This is to certify that the
thesis entitled

EFFECT OF INOCULANT, FUNGICIDAL, AND INSECTICIDAL SEED TREATMENTS ON SOYBEAN GROWTH AND YIELD IN MICHIGAN

presented by

Terry J. Schulz

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Crop and Soil Sciences

Major Professor's Signature

4 June 2007

Date

EFFECT OF INOCULANT, FUNGICIDAL, AND INSECTICIDAL SEED TREATMENTS ON SOYBEAN GROWTH AND YIELD IN MICHIGAN

By

Terry J. Schulz

A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

MASTERS OF SCIENCE

Department of Crop and Soil Sciences

2007

ABSTRACT

EFFECT OF INOCULANT, FUNGICIDAL, AND INSECTICIDAL SEED TREATMENTS ON SOYBEAN GROWTH AND YIELD IN MICHIGAN

By

Terry J. Schulz

In recent years, several new varieties of soybean seed treatments have been introduced to the marketplace, including inoculants and neonicotinoid insecticides. Researchers throughout the Midwest have generated conflicting results regarding the benefit of inoculating soybean seed in successive plantings. One objective of this research was to determine whether inoculation of soybean seed for successive plantings in Michigan. Use of soybean seed inoculant increased soybean grain yields on 6 of 14 sites that had been in a soybean rotation. Another objective was to determine whether interaction between inoculant and Apron Maxx RTA (mefenoxam and fludioxonil) fungicide could be observed. Interaction between inoculant and fungicide was not observed on sites that had been in soybean rotation. However, significant interaction was observed at two sites where soybeans had not been previously grown. The objectives of the neonicotinoid seed treatment research were to a) determine whether neonicotinoid seed treatments increase soybean grain yield, b) determine the duration of soybean aphid control provided by neonicotinoids, and c) determine whether neonicotinoids improve soybean plant health. Yield increases from neonicotinoid seed treatments were only observed in 2005, a year of significant aphid pressure. Significant neonicotinoid effects on soybean aphid populations ended after R2. Neonicotinoid seed treatments did not contribute to soybean plant health on a wide-spread basis

ACKNOWLEDGMENTS

I would like to sincerely thank the members of my graduate committee, Dr. Kurt Thelen, Dr. Karen Renner, and Dr. Chris DiFonzo. Special thanks to Dr. Kurt Thelen who provided me with the assistantship and served as my academic advisor. I got my start in agricultural research thanks in large part to Dr. Karen Renner, who hired me as an undergraduate, and has been a tremendous role model both as a researcher and an instructor.

Special thanks to Bill Widdicombe, John Boyse, and Keith Dysinger, technicians who help coordinate field research. Mike Boring was instrumental in conducting these trials in 2003 and 2004, and this thesis would not be possible without his work. Dr. Sasha Kravchenko was extremely helpful with data analysis. Thanks to the Michigan Soybean Promotion Committee for funding this research project. Thanks to the graduate and undergraduate students who helped collect data for this study, including Steve Wagstaff, Anatoily Kravchenko, Jim Williams, Deanne Sweeney, and Kelly Foote. My wife, Gina, has also helped me along in many ways during this project, and I appreciate everything she has done for me. Finally, I also thank my entire family for their patient support of my educational pursuits over the year.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

CHAPTER 2

LIST OF FIGURES

LITERATURE REVIEW

Introduction

Soybean (*Glycine max* (L.) Merr.) is the most economically important oilseed crop grown in the United States, constituting 90% of U.S. annual oilseed production (Ash et. al. 2006). Major expansion of soybean production in the United States has occurred since World War II, when soybean was promoted as an alternative source of edible oils for the war effort. Since 1923, US annual soybean production has increased from 5 million bushels to 2.97 billion bushels in 2005 (Hymowitz 1990, USDA). Soybean was grown on 28.9 million ha and valued at $17.4 billion (Ash et. al. 2006).

Soybean has become a popular crop in the United States for several reasons. Export markets for US soybeans have been very strong, and soybean production is economically competitive to other cash crops, as a result of relatively lower input costs. Average variable cost per acre of soybean production was estimated to be half that of an acre of corn in 2006 (Dobbins 2006). Differences in variable costs can be attributed to fuel use in tillage, grain drying, and hauling, as well as pesticide costs. However, the most significant difference between corn and soybean cropping budgets is fertilizer usage. For 2006, the fertilizer cost for corn on high productivity land was estimated at \$271 ha^{-1}, compared to \$94 ha^{-1} for soybean (Dobbins and Miller 2006). This difference is increasing due to the high cost of natural gas that is used to synthesize ammonia for nitrogen fertilizer required for high yield corn production. Soybeans do not have a fertilizer N requirement; therefore fertilizer costs in soybean are greatly reduced.

