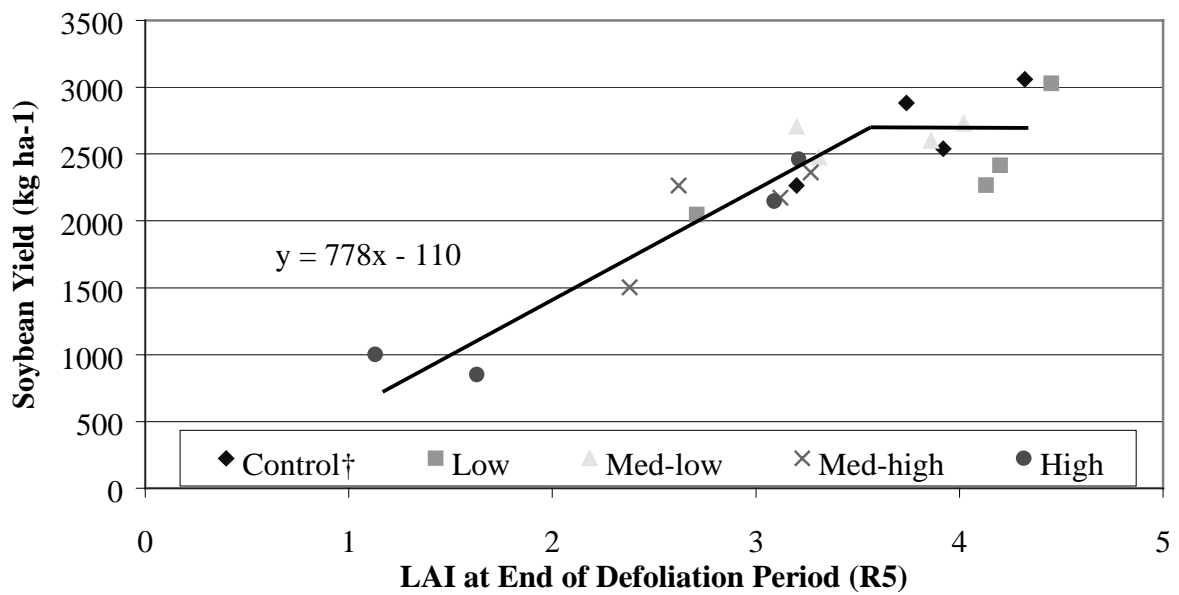


Fig. 3.15. Linear plateau regression of LAI and yield for 2000 Southern States brand cultivar RT-386 soybean planted double-crop in a somewhat poorly drained soil. † The legend indicates level of mechanical defoliation.

