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USING MANUAL DEFOLIATION TO SIMULATE SOYBEAN RUST: EFFECT ON GROWTH AND YIELD FORMATION

Abdullah Mohammad Aqeel
University of Kentucky, abd_aqeel@yahoo.com

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ABSTRACT OF DISSERTATION

Abdullah Mohammad Aqeel

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USING MANUAL DEFOLIATION TO SIMULATE SOYBEAN RUST: EFFECT ON GROWTH AND YIELD FORMATION

ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By
Abdullah Mohammad Aqeel

Lexington, Kentucky

Director: Dennis Egli, Ph.D., Professor of Crop Physiology
Co-Director: Saratha D. Kumudini, Ph.D., Assistant Professor of Crop Physiology

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Field experiments were conducted in Kentucky and Louisiana in 2008 and 2009 (split-plot in a randomized complete block design with four replications) to investigate it is possible to simulate with manual defoliation the effect of soybean rust (SBR) (*Phakopsora pachyrhizi* Syd. and P. Syd) injury on a healthy soybean [*Glycine max*, (L.) Merr.] canopy, understand how defoliation affects the growth dynamics and canopy light interception, and if defoliation affectsleaf senescence and nitrogen remobilization during the seed-filling period. Two manual defoliation treatments based on changes in effective leaf area index (ELAI) (calculated as the reduction in leaf area equivalent to SBR-induced premature leaf abscission, loss in green leaf area, and reduction in photosynthetic capacity of diseased leaves) in infected canopies in Brazil were used to simulate SBR infection at growth stage R2 (full flowering) and R5 (beginning of seed-fill). Both defoliation treatments reduced yield in all experiments and the reduction was larger for the treatments at growth stage R2. The yield losses were equivalent to that observed in infected soybean canopies in Brazil. This suggests that a system of manual defoliation to simulate changes in effective leaf area duration shows promise as a tool to simulate the impact of SBR on soybean yield. The radiation use efficiency and crop growth rate from growth stage R2 to R5 were not influenced by defoliation. Defoliation started at growth stage R2 reduced seed number per unit area, while defoliation started at growth stage R5 reduced seed size due to shortening the seed-fill duration and a lower seed growth rate. There is no evidence that manual defoliation affected leaf senescence or nitrogen redistribution to the seed. This study found that the reduction of light interception by SBR was the main reason for the reductions in soybean growth and yield.

KEY WORDS: Soybean rust, Defoliation, Effective leaf area index, Effective leaf area duration, Nitrogen redistribution.

Abdullah Mohammad Aqeel

July 8, 2011

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Abdullah Mohammad Aqeel

Dennis B. Egli, Ph.D.
Director of Dissertation

Dennis B. Egli, Ph.D.
Director of Graduate Studies

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To my mother for her love and Dow’a
To my lovely wife Muna
To my lovely kids, Bayan and Malaak and Yazeed

بســـــم الله الرحمـــــن الرحيـــــــم

قُلْ يَا عِبَادِيَ الَّذِينَ أَسْرَفُوا عَلَى أَنفُسِهِمْ لا تَقْنَطُوا مِن رَّحْمَةِ اللَّهِ إِنَّ اللَّهَ يَغْفِرُ الذُّنُوبَ جَمِيعًا إِنَّهُ
هُوَ الْغَفُورُ الرَّحِيمُ ﴿۞۞﴾ وَأَنِيبُوا إِلَى رَبِّكُمْ وَأَسْلِمُوا لَهُ مِن قَبْلِ أَن يَأْتِيَكُمُ الْعَذَابُ ثُمَّ لا تُنصَرُونَ
﴿۞۞﴾ وَاتَّبِعُوا أَحْسَنَ مَا أُنزِلَ إِلَيْكُم مِّن رَّبِّكُم مِّن قَبْلِ أَن يَأْتِيَكُمُ الْعَذَابُ بَغْتَةً وَأَنتُمْ لا تَشْعُرُونَ
﴿۞۞﴾ أَن تَقُولَ نَفْسٌ يَا حَسْرَتَىٰ عَلَى مَا فَرَّطتُ فِي جَنبِ اللَّهِ وَإِن كُنتُ لَمِنَ السَّاخِرِينَ ﴿۞۞﴾ أَوْ
تَقُولَ لَوْ أَنَّ اللَّهَ هَدَانِي لَكُنتُ مِنَ الْمُتَّقِينَ

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CHAPTER ONE

LITERATURE REVIEW

Classification, History, and Importance of Soybean

Soybean (*Glycine max* (L.) Merrill) is a legume, so it belongs in the Fabaceae, in the subfamily Faboideae. The genus *Glycine* Wild. is divided into two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *soja* includes the cultivated soybean, *G. max* (L.) Merr., and the wild soybean, *G. soja* Sieb. and Zucc. Both species are annual and diploid with 2n=40 (Palmer et al., 1996).

According to the Food and Agriculture Organization (FAO), the global production of soybean in 2007 was 220 million metric tons [MMT; $8X10^9$ bushels] (FAO, 2010). Soybean is grown in many countries including the United States, Brazil, Argentina, India, China, Japan, Korea, Taiwan and Russia. The U.S. is the world's leading soybean producer and exporter. The total soybean production in the U.S. in 2008 was 80.7 MMT [$3X10^9$ bushels], followed by Brazil (59.2 MMT), Argentina (46.2 MMT), China (15.5 MMT), and India (9.9 MMT) (FAO, 2010). The U.S. exported 27.9 MMT [1.0 billion bushels] of soybean with a value of $12.9 billion in 2007, accounting for 37% of the world's soybean trade. Soybean represents 54% of the world oilseed production. Other oilseed crops such as rapeseed (*Brassica napus* L.), cottonseed (*Gossypium* spp. L.), peanut (*Arachis hypogaea* L.), and sunflower (*Helianthus annuus* L.) accounted for 15, 10, 9, and 8% of world oilseed production, respectively (FAO, 2010).

Physiology of Soybean Yield Production

Understanding the physiology of soybean yield production is important to understand the effect of disease, e.g. soybean rust, on yield. Yield can be defined as a function of the radiation absorbed by the crop canopy over the growing season, the conversion of the absorbed solar radiation into plant dry matter (i.e, radiation use efficiency), and the proportion of total plant dry matter accumulated during the growing season that is allocated to the seed (i.e., harvest index) (Hay and Porter, 2006, p. 145-153). There are many factors that affect the plant's ability to accumulate dry matter including the rate of photosynthesis per unit leaf area, leaf area duration (combination of leaf area and time), intercepted solar radiation, and the plant's ability to tolerate stress. To understand the process of yield formation, focusing on the yield components can be helpful.

Yield Components

The basic soybean yield components are the number of plants per area, number of nodes per plant, number of pods per node, number of seeds per pod, the number of seed per unit area and seed size (weight/seed) (Egli, 1998, p. 86-87). Understanding the role of yield components in yield determination may reveal the answer of how to improve yield. Yield involves the integration of numerous physiological processes over the plant's life cycle. Therefore, it is useful to divide the plant's life cycle into several phases based on the characteristics of the plant.

Murata (1969) divided crop development into three phases: Phase I, formation of organs for nutrient absorption and photosynthesis (vegetative growth); Phase II, formation of flower organs and the yield container (flowering and pod set); and Phase III, production, accumulation and translocation of “yield contents” (seed filling). The phase I represents vegetative growth where the plant produces the leaf area and roots needed to provide and maintain canopy photosynthesis. This phase is represented in the Fehr and Caviness (1977) growth staging system by V stages. Phase II represents the development of reproductive structures and seed set, and it is represented in the Fehr and Caviness (1977) system by the period from growth stage R1 (initial flowering) until approximately shortly after growth stage R6 (seed fills pod cavity at one of the four uppermost nodes on the main stem with a fully developed leaf).

The number of flowers produced during phase II was related to the number of nodes (Egli, 2005), and to environmental conditions (Jiang and Egli, 1993). The number of seeds per unit area is fixed between the beginning of this phase and approximately 4 to 7 days after the beginning of growth stage R6 (Egli, 2010). Seed number per area is related to canopy photosynthesis during flower and pod set (phase II), so the environmental conditions that affect photosynthesis will indirectly affect seed number. The variation in yield due to environmental conditions is usually associated with variability in seed number (Board and Harville, 1993; Hardman and Brun, 1971). Increasing photosynthesis with CO_2 enrichment increases pod and seed number (Hardman and Brun, 1971), while shade, water stress and defoliation reduce pod and seed number (Board and Tan, 1995; Egli and Zhen-wen, 1991; Kumudini et al., 2008a).

The beginning of phase III marks the beginning of the accumulation of yield. This phase is assumed to start at growth stage R5; by shortly after growth stage R6 all pods are established and there will be no changes in the number of pods during the rest of reproductive growth (Egli, 2010). The plants reach physiological maturity at growth stage R7 (at least one pod on main stem has reached mature color) (Fehr and Caviness, 1977). The period from growth stage R5 to physiological maturity (R7) is the seed-filling period. Seed size is determined during this period. In soybean the seed-filling period is usually 30 to 40 days long, which is about 40% of the total growth cycle for most soybean cultivars grown in their area of adaptation (Egli, 1994, 2004).

Seed size may or may not be related to yield (Egli, 1998, p. 76-80). Variation in environmental conditions or defoliation during the seed-filling period, that affect seed size also affect yield. Genetic variation in seed size, however, is not related to yield. Defoliation due to foliar disease during the seed-filling period reduced assimilate supply and therefore seed size was reduced (Board et al., 2010; Kumudini et al., 2008a). Historically there is an inverse correlation between seed number and genetic variation in seed size (Board and Harville, 1993; Hartwig and Edwards, 1970). This is why the yield component approach used by some plant breeders failed to increase yield (Egli, 1998, p. 70-74).

Murata’s (1969) three phases describe yield production as a sequential process and each yield component (seed number and seed size) is determined during a specific phase of plant development (Egli, 1998, p. 70-74). The length of each phase (i.e., time) may affect yield production (Egli, 1998, p. 70-74). There are many crop process and characteristics that affect crop development and yield directly or indirectly, such as LAI, light interception (LI), and canopy photosynthesis, and these affect may be very phase specific. These processes will be discussed in more detail.

Leaf Area Index

The LAI is defined as the ratio of leaf area to land area, and it is related to canopy photosynthesis and crop yield (Evan, 1993, p. 179-189; Loomis and Connor, 1992, p. 36-38). Some researchers estimated that the critical LAI (LAI that intercepted 95% or more of the incident solar radiation) for soybean was between 3 and 4 (Board, 2004; Board and Harville, 1992). Delayed planting of many field crops reduces LAI, as did shortening the vegetative growth period when day length triggered early flower initiation (Hay and Porter, 2006, p. 55-56). With many field crops, higher plant densities tend to have higher LAI (Hay and Porter, 2006, p. 55-56) and generally, plants that suffer water stress have lower LAI than well-watered plants. Nitrogen stress can also reduce LAI (Hay and Porter, 2006, p. 55-56). Insects and foliar diseases also cause severe reductions in the quantity and quality of the leaf area which reduces solar radiation interception and photosynthesis (Hay and Porter, 2006, p. 59).

Integrating LAI over time produces the leaf area duration (LAD) (Evan, 1993, p. 179-189) which is also an important factor determining yield. Foliar diseases affect LAI and LAD (Bassanezi et al., 2001; Waggoner and Berger, 1987). The LAD is a better predictor of yield than disease severity, because LAD (the duration of the healthy or effective leaf tissue) is directly related to yield, (Waggoner and Berger, 1987). The relationship between LAD and yield has been tested in many crops including wheat (*Triticum* spp), maize (*Zea mays* L.), potato (*Solanum tuberosum* L.), and peanut (*Arachis hypogaea* L.) over a range of environmental conditions in many years. In these studies manual defoliation reduced the LAD (Rotem et al., 1983a; 1983b; Waggoner and Berger, 1987; Wilkerson et al., 1984), and yield loss was related to the reduction in LAD. Kumudini et al. (2001) reported that the high yield of newer soybean cultivars was attributed to a longer LAD. Bassanezi et al. (2001) found that foliar diseases of bean plants (*Phaseolus vulgaris* L.) affected the green LAD by reducing the area that was photosynthetically active. Other foliar diseases that caused defoliation also reduced the LAD (Bastiaans, 1993; Jesus Junior et al., 2003; Waggoner and Berger, 1987).

Soybean rust decreases the photosynthetic activity of the leaf area surrounding the lesions (Kumudini et al., 2008b), which can be accounted for with an estimate of the equivalent leaf area called the effective leaf area index (ELAI) (Jesus Junior et al., 2003). The effective leaf area (ELA) is the leaf area that is photosynthetically active. The impact of foliar disease on yield is cumulative throughout seed filling (Bergamin Filho et al., 1997; Bassanezi et al., 2001), and therefore, ELAI must be integrated over the course of the disease interaction with the host to produce the effective leaf area duration (ELAD) (Jesus Junior et al., 2003; Aquino et al., 1992).

The leaves store carbon and nitrogen that is mobilized to the seeds during seed filling. Therefore, reducing LAI during the seed-filling period may have a larger effect on yield than that due simply to the reduction in LI.

Light Interception

Solar radiation is the source of energy for photosynthesis for plant growth and development. Canopy photosynthesis is directly related to LAI and radiation interception. The LI will eventually reach a plateau with increasing LAI (Shibles and Weber, 1965, 1966). Therefore, increasing the LAI above the critical LAI (LAI between 3 and 4) will not increase LI or change the canopy photosynthesis (Shibles and Weber, 1965). When a healthy crop receives adequate water and nutrients, and reaches the critical LAI, dry matter production is at a maximum.

There are several factors affecting LI, including cultural practices (i.e., planting date, row spacing, population density), and leaf angle (Hay and Porter, 2006, p. 66). The cultural practices affect LAI which in return affects LI. Therefore, any change in these traits may affect canopy photosynthesis. Board and Harville (1992) found that growing late-planted soybean in narrow row spacings increased LI and prevented yield loss. Canopy defoliation that reduces LAI may reduce LI.

Reducing critical LAI by biotic and abiotic stress will affect the ELAI, ELAD and may affect LI, and ultimately yield. However, the growth stage (i.e., time) when the stress occurres is very important in determining the affect on yield. Reductions in LAI early in the growing season during the vegetative phase may be compensated for by regrowth; however, if the reduction in LAI was larger than the regrowth, a reduction in LI is likely. Jiang and Egli (1995) reported that stress during phase I before the reproductive phase began does not affect yield if the LAI was large enough to maximize LI by the beginning of flowering. Reducing LAI without affecting LI (i.e., LI ≥ 90%) may not reduce photosynthesis or yield.

Radiation Use Efficiency

The dry matter produced per unit of intercepted solar radiation is radiation use efficiency (RUE). Researchers use RUE to evaluate crop productivity per unit area (Bonhomme, 2000; Kiniry et al., 1989; Purcell et al., 2002; Sinclair and Shiraiwa, 1993). However, estimating RUE requires frequent plant sampling to determine dry matter accumulation; thus, this method is not precise enough to detect small differences between treatments. Some researchers express RUE based on intercepted photosynthetically active radiation, while others express it based on solar radiation (Sinclair and Muchow, 1999). Radiation use efficiency is obviously photosynthesis dependent; therefore, any variation in photosynthesis will affect RUE (Sinclair and Muchow, 1999). The maximum RUE for soybean reported in the literature is 1.1 g MJ^{-1} (based on total solar radiation) (Sinclair and Shiraiwa, 1993).

Foliar pathogens and environmental conditions, such as temperature, water stress, and nutrient availability, directly affect photosynthesis, and therefore, indirectly affect RUE (Sinclair and Shiraiwa, 1993). However, very few studies have investigated the effect of foliar diseases on RUE (Bastiaans, 1993; Beasse et al., 2000; Kumidini et al., 2008a). Kumudini et al. (2008a) reported that the RUE during reproductive phase did not change in SBR infected plants in one year of the study but it changed in the other. The RUE of rice (*Oryza sativa* L.) inoculated with the foliar pathogen (*Pyricularia oryzae* Cav.) was reduced by 55% (Bastiaans, 1993). Beasse et al. (2000) reported a reduction in the RUE of pea plants (*Pisum sativum* L.) after inoculation with a foliar pathogen (*Mycosphaerella pinodes*). All of these studies concluded that the reduction in RUE was attributed to impact of the foliar disease on leaf photosynthetic activity. Foliar disease also increased growth respiration and that reduced the canopy photosynthesis (Beasse et al., 2000).

Cultural practices also affect RUE. Purcell et al. (2002) found that the RUE unexpectedly decreased as soybean population density increased. They proposed that this reduction was due to the fact that biomass samples did not include the fallen leaves and petioles, and the amount of nitrogen obtained from soil was limited by plant competition which affected specific leaf nitrogen concentration and lessened the RUE (Purcell et al., 2002). Increasing nitrogen rate on hybrid maize (*Zea Maize* L.) in semi-arid regions had a significant effect on RUE, so the recommended nitrogen rate is at a level that produces maximum RUE (Khaliq et al., 2008). Sowing date also influenced RUE in faba bean (*Vicia faba* L.) (Confalone et al., 2010). Earlier sowing date increased the RUE, while delaing planting reduced it. Foliar diseases can directly affect RUE and indirectly affect the length of the seed-filling period, by inducing early leaf senescence and reducing the LAD.

Seed-Fill Duration

Murata's phase III is the seed-fill period, when seeds accumulate dry matter and yield is finally produced. Longer seed-fill durations (SFD) have contributed to higher yield in many crops. The SFD represents less than half of the total growth cycle for many crops (Egli, 2004), for example in soybean the SFD represented 26 to 41% of the total crop growth cycle (Egli, 1994; Zeiher et al., 1982).

Many researchers have investigated the impact of abiotic stress on seed-fill duration (de Souza et al., 1997; Egli et al., 1978; Rotundo and Westgate, 2010). de Souza et al. (1997) and Brevedan and Egli (2003) reported that water stress during seed filling shortened the filling period by accelerating leaf senescence and reducing yield. This acceleration did not reverse when the stressed soybean plants were returned to well-watered conditions after 3 to 5 days of stress (Brevedan and Egli, 2003), suggesting that relatively short periods of stress during seed filling may have a greater than expected effect on yield. Variation in the nitrogen supply may also effect soybean SFD. Hayati et al. (1995) reported that nitrogen stress during seed filling shortened the seed-filling period in soybean.

The SFD may be influenced by biotic stress (Kumudini et al., 2008a), but, little information is available on the effect of foliar diseases on SFD. Foliar disease, such as SBR, may result in accelerated leaf abscission which may reduce yield. Board et al (2010) investigated the effect of partial and total defoliation during seed-filling. They reported that the yield loss was associated with a reduction in LI. However, they failed to determine if the defoliation affected the SFD. Kumudini et al. (2008a) proposed that SBR infected canopy at growth stage R2 shortened the soybean life cycle. However, they also did not measure the seed-filling period.

Because foliar diseases reduce LAI, LI, and LAD (Bassanezi et al., 2001; Kumudini et al., 2008a; Waggoner and Berger, 1987), it probably reduces the SFD. Severe defoliation, as caused by SBR, reduces LI and thus may shorten the SFD due to reduced assimilate availability to the seed. Seed filling continues as long as there is assimilate available to the seed and the seed is active in converting the assimilate into storage compounds; when seed growth stops due to a lack of assimilate as result of reduction of canopy photosynthesis, the filling period is terminated (Egli, 2004).

Longer SFD's are associated with a delay in leaf senescence (Egli, 2004). Modern soybean cultivars which have longer SFD, and thus higher yield, also exhibit a slower decline in canopy photosynthesis which is an indicator of delayed senescence (Wells et al., 1982).

Senescence

Leaf senescence is not separate from seed filling in grain crops; both are sequential and synchronous in monocarpic plants. Senescence is a "series of events concerned with cellular disassembly in the leaf and the mobilization of materials released during this process" (Thomas and Stoddart, 1980). Senescence during the seed filling period in soybean results in the remobilization of nitrogen and other nutrients from the vegetative plant parts to the seed (Egli et al. 1983; Zeiher et al., 1982).

Manipulation of leaf senescence could result in changes in seed fill-duration and yield. Stresses such as water deficits or limited nitrogen supplies accelerated leaf senescence and shortened the seed fill period of soybean (de Souza et al., 1997; Hayati et al., 1995). De-podding and seed removal delayed senescence (Neumann et al., 1983; Wittenbach 1982). Wittenbach (1982) found that the delay in senescence and photosynthesis decline started one week after de-podding. Maize genotypes that exhibit delayed leaf senescence usually have higher yields (Tollenaar, 1991). Egli (2004) concluded that “delayed senescence will increase the seed-fill duration only when the seed has the ability to continue growth and increase in size”.

Crop physiologists often use two main hypotheses to explain the cause of senescence in soybean. The first hypothesis states that the pods produce a killing hormone that is transported via phloem to the leaves and initiates senescence (Nooden, 1984, 1985). Leaves closest to the individual pod or pod cluster receive the signal that triggers leaf senescence. Soybean is a monocarpic plant in which de-podding can delay senescence, although it may not prevent the decline in photosynthesis and other parameters that are associated with senescence (Nooden and Leopold, 1988). In monocarpic plants, a tight correlation between the initiation of leaf senescence and development of reproductive organs has been observed which is possibly controlled by a coordinated signaling system (Biswal and Biswal, 1999). Investigations have been carried out to elucidate the genetic mechanism of leaf senescence in the model plant *Arabidopsis*. However, specific genes for induction of senescence have not been identified; the down regulation of photosynthetic genes has been proposed to be the possible signal for up-regulation and induction of senescence.

The second hypothesis is the “self-destruct” hypothesis, proposed by Sinclair and de Wit (1975) where nitrogen must be remobilized from the leaves to meet the high nitrogen demand by the developing seeds when nitrogen uptake from the soil is inadequate. The depletion of nitrogen from the leaves cause leaf senescence and regulates seed-fill duration. Remobilization of nitrogen from the leaves results in destruction of the photosynthetic machinery, reduces the ability of the plant to maintain growth and thus the plant "self-destructs". They proposed that the self-destruct characteristic would limit the length of the seed-filling period and thus limit yield. Their simulation model was based on the “self-destruct” concept and relied on two assumptions. First, seed growth rate (SGR, g m^{-2} d^{-1}) is determined by the amount of available assimilate (gross photosynthesis – respiration); and second, the rate of nitrogen mobilization is dependent on the SGR. In the model, higher assimilate production would increase SGR and cause a more rapid remobilization of nitrogen from the leaves causing an accelerated rate of leaf senescence and the duration of the seed-filling period would be limited by the self-destruct characteristic (Sinclair and de Wit, 1976).