Soybean is a legume, and is therefore able to fix its own nitrogen through an association with symbiotic bacteria. This biological process is known as symbiotic nitrogen fixation. Symbiotic nitrogen fixation is one of the most important plant physiological functions in cropping systems worldwide, accounting for 60% of Earth's fixed nitrogen (Zahran 1999). The ability for leguminous crops to fix atmospheric N_2 through symbioses with rhizobial bacteria allows crops such as soybean, cowpea (*V. unguiculata*), and alfalfa (*M. sativa*) to be grown without added nitrogen fertilizer inputs and on soils with marginal nitrogen reserves. Up to 280 kg ha^{-1} of N can be fixed through symbiotic nitrogen fixation in soybean, accounting for about 70% of total plant nitrogen requirements (Lindemann and Glover 2003, Tien et. al. 2002). One of the reasons soybean is capable of fixing nitrogen is the efficiency of the symbiosis between plant root and the symbiont, a rhizobial bacteria species specific to soybean. Other legumes, such as common bean, do not have a high efficiency symbiont, and therefore require supplemental nitrogen fertilization to obtain the highest yields (Berglund 1997).

The bacterial species that is the most effective symbiont to soybean cultivars in the Americas is *Bradyrhizobium japonicum*. A second species, *Rhizobium fredii* will also form symbioses with soybean (Dowdle and Bohlool 1985). However, this symbiont has a wide range of host legumes, and will only nodulate specific, mostly Asian soybean cultivars (Balatti and Pueppke, 1992; Heron and Pueppke, 1984; Keyser et al. 1982), *B. japonicum* is not ubiquitous; on lands where soybean was not produced in the past, it is unlikely that a resident *B. japonicum* population is present. In cases where a new land area is brought into soybean production, an inoculant is usually necessary to provide the *B. japonicum* required for symbiotic nitrogen fixation. Current inoculation techniques usually involve a commercially produced, highly concentrated rhizobium product in a packaged liquid suspension, a sterilized peat-based powder, or a granular concentrate.

These inoculants can be applied in-furrow near the seed, but are usually placed directly on the seed as a seed treatment at or near planting time.

The yield benefit from use of commercial inoculant on land where inoculation has not occurred previously is substantial, and can reach 100% (Senviratne et. al. 2000, Duong et. al. 1984). However, there are conflicting reports in the Midwest regarding the effectiveness of successive inoculations of soybean plantings. Historically, successive soybean inoculation was deemed unwarranted if healthy soybeans have been grown in a field in recent years (Vitosh 1997). Other recent studies provided evidence that successive inoculation of soybean plantings is usually a profitable practice and encouraged farmers to inoculate their soybean plantings each year. Ohio State University inoculant trials produced average yield increases of 175 kg ha^{-1} in 2004 and 121 kg ha^{-1} in 2005 on ground in soybean rotation (Beuerlein 2004, 2005). As a result, recommendations regarding successive inoculation of soybean plantings differ between states in the Midwest.

Taxonomy and Morphology of Soybean

Cultivated soybean (*Glycine max* (L.) Merr.) is in the soia subgenus of the *Glycine* genus. The soia subgenus consists of the annual soybean species: the cultivated form (*Glycine max* (L.) Merr.), and wild form (*G. soia* Sieb. & Zucc., Newell and Hymowitz 1981). Cultivated soybean, an annual dicot with large cotyledons, produces alternate trifoliate leaves after the V1 stage, where the first pair of true leaves are unifoliate. The flower is a standard papilionoid with five united sepals forming the calyx, ten diadelphous 9+1 stamens forming the androecium, and an apocarpous gynocium. The flowers are predominantly self pollinating, with less than 1% cross pollination, and pods usually contain 2-4 seeds (Carlson and Lersten, 1987). The soybean plant grows approximately 1 meter in height and can have a branched stem. Soybean varieties grown in Northern latitudes of the United States such as Michigan are indeterminate. The root system is characterized between taproot and diffuse types and is usually well nodulated by rhizobacteria (Lersten and Carlson, 1987).

History of Soybean Production

Hymowitz (1990) suggests that soybean was first domesticated in Northeastern China about 3000 years ago, likely during the Shang dynasty. By the first century A.D., soybean production spread throughout mainland China and parts of Korea. Soybean cultivation spread toward other portions of Asia as new trade routes were developed and populations emigrated from China to other lands such as Thailand. By the 1600's, soybean cultivation spread to most of Southeast Asia and India. Soy foods are important sources of protein in this region, and were quickly adopted as a staple in Southeast Asia. References to soy sauce and tofu were mentioned in the diaries of European visitors since the late 16th century. Soybean plantings probably did not occur in Europe until the early 1700's, and North America until the late 1700's. The use of soybean for forage was studied throughout the 19th century in North America. USDA and agricultural experiment stations continued to evaluate soybean cultivars and promoted new uses for soybean grain in the United States. By the 1920's soybean grain production had truly become an industry in the United States (Hymowitz 1990).