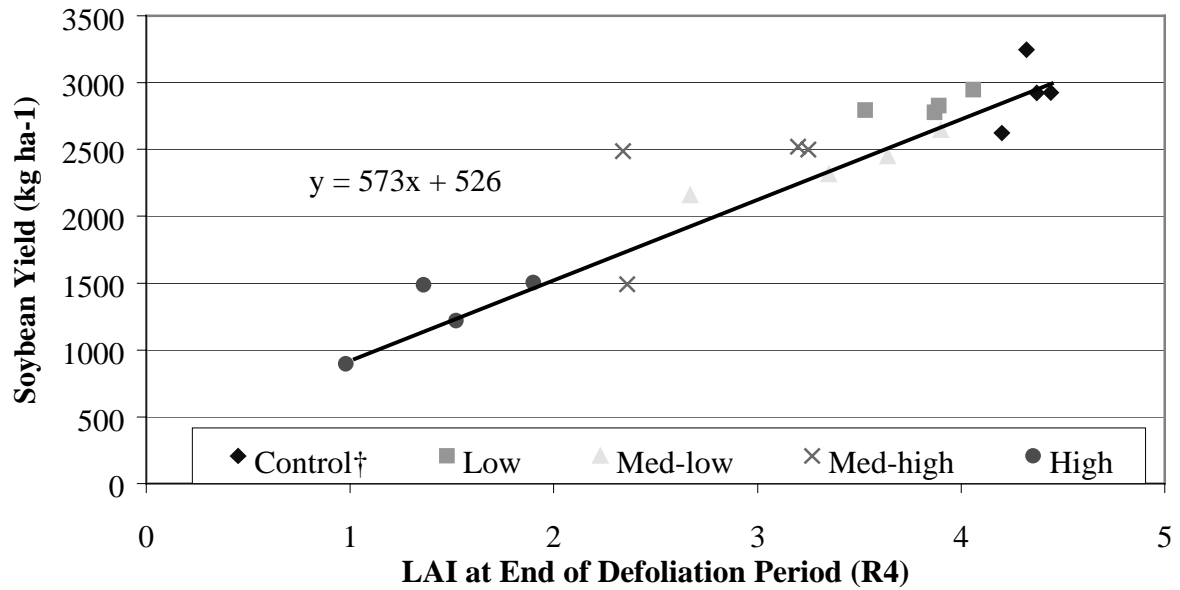


Fig. 3.16. Simple linear regression of LAI and yield for 2000 Southern States brand cultivar RT-446N soybean planted double-crop in a somewhat poorly drained soil. † The legend indicates level of mechanical defoliation.

Appendix A. Images of soybean LAI values (38 cm row centers). Stake height (at the front of each plot) is approximately 25 cm.



Appendix B. Insect populations in the insect defoliation experiments.

Table B.1. Insect pest populations in the Essex County, Virginia soybean test--1999.

Insect	-----Number of Insects per Sample-----		
	6 Aug. (V6)†	17 Aug. (R2)	2 Sep. (R3)
	Mean +/- SD§	Mean +/- SD	Mean +/- SD
<i>Plathypena scabra</i> (small‡)	0.50 +/- 0.76	0.25 +/- 0.46	0.00 +/- 0.00
<i>Plathypena scabra</i> (medium)	0.13 +/- 0.35	0.13 +/- 0.35	1.00 +/- 2.14
<i>Plathypena scabra</i> (large)	0.88 +/- 0.83	0.00 +/- 0.00	1.38 +/- 1.77
<i>Popillia japonica</i>	0.13 +/- 0.35	0.13 +/- 0.35	0.00 +/- 0.00
Pentatomid species	0.50 +/- 0.53	0.13 +/- 0.35	0.00 +/- 0.00
Acridid species	0.25 +/- 0.46	0.13 +/- 0.35	0.13 +/- 0.35
<i>Helicoverpa zea</i> (small)	0.00 +/- 0.00	0.38 +/- 0.74	10.25 +/- 5.75
<i>Helicoverpa zea</i> (medium)	0.00 +/- 0.00	0.00 +/- 0.00	9.13 +/- 2.53
<i>Helicoverpa zea</i> (large)	0.13 +/- 0.35	0.00 +/- 0.00	1.88 +/- 2.17
<i>Spissistilus festinus</i>	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
<i>Cerotoma trifurcata</i>	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
<i>Chaetocnema pulicaria</i>	0.00 +/- 0.00	0.13 +/- 0.35	0.00 +/- 0.00
Meloid species	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00

† Soybean development stage

§ Sampling technique was eight replicates of 15 sweeps with a 38 cm diameter sweep net.

‡ “Small” are instars 1-2; “medium” are instars 3-4; “large” are instars 5-6.

Table B.2. Insect pest populations in the Middlesex County, Virginia soybean test--1999.