Several crop physiologists have evaluated the “self-destruct” hypothesis (Egli, 2004; Hayati et al., 1995; 1996; Kumudini et al., 2002). Many studies of the effect of nitrogen supply on leaf senescence and seed-fill duration used plants grown in liquid media because it was easier to manipulate the nitrogen supply. Streeter (1978) found that removing nitrogen from liquid culture media did not increase the rate of senescence. Also, Egli et al. (1978) reported that increasing the amount of nitrogen in the solution medium did not delay leaf senescence. Contrary to the predictions of the “self-destruct” hypothesis, Hayati et al. (1995) reported that increasing photosynthesis by shade removal at growth stage R5 (beginning seed fill) increased seed growth rate and seed yield but did not accelerate leaf senescence. Hayati et al. (1995, 1996) concluded that there was no seed-nitrogen demand.

According to the Sinclair and de Wit model (1976), yield limitations in soybean can be overcome by prolonging the seed-filling period by lowering the seed growth rate and limiting nitrogen remobilization out of the leaves. However, Kumudini et al. (2002) found that soybean genotypes with higher yield did not remobilize more nitrogen to the seed than lower-yielding genotypes.

There are many concerns about the validity of the “self-destruct” hypothesis as a model of senescence. Some crop physiologists suggested that the “self-destruct” model is not consistent with much of the literature on seed growth and senescence. Thus, it seems that the “self-destruct” hypothesis does not provide useful information about yield limitations in soybean.

Harvest Index

Harvest index (HI) is an indicator of partitioning of dry matter to the seed at maturity in grain crops. The HI is equal to the seed mass (yield) divided by the total above ground biomass [vegetative mass + seed mass] at maturity (Donald, 1968). The HI is a final measure of the dry matter partitioned to the seed. The effects of foliar diseases on the partitioning of dry matter to the seed (i.e., HI) have been reported in only a few studies (Kumudini et al., 2008a). Board et al. (1994, 1997) reported a reduction in HI when manual defoliation was applied during seed-filling. Kumudini et al. (2008a) found that the reduction in HI due to SBR was higher when the infection occurred at growth stage R2, because of the severe reduction in yield as a result of defoliation. They also reported that the defoliation reduced yield through reduction in seed number with SBR onset at growth stage R2, and seed size with SBR onset at growth stage R5 which altered HI (Kumudini et al., 2008a). As seed filling progressed, the reductions in yield and HI due to defoliation diminished. Defoliation at growth stage R7 had no effect on yield and HI (Board et al., 2010). In summary, HI is influenced by the growth stage where the defoliation occurred. Earlier defoliation is expected to reduce HI more due to severe loss in yield.

Soybean Rust

Importance and Threat of Soybean Rust

Phakopsora pachyrhizi Syd., the causal organism of SBR, has a broad host range and it can infect many legume species including lima bean (*Phaseolus lunatus* L.), lupine (*Lupinus* L.), green bean (*Phaseolus vulgaris* L.), jicama (*Calopogonium caeruleum* (Benth.) Suoev.), and the wild legume kudzu (*Pueraria montana* var. *lobat* (Lour.) Merr.) (Ono et al., 1992; Rytter et al., 1984). *Phakopsora pachyrhizi* was restricted for a long time to tropical and sub-tropical countries in Asia, Africa, and South America, where it causes significant yield losses every year (Kawuki et al., 2003a; Levy, 2005; Wrather et al., 2001; Yorinori et al., 2005). Since then, the pathogen has spread to other countries including the U.S., where SBR was first reported in 1994 in Hawaii on islands of Oahu, Kakaha, Kauai, and Hilo (Killgore and Heu, 1994). On 6 November 2004, plants exhibiting SBR symptoms were discovered in a soybean field on a research farm near Baton Rouge, Louisiana (Schneider et al., 2005). A few weeks later, scientists in Alabama, Arkansas, Georgia, Florida, Mississippi, Missouri, South Carolina, and Tennessee confirmed the presence of SBR (Dorrance et al., 2007; Mullen et al., 2006). Soybean rust was confirmed for the first time In Kentucky in 2006 on the alternative susceptible host kudzu (Hershman et al., 2006).

Soybean rust is a devastating disease (Kuchler et al., 1984). It can cause yield losses from 40 to 80% (Hartman et al., 1991; Ogle et al., 1979). The optimum temperature for development of this disease is 15 to 28^{o}C, with 6 to 12 hours of moisture on the leaf required for spore germination (Dorrance et al., 2007). It is possible that SBR infection will spread to the central regions of the U.S. because the weather conditions in these regions are often within the range of conditions favorable for rapid disease development. If the disease is established in the main soybean production states in the U.S., it could cause losses of up to $7.1 billion annually (Livingston et al., 2004).

Pathogen Biology and Disease Cycle

Phakopsora pachyrhizi is an obligate parasite that needs living hosts for survival and reproduction (Agrios, 2005, p. 571-573). Environmental conditions that negatively affect the host's survival can also decrease the ability of the pathogen to reproduce and overwinter. At tropical locations, there is no restriction to *P. pachyrhizi* overwintering except for areas at higher elevations and some heat-stressed areas in South America and central Africa (Pivonia and Yang, 2004). In the U.S., *P. pachyrhizi* is likely to overwinter in the southern states and especially southern Florida (Pivonia and Yang, 2004). In Florida, the pathogen overwinters on an alternative susceptible host kudzu and other susceptible legumes, where it is protected from low temperatures by the plant's foliage (Jurick et al., 2007).

Once soybean is infected, the pathogen produces asexual reproductive structures called uredia, which continues to reproduce for several days (Goellner et al., 2010; Melching et al., 1979). One mature uredinium can produce over 2000 uredinospores in 40 days (Goellner et al., 2010; Yeh et al., 1982). However, environmental conditions affect formation and sporulation of the uredia. Inoculated plants incubated at temperatures of less than 20^{o}C had longer latent periods (infection without symptoms) than plants incubated at 20 to 25^{o}C (Goellner et al., 2010; Kochman, 1979). Development of uredia and uredinospore production are usually more frequent on abaxial surfaces of leaves, which escape exposure to direct sunlight and ultraviolet radiation (Bromfield et al., 1980; Goellner et al., 2010; Isard et al., 2006; Marchetti et al., 1975; Melching et al., 1979, 1988). The uredia are the most common reproductive structures that *P. pachyrhizi* produces. Telia were observed only under laboratory conditions; after incubation for 40 days at a 17–22^{o}C daily temperature cycle under high humidity (Poonpolgul and Surin, 1985).

Fresh uredinospores emerge from the uredia and are dispersed by air. Li et al. (2006a) reported that the clumps of 4 to 30 spores were collected from infected kudzu leaves. The aggregation of spores may reduce the distance of spore movement, but spore clumping probably protects the internal spores from desiccation (Li et al., 2006a).

After landing on susceptible host tissue, uredinospores germinate in the presence of free moisture (Goellner et al., 2010; Magnani et al., 2007; Marchetti et al., 1976). In general, developing germ tubes can elongate up to 185 µm, although they usually exhibited reduced growth under direct light (Koch and Hoppe, 1987). Six hours after germination, the uredinospores develop appressoria (Koch et al., 1983; Magnani et al., 2007; Mclean and Byth, 1981). Twelve hours after inoculation, appressoria were mature and the penetration process was started (Koch et al., 1983). In the appressorium, a funnel-shaped structure called the penetration hypha develops (Koch et al., 1983; Magnani et al., 2007). The hypha penetrates the host’s epidermal cell wall to reach the mesophyl where the fungal colonization begins. This process is usually completed 20 to 24 hours after inoculation (Koch et al., 1983). Obligate parasites, such as *P. pachyrhizi*, develop haustoria structures that are responsible for nourishing the fungus and the maintenance of the parasitic relationship with host cells (Agrios, 2005, p. 87-88; Goellner et al., 2010). Few studies have successfully demonstrated formation of haustoria during *P. pachyrhizi* colonization due to the difficulties associated with staining colonizing hyphae. Seven to nine days after infection, the reproductive structures are formed and the life cycle restarts.

Disease Symptoms

The symptoms of SBR are small (2 to 5 mm) green to brownish or red-brown lesions on lower leaflets with observable pustules (uredia). In most cases, initial lesions can be observed 7 to 9 days after infection; the uredia emerge on abaxial surface 2 days later. In severe infection, the lesions can be observed on stems and petioles (Hartman et al*.,* 1999). Severely infected leaflets will eventually turn yellow, resulting in premature leaf abscission (Kumudini et al., 2008a).

Soybean rust is often confused with other common soybean diseases; especially at early developmental stages before uredia are formed (Dorrance et al. 2007). However, the structure of the uredia is unique to soybean rust. Incubation of leaflets with suspect lesions for a few hours will allow uredia emergence and sporulation (Dorrance et al. 2007). Further diagnostic techniques include observation of uredinospores, serologic tests, and PCR analysis (Lamour et al., 2006).

Effect of Soybean Rust on Soybean

Foliar pathogens such as SBR reduce LAI, LAD, and photosynthetic efficiency (Bastiaans, 1991; Goodwin, 1992; Kumudini et al., 2008b). Consequently, soybean rust affects the plant canopy by accelerating leaf abscission and reducing effective green leaf area due to necrotic and chlorotic lesions on the remaining leaves, which limits photosynthesis and yield by reducing the plant's ability to intercept and absorb solar radiation (Kumudini et al., 2008a). However, the reduction in the photosynthesis of infected leaves extends beyond the area of the lesion.

Bastiaans (1991) proposed the calculation of a virtual lesion as a means of quantifying the effect of foliar disease on photosynthesis, and this concept has been used in several studies (Bassanezi et al., 2001; Bastiaans, 1991, 1993). The term virtual lesion was used to describe the area of the diseased leaf where photosynthesis was negligible. Bastiaans (1991) related the photosynthetic rate of a diseased leaf (P_x) to that of a healthy leaf (P_o) by: $P_x = P_o (1-X)^\beta$, where X is the proportion of the leaf area covered by visible lesions and β is defined as the ratio between the sizes of the virtual and the visual lesions. The value of β is determined experimentally from the relationship between disease severity and the proportionate reduction in photosynthesis. When the β coefficient is equal to 1, the virtual lesion area is the same as the visual lesion area, and there is no effect of the pathogen on photosynthesis of the remaining green tissue. On other hand, when β is larger than 1, the virtual lesion is larger than the visual lesion. The β coefficients of a number of plant pathosystems have been determined to assess the impact of lesions on leaf photosynthetic capacity (Bassanezi et al., 2001; Hartman et al., 1991; Garry et al., 1998; Robert et al., 2005), and substantial variation in β, ranging from 1 to 13, has been reported for pathosystems that included biotrophic or necrotrophic microorganisms. Recently, Kumudini et al. (2010) reported that β ranged from 2.1 to 3.0 for SBR.

Large economic losses are reported in areas where SBR occurs regularly (Bromfield and Hartwig, 1980; Kawuki et al., 2003b; Poonpolgul and Surin, 1980; Poonpolgul and Surin, 1985; Sharma and Mehta, 1996). After pathogen establishment on the North American continent, the expected net economic losses to growers, based on a theoretical model, were predicted to range from $640 million to $1.3 billion (Daberkow, 2004; Livingston et al., 2004) and were expected to vary by region.

The pattern of occurrence of SBR from 2005 until 2009 in the U.S. suggests that the disease may not reach epidemic proportions in the major soybean producing regions in upcoming seasons. The occurrence of SBR seasonal epidemics above 37°N latitude depends upon the build-up of inoculum in southern areas followed by its subsequent northward movement, and on local environmental conditions that are favorable for disease development (Dorrance et al., 2007). The current recommendation to determine if an SBR epidemic is possible in the next growing season is to monitor kudzu and other susceptible legumes for SBR development during winter to early summer period in the southern states.

Control Measures

To date, most, if not all, soybean cultivars grown in the U.S. are highly susceptible to SBR. Therefore, current control of SBR relies primarily on fungicides. Early research showed that three to five applications of mancozeb and benzimidazole fungicides suppressed SBR development (Sinclair and Hartman, 1995). Compounds from the triazole and strobilurin fungicide groups exhibited relatively high levels of SBR control (Levy, 2005; Miles et al., 2007; Patil and Anahosur, 1998). Individual formulations of azoxystrobin, tebuconazole, difenconazole, and mixed formulations of epoxiconazole with pyraclostrobin reduced SBR severity for 4 to 14 days after application (Godoy and Canteri, 2004). In addition to these chemicals, strobilurins, the newest class of fungicides, showed effective control of SBR.

With polycyclic diseases such as SBR, chemical control efficiency depends upon the correct timing of application (Mueller et al., 2009). In the first two seasons after the discovery of SBR in Brazil, severe epidemics were observed in regions where fungicide applications were delayed as a consequence of late disease diagnosis (Yorinori et al., 2005). Limited fungicide supplies, reduced availability of equipment for spraying, and wet weather conditions may also contribute to delayed fungicide applications which increase yield loss.

In Brazil, fungicides are recommended as a preventive measure or after early disease detection in the field (Godoy and Canteri, 2004). In regions of Brazil with extremely high disease pressure due to frequent rainfall, growers make up to five five fungicide applications to control SBR, but yield losses are still observed (Godoy and Canteri, 2004)

Few studies have evaluated the effect of cultural practices on SBR control (Kawuki et al., 2003a). Most SBR epidemics develop from secondary infections, which depend upon environmental conditions. Secondary infections can be reduced by cultural practices that reduce crop exposure to favorable conditions for SBR infection. Early planting of early maturity cultivars reduced the impact of SBR on soybean (Kawuki et al., 2003a; Yorinori et al., 2007). Yorinori et al. (2007) observed that the early planted crops were exposed to smaller amounts of natural inoculum, which delayed the onset of the epidemic. The early planting and early maturity reduced the period that the crop was exposed to environmental conditions favorable for SBR development. However, they did not investigate the impact of inoculum amount (low vs. high) on yield with early planting.

To date, there are six major genes known to confer resistance to SBR in soybean. These single dominant genes are designated Rpp1 (Mclean and Byth, 1981), Rpp2 (Bromfield et al., 1980), Rpp3 (Bromfield and Hartwig, 1980; Bromfield et al., 1980; Hartwig and Bromfield, 1983), Rpp4 (Hartwig, 1986), and Rpp5 (Garcia et al., 2008). There is also a more recently discovered unnamed gene (Monteros et al., 2007). However, some aggressive isolates of *P. pachyrhizi* are able to overcome these sources of single gene resistance (Bonde et al., 2006).To date, there are no commercial soybean cultivar containing all these genes or expressing resistance to all *P. pachyrhizi* races.

The mechanism by which resistant genotypes reduce the effect of SBR on yield is not well known. But, it is well known that SBR reduces the photosynthesis capacity of susceptible genotypes (Kumidini et al., 2008a; Kumudini et al., 2010). Fewer lesions formed on the canopy of resistant accessions (i.e., reduced disease severity) and there was less impact on leaf photosynthesis. Resistant genotypes also showed a lack of pathogen sporulation lesions on the leaf which minimizes the impact of the disease on canopy photosynthesis and yield (Kumudini et al., 2010).

In summary, fungicides are the only effective method available to control SBR. Cultural practices may reduce the SBR severity but they do not prevent yield loss. Incorporating all resistance genes in commercial soybean cultivars is necessary to develop resistant cultivars.

Effect of Defoliation on Yield

Defoliation, caused by biotic and abiotic stress, may occur at any time during Murata’s three phases of yield production. Yield is more sensitive to defoliation during the reproductive phase than the vegetative phase because yield is determined during this phase (Egli, 2004; Murata, 1969; Schaafsma and Ableet, 1994). Defoliation during the vegetative phase may or may not affect yield (Pickle and Caviness, 1984; Weber, 1955). Canopy recovery from leaf regrowth after defoliation during the vegetative phase may compensate for the reductions in leaf area and produce high LI during the flowering and pod set phase (Higley, 1992; Peterson and Higley, 1996), which could maintain maximum photosynthesis rates. However, Hunt et al. (1994) reported that severe defoliation delayed the achievement of the critical LAI, thus LI and dry matter accumulation were limited.

Defoliation during the reproductive phase may reduce LAI, LAD, and LI which, depending upon the amount results in lower yield (Board et al., 1994; Board et al., 2010; Kumudini et al., 2008a) as a result of reductions in canopy photosynthesis (Ingram et al., 1981). This reduction in photosynthesis limited both carbon and nitrogen supplies with the end result a reduction in dry matter accumulation (Kumudini et al., 2008a). Defoliation of the upper canopy reduced yield because of a severe reduction in LI inspite of a stimulation of photosynthesis of the remaining leaves (Higley, 1992; Klubertanz et al., 1996; Li et al., 2006b) due to exposure to more solar radiation. Board et al. (2010) found that narrow row spacings tolerated defoliation better than wide rows spacing because of improved the LI and larger LAI. Recently, Quijano and Morandi (2011) reported that the lateral leaflet removal of every developed trifoliolate increased pod initiation due to improved light penetration to the lower part of the canopy.

Yield loss at a given developmental stage is determined by the amount or percent of LAI removed and if the remaining LAI is below the critical level (Board et al., 1994; Board et al., 2010; Kumudini et al., 2008a; Peterson and Higley, 1996; Pickle and Caviness, 1984; Schaafsma and Ableet, 1994; Weber, 1955). For example, removing 20% of the LAI at growth stage R5 does not have the same effect on yield as removing 20% at growth stage R1, because the canopy at growth stage R1 would probably be below the critical level. Haile et al. (1998) found that high levels of defoliation (about 55%) at growth stage R2 reduced LI and yield and concluded that yield loss was directly related to the reduction in LI after defoliation. Fehr et al. (1981) reported that 80% yield loss occurred when 100% defoliation was applied during the growth stage R5 to R6 period. Board et al. (1994) reported that 100% defoliation at beginning of growth stage R6 resulted in a 40% yield loss, but the yield loss was only 20% for defoliation three weeks after beginning of growth stage R6. Board et al. (1997) found that removal of 41% of the LAI at growth stage R6 caused only an 8% yield loss, while 56% removal reduced yield by 17%. They concluded that, at growth stage R6, a significant yield loss occurred whenever defoliation reduced LI below 95%. In more recently study, Board et al. (2010) reported that soybean yield loss occurred when defoliation is large enough to reduce LI by 18 to 23%. Defoliation at growth stage R7 has no impact on yield. Generally, yield sensitivity to defoliation is high at beginning of reproductive phase and declines as seed filling progresses (Board et al., 2010), so the affect of defoliation is depends upon the reduction in LI and on the proportion of the yield that is accumulated when the defoliation occurs.

Several researchers reported that defoliation altered the reproductive cycle in soybean and corn (Barimavandi et al., 2010; Board et al., 1994; Kumudini et al., 2008a; Ingram et al., 1981; Jones and Simmons, 1983; Tollenaar and Daynard, 1978). One hundred percent defoliation of soybean and corn reduced photosynthesis to near zero and that caused seed growth to stop and reduced the duration of seed filling in both crops (Hunter et al., 1991; Jones and Simmons, 1983; Tollenaar and Daynard, 1978; Vieira et al., 1992).

Soybean compensates for defoliation by delaying senescence and leaf abscission (Higley, 1992); however, the level of compensation depended on the amount of defoliation and the growth stage. Board et al. (2010) reported that defoliation shortened the effective filling period, and reduced crop growth rate from growth stage R1 to R5 (Board 1994; Board and Harville, 1993). Also, Schaafsma and Ablett (1994) documented that removing 66% of navy bean (*Phaseolus vulgaris* L.) canopy at growth stage R6 reduced time to maturity by one week.

Several studies reported that reductions in yield due to defoliation during the reproductive phase were associated with reductions in seed number (Board and Harville, 1993; Board et al., 2010; Caviness and Thomas, 1980; Higgins et al., 1984; Kumudini et al., 2008a), whereas others found that defoliation also reduced seed size (Board et al., 2010; Fehr et al., 1981; Ingram et al., 1981). This apparent controversy was due to the timing of defoliation treatments. If defoliation occurs early during the reproductive phase (Murata's phase II) it will primarily effect seed number (Board and Harville, 1993). When defoliation was applied during seed filling (phase III), a significant reduction in seed size occurred (Ingram et al., 1981).

In summary, the reproductive phase is the most sensitive stage for defoliation, and defoliation during reproductive phase may effect soybean yield and yield components. The amount of defoliation and the growth stage at which defoliation occurs determines which of the yield components is effected; usually only one is affected but not both. Determining the effect of defoliation on SFD and leaf senescence of soybean is warranted to develop effective management practices for SBR control.

Predicting Yield loss

Few studies have simulated foliar disease injury (Vasilas et al., 1989). Any effective simulation method that mimics disease injury would explain the reduction in plant growth. To develop accurate simulation methods that explain yield reductions across a wide range of environmental conditions, it is important to understand the impact of the injury on crop growth and the yield formation process (Savary et al., 2006). Plant injury is defined as a “stimulus producing an abnormal change in a physiological process” (Peterson and Higley, 2001, p. 6-7). Peterson and Higley (2001, p. 6-7) listed several types of canopy injury caused by pests including reductions in plant population, leaf mass, photosynthesis and assimilate supply, alternations in leaf senescence and LI, water balance disruption, fruit destruction and phenological disruption. Yield loss is a function of the magnitude and duration of the injury.