Soybean grown commercially in the United States are generally planted in row crop monocultures. Soybean in Michigan is usually planted in May, with row spacings between 7.5 and 30 inches. Soybean seed can be saved from a previous crop and cleaned by the farmer for use as seed the following year, as long as the variety is not proprietary. Currently, most farmers in the US use some type of commercial proprietary seed source, particularly glyphosate-resistant seed, which is illegal to save for use as seed the following year. In 2005, glyphosate-resistant varieties comprised over 80% of all soybean planted in the United States (Dill 2005). Soybean in Michigan is typically harvested in late September or October using a mechanical combine and grain header. In Michigan during 2004, 2 million acres of soybean was planted, producing 75.24 million bushels, valued at $380 million. Soybean ranks second to corn among all Michigan crop commodities in planted acres and production value (Kleweno and Matthews 2005).

Biological Nitrogen Fixation

Biological nitrogen fixation is a symbiotic relationship requiring a system of molecular signaling between plant roots and a bacterial organism. These few specific species of prokaryotic bacteria are able to reduce atmospheric N_2 into ammonia (NH_3), a form that plants can assimilate into useful biomolecules, such as nucleic acids, amino acids, and coenzymes (Gallon and Chaplin 1987). These specific bacteria are known as diazotrophs. However, not all diazotrophs are symbiotic; some are free living and some are associative. True symbiotic diazotrophs such as *B. japonicum* evolved physiological and morphological adaptations with their symbiont plants, and these systems encourage the continuation of the symbiosis (Gallon and Chaplin 1987).

A population of rhizobial bacteria live and reproduce as free-living organisms in the soil, or on a seed in contact with soil in the case of inoculated soybean. Rhizobia preferentially inhabit the rhizosphere, an environment surrounding the soybean root that is rich in sugars and amino acids produced as root exudates by the plant (Uren 2000). This makes the soybean root area favorable for growth and reproduction of all types of soil bacteria. Soybean roots, like those of other legumes, have the ability to produce chemotactant materials, which attract specific bacteria. In soybean root exudates, organic acids such as succinate, malate, and malonate, and the amino acids glutamate and aspartate elicit chemotactic responses from *B. japonicum* (Barbour et. al. 1991).

Evidence suggests that symbiotic bacteria attach to the legume root hairs via a two step process. Initial attachment can involve root-produced lectins binding to carbohydrate structures present on rhizobial bacteria (Dazzo et. al. 1984). Initial attachment can also result from an adhesive bacterial protein, known as rhicadhesin (Smit et. al. 1992). The second step involves permanent anchoring of the bacteria to the root. In soybean, anchoring is promoted by the pili, fibrous protein structures found on the surface of gram negative bacteria used for bacterial attachment to other organisms (Ottow 1975). Strains of *B. japonicum* lacking pili have reduced attachment to soybean roots, reducing nodule induction (Vesper and Bhuvaneswari 1988). *B. japonicum* preferentially infects emerging root hairs because as root hairs mature, physical defenses are developed that prevent such infection (Siu-Cheong Ho 1994).

Nod factors are signal compunds produced by rhizobia that induce a number of physical changes that condition the root hair to accept the onset of infection and eventual symbiosis. Their production depends on the expression of a set of *nod* genes by the bacteria, which are induced by flavenoids or isoflavenoids produced by the plant (Spaink 1995). These signal molecules are lipochitooligosaccharides, also known as the nod factors. These nod factors elicit responses such as initiation of the nodule primordia in the plant cortex, induction of plant nodulin genes expressed early in the nodulation process, and curling of root hairs (Broughton et. al. 2000). These root responses occur in the presence of nod factors at concentrations as low as picomolars (D'Haeze and Holsters 2002).

After the bacterium attaches itself to the root hair, nod factors elicit a response, causing the hair to curl around the bacteria. This takes place as soon as within 12 hours of bacterial attachment (Bauer and Turgeon 1982). This curling of the root hair traps the bacteria within the curled root. Once the bacterium is encircled by the root hair, an area of swelling develops near the site of infection. Then an area of existing cell wall is hydrolyzed and a new tubular structure is synthesized, called an infection thread. This infection thread allows the bacterium to penetrate the plant cell wall (Callaham and Torrey, 1981). While the infection thread penetrates the root hair, a cell wall is formed around the outside of the thread to contain the bacteria and prevent complete invasion and death of the root hair (Gallon and Chaplin 1987). The infection thread then grows toward the nodule primordium, an area of cells within the root cortex induced to resume active division by nod factors. The nod factors also induce progressive differentiation of cells within the nodule. As the infection thread reaches the nodule primordium, the bacteria are released into the cytoplasm of the plant cell though structures called infection droplets; this process is known as endocytosis (Brewin 1991). As the primordium develops into a nodule, several plant nodulin genes are activated within all the cells within the primordium, which differentiate their functions from typical root and shoot meristems (Mylona et. al. 1995).