Insect	-----Number of Insects per Sample-----		
	4 Aug. (V4-5)†	17 Aug. (R2)	2 Sep. (R3)
	Mean +/- SD§	Mean +/- SD	Mean +/- SD
<i>Plathypena scabra</i> (small‡)	3.63 +/- 1.69	1.75 +/- 1.49	0.13 +/- 0.35
<i>Plathypena scabra</i> (medium)	2.00 +/- 0.93	1.50 +/- 1.31	0.25 +/- 0.46
<i>Plathypena scabra</i> (large)	1.13 +/- 1.46	0.38 +/- 0.74	0.38 +/- 0.52
<i>Popillia japonica</i>	0.50 +/- 1.07	0.38 +/- 0.74	0.00 +/- 0.00
Pentatomid species	0.00 +/- 0.00	0.13 +/- 0.35	0.00 +/- 0.00
Acridid species	0.00 +/- 0.00	0.38 +/- 0.74	0.38 +/- 0.52
<i>Helicoverpa zea</i> (small)	0.00 +/- 0.00	0.38 +/- 0.52	0.63 +/- 0.92
<i>Helicoverpa zea</i> (medium)	0.00 +/- 0.00	0.00 +/- 0.00	2.88 +/- 2.42
<i>Helicoverpa zea</i> (large)	0.00 +/- 0.00	0.00 +/- 0.00	0.50 +/- 0.76
<i>Spissistilus festinus</i>	0.13 +/- 0.35	0.00 +/- 0.00	0.13 +/- 0.35
<i>Cerotoma trifurcata</i>	0.00 +/- 0.00	0.00 +/- 0.00	0.13 +/- 0.35
<i>Chaetocnema pulicaria</i>	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
Meloid species	0.00 +/- 0.00	0.00 +/- 0.00	0.13 +/- 0.35

† Soybean development stage

§ Sampling technique was eight replicates of 15 sweeps with a 38 cm diameter sweep net.

‡ “Small” are instars 1-2; “medium” are instars 3-4; “large” are instars 5-6.

Table B.3. Insect pest populations in the New Kent County, Virginia soybean test--1999.

Insect	-----Number of Insects per Sample-----		
	6 Aug. (R1)†	17 Aug. (R3)	2 Sep. (R5)
	Mean +/- SD§	Mean +/- SD	Mean +/- SD
<i>Plathypena scabra</i> (small‡)	1.25 +/- 1.28	0.50 +/- 0.53	1.88 +/- 2.42
<i>Plathypena scabra</i> (medium)	1.88 +/- 1.46	1.13 +/- 1.13	1.00 +/- 1.41
<i>Plathypena scabra</i> (large)	0.38 +/- 0.52	0.25 +/- 0.71	1.38 +/- 1.60
<i>Popillia japonica</i>	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
Pentatomid species	0.25 +/- 0.46	0.38 +/- 0.74	0.00 +/- 0.00
Acridid species	0.50 +/- 0.76	0.38 +/- 0.52	1.00 +/- 1.31
<i>Helicoverpa zea</i> (small)	0.00 +/- 0.00	0.13 +/- 0.35	2.25 +/- 1.67
<i>Helicoverpa zea</i> (medium)	0.00 +/- 0.00	0.00 +/- 0.00	1.50 +/- 1.07
<i>Helicoverpa zea</i> (large)	0.00 +/- 0.00	0.00 +/- 0.00	0.25 +/- 0.71
<i>Spissistilus festinus</i>	0.25 +/- 0.71	0.13 +/- 0.35	0.25 +/- 0.46
<i>Cerotoma trifurcata</i>	0.13 +/- 0.35	0.00 +/- 0.00	0.50 +/- 0.76
<i>Chaetocnema pulicaria</i>	0.13 +/- 0.35	0.00 +/- 0.00	0.00 +/- 0.00
Meloid species	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00

† Soybean development stage

§ Sampling technique was eight replicates of 15 sweeps with a 38 cm diameter sweep net.

‡ “Small” are instars 1-2; “medium” are instars 3-4; “large” are instars 5-6.

Table B.4. Insect pest populations in the Prince George County, Virginia soybean test--1999.