Some crop-loss assessments depend upon the relationship between yield and disease incidence or severity, and the area under the disease progress curve (AUDPC) (Jeger and Viljanen-Rollinson, 2001; Nutter et al., 2002; Schoeny et al., 2001; Subba Rao et al., 1990). The AUDPC is the intensity of disease integrated between two times of interest which is the quantitative disease injury over time (Jeger and Viljanen-Rollinson, 2001). Plant pathologists use AUDPC for yield loss assessment (Schoeny et al., 2001; Subba Rao et al., 1990). The AUDPC was used to quantify the level of resistance to many plant diseases, where higher AUDPC represented susceptible plants. It provides an effective, fast, and robust method to assessing disease progress because it makes it possible to characterize the disease intensity on plant canopy during the time period of interest (Jeger and Viljanen-Rollinson, 2001; Schoeny et al., 2001; Subba Rao et al., 1990). However, Waggoner and Berger (1987) reported that using disease severity and AUDPC to explain yield reductions did not provide a valid comparison among the treatments or between seasons because the LAI was not the same. Also, Jesus Junior et al. (2003) and Waggoner and Berger (1987) found that the AUDPC and disease's severity was not directly related to LI.

In the late 1980s and early 1990s, scientists started studying yield loss by investigating the relationship between disease infection and the physiology of yield formation. Some researchers used defoliation to estimate the relationship between the amounts of vegetation lost or the remaining healthy green leaf area, and yield (Bancal et al., 2007; Haile et al., 1998). Others accounted for disease effects on photosynthesis, assimilate supply, and biomass accumulation to estimate yield loss (Aggarwal et al., 2006; Bancal et al., 2007). Boote et al. (1983) and Johnson (1992) used the relationship between the LAI, LI and RUE to estimate yield loss from pests. Boote et al. (1983) developed a crop growth simulation model that provided a framework for estimating the effects of pests on crop growth and yield. Johnson (1992) used the relationship between the pest damage and yield to develop an empirical model to estimate the impact of pests on potato (*Solanum tuberosum* L.) productivity. Bancal et al. (2007) investigated the relationship between green leaf area and growth and developed a simple yield loss model for foliar diseases of wheat (*Triticum aestivum* L.).

Disease lesions on the leaf surface can compromise the ability of the canopy to absorb radiation. Bastiaans (1991; 1993) found that rice blast disease (*Magnaporthe grisea* [T.T. Hebert] M.E. Barr) reduced LI as a result of reduction in leaf area, and reduced photosynthesis of the remaining healthy leaf area which affect the RUE, both factors were related to the yield loss. Jesus Junior et al. (2003), Kumudini et al. (2008a), and Waggoner and Berger (1987) suggested that healthy leaf area (i.e., total leaf area minus the area of the leaves that is diseased), healthy LAD, ELAI, and ELAD are good estimates the impact of the disease on yield. Indeed, healthy LAD and the absorption of incident solar radiation by healthy leaf area have been shown to be excellent predictors of yield (Bancal et al., 2007; Bergamin Filho et al., 1997; Leite et al., 2006; Jesus Junior et al., 2003; Waggoner and Berger, 1987).

In summary, simulating disease injury could help predict yield loss. Investigation of the relationship between the disease injury and yield is essential to developing yield loss prediction tools. There are several factors involved in these relationships including LAI, LAD, and RUE, and any reduction in these traits may reduce yield.

Simulating Foliar Disease Injury

Many researchers investigated the impact of insects (Browde et al., 1994; Gustafson et al., 2006a, 2006b; Haile et al., 1998; Hammond et al., 2000; Herbert et al., 1992; Higgins et al., 1984; Hunt et al., 1994; Ingram et al., 1981; Peterson and Higley, 2001, p. 6-7; Talekar and Lee, 1988) and diseases (Schoney et al., 2001; Vasilas et al., 1989; Waggoner and Berger, 1987) on crop productivity. However, the objective of many of these investigations was to simulate the insect's impact, but not the disease's, on yield (Browde et al., 1994; Gustafson et al., 2006a; Haile et al., 1998; Hammond et al., 2000; Herbert et al., 1992; Higgins et al., 1984; Hunt et al., 1994; Ingram et al., 1981). Some researchers simulated insect injury with crop growth models (Yadav and Chander, 2010), while other developed models based on the epidemiology of the insect (Berger et al., 1995).

Most of the simulation methods relied on manual defoliation to investigate the relationship between crop injury and yield loss (Gustafson et al., 2006a, 2006b; Hammond 1989; Hammond et al., 2000; Herbert et al., 1992; Hunt et al., 1994; Talekar and Lee, 1988). Many of these researchers found that manual defoliation was an efficient and reliable method to simulate insect injury (Gustafson et al., 2006a; Hammond 1989; Hammond et al., 2000; Herbert et al., 1992; Higgins et al., 1984; Hunt et al., 1994), and helped to understand how insect caused yield loss (Gustafson et al., 2006a; Hammond et al., 2000).

Gustafson et al. (2006a) simulated the early season insect defoliation that is associated with weed interference to determine the effect on soybean growth and yield. They used three defoliation levels (0, 33, and 66%) conducted sequentially over a period of 7 days starting at growth stage VC. The simulation was effective in estimating yield loss from weed interference and insect infestation (Gustafson et al., 2006a). Hammond et al. (2000) used defoliation to develop a model to simulate insect injury. They found that the relationship between insect injury and yield loss was similar across the row widths tested, and the simulation accurately explained the yield loss (Hammond et al., 2000).

Very few studies used defoliation to simulate the impact of foliar diseases on crop productivity (Kumudini et al., 2008a; Vasilas et al., 1989). Vasilas et al.

(1989) simulated the impact of *Septorla* brown spot disease on soybean yield by manually defoliating from the bottom of the canopy upward to mimic the natural infection of the disease. The defoliation was applied at growth stage R5 and one week later by removing the leaf blades and petioles from the lowest three or four leaf-bearing nodes four times at four days intervals so that only three or four nodes at the top of the plant remained leaf-bearing when defoliation ceased. They found that yield responded to manual defoliation in similar way that it respond to diseased canopies. The defoliation at growth stage R5 reduced yield by 19% and 9% when defoliation was applied one week after R5.

One general approach to simulating defoliation can be extracted from the literature. This approach involves reducing the leaf area to the level caused by the pest; this reduction was expressed as percent of LAI removal or percent of the remaining leaf area (Bancal et al., 2007; Board et al., 2010; Fehr et al., 1977; Gustafson et al., 2006a, 2006b; Hammond 1989; Hammond et al., 2000; Herbert et al., 1992; Hunt et al., 1994; Kumudini et al., 2008a; Talekar and Lee, 1988). However, Herbert et al (1992) reported that defoliation based on reducing LAI did not predict yield loss as well as the total leaf area remaining after defoliation.

Cultural practices, such as row width, may affect the effectiveness of the simulation method through indirect effects on LI. Hammond et al. (2000) found that the relationship between the LAI or percent of LI and yield was dependent on row width. Also, they reported that large canopies (i.e., high LAI) tolerated larger amounts of defoliation without significant reductions in LI and, therefore, no significant reduction in yield. On the other hand, defoliation in small canopies (i.e., low LAI) reduced yield more, because there were significant reductions in LI (Hammond et al., 2000). However, the growth stage at which the pests infect crop is important when determining the relationship between a defoliation and yield loss. Kumudini et al. (2008a) found that the relationship between yield loss and the defoliation due to SBR at growth stage R2 and R5 was explained better than defoliation at growth stage R6.

Some studies used a single defoliation to simulate pest injury (Hammond 1989; Talekar and Lee, 1988) while other used sequential defoliation treatments (Gustafson et al., 2006a, 2006b; Kumuduni et al., 2008a). Comparison of single and sequential defoliation revealed that the sequential defoliation provided a better simulation of pest injury (Board et al., 2010; Fehr et al., 1977; Gustafson et al., 2006a, 2006b; Kumudini et al., 2008a). To develop an effective injury simulation method, an understanding of the mechanism of how the pest caused yield loss is needed.

In summary, many of the insect and disease simulations used manual defoliation to study the impact on yield. The effectiveness of defoliation depended on the ability to reduce LAI to similar levels as caused by the insects or plant diseases. Sequential defoliation probably is a better method for canopy defoliation because it simulates injury over time. There is very little information available about the impact of SBR on LAI and LAD. To our knowledge, no one has simulated SBR injury or investigated the impact of canopy defoliation on crop and seed growth rate or nitrogen mobilization.

Statement of Purpose

Soybean rust (SBR) (caused by *Phakopsora pachyrhizi* Syd. and P. Syd) is a devastating soybean disease, causing yield loss of up to 80%. If SBR is established in the soybean production regions of the U.S., severe yield losses could occur. Fungicides are the only means of control, since all currently available commercial soybean cultivars grown in the U.S. are susceptible to SBR. Understanding how SBR affects the soybean canopy and the yield formation process is critical to developing effective strategies to control SBR. Soybean rust affects the plant canopy by three mechanisms: i) it accelerates leaf abscission and reduces green-leaf area which limits yield by reducing the plant’s ability to intercept and absorb solar radiation, and ii) SBR lesions reduce photosynthesis by reducing the green leaf area, but the reduction in photosynthesis of green leaf area extends beyond the actual lesion area.

The impact of foliar disease on yield is cumulative throughout the seed formation process. Previous research showed that foliar pathogens, such as *P. pachyrhizi*, affect the leaf area duration (LAD) and effective leaf area index (ELAI). Reduction in LAI reduces the carbon and nitrogen available for remobilization to the seed during the seed-filling period. Therefore, understanding how SBR impacts LAD and ELAI is critical to managing SBR.

In the U.S. field inoculation of plants with SBR that is necessary to study the impact of SBR on soybean growth and yield production is difficult due to possibility of spreading the pathogen inoculum into neighbor field or states. Therefore, developing a method to simulate SBR injury and estimate yield loss was warranted. Reliable estimates of yield losses from SBR infestation in field conditions is a prerequisite for the rational development of any protection program. In this dissertation, healthy soybean canopies were artificially defoliated to simulate the impact of SBR on yield by changing the leaf area index based on data obtained from diseased canopies in Brazil. The purpose of this dissertation is (i) to develop a method to simulate SBR injury under field conditions, (ii) to investigate how SBR affects the growth dynamic of soybean, and (iii) to study how defoliation affects nitrogen remobilization to the seed during seed filling period.

CHAPTER TWO

SIMULATION THE IMPACT OF SOYBEAN RUST ON SOYBEAN YIELD WITH MANUAL DEFOLIATION

Introduction

Soybean rust (SBR), a destructive foliar disease caused by *Phakopsora pachyrhizi* Syd. and P. Syd, is major threat to U.S. soybean production (Hartman et al., 1991; Ogle et al., 1979). In areas where this disease is endemic, yield losses can be as high as 80%. Since its introduction to the U.S. in 2004, yield-damage levels of SBR have been restricted to the Southeastern production regions, but it could cause serious yield losses in the Midwest if the disease spreads to the Midwest with the right climatic conditions (Dorrance et al., 2007). Coping with this disease threat requires reliable estimates of yield losses from SBR under field conditions, which will help us understanding how the disease impacts yield production. Estimating yield losses is hampered by restrictions of spreading SBR to healthy neighbor field or states. An alternative approach is to use manual defoliation to simulate the impact of SBR on yield.

Previous levels and timings of defoliation research produced variable yield responses among environments and genotypes (Timsina et al., 2007). However, when defoliation effects across genotypes and environments were analyzed through their effect on light interception (i.e., their effect on canopy photosynthetic activity), genotype and environments effects disappeared (Board, 2004; Board et al., 2010; Browde et al., 1994; Ingram et al., 1981). In the current study we used a similar approach that involved the effective leaf area index

(ELAI) (Jesus Junior et al., 2003), which accounts for the effect of SBR on defoliation, the reduction in green leaf area (due to necrotic and chlorotic lesions) and the reduced photosynthetic rate of the remaining green leaves (Kumudini et al., 2008a, 2010). Because foliar diseases affect yield formation across an extended period rather than at one specific time (Aquino et al., 1992; Bassanezi et al., 2001; Bergamin et al., 1997; Jesus Junior et al., 2003), yield losses from SBR are best analyzed by integrating ELAI across time into the effective leaf area duration (ELAD) (Omielan et al., 2009).

Our hypothesis is that field plants manually defoliated to achieve ELAD that simulate the progress of SBR will have yields similar to those from the SBR-infested plants. Field inoculation of soybean with SBR that is necessary to study the impact of SBR on soybean growth and yield production and help determine the effective management practices to control SBR. Validity of this hypothesis would indicate that yield losses from possible SBR attacks in the U.S. could be approximated by manual defoliation. Although such a hypothesis is best tested in side-by-side studies with SBR-infested plots, this option is difficult in absent of SBR. Therefore, our objective was to test the aforementioned hypothesis by comparing ELAD and yields from a SBR-infested field in Brazil with manually-defoliated trials in the U.S. designed to simulate the ELAD of the Brazilian tests.

Materials and Methods

Culture

Field studies were carried out in Kentucky and Louisiana during the 2008 and 2009 growing seasons.

Kentucky Site

Studies at Lexington, Kentucky (38^o N Latitude) were conducted at the Spindletop Research Farm on a Maury silt loam soil (fine, mixed, semiactive, mesic Typic Paleudalfs). Seed of ‘AG3905’ (maturity group III, indeterminate) was machine planted on 13 May 2008 and 21 May 2009 into 38-cm rows 8-m long. Experimental units were nine-rows wide. Fertilizer was applied pre-plant only in 2008 according to soil test recommendations at a rate of 0-0-112 (kg ha^{-1} N-P-K). Lime was also added at a rate of 3362 kg ha^{-1} in 2008 and 2242 kg ha^{-1} in 2009. Seed were sown at a rate of 58 seed m^{-2} in 2008 and 67 seed m^{-2} in 2009. Recommended practices were used for control of weeds, diseases, and insects. Sprinkler irrigation was applied as needed to avoid drought stress.

Louisiana Site

Studies in Louisiana were conducted at the Ben Hur research station near Baton Rouge, LA (30^o N Latitude) on a Commerce silty clay loam soil (fine-silty, mixed, superactive, nonacid, thermic Fluvaquent Endoaquepts). Seed of ‘DP4331’ (maturity group IV, indeterminate) was machine planted on 17 April 2008 and 16 April 2009 into 97-cm rows 7.3-m long. Experimental units were four rows wide. Fertilizer was applied prior to planting according to soil test recommendations at the rate of 0-15-56-21 kg ha^{-1} N-P-K-S. Seed were sown at a rate of 28 seed m^{-2}. Recommended practices were used to control weeds, diseases, and insects. Sprinkler irrigation was applied as needed to avoid drought stress.

Defoliation Treatments

Defoliation during reproductive development was used to simulate the effect of SBR on soybean growth and yield starting at full bloom (growth stage R2, Fehr and Caviness, 1977) and the beginning of the seed-filling period (growth stage R5). The treatments were defoliation starting at growth stage R2, defoliation starting at growth stage R5, and an undefoliated control (Table 2.1). All defoliations were applied by removing leaves (leaving the petioles on the plant) from the bottom of the canopy upward to mimic the natural progression of leaf loss due to SBR (Kumudini et al., 2008a).

Table 2.1. Defoliation treatments used to simulate the effect of soybean rust (SBR) on leaf area in Kentucky and Louisiana.

Growth Stage	LAI removal to simulate SBR infection	
	at growth stage R2	at growth stage R5
	Percent of control[†]	
R2	2	---
R3	3	---
R4	3	---
R5	39	15
R5+1week	43	10
R5+2weeks	10	31
R5+4weeks	---	44
Total removal	100	100

[†] Percent of control LAI.

Briefly, the leaf removal levels in the defoliation treatments were determined as follows: 1) Leaf abscission and disease severity in an infected soybean canopy in Brazil (Kumudini et al., 2008a) were used to calculate the effective leaf area index (ELAI) during reproductive growth; 2) This ELAI and the LAI on the fungicide-protected control in Brazil were used to estimate the reduction in the ELAI resulting from SBR infection (Table 2.1, Fig. 2.1); and 3) The LAI of the defoliation treatments in the experiments reported here was reduced below the control at frequent intervals by the amount in Table 2.1 to simulate SBR infection starting at growth stage R2 and R5.

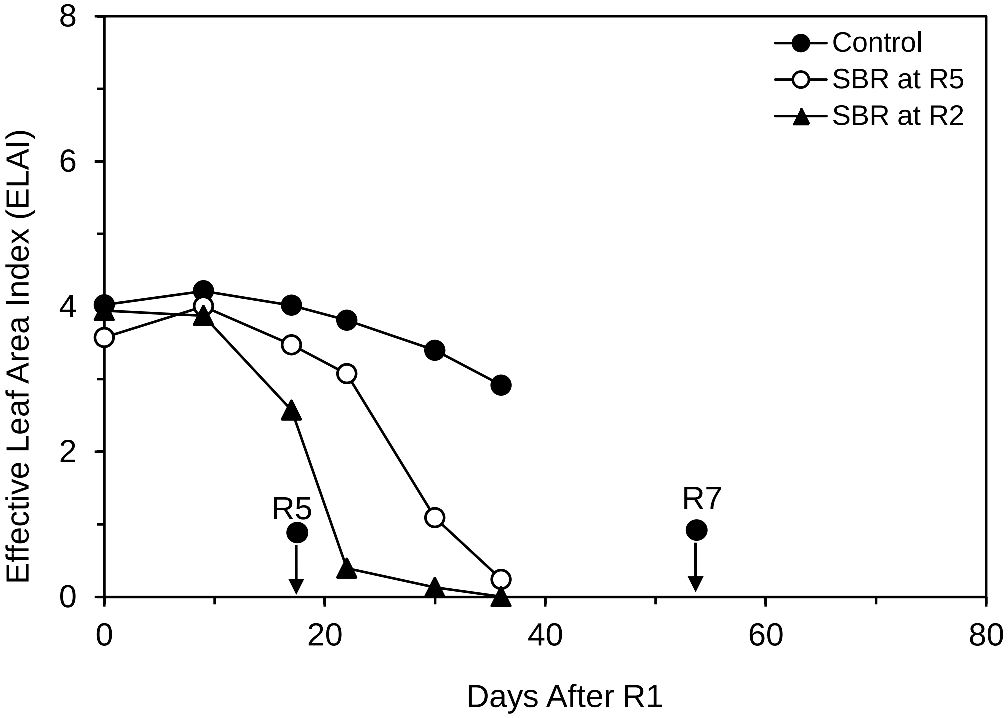

Figure 2.1. Effect of soybean rust (SBR) on effective leaf area index (ELAI) in a field trial with plants inoculated with *Phakopsora pachyrhizi* in Brazil in 2006/2007. Plants were inoculated at growth stage R2 and R5. Symbols and arrows at the x-axis indicate when the control reached growth stage R5 and R7.

The LAI of the control plots in Kentucky and Louisiana was determined four times at weekly intervals during growth stage R2 to R5 by removing all the leaflets from all plants in 0.5 m^2 from interior portions of the plot. The leaf area of a sub-sample of 150 leaflets selected randomly from lower-, mid-, and upper-locations in the canopy was measured with a LI-3000 leaf area meter (LI-COR Corp., Lincoln, NE). The fresh and dry weight of the sub-sample and the leaves remaining in the 0.5-m^2 sample were determined and used to estimate the leaf area of the entire sample. The sub-sample represented approximately 30% of the total sample, on average.

The maximum LAI occurs approximately at growth stage R5 (Carpenter and Board, 1997), and the LAI of the standing crop in the control plots from this point until physiological maturity (growth stage R7) was determined weekly by subtracting the LAI of the abscised leaves lost due to natural senescence from the LAI of control plots at growth stage R5. One m^2 wire enclosures were installed in the control plots to collect fallen leaves from growth stage R5 to R7 and the LAI of the fallen leaves was calculated as (number of fallen leaves) x (average area per leaf) per m^2.

The affect of SBR on leaf area and function was estimated in Brazil by the effective leaf area index (ELAI) which includes the reduction in green leaf area index as function of disease severity (Fig. 2.1) (Godoy et al., 2006) and the reduction in photosynthesis due to the SBR lesions (Bassanezi et al., 2001). The ELAI over time (Bassanezi et al., 2001; Jesus Junior et al., 2003) was calculated from $ELAI = LAI\ (1-X)^{\beta}$ where X is the disease severity and the β is the ratio between virtual and visual lesions. We used a β value of 3.0 based on estimates on field grown soybean genotypes (Kumudini et al., 2008a, S. Kumudini, personal communication, 2008). The effect of SBR was determined by comparing the ELAI of infected plants with LAI of fungicide protected controls and this ratio was used to calculate the defoliation level in Kentucky and Louisiana (Table 2.1). In the non-infected canopies in Kentucky and Louisiana the ELAI and LAI were equal.

Experiment Design

The experiments at both locations were randomized complete block designs with split-plot treatment arrangement with four replications. Main plots were the eight sampling periods simulating onset of SBR at growth stages R2 and R5, and split plots were the control and two defoliation treatments. Because yield was measured at maturity (growth stage R8) it was analyzed as RCBD with four replications.

Plant Phenology

Plant growth stage of 10 consecutive plants in the row was recorded twice a week (Fehr and Caviness, 1977). The same plants were measured throughout the season. A plot was considered to be at a particular growth stage when 50% or more of the plants had reached that stage.

Kentucky Yield

Plants from 4.6 m^2 of bordered rows were hand-harvested and threshed and weighted. Seed moisture concentration was determined and yield was adjusted to 130 g kg^{-1} moisture concentration.

Louisiana Yield

Yield was determined at maturity by sampling 4 m^2 of interior rows and determining the total fresh weight of the entire sample using a field scale. A 0.5-

m^2 sample was harvested to determine fresh weight and dry weight (oven dried at 60°C for 4 days), then threshed to determine seed weight, leaf and petiole dry weight, and HI. Yield calculated as follows: Yield = [(total fresh weight of 4-m^2) x (dry weight of 0.5-m^2/fresh weight 0.5-m^2) – leaf and petiole dry weight] x harvest index (fraction) (Board et al., 2010). Yield was adjusted to 130 g kg^{-1} moisture concentration. Yield data from the Louisiana-2009 experiment was not available.

Effective Leaf Area Duration

At both locations, the effective leaf area duration (ELAD) was calculated by integrating ELAI over time (Jesus Junior et al., 2003) as in the equation ELAD = $\sum_{1}^{n=i} [(ELAI_i + ELAI_{i+1})/2] \times (t_{i+1} - t_i)$, where n is the number of assessments, t_i is the time at which ELAI was evaluated, and $(t_{i+1} - t_i)$ is the interval (days) between two consecutive assessments.