Nodule primordia become functional nodules after rhizobial bacteria move from the infection thread into nodule primordial cells. As bacteria enter the cytoplasm of the nodule primordial cells these cell are converted for use in nitrogen fixation. These converted cells are known as bacteroids. Bacterioids are surrounded by a plant derived membrane, the peribacteroid membrane. This membrane protects the bacteroid from plant defensive responses and serves as the interface where exchange of metabolites between plant and bacterial cells occur (Mylona et. al. 1995). The ureides allantoin and allantoic acid are the final N metabolites; these are produced at the perioxisomes of uninfected soybeans nodule cells and then exported through the peribacteroid membrane and transported to the plant (Streeter 1972, McClure and Israel 1979, Hanks et al 1983). Sucrose is the carbon metabolite transported from leaves through the vascular tissue to the nodule. Sucrose is then hydrolyzed by sucrose synthase into the products fructose and UDP-glucose. These products are used for starch and cellulose synthesis within the nodule, or are further metabolized (Kavroulakis et. al. 2000, Day and Copeland 1991).

The conversion of N_2 gas into NH_3 ammonium ions is catalyzed by the enzyme nitrogenase. For this reaction to take place, a low oxygen content must be present within the nodule, because a vital cofactor of the nitrogenase enzyme is deactivated in the presence of oxygen (Shah and Brill 1977). To accomplish this, a nodule parenchyma layer is formed around the nodule, eliminating oxygen diffusion. Oxygen is then delivered to bacteria by leghemoglobin carrier proteins; this allows the bacteria to continue aerobic respiration (Mylona et. al. 1995).

Life Span of Soybean Nodules

The time between first soybean root exposure to *B. japonicum* and the onset of symbiotic nitrogen fixation is very dependent of the root zone temperature. Soybean nodulation and nitrogen fixation is optimal at 25 degrees centigrade, and is significantly reduced below 17°C, (Lynch and Smith 1993, Dart and Day 1971, Jones and Tisdale 1921). A laboratory study determined that at optimal temperature, soybeans can fix nitrogen approximately 20 days after initial exposure to *B. japonicum*. At 15°C, this process takes between 39 and 45 days (Zhang and Smith, 1994).

Soybean nodules have a limited life span. Nodules first form on soybean roots around the V2 stage of development, and will continue to form until approximately seven weeks after planting (Klukas 1974). Nodules of annual plants only fix nitrogen at highest capacity for about three to five weeks (Puppo et. al. 2005). An experiment observing timing of nodule senescence of a group II variety soybean found that nitrogen fixation declined sharply between 58 and 65 days after planting, which was between two and three weeks after flowering in this study (Klucas 1974). As nitrogen fixation slows, the nodule will begin to senesce starting at the central tissue and working toward the outside of the nodule over a few weeks. This makes the total life span of a nodule about 10-12 weeks in length (Puppo et. al. 2005).

About half of the N used for seed fill is remobilized from plant leaves, rather than being fixed and directly supplied by nodules or taken up as mineral N by roots (Imsande and Touraine 1994). Over half of soybean leaf N content is moved to the developing seed during podfill (Hanaway and Weber 1971). Lawn and Brun observed that as soybean pod fill begins, N fixation by nodules peaks and then rapidly decreases. Plant depodding allowed nodule activity to be maintained longer, while plant defoliation caused N fixation in nodules to decline earlier (Lawn and Brun 1974). Others have found N fixation to peak later than early pod fill. (Thibodeau and Jaworski 1975, Abendroth and Elmore 2006). One hypothesis to explain nodule death is that the plant-nodule relationship may be a source-sink relationship, and that as photosynthate is supplied to new seeds, it is diverted from nodules, particularly if resources are limited under stressful conditions. While indirect evidence supporting this hypothesis has been observed (Lawn and Brun 1974, Schulze 2003), this hypothesis has not been supported with direct evidence relating carbon assimilate competition by nodules and nitrogen fixation (Schulze et. al. 2000, Puppo et. al. 2005).