Insect	-----Number of Insects per Sample-----		
	6 Aug. (V4)†	17 Aug. (V7)	2 Sep. (R3)
	Mean +/- SD§	Mean +/- SD	Mean +/- SD
<i>Plathypena scabra</i> (small‡)	0.00 +/- 0.00	6.11 +/- 3.33	1.56 +/- 2.24
<i>Plathypena scabra</i> (medium)	0.67 +/- 0.71	4.22 +/- 3.46	1.78 +/- 1.48
<i>Plathypena scabra</i> (large)	0.11 +/- 0.33	0.67 +/- 1.00	2.00 +/- 1.50
<i>Popillia japonica</i>	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
Pentatomid species	0.00 +/- 0.00	0.00 +/- 0.00	0.22 +/- 0.67
Acridid species	0.22 +/- 0.44	0.00 +/- 0.00	0.33 +/- 0.71
<i>Helicoverpa zea</i> (small)	0.00 +/- 0.00	0.00 +/- 0.00	0.78 +/- 0.83
<i>Helicoverpa zea</i> (medium)	0.00 +/- 0.00	0.00 +/- 0.00	0.33 +/- 0.71
<i>Helicoverpa zea</i> (large)	0.00 +/- 0.00	0.00 +/- 0.00	0.11 +/- 0.33
<i>Spissistilus festinus</i>	0.22 +/- 0.44	0.33 +/- 0.50	0.33 +/- 0.71
<i>Cerotoma trifurcata</i>	0.22 +/- 0.44	0.33 +/- 0.50	0.00 +/- 0.00
<i>Chaetocnema pulicaria</i>	0.00 +/- 0.00	0.11 +/- 0.33	0.00 +/- 0.00
Meloid species	0.00 +/- 0.00	0.00 +/- 0.00	0.67 +/- 0.87

† Soybean development stage

§ Sampling technique was nine replicates of 15 sweeps with a 38 cm diameter sweep net.

‡ “Small” are instars 1-2; “medium” are instars 3-4; “large” are instars 5-6.

Appendix C. Response of double-crop soybean yields to reductions in LAI through insect defoliation.

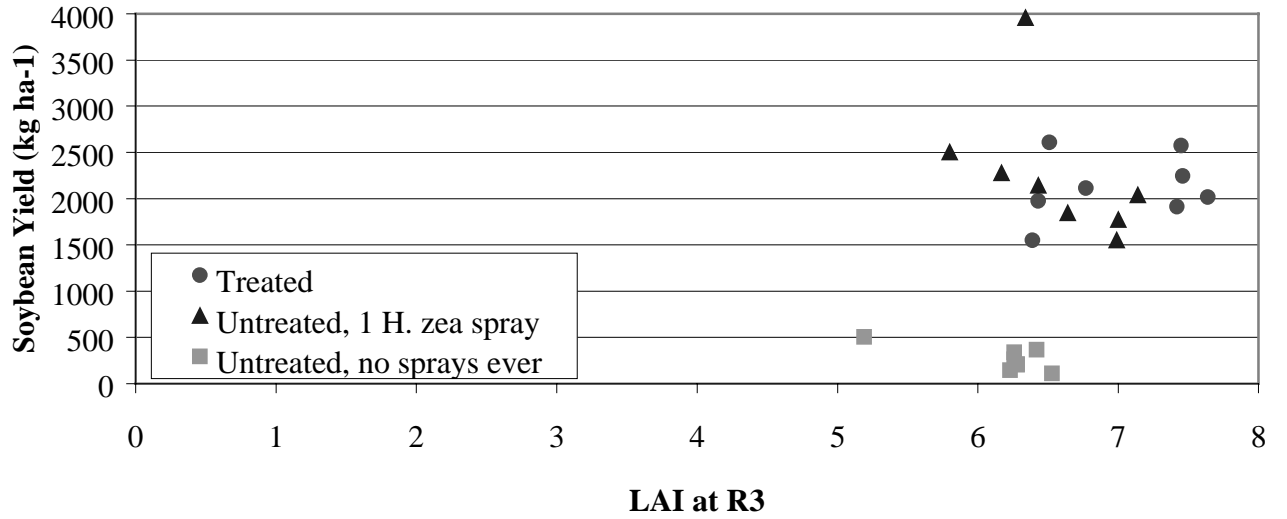


Fig. C.1. Yield response of double-crop soybean to reduction of LAI by insect defoliators in Essex County, Virginia--1999.