Data Analysis

To verify assumptions of independently and normally distributed error, the means were plotted against their variance to confirm the errors homogeneity. Two-tailed *F*-test test was conducted to determine the homogeneity of variance among the experiments before the data were analyzed. The data were analyzed using Proc MIXED and significant means differences were determined by the SAS LSMEANS procedure (SAS 9.1; SAS Institute Inc., Cary, NC). Year and location were considered random effects, while treatments were considered a fixed effect.

Results and Discussion

In this study healthy soybean canopies were artificially defoliated to simulate the impact of SBR on the yield. Previous reports have shown that accelerated leaf abscission, reduction in the green leaf area, and reduced photosynthesis are all SBR-induced factors that can influence yield (Kumudini et al., 2008a; 2008b; 2010). There are many reports of the effect of defoliation on soybean yield (Board et al., 1994; Goli and Weaver, 1986; Haile et al., 1998; Vasilas et al., 1989); however, simulating SBR through modification of the canopy's ELAD has not been previously reported.

There was variability in maximum LAI among the four location/years of the experiment (Fig. 2.2 and 2.3). Leaf area index of healthy canopies increases until the crop reaches growth stage R5 (Fig. 2.2 and 2.3). This is in agreement with Board and Harville (1992), and Carpenter and Board (1997) who reported that soybean reaches maximum LAI approximately at growth stage R5, after which natural senescence causes a slow decline (Fig. 2.2 and 2.3). The larger LAI in Kentucky in 2009 compared with 2008 may be due to variation in ambient air temperatures between the two years, and the precipitation during vegetative growth (Fig. 2.4). The cumulative precipitation from planting until the plants reached growth stage R5 was 252 mm in 2008 and 344 mm in 2009 which, in combination with the cooler temperature and supplemental irrigation, may have created a more favorable environment for growth.

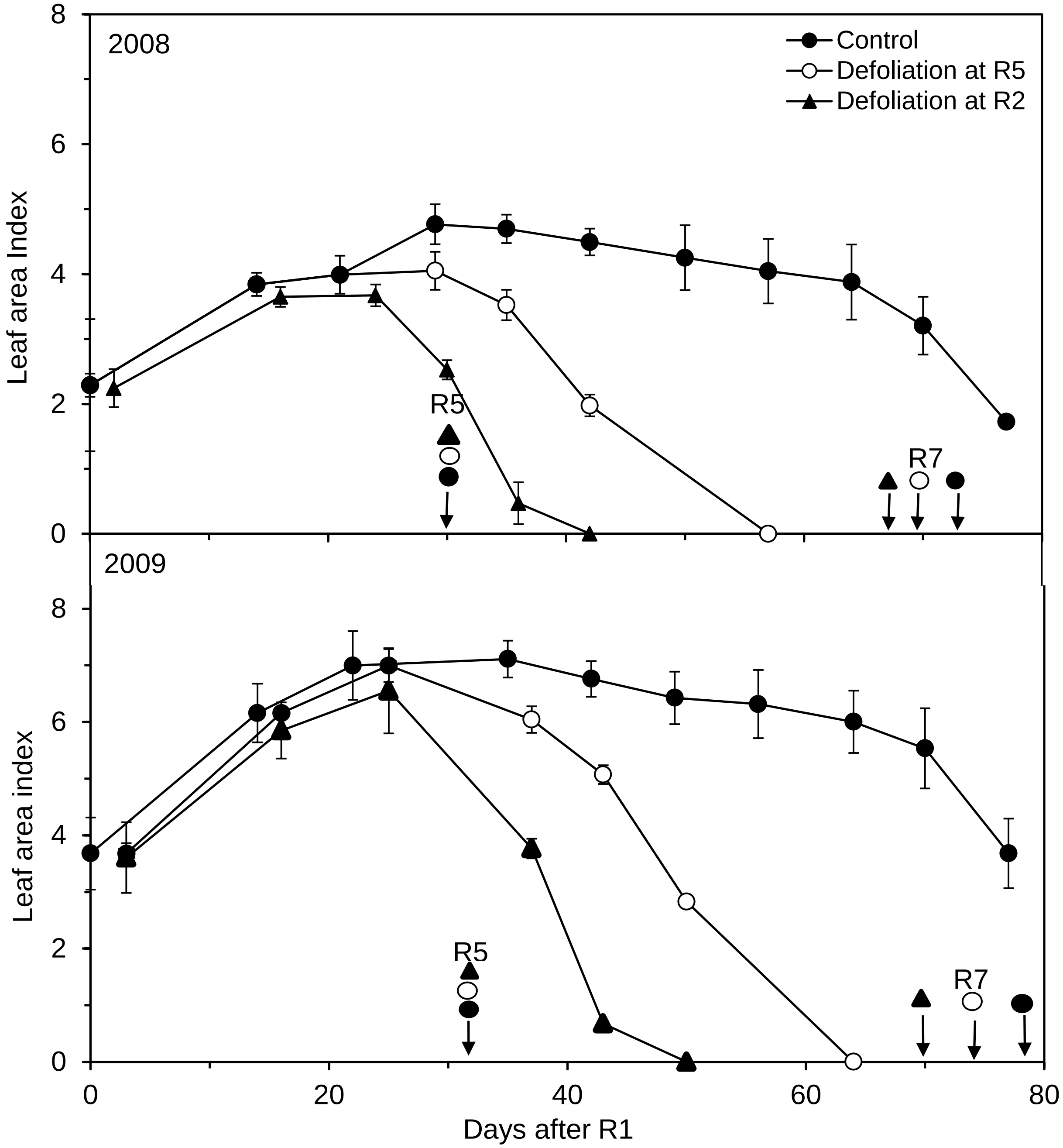

Figure 2.2. Leaf area index from flowering until physiological maturity for control and manual defoliation treatments simulating soybean rust onset at growth stage R2 and R5 in Kentucky in 2008 and 2009. Arrows on the x-axis indicate the occurrence of beginning seed fill (growth stage R5), and physiological maturity (growth stage R7). Vertical bars represent ± standard error of the mean.

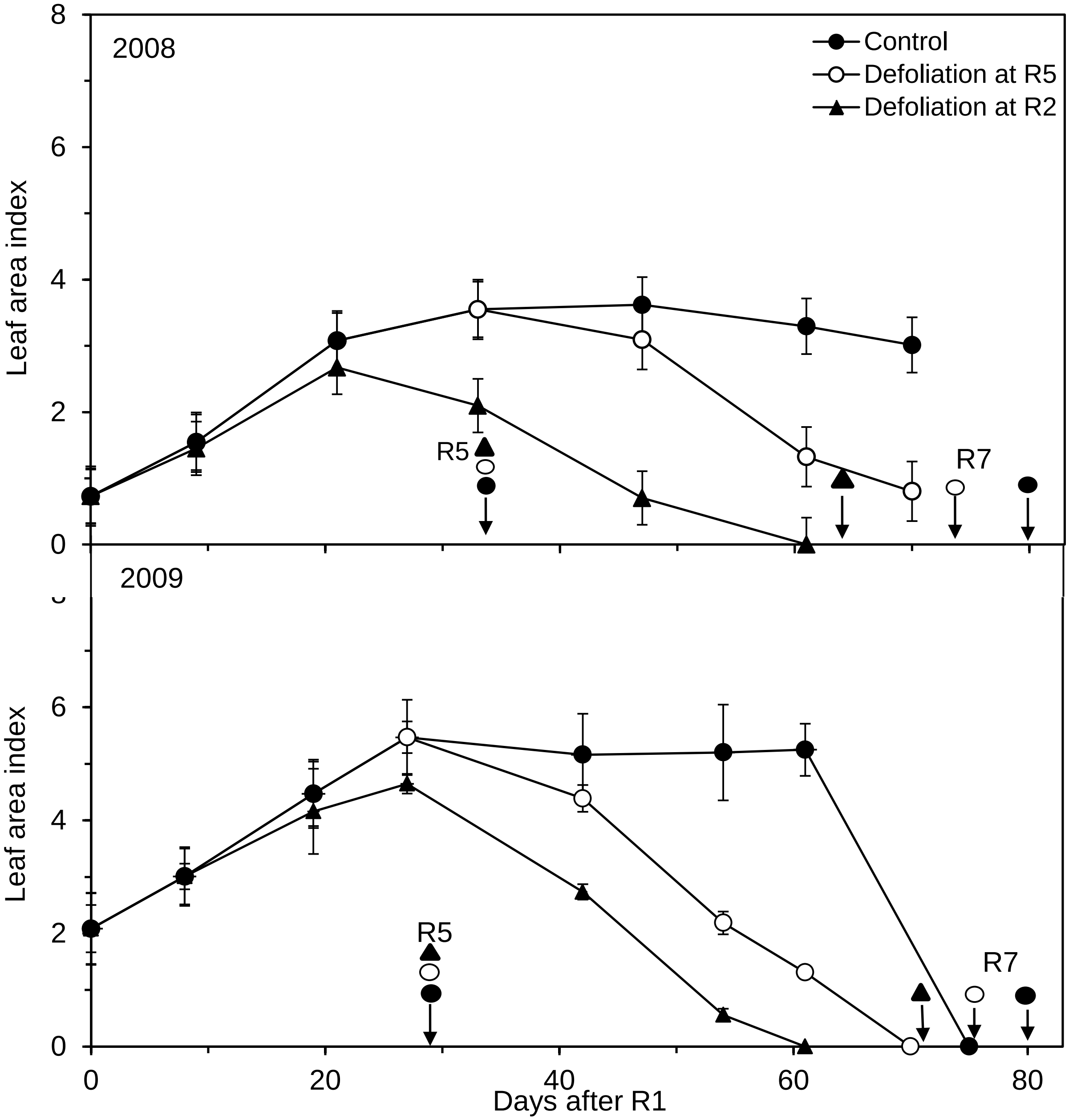

Figure 2.3. Leaf area index from flowering until physiological maturity for control and manual defoliation treatments simulating soybean rust (SBR) onset at growth stage R2 and R5 in Louisiana in 2008 and 2009. Arrows on the x-axis indicate the occurrence of beginning seed fill (growth stage R5), and physiological maturity (growth stage R7). Vertical bars represent ± standard error of the mean.

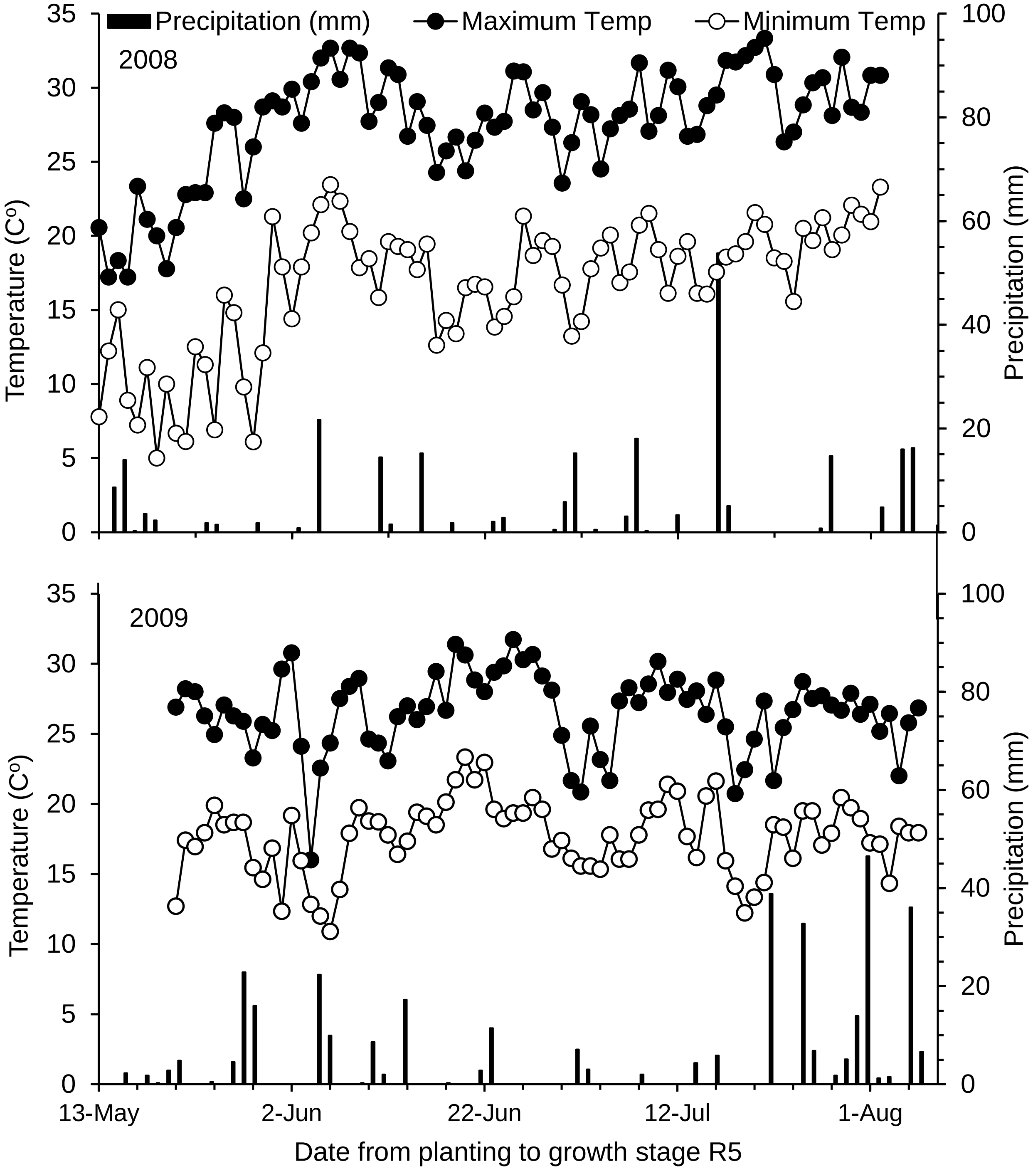

Figure 2.4. Temperature and precipitation for vegetative growth period (planting until growth stage R5) in 2008 and 2009 in Kentucky, data from weather station installed at the site of the field plots.

The defoliation treatments simulated the change in ELAI due to SBR, and were based on the percentage change in ELAI of infected plants relative to the control (Fig. 2.1). Therefore there was a proportionate reduction in LAI, although the absolute LAI values varied among the four years/locations (Fig. 2.2 and 2.3). The LAI declined faster when defoliation started at growth stage R2 compared with defoliation starting at growth stage R5 at both locations across years (Fig. 2.2 and 2.3). The trends were similar to those observed due to leaf abscission in the SBR-infected treatments (Fig. 2.1). The defoliation treatments also produced differences in leaf area duration (LAD) (Table 2.2); the LAD of defoliation treatment started at growth stage R2 was always significantly less than defoliation treatment started at R5. Leaf area duration of both treatments was much lower than the control.

There was also variation in the time to reach growth stage R7 among the years/locations; plants in Kentucky reached growth stage R7 sooner than plants in Louisiana. Interestingly, the time that plants took to reach physiological maturity varied among treatments. Plants where defoliation started at growth stage R2 reached growth stage R7 sooner than when defoliation started at growth stage R5 which, in turn, reached growth stage R7 sooner than control (Fig. 2.2 and 2.3). Similar trends occurred in both locations and both years (Fig. 2.2 and 2.3). Thus, the time to reach physiological maturity was directly associated with the LAD. In soybean the length of seed filling period (R5 to R7) is related to the yield; shortening in the seed-filling period would have negative effect on yield (Egli, 1998, 2004). These results are in agreement with

Table 2.2. Effect of simulating soybean rust injury using manual defoliation on effective leaf area duration (ELAD) from R5 to R7 in Kentucky and Louisiana.

	Effective Leaf Area Duration (ELAD)													
	Brazil[†]		Kentucky[‡]						Louisiana[‡]					
Treatment	2007		2008		2009		Mean		2008		2009		Mean	
	Days	% of control	Days	% of control	Days	% of control	Days	% of control	Days	% of control	Days	% of control	Days	% of control
Control	81 a[§]	100	173 a	100	216 a	100	195 a	100	137 a	100	217 a	100	177 a	100
Manual defoliation at														
Growth stage R2	---		11 c	6	16 c	7	13 c	7	26 c	19	72 c	33	49 c	28
Growth stage R5	---		60 b	34	82 b	38	71 b	36	63 b	46	110 b	50	87 b	49
Infected with SBR at														
Growth stage R2	10 c	12	---	---	---	---	---	---	---	---	---	---	---	---
Growth stage R5	39 b	48	---	---	---	---	---	---	---	---	---	---	---	---

[†] Plants growing in field where plants inoculated with SBR causal organism (*P. pachyrhizi*) in 2007, and the control plots were protected with fungicides. From Kumudini et al. (2008a).

[‡] In 2008 and 2009 SBR effects were simulated by matching the change in ELAI observed in Brazil with manual defoliation in Kentucky and Louisiana.

[§] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.

Kumudini et al. (2008a) who reported that SBR changed the natural cycle of soybean canopy development and caused rapid premature leaf abscission.

Field studies with SBR infection in Brazil showed that inoculations earlier in reproductive growth had a greater impact on yield than inoculation later in reproductive growth (Kumudini et al., 2008a). Yield was reduced by both SBR and manual defoliation (Table 2.3). Defoliation starting at growth stage R2 caused larger yield reductions than defoliation at growth stage R5 and this response was consistent across year and locations (Table 2.3). These finding were in agreement with Hartman et al. (1991) and Yang et al. (1992) who found yield reductions up to 80% for SBR infection at R1, and up to 50% for SBR infection at R5. Board et al. (2010) reported that partial defoliation after R5 resulted in 78% of yield reduction. This response was nearly identical to the response to SBR in Brazil (Table 2.3). The greater effect of the earlier defoliation is probably due to less of light intercepted by the canopy (chapter three) leading to reduction in canopy photosynthesis and the assimilate available for seed filling (Board et al., 2010), as shown by the treatments affect on LAD and the duration of the seed-filling period (i.e., earlier occurrence of growth stage R7).

Omielan et al. (2009) developed a simple model to estimate the yield loss from SBR infestations. The data obtained from the study using field inoculation of SBR and simulated SBR injury at growth stage R1 and R5 in Brazil (Fig. 2.1) showed that there was a significant relationship between proportional decreases in ELAD and proportional decreases in yield (Fig. 2.5A) (Kumudini et al., 2008a). The ELAD data obtained from the Kentucky and Louisiana also predicted a

Table 2.3. Effect of soybean rust (SBR) and manual defoliation that simulates SBR on soybean yield at three locations.

	Brazil[†]		Kentucky						Louisiana	
Treatment			2008		2009		Mean		2008	
	Kg ha^{-1}	% of control	Kg ha^{-1}	% of control	Kg ha^{-1}	% of control	Kg ha^{-1}	% of control	Kg ha^{-1}	% of control
Control	3166 a[‡]	100	4514 a	100	4765 a	100	4640 a	100	4442 a	100
Simulate SBR at										
Growth stage R2	---	---	1235 c	27	1700 c	36	1468 c	32	1381 c	31
Growth stage R5	---	---	2614 b	58	3410 b	72	3012 b	65	2466 b	56
Infected with SBR at										
Growth stage R2	1078 c	34	---	---	---	---	---	---	---	---
Growth stage R5	2070 b	65	---	---	---	---	---	---	---	---

[†] Plants growing in field were inoculated with the SBR causal organism (*P. pachyrhizi*) , and the control plots were protected with fungicides. From Kumudini et al., 2008a.

[‡] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.

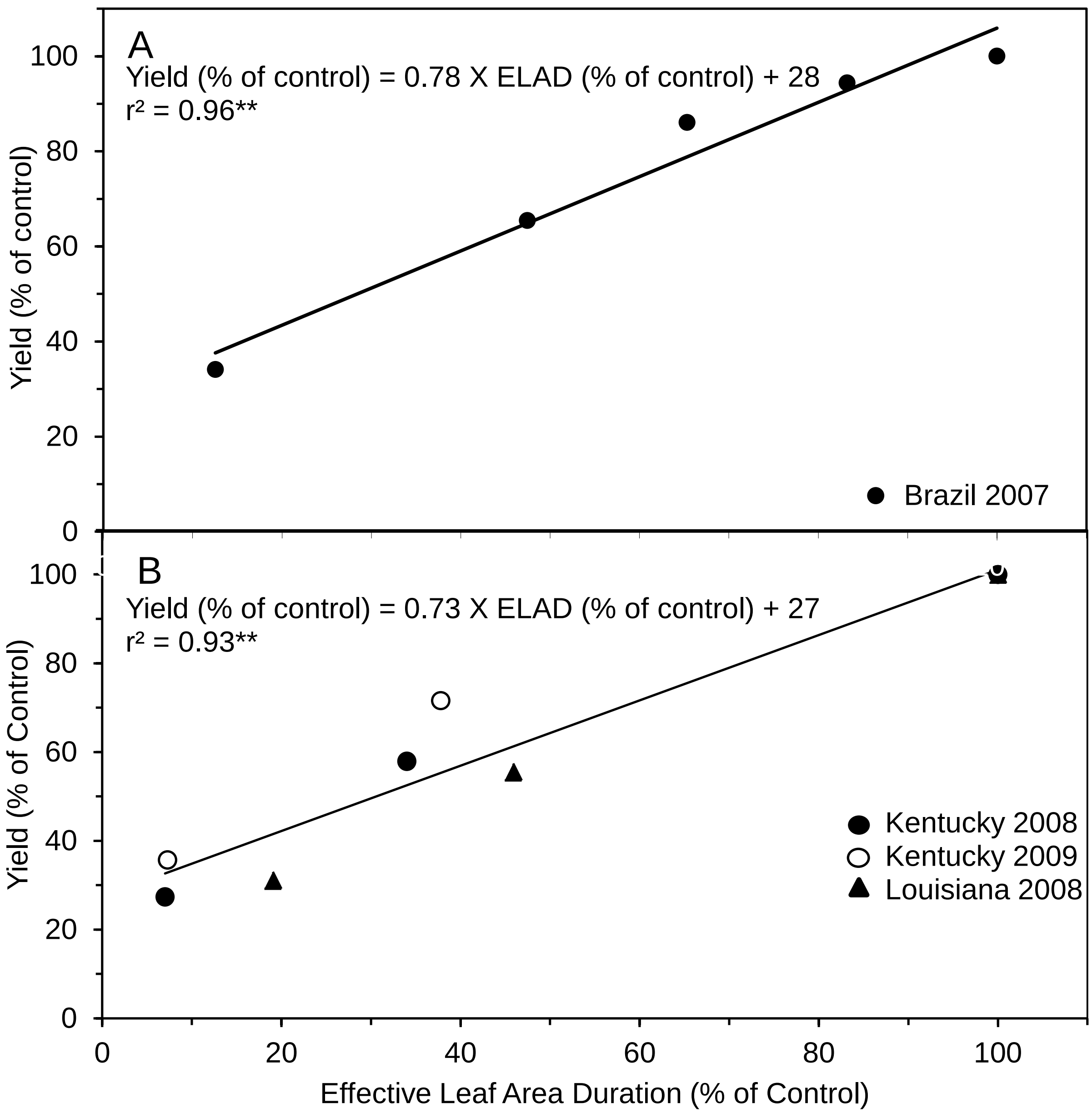

Figure 2.5. Relationship between effective leaf area duration (integrated from R5 to R7) and yield in A) Brazil, and B) Kentucky and Louisiana. The Brazil data are from a field trial when plants inoculated with *Phakopsora pachyrhizi* and simulated SBR by manual defoliation at growth stage R2 and R5 in 2006/2007 were compared with fungicide protected controls. In Kentucky and Louisiana the change in effective leaf area duration was created by manual defoliation that simulated SBR injury.

significant relationship between the relative ELAD and relative yield (Fig. 2.5B). The regression equation from the Kentucky and Louisiana data agreed very well with the model from the Brazil data (Fig. 2.5A, Table 2.2). Thus the model derived from the SBR infected field trial conducted in Brazil in 2007 (Fig. 2.5A) could predict the yield losses from the manual defoliation in Kentucky and Louisiana. The fact that the model derived from SBR infected data in Brazil could be used to accurately predict the yield losses in trials with manual defoliation confirms the effectiveness of the use of ELAD to simulate the damage caused by SBR (Fig. 2.5B). For both locations, the models affirm the relationship between the ELAD and yield across environments, cultivars, and production practices. The fact that ELAD is related to yield loss is in agreement with Bassanezi et al. (2001), Jesus Junior et al. (2003), and Waggoner and Berger (1987).