The signal to initiate nodule senescence can be triggered by shoot stresses. However the exact signal that triggers the shutdown of symbiosis and senescence of nodules has not been characterized. A possible trigger for nodule senescence may involve the reduced levels of antioxidants within nodules as they age. Antioxidant compounds glutathione and ascorbate have been shown to affect the nitrogenase activity in nodules (Bashor and Dalton 1999, Ross et. al. 1999). These antioxidants may be involved in signal transduction in root nodules as they are in plant leaves (Kiddle et. al. 2003, Arora et. al. 2002). Oxidative stress can occur in nodules in the absence of these and other metabolites, which could damage the both the parenchyma layer that cordons oxygen out of the nitrogen fixing region of the nodule and infected nodule cells, which could also adversely affect nodule function (Becana et. al 2000).

Environmental Factors

Many environmental factors are known to affect rhizobial populations and biological nitrogen fixation. Several studies concluded that acidic soils can reduce the population of rhizobia and interfere with the symbiotic process even when a viable population is present (Taylor et al. 1991, Graham 1992). A soil pH of 5.5 or less decreases free living rhizobial populations (Daniels 1999). Initial contact and attachment of rhizobium to root hairs can be affected by soil acidity, as low pH appears to destabilize rhizobial binding to root hairs in alfalfa (Caetano-Anolles et. al. 1989).

Soil moisture is another environmental factor that affects biological nitrogen fixation. Soybean nodule function is much more susceptible to low water stress than plant roots and shoots (Albrecht et. al. 1994). Deficit water stress can lower rhizobial population densities, reduce the formation of infection threads, and reduce the number and size of nodules on soybean roots (Tate 1995, Williams and DeMallorca 1984, Worrall and Roughly 1976). Soybean nodule functions are also more susceptible to flooding stress than root and shoot tissue, as inhibition of N fixation and N accumulation in plant tissue occurs earlier and more drastically than plant tissue biomass accumulation. Supplementation of N fertilizer to flood stressed plants reduces plant tissue biomass responses, so soybean nodule functions appear to be more sensitive to flooding than soybean itself (Bacanamwo and Purcell 1999).

Agronomists consider conditions such as low soil pH, high sand content, and soil flooding in recommending where soybean seed should be inoculated (Pederson 2003, Abendroth 2006). Rhizobia are heterotrophic, so they depend on soil organic matter for nutrition when they are free living. Therefore, soil organic matter levels play a role in maintaining *B. japonicum* in the soil. Other soil conditions that influence the effectiveness of inoculation include use of no-till practices, use of seed applied fungicides in previous soybean crops, planting date, and the frequency of water stresses on past soybean crops (Abendroth and Elmore 2006).

Inoculant Rate Effects

Soybean grain yield generally responds positively to increased inoculant rates. Depending on soybean size and the bacterial concentration of the inoculant, a typical commercial inoculant rate provides between $8.5*10^5$ and $1.4*10^6$ bacteria per seed. Hume and Blair performed a study of inoculant rate effects and soybean grain yield on virgin soybean fields by performing a set of logarithmic dilution applications of commercial inoculant to soybean seed. Rates of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} of the recommended rate showed significant and regressing yield effects compared to the standard inoculant rate, which was estimated to be approximately 10^6 bacteria per seed (Hume and Blair 1992). Based on the regression curves relating inoculant rate and grain yield produced in this study, use of a 10% standard inoculant rate would reduce grain yield between 4 and 11%. Use of a 2X standard inoculant rate would increase grain yield by 1.6 to 4.2% (Hume and Blair 1992).

Researchers have had difficulty determining the amount of inoculant required to provide maximum yield on new soybean ground. More inoculant has typically provided increased yield, yet in some studies, yield plateaus have been reached at varying inoculant levels (Hume and Blair 1992, Catroux et. al. 2001). A greenhouse study determined that while 10^5 rhizobium per seed did not provide maximum nodulation of roots, it did provide maximum total N content in soybean plants (Weaver and Fredrick 1972). These studies have all been performed on soils that had no resident *B. japonicum* population, and it is difficult to extrapolate effects seen in these trials to expected effects in soils that have been in soybean rotation. Also, very high inoculation rates are constrained by economic cost to growers, seed adhesion difficulty, and issues with seed metering and monitoring equipment.

Results in past trials

As nitrogen costs have risen dramatically in recent years, soybean seed inoculant treatments have received increased attention in commercial production and in agronomic research trials. Several field trials investigating the efficacy of soybean seed inoculant treatments were conducted throughout the Midwest in the past decade. The common recommendation of inoculating soybean seed only if a site has been out of soybean production for three years or more has been challenged by some researchers in recent years. Six field trials performed in Nebraska between 2001 and 2004 on rotational soybean ground show no yield differences between inoculated and non-inoculated plots (Abendroth and Elmore 2006). Therefore, the University of Nebraska recommends inoculation only when soybeans have not been grown in a field for four years unless environmental factors such as low soil pH, flooding, drought, high soil sand content, etc. are a factor. Soybean inoculant trials yielded similar results at Iowa State University in 2004 and 2005, and their recommendations are similar to the University of Nebraska regarding consecutive years without soybean production in a field (Pederson 2006).