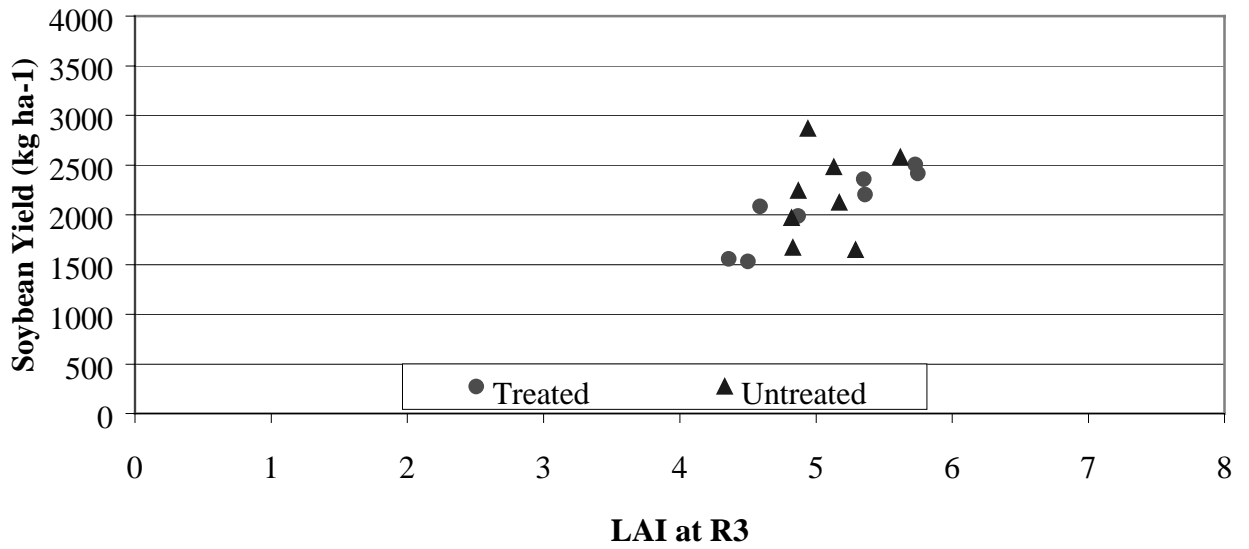


Fig. C.2. Yield response of double-crop soybean to reduction of LAI by insect defoliators in Middlesex County, Virginia--1999.

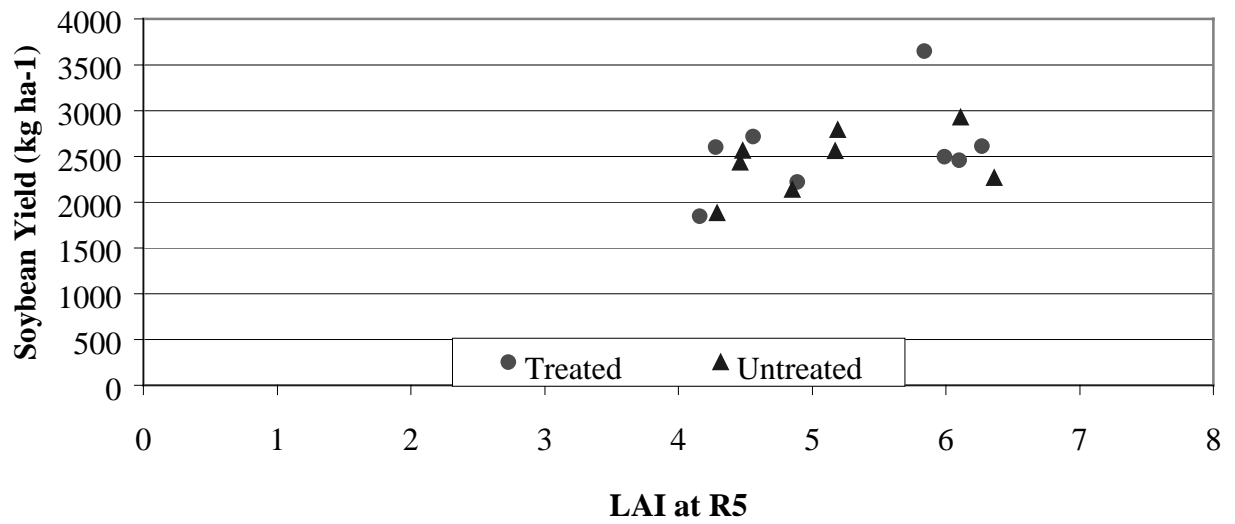


Fig. C.3. Yield response of double-crop soybean to reduction of LAI by insect defoliators in New Kent County, Virginia--1999.