Conclusions

Manual defoliation of soybean canopy from the bottom simulating soybean rust accelerated the decline in leaf area index compared to the control and reduced yield. Defoliation starting at growth stage R2 caused larger yield loss than defoliation starting at growth stage R5. The ELAD (R5 to R7) was closely associated with yield loss in diseased canopies and in manually defoliated healthy canopies where the disease did not exist.

This study provides clear evidence that manual defoliation impacts yield in similar manner as SBR when the ELAI is used to represent the effect of the disease on the plant. Thus, manual defoliation can be used to simulate soybean rust injury. This simulation method can be applied in any region where the soybean crop is at risk from SBR. This method was efficient and reliable and helps us understand how soybean rust reduces yield. These finding open an avenue to study the impact of the foliar disease on yield reduction without the need for field inoculation. The use of ELAD could be an effective way to develop a yield loss prediction model for other foliar crop diseases.

Abstract

The field inoculation necessary to study the impact of soybean rust (SBR) caused by *Phakopsora pachyrhizi* Syd. and P. Syd on soybean [*Glycine max*, (L.) Merr.] production in the United States. Inspite there is no regulation prevent soybean field inoculation with *P. pachyrhizi*, the scientists cannot do that because the highly possibility of spread the inoculum to neighbor healthy fields or carried by wind to neighbor states. One alternative is to simulate the impact of the disease on soybean yields. The objective of this study was to determine whether simulated injury on a healthy soybean canopy can reduce yields to the same level as SBR injury. Experiments (split-plot in a randomized complete block design with four replications) were carried out in Kentucky and Louisiana in 2008 and 2009. Manual defoliation based on changes in effective leaf area index (ELAI) (calculated as the reduction in leaf area equivalent to SBR-induced premature leaf drop, loss in green leaf area, and reduction in photosynthetic capacity of diseased leaves) in infected canopies in Brazil was used to simulate SBR infection at growth stage R2 and R5. The yield loss in Kentucky and Lousiana was then related to the yield loss of SBR infected crops in Brazil. Defoliation of healthy soybean canopies reduced crop yield an average of 68%

and 69% when imposed at growth stage R2, and 35% and 44% when imposed starting at growth stage R5 in Kentucky and Louisiana, respectively. These yield losses were equivalent to losses observed in infected soybean canopies in Brazil. There was a close association between leaf area duration and yield at all locations. The proportionate reduction in yield was similar across the years, locations, and cultivars tested. These results suggest that a system of manual defoliation to simulate changes in effective leaf area duration shows promise as a tool to simulate the impact of SBR on soybean yield.

CHAPTER THREE

INFLUENCE OF DEFOLIATION ON INTERCEPTED SOLAR RADIATION AND SOYBEAN GROWTH DYNAMIC

Introduction

Solar radiation is the source of energy for photosynthesis for plant growth, and canopy photosynthesis is directly related to LAI and light interception (LI). The LI will eventually reach a plateau with increasing LAI (Shibles and Weber, 1965; Shibles and Weber, 1966); therefore, increasing the LAI above the critical LAI (usually between 3 and 4) will not increase LI or change canopy photosynthesis (Shibles and Weber, 1965). When a healthy crop receives adequate water and nutrients, and reaches the critical LAI, dry matter production reaches a maximum. Reducing LAI by biotic and abiotic stress affects LI, leaf area duration (LAD), and ultimately yield. However, this effect depends upon the level of the stress and the growth stage (i.e., time) when the stress occurs.

The dry matter produced per unit of intercepted solar radiation is radiation use efficiency (RUE). Researchers use RUE to evaluate crop productivity per unit area (Bonhomme, 2000; Kiniry et al., 1989; Purcell et al., 2002; Sinclair and Shiraiwa, 1993). Radiation use efficiency depends upon photosynthesis; therefore, any variation in photosynthesis will affect RUE (Sinclair and Muchow, 1999).

Foliar pathogens such as soybean rust (*Phakopsora pachyrhizi* Syd. and P. Syd.), and environmental conditions, such as temperature, water stress, and nutrient availability, directly affect photosynthesis, and therefore, indirectly affect

RUE (Sinclair and Shiraiwa, 1993). However, few studies have investigated the effect of foliar diseases on RUE (Bastiaans, 1993; Beasse et al., 2000; Kumidini et al., 2008a). Kumudini et al. (2008a) reported that the RUE during the reproductive phase was not affected by SBR infection in one year of the study but it was in a second year. The RUE of rice (*Oryza sativa* L.) inoculated with a foliar pathogen (*Pyricularia oryzae* Cav.) was reduced by 55% (Bastiaans, 1993). Beasse et al. (2000) reported a reduction in the RUE of pea plants (*Pisum sativum* L.) after inoculation with the foliar pathogen *Mycosphaerella pinodes*. All of these studies concluded that the reduction in RUE was a result of the impact of the foliar disease on leaf photosynthetic activity.

Yield is a function of the amount of solar radiation intercepted and the efficiency of its use in biomass production (i.e., RUE). In soybean, the RUE in non-stress environments was found to be within a range from 0.70 to 0.90 g MJ^{-1} of intercepted solar radiation (Egli, 1993; Egli and Bruening, 2000; Board et al., 1994b). Many researchers used RUE (De Bruin and Pederson, 2009; Muchow et al., 1993; Purcell et al., 2002; Sinclair and Shiraiwa, 1993) and crop growth rate (CGR) to study crop growth (Egli and Zhen-wen, 1991; Karimi and Siddique, 1991), and the effects of environmental modification and abiotic stress on crop development (Egli and Zhen-wen, 1991; Egli and Bruening, 2001; Jiang and Egli, 1995).

Defoliation, caused by biotic and abiotic stress, may occur at any time during the plant's growth cycle. The reproductive phase is more sensitive to defoliation than the vegetative phase because yield is determined during this phase (Egli, 2004; Murata, 1969; Schaafsma and Ableet, 1994). Defoliation during the vegetative phase may or may not affect yield (Pickle and Caviness, 1984; Weber, 1955) depending on the ultimate effect on LI during reproductive growth (Board et al., 2010). Defoliation during the reproductive phase that reduces LAI, LAD, and LI decreases yield (Board et al., 1994a; Board et al., 2010; Kumudini et al., 2008a) as a result of reduction in photosynthesis (Ingram et al., 1981; Klubertanz et al., 1996). Reduction in photosynthesis during the seed-filling period, due to SBR infection that caused premature leaf drop, limited both carbon and nitrogen supplies, with the end result a reduction in CGR and RUE (Kumudini et al., 2008a).

A method to simulate injury from soybean rust (SBR) was developed using manual leaf defoliation (chapter two). The defoliation was from the bottom of the canopy upward to mimic the natural progress of leaf loss in canopies infected with SBR. To our knowledge there is no published research that characterized the effect of defoliation, to similar level as that caused by SBR, during the reproductive phase on CGR. Identifying the impact of defoliation on RUE, CGR, and LI would help crop physiologists understand how SBR impacts the crop growth dynamic.

Since the SBR simulation method was reliable and accurately simulated SBR injury, we hypothesized that the defoliation technique could be used to develop a yield loss model that could be used to develop management practices that minimize the effect of SBR on yield. The objectives of this study, therefore, were to characterize the effect of defoliation at growth stage R2 and R5 on the yield production process by determining its effect on i) LI, CGR, and RUE, and ii) seed number and size, and seed growth rate.

Materials and Methods

Culture

Field studies were carried out in Kentucky and Louisiana during the 2008 and 2009 growing seasons.

Kentucky Site

Studies at Lexington, Kentucky (38^{o} N Latitude) were conducted at the Spindletop Research Farm on a Maury silt loam soil (fine, mixed, semiactive, mesic Typic Paleudalfs). Seed of ‘AG3905’ (maturity group III, indeterminate) was machine planted on 13 May 2008 and 21 May 2009 into 38-cm rows 8-m long. Experimental units were nine-rows wide. Fertilizer was applied pre-plant only in 2008 according to soil test recommendations at a rate of 0-0-112 (kg ha^{-1} N-P-K). Lime was also added at a rate of 3362 kg ha^{-1} in 2008 and 2242 kg ha^{-1} in 2009. Seed were sown at a rate of 58 seed m^{-2} in 2008 and 67 seed m^{-2} in 2009. Recommended practices were used to control weeds, diseases, and insects. Irrigation was applied as needed to avoid drought stress.

Louisiana Site

Studies in Louisiana were conducted at the Ben Hur research station near Baton Rouge, LA (30^{o} N Latitude) on a Commerce silty clay loam soil (fine-silty, mixed, superactive, nonacid, thermic Fluvaquent Endoaquepts). Seed of ‘DP4331’ (maturity group IV, indeterminate) was machine planted on 17 April 2008 and 16 April 2009 into 97-cm rows 7.3-m long. Experimental units were four-rows wide. Fertilizer was applied prior to planting according to soil test recommendations at the rate of 0-15-56-21 kg ha^{-1} N-P-K-S. Seed were sown at a rate of 28 seed m^{-2}. Recommended practices were used to control weeds, diseases, and insects. Irrigation was applied as needed to avoid drought stress.

Statistical Design

The experiments at both locations used randomized complete block designs with a split-plot treatment arrangement with four replications. Main plots were the eight sampling periods simulating onset of SBR at growth stages R2 and R5, and split plots were the control and two defoliation treatments. Because yield was measured at maturity (growth stage R8) it was analyzed as RCBD with four replications.

Defoliation Treatments

Defoliation during reproductive development was used to simulate the effect of SBR on soybean growth and yield starting at full bloom (growth stage R2, Fehr and Caviness, 1977) and the beginning of the seed-filling period (growth stage R5). The treatments were defoliation starting at growth stage R2, defoliation starting at growth stage R5, and an undefoliated control (Table 3.1). All defoliations were applied by removing leaves (leaving the petioles on the plant) from the bottom of the canopy upward to mimic the natural progression of leaf loss due to SBR (Kumudini et al., 2008a).

Briefly, the leaf removal levels in the defoliation treatments were determined as follows: 1) Leaf abscission and disease severity in an infected soybean canopy in Brazil (Kumudini et al., 2008a) were used to calculate the effective leaf area index (ELAI) during reproductive growth; 2) This ELAI and the LAI on the fungicide-protected control in Brazil were used to estimate the reduction in the ELAI resulting from SBR infection (Table 3.1); and 3) The LAI of the defoliation treatments in the experiments reported here was reduced below the control at frequent intervals by the amount in Table 3.1 to simulate SBR infection starting at growth stage R2 and R5.

Table 3.1. Defoliation treatments used to simulate the effect of soybean rust (SBR) on leaf area in Kentucky and Louisiana.

Growth Stage	LAI removal	
	Simulate SBR at R2	Simulate SBR at R5
	Percent of control[†]	
R2	2	---
R3	3	---
R4	3	---
R5	39	15
R5+1week	43	10
R5+2weeks	10	31
R5+4weeks	---	44

[†] Percent of control LAI.

The LAI of the control plots in Kentucky and Louisiana was determined at four weekly intervals during growth stage R2 to R5 by removing all the leaflets from all plants in 0.5 m^2 from interior portions of the plot. The leaf area of a sub-sample of 150 leaflets selected randomly from lower-, mid-, and upper- locations in the canopy was measured with a LI-3000 leaf area meter (LI-COR Corp., Lincoln, NE). The fresh and dry weight of the sub-sample and the leaves remaining in the 0.5-m^2 sample were determined and used to estimate the leaf area of the entire sample. The sub-sample represented approximately 30% of the total sample, on average.

The maximum LAI occurs approximately at growth stage R5 (Carpenter and Board, 1997) and the LAI of the standing crop in the control plots from this point until physiological maturity (growth stage R7) was determined weekly by subtracting the LAI of the abscised leaves lost due to natural senescence from the LAI of control plots at growth stage R5. One m^2 wire enclosures were installed in the control plots to collect fallen leaves from growth stage R5 to R7 and the LAI of the fallen leaves was calculated as (number of fallen leaves) x (average area per leaf) per m^2.

The affect of SBR on leaf area and function was estimated in Brazil by the effective leaf area index (ELAI) which includes the reduction in green leaf area index as function of disease severity (Godoy et al., 2006) and the reduction in photosynthesis due to the SBR lesions (Bassanezi et al., 2001). The ELAI over time (Bassanezi, et al., 2001; Jesus Junior et al., 2003) was calculated from $ELAI = LAI\ (1-X)^{\beta}$ where X is the disease severity and the β is the ratio between virtual and visual lesions. We used a β value of 3.0 based on estimates on field grown soybean genotypes (Kumudini et al., 2008a, S. Kumudini, personal communication, 2008). In the non-infected canopies in Kentucky and Louisiana the ELAI and LAI were equal.

Crop Growth Measurements

The growth stage of 10 consecutive plants in the row was recorded twice a week (Fehr and Caviness, 1977). The same plants were measured throughout the season. A plot was considered to be at a particular growth stage when ≥ 50% of the plants reached that stage.

Light Interception

Light interception (LI) in Kentucky and Louisiana was estimated by first measuring photosynthetic active radiation (PAR) at the soil surface using a 1-m-long Line Quantum Sensor (LI-191, LI-Cor, Lincoln, NE) connected to a LI-1000 data logger (average of three measurements made by placing the bar diagonally across the inter-row space) (Board, 2000; Board, 2004). The ambient light intensity at the top of the canopy was then measured using a Quantum Sensor (LI-190, LI-Cor, Lincoln, NE) and LI was calculated (Board, 2000; Board, 2004). All measurements were made between 1100 and 1300 h under full-sun conditions at weekly intervals from growth stage R2 until growth stage R7.

Crop Growth Rate

After each defoliation (and before the first defoliation) all plants 2 m^2 from interior portions of bordered rows were harvested by cutting the main stem at ground level, dried to constant dry weight and weighed. Crop growth rate (CGR, g m^{-2} day^{-1}) in Kentucky and Louisiana was estimated by linear regression of the weight of biomass from growth stage R1 to R5 versus time (Hunt and Parsons, 1981). All regression model were significant (P <0.001) and all r^2 were > 0.9.

Seed Growth Rate

In Kentucky, a sub-sample of five arbitrarily chosen plants was taken from each 2 m^2 sample during seed-filling period. The seeds were separated and dried to constant dry weight and weight. Seed growth rate (SGR, g m^{-2} day^{-1}) was estimated as the slope of linear regression of seed weight and time (Munier-Jolain et al., 1993). All regression model were significant (P <0.001) and all r^2 were > 0.7.

Radiation Use Efficiency

In Kentucky, the daily incident solar radiation (MJ m^{-2} day^{-1}) was measured at a weather station located beside the plots. In Louisiana, the incident solar radiation was obtained from a weather station of the agriclimatic information system at the Ben Hur research station. The RUE was estimated for the period between R2 and R5 for all treatments. Intercepted solar radiation was calculated as the product of LI and incident solar radiation. The RUE (g MJ^{-1}) was estimated as the slope of the linear regression between cumulative biomass and cumulative intercepted solar radiation (Sinclair and Muchow, 1999). All regression models were significant (P <0.001) and all r^2 were > 0.7.

Yield and Yield Components

In Kentucky, yield was measured by harvesting the plants from 4.6 m^2 at maturity and threshing the plants in small plot thresher. Seed moisture concentration was determined and yield was adjusted to 130 g kg^{-1} moisture concentration. Yield in Louisiana was determined at maturity by sampling 4 m^2 of interior rows and determining the total fresh weight of the entire sample using a field scale. A 0.5 m^2 sample was harvested to determine fresh weight and dry weight (oven dried at 60^{o}C for 4 days), then threshed to determine seed weight, and leaf and petiole dry weight. Yield calculated as follows: Yield = [(total fresh weight of 4-m^2 sample) x (dry weight of 0.5-m^2/fresh weight 0.5 m^2) - leaf and petiole dry weight] x harvest index (fraction) (Board et al., 2010). The harvest index was determined from 0.5 m^2 (HI = seed dry weight/total dry weight [exclusive of leaf and petiole material]). Yield was adjusted to 130 g kg^{-1} moisture concentration. Yield data from the Louisiana-2009 experiment were not available. In both locations, seed size (weight/seed) was determined on all plots by counting 300 seed from each yield sample, drying them to constant weight in a 70^oC oven for 7 days, and weighing the sample. Also, seed number was determined on a dry weight basis by dividing yield (g m^{-2}) by weight per seed (g $seed^{-2}$).

Data Analysis

To verify assumptions of independently and normally distributed error, the means were plotted against their variance to confirm the homogeneity of error. Two tailed *F*-test was conducted to determine the homogeneity of variance among the experiments before the data were analyzed. The data were analyzed using Proc MIXED and significant means differences were determined by the SAS LSMEANS procedure (SAS 9.2; SAS Institute Inc., Cary, NC). Year and location were considered random effects, while treatments were considered a fixed effect.

Results

Light Interception

The maximum LI occurred at approximately growth stage R5 except for KY 2009 when it was nearly 100% a few days after growth stage R1 (Fig. 3.1 and 3.2). There was very little affect of defoliation on LI growth stage R1 to R5. The decline in LI was larger and occurred sooner when defoliation began at growth stage R2 compared with growth stage R5 (Fig. 3.1 and 3.2) and that was consistent across locations and years. The LI of the growth stage R2 treatment was less than 50% (control > 80% LI) one week after plants reaches growth stage R6 (45 to 53 days after R1) in all experiments which was much lower than the growth stage R5 treatment. In Kentucky in 2009 the LI was relatively high from growth stage R1 until growth stage R6 in all treatments because of excessive growth (the control LAI was 7, see Fig. 2.2 in chapter two) that caused substantial lodging. In all locations and years plants from the defoliation starting at growth stage R2 reached physiological maturity (growth stage R7) sooner than those in the defoliation at growth stage R5 treatment.