Conley and Christmas compiled ten years of soybean inoculant trial data in Indiana on soils in a corn/soybean rotation (Conley and Christmas 2006). These trials tested several inoculant products throughout the state over this period. Inoculated soybeans yielded an average of 67 kg ha^{-1} per acre higher than those that were not treated. Some sites showed significant yield increases from inoculation, while others did not. They suggested that soybean seed inoculation may be an effective method of increasing yields, and that farmers ought to create replicated strip trials on their own farm to determine if inoculation enhances yield (Conley and Christmas 2006).

Beuerlein (2005) recommends inoculating soybean seed for every planting based on trials conducted in Ohio. More consistent yield increases have been observed in these trials than in other states. Results from the 2005 inoculant trial resulted in statistically significant yield increases for 17 out of 19 inoculant products and an average yield increase of 121 kg ha^{-1} above the untreated control over six sites (Beuerlein 2005). In 2004, a similar study resulted in a 175 kg ha^{-1} yield increase with 18 products tested at six sites. The average yield increase of the Ohio inoculation studies between 1995 and 2004 was 135 kg ha^{-1} (Beuerlein 2004).

Vitosh (1997) conducted several on-farm soybean inoculant trials around Michigan between 1990 and 1995, as well as a set of six trials conducted at the Michigan State University research farm between 1993 and 1996. No yield differences were observed for any of these trials, individually or collectively. Another set of eight on-farm replicated strip trials performed between 1990 and 1995 tested the yield effect of soybean seed inoculant in fields that had a history of soybean production. Only one of these eight trials showed a significant yield increase for seed inoculant treated strips versus the untreated checks. The average yield difference for these eight trials was only 6.7 kg ha^{-1}(Vitosh 1997).

Clearly, conflicting results have been observed among university soybean inoculant trials in recent years. It appears that some of these results may be associated with geography, as inoculants have increased yields in eastern areas of the soybean belt, including Ohio and Indiana. Studies in western states, including the Dakotas, Minnesota, Iowa, and Nebraska have shown no effect from soybean seed inoculation (Abendroth and Elmore 2006).

Advances in Soybean Inoculants

Soybean seed inoculant is available in three forms: peat-based, liquid, and granular. Historically, peat based powdered inoculants have been the most popular commercial form. Finely powdered peat provides adequate adhesion to soybean seed if a sticking agent is included in the carrier. Most peat carriers in commercial products are sterilized because sterilized peat provides advantages for sustaining rhizobial levels over a longer period of time, thus increasing the product's shelf life. However, peat sterilizing treatments such as heat and radiation can create byproducts which can interfere with rhizobial growth within the media. Liquid inoculants are either aqueous, oil, or polymer based, and have become more popular in recent years because of their ease of use (Xavier et. al. 2004).

In recent years, several new commercial soybean inoculant products of all three forms have been introduced to the market. Newer products claim higher efficacies than past products for various reasons, such as the use of multiple strains of *B. japonicum*, which are intended to be more competitive nodulators in different environments. Not all strains of *B. japonicum* are equally effective in nodulating soybean roots. Indigenous *B. japonicum* strains belonging to the serogroup USDA 123 are very competitive nodulators, but much less effective at nitrogen fixation than those used in soybean inoculants (Cregan et. al. 1989). Soybeans grown where *B. japonicum* serogroup 123 is the dominant symbiont yield significantly less than soybeans grown where higher efficiency strains are predominant (Ham 1980). Introduction of more efficient strains of *B. japonicum* could increase soybean yields if these introduced strains can effectively compete with serogroup 123 strains for nodulation sites.

Other advancements in soybean inoculation include the increase in rhizobial concentrations of commercial inoculant products, and the use of bacterial life extenders. Concentrations of 2 billion or more bacteria per gram are now common in many inoculant products, allowing for decreased application rates and increased efficiency of use. The use of bacterial life extenders in liquid and granular inoculants has also been recently introduced to the marketplace. These "extenders" provide a nutrient source for the bacteria to allow them to live on the seed for a longer period of time. These nutrient sources are a proprietary product; it is difficult to know what nutrient concentrations are in each of these products. Some of the newest encapsulated granular (ex. Excalibre, Advanced Biological Marketing, VanWert, OH) and liquid products (ex. Optimize, EMD Crop Bioscience, Brookfield, WI) claim a 120-day application to planting window. This time period allows for inoculant application by the seed dealer rather than the farmer in some cases, which is of significant convenience.