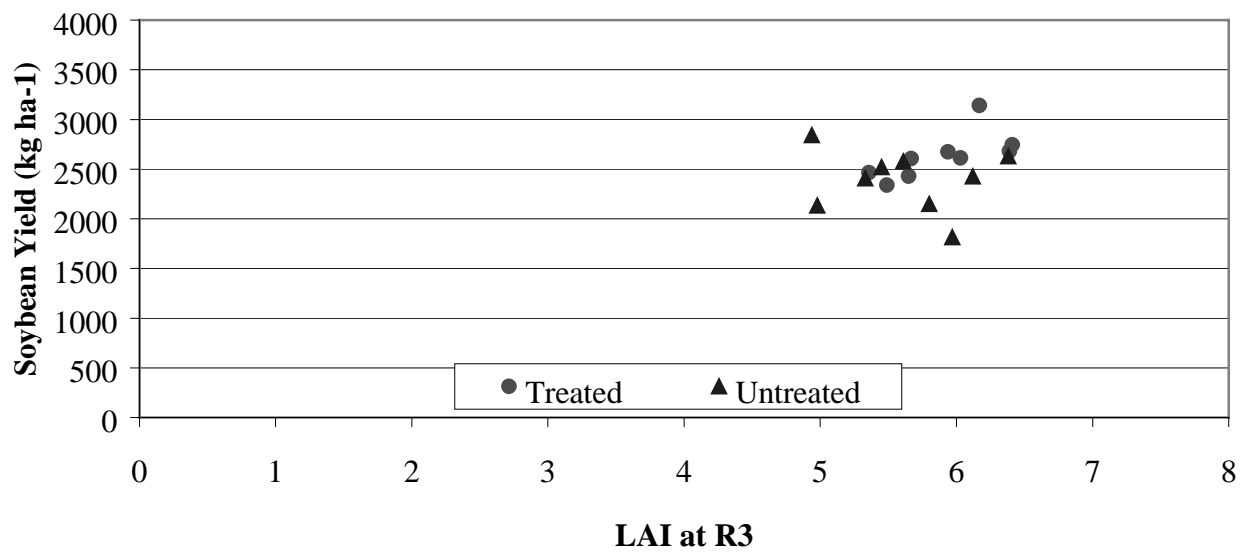


Fig. C.4. Yield response of double-crop soybean to reduction of LAI by insect defoliators in Prince George County, Virginia--1999.

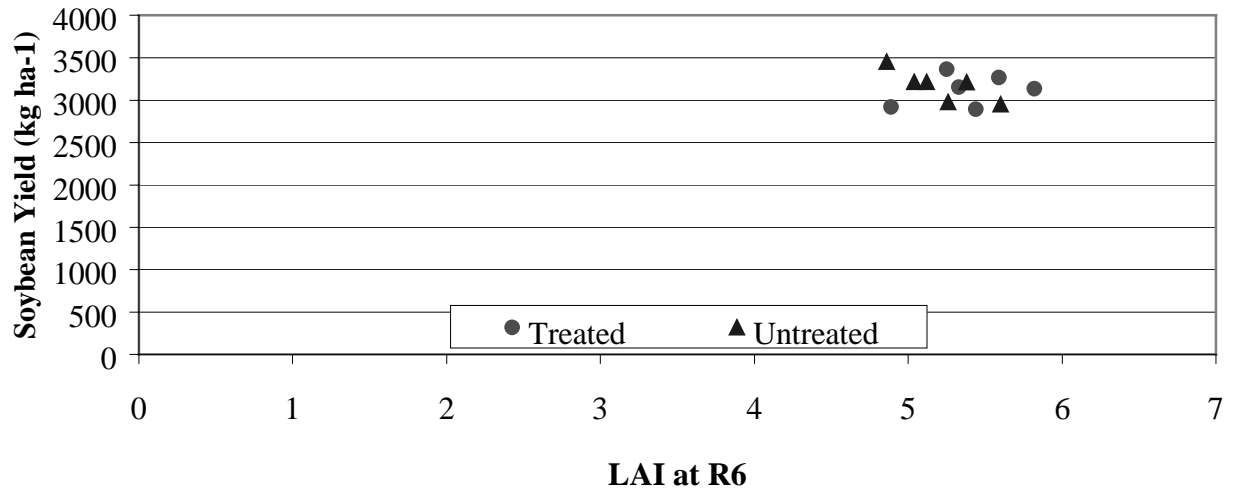


Fig. C.5. Yield response of double-crop soybean to reduction of LAI by insect defoliators in Essex County, Virginia--2000.