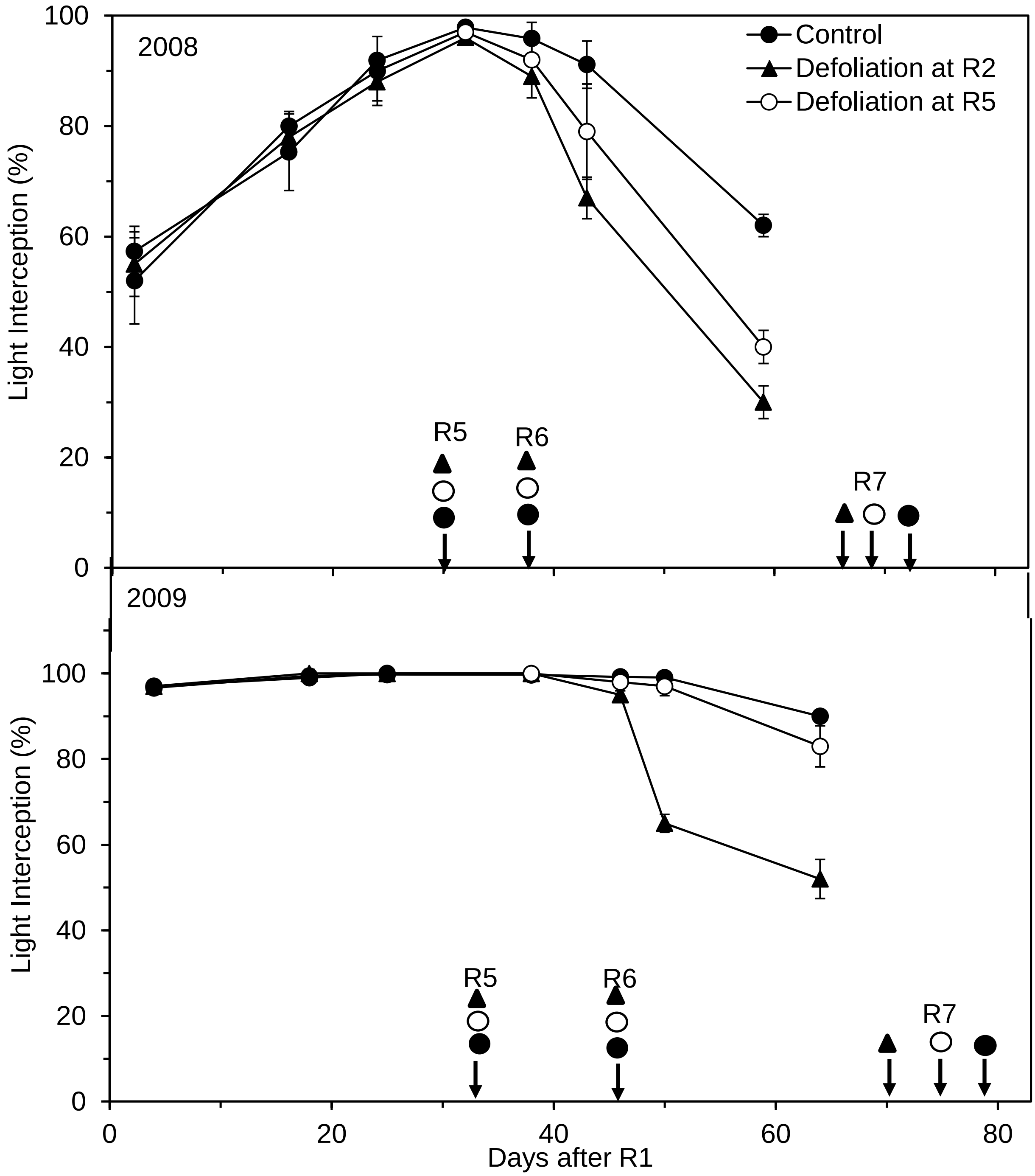

Figure 3.1. The effect of defoliation on light interception (LI) in Kentucky during reproductive growth in 2008 and 2009. Vertical bars represent ± standard error of the mean. Arrows on the x-axis indicate the occurrence of beginning seed fill (growth stage R5), growth stage R6, and physiological maturity (growth stage R7).

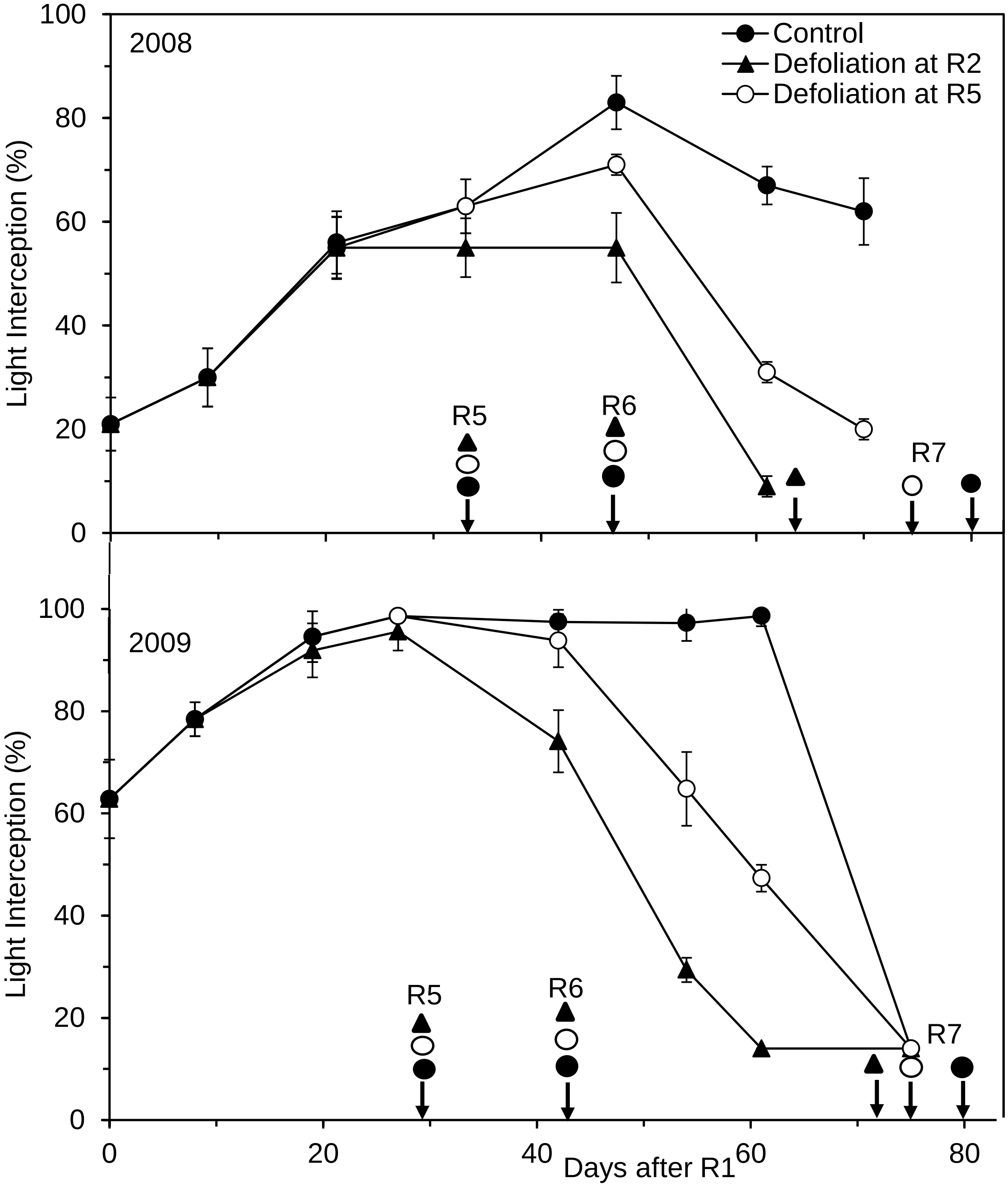

Figure 3.2. The effect of defoliation on light interception (LI) in Louisiana during reproductive growth in 2008 and 2009. Vertical bars represent ± standard error of the mean. Arrows on the x-axis indicate the occurrence of beginning seed fill (growth stage R5), growth stage R6, and physiological maturity (growth stage R7).

Yield and Yield Components

Yield was reduced by defoliation treatments (Table 3.2). Defoliation starting at growth stage R2 caused larger yield reductions (64 to 73%) than defoliation at growth stage R5 (28 to 44%) and this response was consistent across years and locations (Table 3.2). Defoliation at growth stage R2 reduced seed number per area, while defoliation at growth stage R5 had no effect. The defoliation at growth stage R5 reduced seed size much more than defoliation at growth stage R2 (Table 3.2).

Table 3.2. Effect of defoliation that simulated soybean rust (SBR) on yield and yield components of soybean in two locations.

Treatment[†]	Kentucky						Louisiana		
	2008			2009			2008		
	Yield Kg ha^{-1}	Seed No. No. m^{-2}	Seed Size mg seed^{-1}	Yield Kg ha^{-1}	Seed No. No. m^{-2}	Seed Size mg seed^{-1}	Yield Kg ha^{-1}	Seed No. No. m^{-2}	Seed Size mg seed^{-1}
Control	4514 a[‡]	2510 a	180 a	4765 a	2650 a	180 a	4443 a	2880 a	154 a
Defoliation at R2	1235 c	1280 b	96 c	1700 c	1620 b	105 c	1382 c	1970 b	70 c
Defoliation at R5	2614 b	2425 a	108 b	3411 b	2350 a	145 b	2466 b	2645 a	93 b

[†] In 2008 and 2009 SBR effects were simulated by matching the change in ELAI observed in Brazil with manual defoliation in Kentucky and Louisiana. Defoliation started at growth stage R2 or R5.

[‡] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.

Radiation Use Efficiency, Crop Growth Rate, and Seed Growth Rate

The radiation use efficiency (RUE) and crop growth rate were measured from growth stage R1 to R5 (Table 3.3). There was no affect of defoliation on RUE or crop growth rate (CGR). The seed growth rate (SGR) was measured only in Kentucky, and both defoliation treatments reduced the SGR (Table 3.3). Defoliation at growth stage R2 reduced SGR more than defoliation at growth stage R5.

Seed Filling Duration

Defoliation shortened the seed-fill duration (Table 3.2). Defoliation at growth stage R2 reduced the seed-filling period by 6 to 20 days, while defoliation at growth stage R5 shortened the seed-filling period by 2 to 9 days. Defoliation at growth stage R2 had no affect on time that plants reached beginning of seed filling (growth stage R5) (Fig. 3.1 and 3.2).

Table 3.3. Effect of defoliation simulating soybean rust (SBR) on soybean growth dynamic.

Treatment[†]	RUE[‡]	CGR[§]	R1-R5	SFD[††]	SGR
	g MJ^{-1}	g m^{-2} day^{-1}	days		g m^{-2} day^{-1}
Kentucky 2008					
Control	0.82	12.3	30	43 a[#]	6.89 a
Defoliation at R2	0.72	11.1	30	37 b	2.95 c
Defoliation at R5	0.81	11.0	30	41 a	4.95 b
	NS[¶]	NS	NS		
Kentucky 2009					
Control	1.06	15.2	33	46 a	9.35 a
Defoliation at R2	0.97	14.7	33	37 c	1.95 c
Defoliation at R5	0.98	14.2	33	42 b	4.38 b
	NS	NS	NS		
Louisiana 2008					
Control	0.80	10.1	33	54 a	---
Defoliation at R2	0.72	7.5	33	34 c	---
Defoliation at R5	0.80	9.3	33	45 b	---
	NS	NS	NS		
Louisiana 2009					
Control	0.87	15.9	28	53 a	---
Defoliation at R2	0.85	15.4	28	44 c	---
Defoliation at R5	0.85	15.7	28	47 b	---
	NS	NS	NS		

[†] In 2008 and 2009 SBR effects were simulated by matching the change in ELAI observed in Brazil with manual defoliation in Kentucky and Louisiana. Defoliation started at growth stage R2 or growth stage R5.

[‡] Radiation use efficiency, the RUE was determined from growth stage R2 to R5 using total solar radiation.

[§] Crop growth rate, CGR determined from growth stage R1 to R5.

[¶] NS, not significant at $P < 0.05$.

[#] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.

[††] Seed-fill duration is the days from growth stage R5 to R7.

Discussion

The hypothesis of this study was that defoliation could be used to understand the impact of soybean rust (SBR) on yield production. Characterizing the effect of defoliation during reproductive growth on the yield production process (i.e., on light interception (LI), crop growth rate (CGR), radiation use efficiency (RUE), seed number and size, and seed growth rate) could help develop a yield loss model, which would facilitate the development of management practices that minimize SBR impact on yield.

Defoliation beginning in phase II (growth stage R2 treatment) (Murata, 1969) reduced yield, seed number per unit area and seed size in all experiments. Defoliation had no affect on LI before growth stage R5, but LI started to decline after growth stage R5 and continued declining until maturity.

The CGR (10-15 g m^{-2} d^{-1}) and RUE (0.7-1.0 g MJ^{-1}) reported in this study are in the range of some previous reports by Egli (1993), Egli and Bruening (2000), Egli and Zhen-wen (1991), and Board et al. (1994b), but lower than others (maximum of 28 g m^{-2} d^{-1}, and 1.1 g MJ^{-1}) (Egli and Zhen-wen, 1991; Sinclair and Muchow, 1999). There are no reports of RUE determined under stress conditions, such as defoliation caused by a foliar disease. Defoliation did not affect CGR or RUE (determined between growth stage R1 to R5) since it had no affect on LI during this period. Kumudini et al (2008a) found differences in RUE between manual defoliation and SBR-infected canopies; the RUE (during growth stage R2 to R7) of the inoculated plants was 40% lower than the manually defoliated plants. Radiation use efficiency was not influenced by defoliation treatments at growth stage R5 simply because no leaf removal occurred until growth stage R5.

Defoliation starting at growth stage R2 did not affect CGR but it always reduced seeds number m^{-2}, which was unexpected given the close association between CGR and seed number reported by Egli and Zhen-wen (1991) and others (Charles-Edward et al., 1986; Herbert and Litchfield, 1984, Ramseur et al., 1985). The critical period for the determination of seed number per unit area is from initial flowering to shortly after the beginning of growth stage R6 (Egli, 2010). Defoliation that started at growth stage R2 covered the critical period (Egli, 2010), but LI was not reduced until after growth stage R5 and this reduction no doubt reduced the assimilate supply to the seed while the plants was still in the critical stage, thus reducing seed number. Some of the reduction in yield from defoliation starting at growth stage R2, can therefore be attributed to lower LI between growth stage R5 and R6. Many researchers reported that reductions in yield were attributed to reductions in LI (Board et al., 1994a, 2010; Browde et al., 1994; Haile et al., 1998; Higley, 1992; Ingram et al., 1981; chapter three). Our results are in agreement with Hardman and Brun (1971), Egli and Zhen-wen (1991), Board and Tan (1995), and Schou et al. (1978) who reported that modification of photosynthesis during the flowering and pod set phase (i.e., from initial flowering until growth stage R6) with CO_2 enrichment, shade, defoliation, and extra light caused corresponding changes in pods and seeds per unit area.

Another possible explanation for the reduction in seed number when there was no affect on CGR in the R2 treatment is that defoliation from the bottom of the plant increased pod abortion without affecting LI. Heitholt et al. (1986) reported that defoliation increased flower abortion, but the processes that control pod abortion are more complex than simple relationships between pod survival and assimilate supply especially when time and pod location are included. Egli and Bruening (2006) found that removing the leaf to eliminate the assimilate supply to an individual node had minimal impact on pod abortion at that node, but removing three adjacent leaves reduced pod number on the target node. In our study, at least three leaves were removed from the bottom of the canopy at growth stage R2 and this number increased as LAI increased and defoliation continued. The affect of local defoliation and inter-node interactions may influence the processes that control pod abortion, so removing the assimilate sources (i.e., the leaves) from the bottom nodes may make pods at that node more likely to abort even though CGR is not affected. This scenario agrees with Heitholt et al. (1986) who suggested that abortion was regulated by processes occurring at individual nodes, rather than responding to the whole plant assimilate supply.

Defoliation during phase III (Murata, 1969) reduced seed size of both defoliation treatments by reducing LI, the leaf area duration, which probably reduced the assimilate supply, the SGR, and shortening the seed-fill duration. Seed size of R2 defoliation treatment was smaller than the treatment starting at growth stage R5.

Defoliation reduced the SGR (the rate of dry matter accumulation in the seeds) because of a reduction in LI. Defoliation beginning at growth stage R2 caused a larger reduction in SGR than defoliation starting at growth stage R5 because the decline in LI occurred earlier in the seed-filling period (Fig. 3.1 and 3.2). The reduction in LI was less and LAI reached zero (Chapter two) later in the seed-filling period for the defoliation treatment beginning at growth stage R5 and the effects on seed size (Table 3.2) were also less. Defoliation probably limited both carbon and nitrogen supplies to the seeds with the end result a reduction in SGR. Egli (1997) found that the shade that reduced photosynthesis resulted in reduced SGR and seed size. These findings also agree with Board et al. (2010) who reported that partial defoliation (33% leaf removal) after R5 resulted in 78% of yield reduction due to reduction in seed size.

In soybean, the length of seed filling period (R5 to R7) is related to yield; shortening the seed-filling period would have a negative effect on seed size and yield (Egli, 2004). Defoliation beginning at growth stage R2 and R5 that reduced leaf area and leaf area duration (LAD) (chapter two) also shortened the seed-fill duration. Several researchers also reported that defoliation shortened the reproductive cycle in soybean and corn (*Zea mays* L.) (Barimavandi, 2010; Ingram et al., 1981; Jones and Simmons, 1983; Tollenaar and Daynard, 1978). Kumudini et al. (2008a) reported similar findings with soybean.

Seed filling continues as long as there is assimilate available to the seed and the seed is active in converting the assimilate into storage compounds; when seed growth stops as a result of a reduction in canopy photosynthesis and a lack of assimilate, the filling period is terminated (Egli, 2004). The amount of assimilate available to the seed in these experiments was reduced by defoliation which shortened the seed-filling period. These results suggest that defoliation during the seed-filling period affects yield by a reduction in LI and photosynthesis which agrees with Board et al. (2010) who reported similar findings.

In conclusion, the affect of defoliation on yield production depended upon the growth stage when it occurred and the magnitude of the defoliation. If defoliation began earlier in the flowering and seed set stage (growth stage R1 to R6), seed number per unit area was reduced, while defoliation during the seed-filling period reduced seed size. Canopy assimilate availability (as estimated by CGR and RUE) is not the only factor determining seed number, local assimilate availability to specific nodes may also be important. Defoliation during the seed-filing period that was enough to reduce assimilate supply to the seeds reduced SGR and shortened the seed-fill duration. Soybean rust reduced photosynthesis and causes defoliation from the bottom of the plant (Kumudini et al., 2008a) so the mechanisms by which it would affect soybean growth dynamics and yield if infection occurs during reproductive phase, should be similar to the responses to manual defoliation reported here.

Abstract

Defoliation of a soybean canopy, as caused by soybean rust (SBR) (*Phakopsora pachyrhizi* Syd. and P. Syd) reduces yield, but how defoliation impacts yield components is not fully understood. The aim of this research was to determine the effect of manual defoliation on the interception of solar radiation and growth dynamics of the soybean canopy. Defoliation treatments beginning at growth stage R2 (full bloom) and R5 (beginning of seed-fill) that mimicked

defoliation measured in infested soybean canopies were applied in the field in Kentucky and Louisiana for two years. All leaf removal was from the bottom of the canopy. Both defoliation treatments reduced yield in all experiments and the reduction was larger for the treatments at growth stage R2. The radiation use efficiency and crop growth rate from growth stage R2 to R5 were not influenced by defoliation since there was no effect on light interception. Defoliation started at growth stage R2 reduced seed number per unit area due to reduction in light interception between growth stage R5 and R6. The reduction in light interception reduced the assimilate needed for successful pod set. Defoliation during seed filling (in both treatments) also reduced seed size due to a shortened seed-fill duration and lower seed growth rate.

CHAPTER FOUR

EFFECT OF DEFOLIATION ON PHOTOSYNTHESIS AND NITROGEN REMOBILIZATION DURING THE SEED-FILLING PERIOD IN SOYBEAN

Introduction

Manual defoliation was recently used to simulate soybean rust (SBR) injury by determining the changes in effective leaf area in diseased canopies and imposing these changes onto healthy canopies where the disease was not present (chapter two). With this method, SBR infection during the reproductive phase was simulated under field conditions. That experiment provided evidence that manual defoliation could be used to simulate the impact of SBR on yield.

Soybean rust reduces soybean yield through a number of mechanisms. First, it impacts the plant’s ability to intercept and absorb radiation by accelerated leaf abscission and a reduction in green leaf area (due to necrotic and chlorotic lesions on the remaining leaves). Secondly, it impacts the photosynthetic efficiency of the apparently healthy remaining green leaves (Kumudini et al. 2008a). Leaf loss reduces the nitrogen stored in the leaves, leaf area duration (LAD), effective leaf area index (ELAI), and light interception (LI) which results in decreased canopy photosynthesis and yield (Board et al., 2010; Burton et al., 1995; Kumudini et al., 2001, 2008a; Li et al., 2009). Reduction in photosynthesis during the seed-filling period, due to leaf loss, limits both carbon and the nitrogen supply to the seed (Burton et al., 1995; Li et al., 2009).

Nitrogen affects crop growth through its effect on leaf growth and photosynthesis which is dependent upon leaf nitrogen concentration. The enzymes involved in photosynthesis, including RUBISCO and the other light harvesting complex proteins, represent a large proportion of leaf nitrogen (Staswick, 1994). The leaf nitrogen distribution within the canopy affects canopy photosynthesis and radiation use efficiency (RUE) (Sinclair and Horie, 1989). Leaves with high nitrogen concentrations have been shown to accumulate more assimilate and have high RUE (Sinclair and Horie, 1989). Reducing leaf mass reduces the nitrogen stored in leaf tissue which could be remobilized to the seed during the seed-filling period. This loss of nitrogen could contribute to the seed yield reduction.

Soybean seed nitrogen comes from nitrate uptake from soil, N_2 fixation, and redistribution from vegetative plant parts and pod walls. Redistribution occurs as the protein in the vegetative plant parts are broken down and the amino acids are remobilized to the seed (Liu et al., 2008; Staswick, 1994). The contribution of redistributed nitrogen to the seed nitrogen varied from 30 to 100% (Egli et al., 1978, 1983; Zeiher et al., 1982). This loss of nitrogen from leaves is a sign of senescence which is associated with a decline of photosynthesis.

A model to explain nitrogen uptake and partitioning in soybean was developed by Sinclair and de Wit (1975). They proposed that the high nitrogen demand of the developing soybean seed could not be sustained by nitrogen uptake from the soil and therefore, nitrogen must be remobilized from the vegetative organs to meet the seed nitrogen demand. This remobilization process results in the destruction of the photosynthetic capacity and accelerates leaf senescence. This hypothesis was called the “self-destruct” hypothesis.

Sinclair and de Wit (1976) believed that the self-destruction phenomena limited the length of the seed filling-period and yield.

Several scientists evaluated the validity of the “self-destruct” hypothesis (Egli, 2004; Egli and Leggett, 1976; Egli et al., 1978; Hayati et al., 1995; 1996; Kumudini et al., 2002; Streeter, 1978). Streeter (1978) found that removing nitrogen from a hydroponics system did not increase the rate of senescence in soybean. Also, Egli et al. (1978) reported that increasing the amount of nitrogen in the solution did not delay leaf senescence. Hayati et al. (1996) invistigated the effect of nitrogen supply on soybean seed growth *in vitro*. They found that soybean seeds can sustain dry matter accumulation with only minimal supplies of ntrogen in the media. These data support the concept that there is no seed nitrogen demand, and are in direct conflict with the “self-destruct” model. Some researchers reported that defoliation shortened the reproductive cycle in soybean (Kumudini et al., 2008a; Ingram et al., 1981). However, no one quantified the impact of defoliation on the rate of senescence of the remaining leaves.