Genetic advances have also led to improvements in soybean inoculant technology. One example is the development of *B. japonicum* strain Ta11Nod+ as a result of mutagenesis of the common I-110 strain (Kuykendall and Hunter 1991). Compared to the I-110 strain, this mutated strain increased soybean root nodulation by 44%, and increased yields by 135 to 202 kg ha^{-1}. This mutant strain was patented in 1991, and is still being produced commercially, more commonly known as "Nod+" or the USDA strain (Suszkiw 1992).

Inoculant manufacturers have applied knowledge of the biochemistry involved in symbiotic nitrogen fixation by incorporating lipochitooligosaccarides produced by *B. japonicum* into their product. The lipochitooligosaccharide class of molecules comprise the nod factor signal molecules produced by *B. japonicum* that elicit responses such as root hair curling, infection thread formation and nodule formation. Optimize (EMD Crop BioScience Inc., Brookfield, WI) inoculant is formulated with this molecular additive. Nitragin Inc. (now EMD Crop BioScience Inc.) has also applied for a patent to use the manufactured nod factor molecules in combination with insecticidal seed treatments such as thiamethoxam and imidacloprid (Smith et. al. 2004).

Insecticidal Seed Treatments for Soybean

Neonicotinoids are the most recent class of insecticides introduced for crop protection. Neonicotinoids are systemic insecticides, absorbed by plants from foliar or soil application, or seed treatment. Generally, the neonicotinoids have lower logP solubility values than organophosphates and other insecticides, meaning they exhibit higher water solubility, an important property of systemic insecticides (Tomizawa and Casida 2005, Elliot 1977). Neonicotinoids move to actively growing tissue, where they are ingested by insect pests. The site of activity for neonicotinoids is the nervous system of insects, where they act upon the acetylcholine receptor. As neonicotinoids bind to the acetylcholine receptor, giant interneurones are depolarized, leading to paralysis and death of the insect (Matsuda et. al. 2001, Rice 2004).

The neonicotinoids preferentially bind to insect acetylcholine receptors rather than vertebrate acetylcholine receptors (Yamamoto and Casida 1999). This property makes neonicotinoids less toxic to mammals than the organophosphates and methylcarbamates, the two other common classes of soil-applied insecticides. Neonicotinoids have the highest insect selectivity of any major class of systemic insecticide, with an insect to mammal selectivity factor 14 and 28 times greater than the organophosphates and carbamates (Tomizawa and Casida 2005, Elliot 1977). This favorable property has made the neonicotinoids popular options for insect control in many crops.

Since 2004, two new neonicotinoid seed treatments for soybean were approved by the EPA; Cruiser (thiamethoxam, Syngenta, Greensboro, NC), and Gaucho (imidacloprid, Bayer Crop Science, Research Triangle Park, NC). These chemicals provide early season control or suppression of insects such as soybean aphid, bean leaf beetle, leafhoppers, thrips, Mexican bean beetle, and wireworms. The products advertise crop benefits such as increased plant density, plant height, root, stem and leaf development, as well as increased grain yields.

Results in past trials

Bean leaf beetle feeding in soybean causes significant leaf defoliation and transmits bean pod mottle virus, which can significantly reduce soybean grain yield and quality. Overwintering and first generation bean leaf beetles feed on soybean only through early June, and early feeding causes the highest risk for bean pod mottle virus transmission (Ross 1969, Hopkins and Muller 1984, Krell et. al. 2004). Thiamethoxam or imidacloprid seed treatment may be ideal to control early season bean leaf beetle infestation and bean pod mottle virus transmission.

A pair of trials conducted at Janesville, WI in 2003 tested neonicotinoid control of bean leaf beetle feeding and effects on soybean yield (Cullen et. al. 2004a). Bean leaf beetle feeding was virtually absent in both thiamethoxam and imidacloprid treated plots 19 days after planting compared to over 60% of plants with feeding damage in untreated plots. Although increases of 492 and 485 kg ha^{-1} were observed in one of two trials where high rates of thiamethoxam (50 g a.i./100 kg) and imidacloprid (62.5 g a.i./100 kg) were used, these yield increases were likely a result of soybean aphid suppression later in the growing season, rather than the below threshold bean leaf beetle pressure observed (Cullen et. al. 2004a). An Iowa trial tested the effects of thiamethoxam seed treatment on soybean yield and bean leaf beetle control (Schmitt and VanDee 2005). Significant decreases in late August bean leaf beetle populations were observed, but this did not lead to significant yield differences.