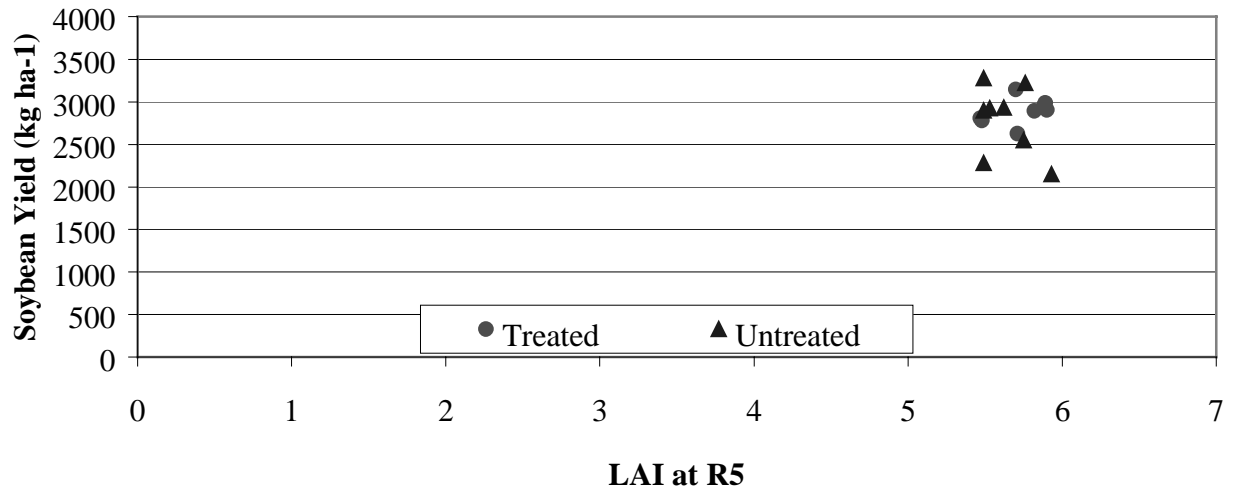


Fig. C.6. Yield response of double-crop soybean to reduction of LAI by insect defoliators in Middlesex County, Virginia--2000.