There is no information available describing how defoliation by SBR effects nitrogen redistribution to the soybean seed. The objective of this study was to determine if defoliation as caused by SBR affects the rate of leaf senescence and nitrogen remobilization during seed filling.

Materials and Methods

Culture

Field studies were carried out during the 2010 growing season at Spindletop Research Farm (Lexington, Kentucky, 38^{o} N Latitude) on a Maury silt loam soil (fine, mixed, semiactive, mesic Typic Paleudalfs). Seed of ‘AG4005’ (MG IV, indeterminate) was hand planted on 26 May 2010 at a 38-cm row spacing in 6-m long rows. Experimental units were eleven-rows wide. Seed were sown at the rate of 52 seed m^{-2}. Herbicides and hand cultivation were used to control weeds. Soil moisture was monitored by placing two tensiometers in the soil at depth of 0.20 m. The plots were irrigated when the tensiometer reading was above -0.05 MPa with an overhead sprinkler irrigation system to minimize moisture stress. Reproductive stages were determined from initial flowering (growth stage R1) until maturity (growth stage R8) using the Fehr and Caviness (1977) system. Ten consecutive plants in the row in four plots, (one plot in each replication), were marked with flags and were staged twice a week, and every other day as physiological maturity (growth stage R7) approached.

Defoliation Treatments

The treatments were a single defoliation, sequential defoliation, and an undefoliated control. Both defoliation treatments involved removing leaves manually (leaving petioles on the plant) from the bottom of the canopy upward to mimic leaf loss of plants infected with SBR (Kumudini et al., 2008a). The sequential defoliation treatment was initiated at the beginning of the seed-filling period (growth stage R5) (Table 4.1) to simulate defoliation of a natural infestation of SBR in Brazil (chapter two). The single defoliation treatment, designed to reduce the amount of vegetative nitrogen available for redistribution, was applied at the beginning of growth stage R6.

Table 4.1: The defoliation level used in the sequential defoliation treatment applied at the beginning of seed-filling (growth stage R5).

Growth Stage	LAI removal (% of control)
R5	15
R5+1week	10
R5+2weeks	31
R5+4weeks	44

Briefly, the level of leaf removal in the sequential defoliation treatment was determined as follows: 1) Leaf abscission and disease severity in an infected soybean canopy in Brazil (Kumudini et al., 2008a) was used to calculate the effective leaf area index (ELAI) during seed filling, 2) This ELAI and the LAI on the fungicide protected control in Brazil was used to estimate the reduction in the ELAI resulting from SBR infection (Table 4.1), and 3) The LAI of the sequential defoliation treatment was reduced below the control at frequent intervals by the amount in Table 4.1.

The LAI for the control plots during the growth stage R2 to R5 period was determined as previously described in chapter two by removing the leaflets from all plants in 0.5 m^2 from interior portions of the plot. The leaf area of a sub-sample was measured with a LI-3000 leaf area meter (LI-COR Corp., Lincoln, NE).

The maximum LAI occurs approximately at growth stage R5 (Carpenter and Board, 1997). The LAI of the standing crop in the control plots was determined weekly from beginning of seed-filling period (growth stage R5) to physiological maturity (growth stage R7) by subtracting the LAI of the abscised leaves from the LAI of control plots at growth stage R5 (chapter two). The LAI lost due to natural senescence was measured as previously described in chapter two using 1 m^2 wired enclosures to collect fallen leaves.

The affect of SBR on leaf area was estimated in Brazil by the effective leaf area index (ELAI) which includes the reduction in green leaf area index as function of disease severity (Godoy et al., 2006) and the reduction in photosynthesis due to the SBR lesion (Bassanezi et al., 2001). Determination of the ELAI in an infected canopy was discussed in chapter two.

The single defoliation treatment was applied early in the seed-filling period (beginning growth stage R6) to reduce vegetative mass and stored nitrogen as much as possible without reducing LI (i.e., LI maintained at ≥ 90%). The critical period for seed number determination in soybean is between flowering (growth stage R1) and a little after beginning of growth stage R6 (Egli, 2010). Therefore, defoliation was applied at beginning of growth stage R6 to avoid any effect of defoliation on seed number. Practice defoliation on rows outside the experimental plot was used to determine the leaf area to remove. After removed the desired LAI from the experimental plots the LI was measured one day after defoliation using Quantum Sensors (LI-190 above canopy, and LI-191 below canopy LI-Cor, Lincoln, NE) to confirm that the LI was ≥ 90%.

Photosynthesis

Single leaf carbon exchange rate (CER) was measured with an open-path, portable photosynthesis system (LI-6400-02B, LI-Cor, Lincoln, NE) fitted with a LI-6400 fluorometer chamber, a red/blue LED light source (LI-6400–02B) and a 6 cm^2 leaf cuvette. Measurements were made in the field between 1000 and 1500 h on clear sunny days at weekly intervals from growth stage R2 until growth stage R7. The relative humidity in the cuvette was maintained as closely as possible to ambient air with the LI-6400 desiccant tube. All of the measurements were taken at photosynthetic active radiation (PAR) of 1500 μmol m^{-2} s^{-1} and at a constant airflow of 400 μmol s^{-1}. The concentration of CO_2 in the cuvette was 400 ± 2 μmol CO_2 mol air^{-1}, and the air temperature was maintained at 28 ± 2°C. Carbon exchange rate was determined on illuminated upper trifoliolioate leaves on the main stem. Three or four measurements were taken from each plot from separate plants. Chlorophyll levels of the same leaves were estimated with a SPAD-502 hand-held chlorophyll meter (Minolta Corp.) after the CER was determined.

Light Interception

Light interception (LI) was estimated by first measuring light intensity at the soil surface using a 1-m-long Line Quantum Sensor (LI-191, LI-Cor, Lincoln, NE) connected to a LI-1000 data logger (average of three measurements made by placing the bar diagonally across the inter-row space) (Board, 2000; Board, 2004). The ambient light intensity at the top of the canopy was then measured with a Quantum Sensor (LI-190, LI-Cor, Lincoln, NE) and LI was calculated. All measurements were made between 1100 and 1300 h under full-sun conditions at weekly intervals from growth stage R2 until growth stage R7.

Nitrogen Redistribution

Plants were harvested from a 1 m^2 of interior rows of the plots at the beginning of the seed-filling period (growth stage R5) on the sequential defoliation treatment, when single defoliation was applied (growth stage R6), and at physiological maturity (growth stage R7). The plants were separated into leaves, petioles, stems, pod walls, and seeds, and dried at 60^{o}C to determine dry weight. The leaves removed in the defoliation treatments were collected after each defoliation and dried at 60^{o}C to determine dry weight. The abscised leaves and petioles were collected from the cages every other day and combined over the period from growth stage R5 or R6 to R7 and dried at 60^{o}C to determine dry weight. After drying, the samples from all plant parts were ground to estimate total nitrogen concentration in the tissue based on the Berthelot reaction using Dual Technicon System II Autoanalyzer (wavelength was 660nm) (Chaney and Marbach, 1962).

Redistributed nitrogen is the nitrogen translocated from all vegetative plant parts and pod walls to the seed during the seed-filling period. The amount of redistributed nitrogen was calculated from the differences in the nitrogen content (g m^{-2}) of each plant part at beginning of the seed-filling period (growth stage R5 or R6) and at physiological maturity and in the abscised leaves (including the leaves removed by defoliation) and petioles as described previously by Zeiher et al. (1982) and Egli et al. (1983). Thus, the nitrogen redistributed from the leaves was represented by the total nitrogen in the leaf blades at growth stage R5 minus the total nitrogen in the abscised and defoliated leaves and in the leaf blades on the plant at growth stage R7. Similar calculations were performed for the petioles, stems, and pod wall, except that there was no abscised materials for the stem and pod walls. It was assumed that nitrogen lost from the vegetative plant parts and the pod walls was redistributed to the seeds. The total nitrogen (g m^{-2}) in the seed at the beginning of seed-filling period (growth stage R5) was minimal and therefore was not subtracted from the total nitrogen in the seed at the final harvest to give the net gain in seed nitrogen.

Yield and Yield Components

Yield was measured by harvesting 4 m^2 from bordered rows at maturity and threshing the plants in a small plot thresher. Seed moisture concentration was determined and yield was adjusted to 130 g kg^{-1} moisture concentration. Additional plants from 1 m^2 area were cut and threshed manually to estimate the apparent harvest index (HI). The apparent HI was calculated on a dry basis as follows: seed mass divided by [seed mass + vegetative mass + abscised leaves and petioles] at physiological maturity. Seed size (weight/seed) was determined on all plots by counting 300 seed from each yield sample, drying them to constant weight in a 70^{o}C oven for 7 days, and weighing the sample. Seed number was determined on a dry weight basis by dividing yield (g m^{-2}) by weight per seed (g $seed^{-2}$).

Statistical Design and Analysis

The statistical design was a randomized complete block with four replications. Data were analyzed using PROC MIXED (SAS ver. 9.2, SAS Institute, Cary, NC). Mean comparisons were made with F-test at $\alpha=0.05$.

Results

Light Interception

Light interception (LI) was influenced by the time and level of defoliation (Fig. 4.1). The rate of LI decline was higher with sequential defoliation than with the single defoliation treatment, which was similar to the control. The LI of the sequential defoliation treatment reached 50% at physiological maturity which was much lower than the single defoliation treatment and the control (both > 90%). The LI at physiological maturity was still relatively high in all treatments because of the excessive growth that occurred during the season which caused substantial lodging.

Plants in the sequential defoliation treatment reached growth stage R7 four days earlier than the single defoliation treatment, which reached growth stage R7 at the same time as the control (Fig. 4.1).

Photosynthesis

The carbon exchange rate (CER) of an upper leaf declined slowly early in seed-filling, and then declined rapidly after the plants reached growth stage R6, 51 days after R1 (Fig. 4.2). However, there was no difference among the treatments. Leaf chlorophyll level, estimated with a SPAD chlorophyll meter, started to decline about 60 days after growth stage R1, again with no difference among the treatments (Fig. 4.3).

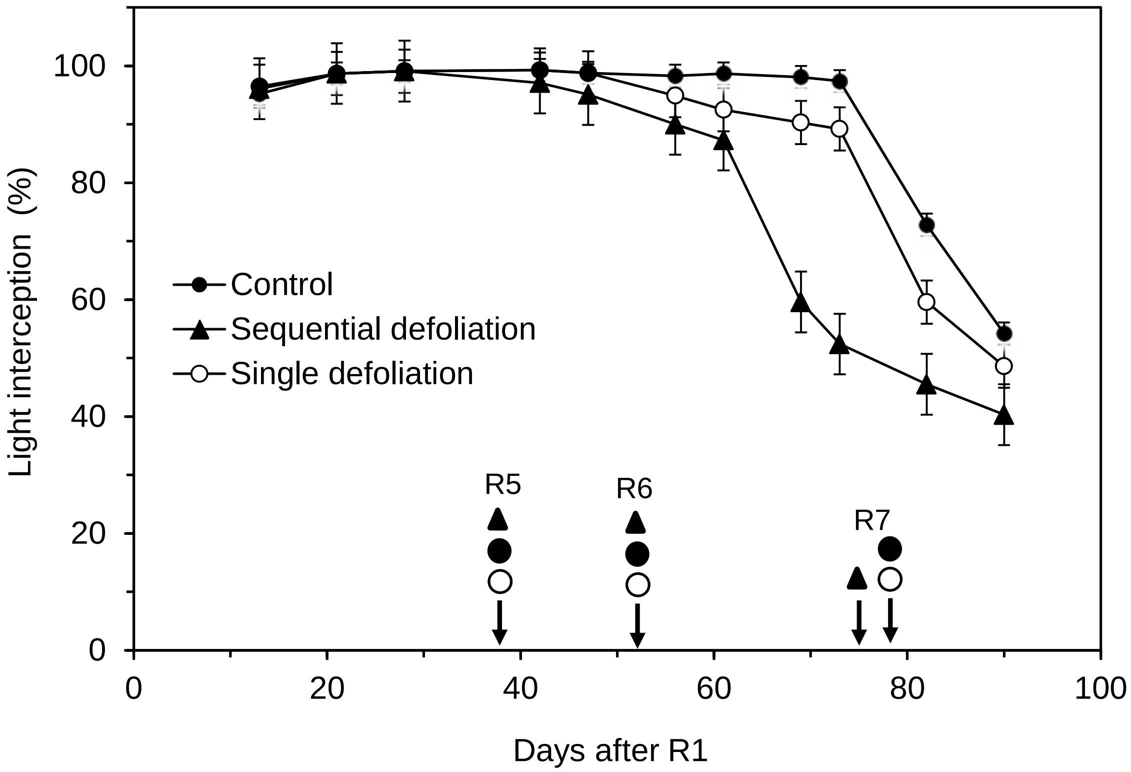

Figure 4.1. The effect of defoliation on light interception (LI) during reproductive growth, 2010. Vertical bars represent ± standard error of the mean. Arrows on the x-axis indicate the occurrence of beginning seed fill (growth stage R5), growth stage R6, and physiological maturity (growth stage R7).

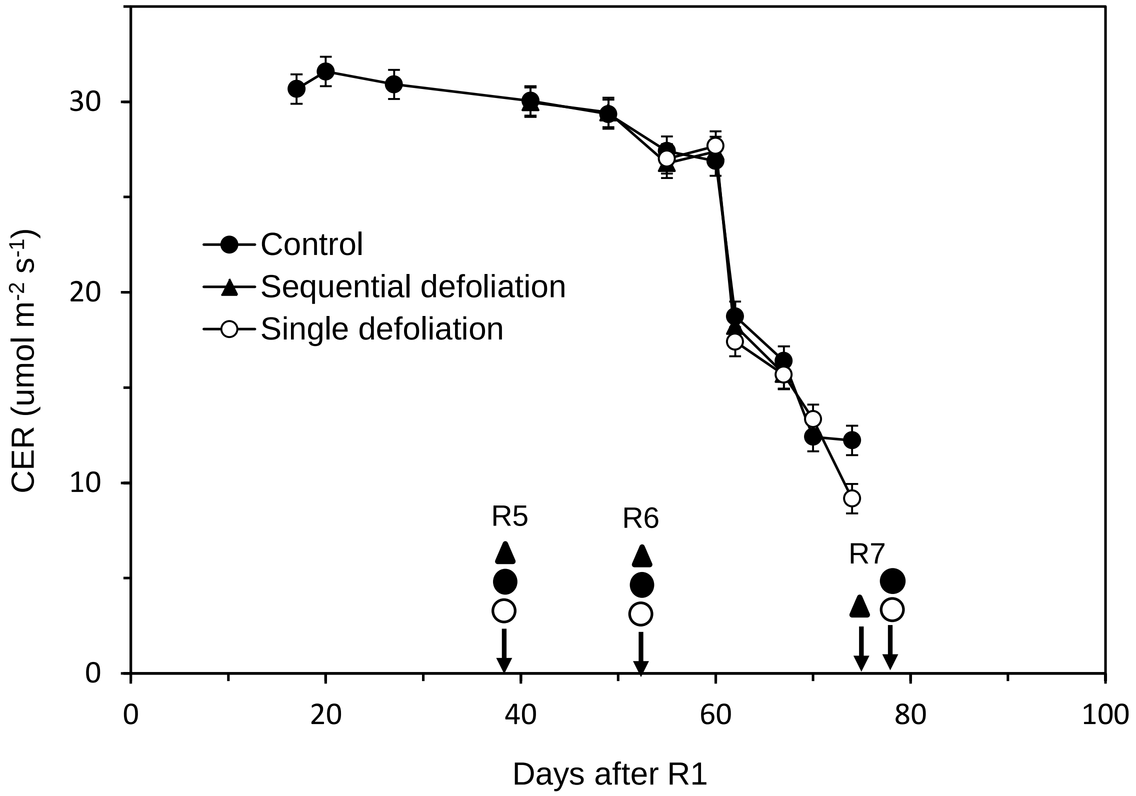

Figure 4.2. The effect of defoliation on carbon exchange rate (CER) during reproductive growth, 2010. Vertical bars represent ± standard error of the mean. Arrows on the x-axis indicate the occurrence of beginning seed fill (growth stage R5), growth stage R6, and physiological maturity (growth stage R7).

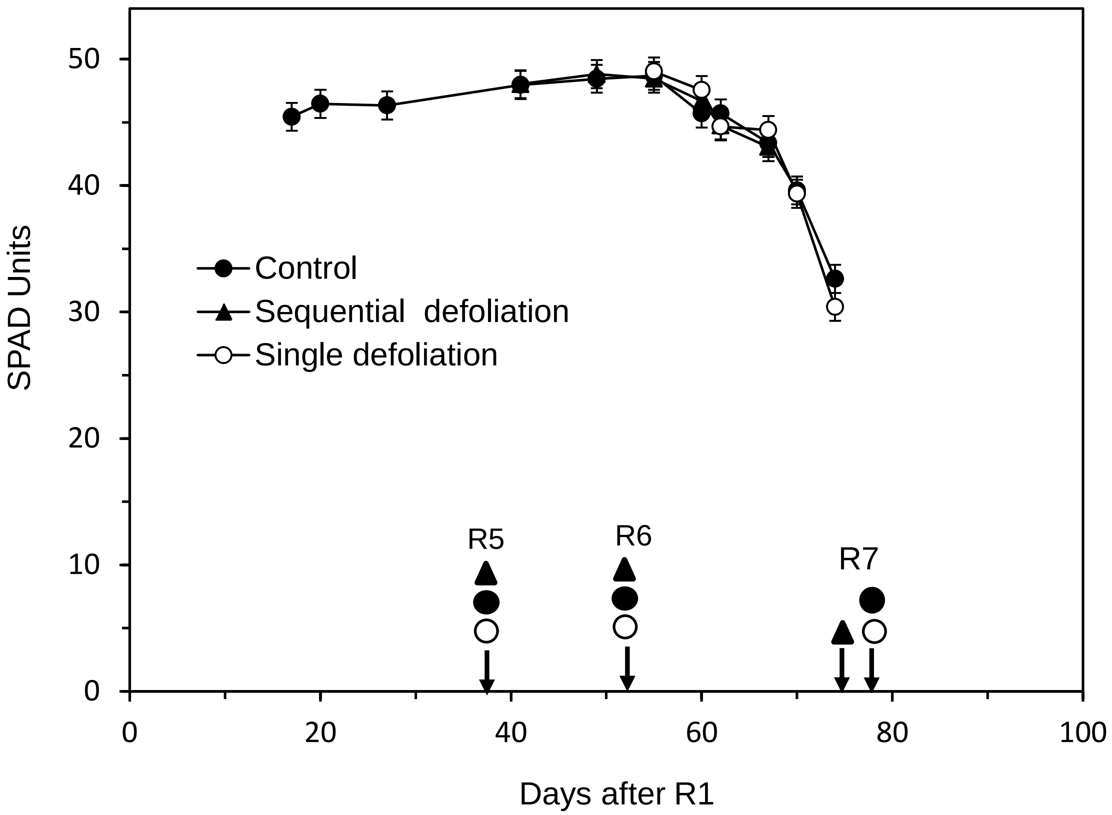

Figure 4.3. The effect of defoliation on leaf chlorophyll levels (estimated by a SPAD meter) during reproductive growth, 2010. Vertical bars represent ± standard error of the mean. Arrows on the x-axis indicate the occurrence of beginning seed fill (growth stage R5, and R6), and physiological maturity (growth stage R7).

Yield and Yield Components

Sequential defoliation reduced yield by 33% because of fewer seeds (18% less than control) and smaller seeds (18% less than control) and a shortened seed-filling duration (10% less than control), while the single defoliation had no significant effect on yield (Table 4.2). The sequential defoliation treatment also reduced the apparent HI by 30%.

Nitrogen Redistribution

The nitrogen concentration in the vegetative plants parts and the pod walls at growth stage R5 of the sequential defoliation treatment was equal to the control, but the single defoliation treatment tended to be less than control (significant only for stems and pod walls) at growth stage R6 because it was collected later than the control (Table 4.3). The nitrogen concentration in vegetative plant parts and pod walls decreased from the beginning of the seed-filling period (growth stage R5 or R6) to physiological maturity (growth stage R7) (Table 4.3) for all treatments. This lost nitrogen is assumed to be remobilized to the seed. The nitrogen concentration was higher in the leaves removed by defoliation than in the leaves still attached to plant at physiological maturity for all treatments. This difference occurred because the leaves were removed early in the seed-filling period when they were still green and functional. There was, however no effect of defoliation on the nitrogen concentration in the leaves that remained attached to the plants at physiological maturity or in the abscised leaves (Table 4.3). Defoliation had no effect on nitrogen concentration in the stem, pod walls, and the seed at physiological maturity.

Table 4.2. Effect of defoliation during seed filling on total dry matter, yield and yield components.

Treatments	Vegetative mass		HI[¶]	SFD[#] R5-R7	Seed number	Seed size	Yield
	Beginning seed-filling (R5 or R6)	Physiological maturity (R7)[†]					
	——g m^{-2}——		%	days	Seed m^{-2}	mg seed^{-1}	g m^{-2}
Control	602	508	50 a[††]	41 a	3110 a	160 a	495 a
Sequential defoliation[‡]	611	437	35 b	37 b	2542 b	131 b	335 b
Single defoliation[§]	776	457	48 a	41 a	3004 a	155 a	465 a
	NS[‡‡]	NS					

[†] Leaves, petioles, and stem dry weight, not including the leaves removed by defoliation or the abscised leaves and petioles.

[‡] Four defoliations at weekly intervals based on defoliation targets calculated from a SBR infected soybean canopy (Kumudini et al., 2008a). Plants were harvested at beginning of the seed-filling period (growth stage R5) after the treatment was applied.

[§] One defoliation at the beginning of growth stage R6. Plants were harvested at beginning of growth stage R6 after the treatment was applied.

[¶] HI, harvest index: seed mass divided by [seed mass + vegetative mass + abscised leaves and petioles]. Mass determined at physiological maturity (growth stage R7) and expressed on dry mass basis.

[#] SFD, seed-filling duration.

[††] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.

[‡‡] NS, not significant at $P < 0.05$.

Table 4.3. The effect of defoliation on tissue nitrogen concentrations at beginning seed filling (R5 or R6) and physiological maturity (R7).

Treatments	Leaves			Petioles		Stem	Pod wall	Seeds
	mg g^{-1}							
Beginning seed filling (R5 or R6)								
Control		48		14		19 a[#]	31 a	56
Sequential defoliation[†]		48		14		20 a	30 a	57
Single defoliation[‡]		45		13		16 b	26 b	56
		NS[††]		NS				NS
Physiological maturity (R7)[§]								
	On plant	Defoliated	Abscised[¶]	On plant	Abscised[¶]			
Control	26	---	18	9	5	5	11	62
Sequential defoliation	---	37	19	6	6	5	9	61
Single defoliation	22	36	18	7	6	4	8	62
	NS	NS	NS	NS	NS	NS	NS	NS

[†] Four defoliations at weekly intervals based on defoliation targets calculated from a SBR infected soybean canopy (Kumudini et al., 2008a). Plants were harvested at beginning of the seed-filling period (growth stage R5) after the treatment was applied.

[‡] One defoliation at the beginning of growth stage R6. Plants were harvested at beginning of growth stage R6 after the treatment was applied.

[§] Plants were harvested at physiological maturity (growth stage R7).

[¶] The abscised leaves and petioles represent material collected between the beginning of the seed-filling period (growth stage R5) and physiological maturity (growth stage R7).

[#] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.

[††] NS, not significant at $P < 0.05$.

Sequential defoliation starting at growth stage R5 reduced the nitrogen content of the remaining leaves by 13% (Table 4.4). Defoliation had no effect nitrogen content (g m^{-2}) in stems and pod walls at physiological maturity (Table 4.4), but the abscised leaves (includes leaves removed by defoliation) from the sequential defoliation treatment contained more nitrogen (Table 4.4). However, at physiological maturity the nitrogen content of the abscised petioles from the defoliation treatments was similar to the control.

Sequential defoliation reduced seed nitrogen content by 23% at physiological maturity (Table 4.4). Sequential defoliation also, reduced the nitrogen that was redistributed to the seed by 22% (Table 4.5); however, this reduction was not significantly different from the control (P=0.44). The proportion of the seed nitrogen that came from redistribution (39 to 42%) was not significantly affected by the defoliation treatments (Table 4.5).

Table 4.4. Effect of defoliation on nitrogen content in soybean vegetative parts and pod wall.

	Beginning seed fill (R5 or R6)[†]					Physiological maturity (R7)					R5 to R7[§]	
Treatment	Leaf	Petioles	Stem	Pod wall	Seed	Leaves[‡]	Petioles	Stem	Pod wall	Seed	Abscised Leaf	Abscised Petioles
	g m^{-2}											
Control	7.89 a[#]	1.44	4.41	2.28	1.75 b	0.30 a	0.30 a	1.13	1.11	25.63 a	2.11 b	0.38
Sequential defoliation[¶]	6.87 ab	1.54	4.88	2.44	2.06 b	---	0.20 ab	1.11	1.03	19.67 b	4.70 a	0.44
Single defoliation	5.63 b	1.58	3.95	2.73	2.75 a	0.14 b	0.10 b	0.96	1.06	24.16 a	1.66 b	0.46
		NS[††]	NS	NS				NS	NS			NS

[†] For the control and sequential defoliation treatment the plants were harvested at the beginning of the seed-filling period (growth stage R5) after the treatment was applied. For the single defoliation treatment the plants were harvested at the beginning of growth stage R6 after the treatment was applied.

[‡] Leaves on plant.

[§] Abscised material collected between the beginning of the seed-filling period (growth stage R5) and physiological maturity (growth stage R7) and it includes the leaves removed by defoliation.

[¶] Four defoliations at weekly intervals based on defoliation target calculated from SBR infected soybean canopy (Kumudini et al., 2008a).

[#] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.

[††] NS, not significant at $P < 0.05$.

Table 4.5. Nitrogen redistribution to the seed from vegetative plant parts and pod walls during seed filling.

	Source of redistributed N					Proportion of seed N from N redistributed
Treatment	Leaves	Petioles	Stem	Pod wall	total	RN/SN[‡]
	% of total[†]				g m^{-2}	%
Control	51 a[#]	7 b	31 b	11 b	10.7	42
Sequential defoliation[§]	26 b	11 a	46 a	17 a	8.3	42
Single defoliation[¶]	40 ab	11 a	31 b	18 a	9.5	39
					NS[††]	NS

[†] The amount of redistributed nitrogen contributed by each plant part, as a percent of the total nitrogen redistributed.

[‡] Proportion of the total seed nitrogen (SN) that came from redistributed nitrogen (RN).

[§] Four defoliations at weekly intervals based on defoliation targets calculated from a SBR infected soybean canopy (Kumudini et al., 2008a). Plants were harvested at beginning of the seed-filling period (growth stage R5) after the treatment was applied.

[¶] One defoliation at the beginning of growth stage R6. Plants were harvested at beginning of growth stage R6 after the treatment was applied.

[#] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.

[††] NS, not significant at $P < 0.05$.

Discussion

Soybean rust (SBR) causes defoliation from the bottom of the canopy upward, which reduces LI and therefore yield. Defoliation may also influence senescence and nitrogen redistribution to the seed by reducing the nitrogen available for redistribution, and that may limit yield. The objective of this study was to investigate the affect of defoliation on the rate of leaf senescence and nitrogen redistribution during seed filling.

Yield and Seed-Filling Duration

Defoliation reduced yield. Seed number per unit area and seed size were both reduced when sequential defoliation was applied at growth stage R5 due to a reduction in LI and the assimilate supply as reported in chapter three. A single defoliation early in seed-filling, which as intended, had no affect on LI, did not affect yield. The yield reduction from the sequential defoliation treatment was the result of fewer and smaller seeds suggesting that events during flowering and pod set, and seed filling were responsible. Many researchers reported that reductions in yield were attributed to reductions in LI (Board et al., 1994, 2010; Ingram et al., 1981; Browde et al., 1994; Higley, 1992; Haile et al., 1998; chapter three). Our results are also in agreement with those of Vasilas et al. (1989) who reported that severely sequential defoliation from the bottom of the canopy upward reduce yield. They found that the sequential defoliation started at growth stage R5 and a week after R5 reduced yield by 18% and 9%, respectively. The failure of the single defoliation treatment, that did not reduce LI, to affect yield supports the contention that yield reduction from sequential defoliation was a result of a lower LI. The sequential defoliation treatment started at growth stage R5, there was reduction in LI occurred before the end of critical phase for determining seed number (Egli, 2010), in contrary to the results in chapter three the seed number was reduced. More research is needed to investigate these results.

The sequential defoliation shortened the seed-fill duration by four days (about 10% of control). This result agrees with the results in chapter three for 2008 and 2009, where seed-fill duration was reduced by the same sequential defoliation treatment. Several researchers reported that defoliation shortened the reproductive cycle in soybean and corn (*Zea mays* L.) (Barimavandi, 2010; Ingram et al., 1981; Jones and Simmons, 1983; Tollenaar and Daynard, 1978). In chapter two we found that sequential defoliation at beginning of seed-filling period (growth stage R5) reduced the leaf area duration (LAD) as did Kumudini et al. (2008a). Thus, in this study, the reduction in LAI most likely reduced the LAD which caused a shorter seed-fill duration.

The sequential defoliation treatment reduced LI and shortened the seed-filling duration due to the reductions in the assimilate available to the seed. Seed filling continues as long as there is assimilate available to the seed and the seed is active in converting the assimilate into storage compounds; when seed growth stops due to a lack of assimilate as result of reduction of canopy photosynthesis, the filling period is terminated (Egli, 2004). The single defoliation treatment did not reduce the assimilate supply and had no affect on seed-fill duration. These results suggest that, as reported in chapter three, defoliation affects yield only by reduction in LI.

Senescence

The chlorophyll content and carbon exchange rate (CER) of upper leaves were measured to characterize potential affects of defoliation on senescence of the remaining leaves. Defoliation during the reproductive growth phase also reduces the nitrogen potentially remobilizable to the seed. This reduction in nitrogen could accelerate leaf senescence and shorten the seed-fill duration according to the model of Sinclair and de Wit (1975, 1976). There was, however, no effect of defoliation on CER and leaf chlorophyll level as estimated by SPAD. In this study, the leaf chlorophyll level and CER started to decline at approximately the beginning of growth stage R6 and reached minimal levels near physiological maturity. These results agree with Crafts-Brander and Egli (1987a, 1987b), and Egli and Bruening (2003). Our finding is also in agreement with Peterson and Higly (1996) who reported that removal of a portion of a soybean leaflet does not change the photosynthesis rate of the remaining leaf. Haile (2001) proposed that the impact of defoliation on photosynthesis was explained by plant-water relations. He found that the defoliation had no effect on the photosynthesis rate of well-watered soybean plants. Instead, defoliation improved the leaf water potential of soybean under water stress. In this study the plants in the field plots were well-watered, so our results are consistent with Hail finding (2001). In opposition to the “self-destruct” hypothesis (Sinclair and de Wit, 1975, 1976) and to our findings, Klubertanz et al. (1996) found that defoliation delayed the senescence of lower leaves. However, in that study, the defoliation occurred at growth stage R2 at the top of the canopy and exposed the lower leaves to higher levels of solar radiation, which probably accounted for the delay in senescence.

Our results provide no evidence that defoliation accelerates leaf senescence. This provides additional support to the proposition that the main effect of defoliation on yield is by reducing LI.

Nitrogen Redistribution

During the seed-filling period nitrogen in the leaves translocated to the seed from vegetative plant parts and pod walls. Sinclair and de Wit (1975, 1976) proposed that the amount of nitrogen in the vegetative plant parts at growth stage R5 is important to the yield production process.

In this study the nitrogen in the abscised leaves and those leaves removed by defoliation, in the abscised petioles, and in the stem at physiological maturity was not available for redistribution to the seed. Thus, the amount of nitrogen redistributed was the nitrogen in the vegetative plant parts at the initial harvest minus the amount not available for redistribution. Defoliation increased the amount that was not available for redistribution. The initial harvest was earlier in the control and sequential defoliation treatment (by 12 days) than in the single defoliation treatment. The rate of leaf senescence from growth stage R5 to R6 was slow (Fig. 4.2 and 4.3), therefore, the amount of nitrogen that was redistributed between the imposition of the sequential and single defoliation treatments to the seed was minimal. Most of the nitrogen redistribution occurred after growth stage R6 suggesting that comparison of the defoliation treatments that began at growth stage R5 and R6 was valid.

The primary source of redistributed nitrogen was the leaves, followed by stems and pod walls. Similar observations have been reported for soybean by Egli et al. (1983) and Zeiher et al. (1982). Sequential defoliation reduced the leaf nitrogen available for redistribution (increased the amount in abscised leaves, including those removed by defoliation), the leaf nitrogen content (g m^{-2}) at beginning of the seed filling, seed nitrogen content at physiological maturity (due to lower yield), and the total nitrogen redistributed to the seed; however the latter reduction was not significantly different from the control (Table 4.5, $P=0.44$). The nitrogen redistributed from stem, petioles, and pod walls, however, was higher than the control, which with the reduction in total seed nitrogen (due to the lower yield) maintained the same proportion of nitrogen that came from redistribution as the control.

The proportion of seed nitrogen that came from redistribution ranged from 39 to 42% across treatments, which falls in the range (33 to 100%) reported by Egli et al. (1983) and Zeiher et al. (1982). Our findings indicate that when the soybean plant experiences accelerated defoliation stress during the seed-filling period, such as that caused by SBR, yield and seed nitrogen content (g m^{-2}) are reduced, so less nitrogen from redistribution is needed, but, the plant also remobilizes more nitrogen from stem, petioles, and pod walls to help sustain the contribution from redistribution. Interestingly, in this study the sequential defoliation which shortened the seed-fill duration and reduced yield had similar proportion of seed nitrogen from nitrogen redistribution as the control.

The single defoliation treatment reduced leaf nitrogen content and caused a reduction (11%) in the total nitrogen redistributed to the seed, but the reduction was not significantly different from the control (*P*=0.44). On other hand, the seed nitrogen content at physiological maturity was similar to the control because the nitrogen content of the leaves removed by defoliation had only minimal effect on the nitrogen redistributed to the seed and the plant remobilized more nitrogen from petioles, and pod wall.

Sequential defoliation reduced LI and seed yield, but it did not change the nitrogen concentration in the seed. The single defoliation treatment that reduced the vegetative mass (stored nitrogen and carbon) without reducing LI below 90% (i.e., did not affect assimilate production) had no effect on yield or seed nitrogen concentration. These results suggest that carbon assimilation limits yield more than nitrogen does because manipulation of the carbon supply had a greater effect than manipulation nitrogen. Our results agree with Hayati et al. (1996) who found that carbon and nitrogen metabolism in soybean seeds were not tightly linked, and that the accumulation nitrogen in the seed was not an absolute requirement for seed dry matter accumulation.

According the to “self-destruct” model, increasing seed nitrogen per unit area (i.e., increasing yield without changing seed nitrogen concentration) without increasing vegetative mass will accelerate leaf senescence and shorten the seed-filling period (Sinclair and de Wit, 1976). Contrary to the “self-destruct” hypothesis, the single defoliation reduced the vegetative nitrogen but had no effect on yield, seed nitrogen content or seed fill duration comparing it to the control. This finding agrees with Egli and Bruening (2007a; 2007b) who reported that there was no evidence that the accumulation of a larger amount of nitrogen in the seed accelerated leaf senescence or shortened the seed-filling period.

In summary, defoliation that mimicked SBR at growth stage R5 did not accelerate leaf senescence in the remaining leaves (i.e., did not affect leaf photosynthesis or chlorophyll level), but it reduced yield, shortened the seed-filling duration and reduced the nitrogen redistributed to the seed but not its contribution to seed nitrogen. The main effect of defoliation was reduction in the assimilate supply as result of reduction in the leaf area and LI, which ultimately shortened the seed-filling duration and reduced yield.

Abstract

Defoliation of a soybean canopy may affect leaf senescence and nitrogen remobilization during the seed-filling period. Two defoliation treatments-sequential removal of leaf area between growth stage R5 and R7 and a single defoliation at growth stage R6 that did not reduce light interception below 90%-were applied in the field. All leaf removal was from the bottom of the canopy. The rate of senescence during seed filling of an upper leaf on the defoliation treatments, as estimated by the change in leaf carbon exchange rate and chlorophyll concentration, did not differ from the control plants. Thus there was no evidence that defoliation accelerated leaf senescence. The single defoliation treatment did not reduce yield or the total nitrogen redistributed to the seed.

Sequential defoliation reduced light interception, seed number, seed size, yield, and shortened the seed-fill period by 10%. Sequential defoliation reduced the nitrogen redistributed to the seed (not significantly different from the control). The amount of nitrogen redistributed from stems, petioles, and pod walls in the sequential defoliation treatment was slightly higher than the control, which, coupled with the reduction in yield and seed nitrogen content, resulted in the same proportion of redistributed nitrogen to total seed nitrogen as the control. The primary effect of defoliation, such as that caused by soybean rust, was to reduce the assimilate supply as result of reduction in the leaf area and light interception, which ultimately shortened the seed-filling duration and reduced yield. The effects on leaf senescence and nitrogen redistribution were minimal.

CHAPTER FIVE

CONCLUDING REMARKS

Soybean rust (SBR) (caused by *Phakopsora pachyrhizi* Syd. and P. Syd) is a serious threat to soybean [*Glycine max* (L.) Merr.] production in the U.S. All currently available commercial soybean cultivars grown in the U.S. are susceptible to SBR and cultural controls are very limited in effectiveness, consequently, fungicides are the only means of control if this disease invades the major soybean growing regions of north-central United States. The disease accelerates leaf abscission from the bottom of the plant and reduces green leaf area (due to necrotic and chlorotic lesions on the remaining leaves) which limits yield by reducing the plant's ability to intercept and absorb solar radiation.

In the United States, field inoculation study that is necessary to study the effect of the disease on soybean is prohibited due to possibility of spread the inoculum to healthy neighbor fields or states. Thus, artificial simulation of the disease's impact is the only option available to United States researchers. When the project described in this dissertation began there was no method available to simulate SBR effects on plant growth and yield. Developing a simulation method to estimate yield loss under field conditions would be a vital step toward developing effective SBR control practices. A reliable and accurate method of simulating SBR injury should also contribute to the development of a yield loss prediction tool.

The objectives of this study were i) to evaluate the use of manual defoliation to simulate SBR damage to a healthy soybean canopy; ii) to characterize the effect of defoliation on the yield production process by determining its effect on light interception, crop growth rateand radiation use efficiency, seed number and size, and seed growth rate; and iii) to determine if defoliation affects the rate of leaf senescence and nitrogen remobilization during the seed-filling period.

To effectively simulate SBR injury we should understand the mechanisms responsible for the reductions in plant growth and yield. It was confirmed here that manual defoliation that mimicked SBR damage reduced light interception by the soybean canopy, and this reduction was the driving force for all of the consequent events in the reduction of plant growth and yield loss. The main affect of the reduction in light interception during seed filling was a reduction in the seed growth rate and the seed-fill duration leading to smaller seed, and lower yield. There was evidence that manual defoliation reduced seed number, but light interception was not reduced during the critical period between growth stage R2 and R5 and there was no reduction in crop growth rate during this period. Thus, the mechanism of how seed number was reduced was not clear. Light interception was significantly lower only at the end of the critical period (i.e., between growth stage R5 and the beginning of R6) which could have accounted for the lower seed number. Alternatively, removal of the lower leaves could have reduced pod set of lower nodes without any affect on light interception. Additional research is needed to resolve these issues.

Simulation of SBR by manual defoliation predicted that SBR infestation at growth stage R2 would cause a larger yield loss (about 70%), due to reduced seed number per unit area and seed size, than onset of SBR at the beginning of the seed-filling period, i.e., growth stage R5 (about 40% due to primarily to small seed). Thus, maintaining soybean fields free of SBR from flowering (growth stage R2) until physiological maturity (growth stage R7) is warranted to avoid any yield loss.

There are some theories in the literature suggesting that defoliation, especially from the bottom of the canopy, which reduces the total plant nitrogen, might accelerate leaf senescence, shorten the seed-fill duration, and reduce yield. However, in this research we found no evidence that these mechanisms contributed to the reduction in yield from simulated SBR, suggesting that there may be more than one factor controlling leaf senescence and that it is not just simply controlled by nitrogen supply and demand.

Manual defoliation was very effective in simulating the impact of SBR on yield. Defoliation simulated the change in effective leaf area index due to SBR, and was based on the percentage change in effective leaf area index of infected plants relative to the control. The relationship between the effective leaf area duration and yield loss as percent of control was significant and strong. The relative reduction in the effective leaf area duration predicted the relative reduction in yield very well across two states (Kentucky and Louisiana) using different cultural practices (i.e., row spacing, maturity group, and cultivars). The reductions were close to what was found with diseased soybean plants in Brazil. This correlation model can be used to predict yield loss in areas where SBR infection is expected and it will help growers and the extension service allocate their efforts to effectively control SBR.

This association between the effective leaf area duration and yield loss was used to develop a yield loss prediction model that is available to growers and extension agents. This model includes a fungicide spray decision tool based on our understanding of how SBR develops and damages soybean plants. The tool calculates yield losses based on three SBR infection levels or epidemic type (low, medium, and high) and soybean growth stage, and estimates the economic benefits of spraying based on the yield response, the cost of spraying and other related costs. The model is available at http://dept.ca.uky.edu/sbrtool/.

The data presented in this dissertation are novel and are valuable to help to control SBR by develop a model to estimate the yield-loss. This research could have been improved if we had been able to compare the manual defoliation to a natural SBR infected canopy in the U.S. Also, we did not compare low vs. high SBR infestation in Brazil, thus we don't know for sure how the level of infestation will affect leaf abscission and the seed-fill duration. The manual defoliation process *per se* caused plant injury and that would expose plants to pathogenic microorganism infestation which could impact leaf abscission.

Suggestions for future studies include investigation of the mechanism(s) by which defoliation reduces seed number per unit area. Is this reduction due to increased a pod abortion, decreased flower production, or reduced number of seed per pod? Also, we recommend investigating the affect of manual defoliation on nitrogen redistribution in soybean plant for second year to confirm our preliminary results.

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VITA

Author's Name Abdullah Aqeel

Birthplace AL-Ramtha, Jordan

Birthdate February 21, 1976

Education

Master of Science in Agronomy.
Jordan University of Science and Technology
May – 2001

Bachelor of Science in Plant Production.
Jordan University of Science and Technology
May – 1998

Research Experience
Graduate Research Assistant
University of Kentucky
Lexington, KY
June/2008 – July/2011.

Graduate Teaching Assistant, and
Graduate Research Assistant
Jordan University of Science and Technology
Department of Plant Production
October 1998 – May 2001.

Peer Reviewed Publications

Aqeel A.M., Hameed, K., and Alaudat, M. 2007. Effect of olive mill by-products on mineral status, growth, and productivity of faba bean. J. of Agronomy 6:403-408.

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Published Abstracts

Aqeel A.M., E. Prior, J. Omielan, J. Board, and S. Kumudini. Can we simulate the impact of soybean rust in the south central soybean production regions of the United States?. Abstract. Proceedings of the American Society of Agronomy Annual Meeting – The American Society of Agronomy, 01-05 Nov 2009. Pittsburgh, PA, USA; Nov 2010 Supplement.

J. Omielan, **Aqeel A.M**. E. Prior, J. Board, C. Godoy, D. Wright, B. Kemerait, W. Dong, and S. Kumudini. The development and validation of a simple yield loss prediction model for soybean rust. Abstract. Proceedings of the American Society of Agronomy Annual Meeting – The American Society of Agronomy, 01-05 Nov 2009. Pittsburgh, PA, USA; Nov 2009 Supplement.

Honors, Awards, and Activities

- 3rd place, graduate student competition poster, ASA meeting, Pittsburgh 2009
- Two years on university honor list 1999, and 2000, Jordan Univ. of Sci and Technology.
- Member of the American Society of Agronomy (ASA).