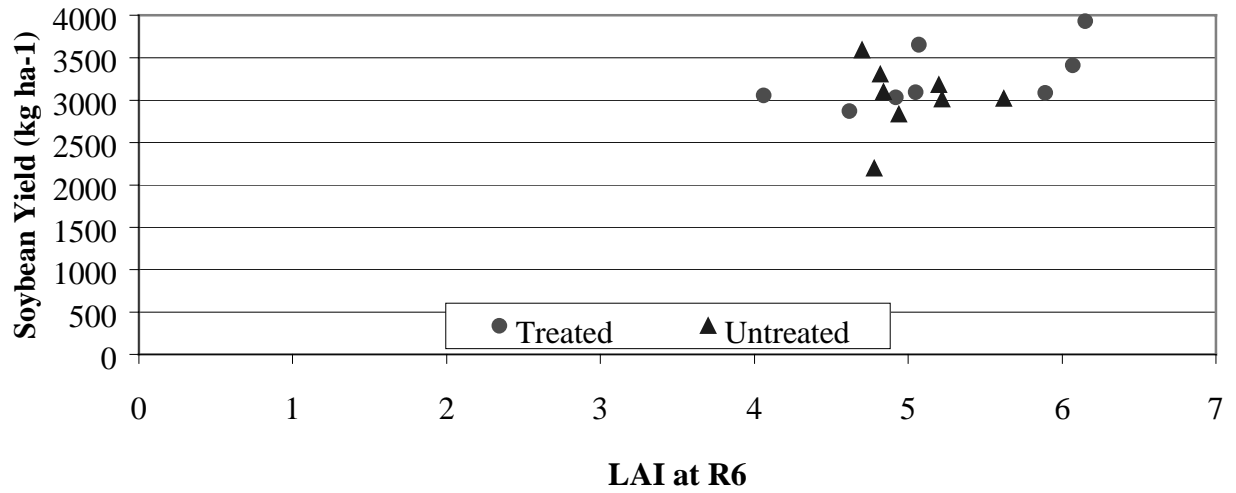


Fig. C.7. Yield response of double-crop soybean to reduction of LAI by insect defoliators in New Kent County, Virginia--2000.

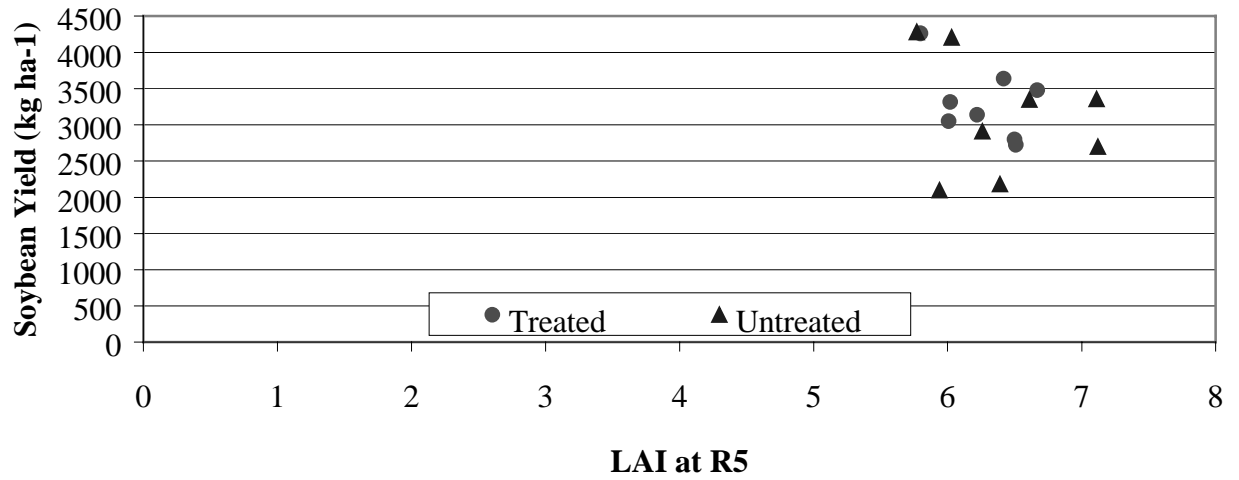


Fig. C.8. Yield response of double-crop soybean to reduction of LAI by insect defoliators in Prince George County, Virginia--2000.

Summary

Estimates of leaf area index (LAI) in soybean with the LAI-2000 Plant Canopy Analyzer are generally reasonable compared to directly determined LAI values, provided proper techniques are used. Like full-season soybean, double-crop soybean were found to require LAI values of at least 3.5 to 4.0 by mid-reproductive developmental stages for maximum yield potential. Average yield decreases approximately 847 kg ha⁻¹ for every unit decrease in LAI below the critical 3.5 to 4.0 level. Above this level, yields tend to plateau. Although current insect management thresholds are based on percent defoliation, including LAI in management decisions is more meaningful; therefore, LAI meters could play a role in soybean insect pest management. Locating portions of soybean fields with sub-critical LAI values indicates the “sick” areas of the field that should be monitored more closely for insect leaf feeders and could require protection. For example, a field that has defoliating insects present and an LAI of 3.5 or less could require pest control to prevent a yield loss. Therefore, redefining insect management strategies based on LAI should facilitate management of pest complexes in soybean.

Vita

Sean M. Malone received his Ph.D. degree in Entomology from Virginia Tech in Fall 2001.