Another trial conducted in Southern Minnesota tested effects of thiamethoxam and imidacloprid on soybean stand, plant height, soybean aphid population, and bean leaf beetle populations on two planting dates, May 2 and May 23 (Potter 2002). Soybean stand was lower in untreated early-planted soybean, but plant populations later in the growing season were not significantly different. Early-planted soybean treated with high rates of thiamethoxam and imidacloprid (50 g and 124 g/100 kg seed) showed respective plant height increases of 7.7 and 12.0 cm at R3. Bean leaf beetle defoliation was significantly reduced at V2 where soybean seed was treated with thiamethoxam or imidacloprid. Soybean aphid population reduction was observed in early planted R3 soybean but not at R4, or in late planted soybean (Potter 2002).

Soybean aphid infestations occur later into the growing season relative to bean leaf beetle. Soybean aphid pressure usually increases until late July or early August in Michigan (DiFonzo 2003, 2005). This poses a challenge for the neonicotinoid seed treatments, as the insecticide must persist longer and maintain potency in a much larger soybean plant to provide any level of aphid control. Planting date may play an important role as to whether seed treatment can control soybean aphid. Thiamethoxam has been shown to provide systemic activity and significant SBA mortality for 35 days after planting and a reduction in nymph production up to 49 days after planting (McCornack and Ragsdale 2006). Therefore, insecticide treated soybeans planted in late May or early June may maintain some systemic activity through July, while early planted soybeans will likely lose systemic activity before the highest aphid pressure occurs.

Two Wisconsin trials in 2003 determined the effectiveness of neonicotinoid seed treatments in controlling soybean aphid (Cullen et. al. 2004b). Soybean was planted in these trials on June 9, and throughout the six aphid sample dates in both studies, thiamethoxam and imidacloprid treated soybean had significantly reduced aphid populations. Untreated soybean averaged 1700 and 2600 aphids per plant on August 4, while treated soybean averaged less than 700 aphids per plant, and less than 250 aphids per plant for imidacloprid in one trial. Aphid pressure began to fall after August 4, while aphid populations in treated soybean continued to rise through August 13, indicating that aphid control was weakening (Cullen et. al. 2004b).

A study conducted at Beresford, SD in 2003 determined the effectiveness of neonicotinoid seed treatments in controlling soybean aphid (Catangui et. al. 2003). These trials were planted on June 12, and aphids were counted on August 18, at the R5 stage. High aphid pressure was observed at this site, with an average of 1,051 aphids per untreated plant. No significant difference between untreated and insecticide treated plots in mean number of soybean aphids per plant was observed, nor was any resulting yield advantage observed.

Trials in Iowa from 2002-2005 determined the effectiveness and duration of soybean aphid control from neonicotinoid seed treatments (Pederson and Lang 2006). In 2003, soybean aphid pressure was high, with over 3,000 aphids per untreated plant by July 27. The economic threshold was not reached in treated plots until July 20, compared to June 30 for untreated soybean. In 2005, thiamethoxam and imidacloprid held soybean aphid pressure under threshold through July 18, about one week longer than untreated soybean. Pederson and Lang concluded that seed treatments can control soybean aphid approximately 60 days after planting, which may be useful in some late planting, replant, or double-crop situations (Pederson and Lang 2006).

Insecticide-Rhizobium Interactions

Research is limited regarding potential interactions between neonicotinoids and rhizobial bacteria. Thiamethoxam had little toxicity invitro to entomopathogenic bacteria (Filho et. al. 2001). Imidacloprid seed treatments had no antagonistic effect on soil bacteria populations (Singh and Singh 2005).

Research has been conducted regarding rhizobial interactions with insecticides from the carbamate and organophosphate groups. The soil applied insecticides malathion, diazinon, acephate, carbaryl, and toxaphene at 5 and 10 times the recommended rates had no effect on plant growth and total nodulation, although nitrogen fixation and nodulation sites were affected (Mallik and Tesfai 1985). Carbosulfan had little effect on *B. japonicum* survival on soybean seed (Martyniuk et. al. 2002). Tandem seed treatment of omethoate and inoculant reduced survival of rhizobium on subterranean clover and alfalfa seeds, although little to no effect was seen when insecticide was applied 16 h prior to inoculation (Evans et. al. 1991). Aldicarb inhibited in-vitro growth of rhizobium bacteria at 5 and 10 ppm levels, and reduced nodulation and nitrogen content of cowpea, although plant growth was enhanced (Sekar and Balasubramanian 1979). Lin et. al. conducted a study of nine different carbamate and organophosphate insecticides on in-vitro rhizobium growth of four different species, including *B. japonicum* (Lin et. al. 1972). *B. japonicum* was the least inhibited by the insecticide; only 2 of 9 chemicals tested at 2u concentration inhibited colony growth. Both of these chemicals were organophosphates.

References

Abendroth, L. and R. Elmore. 2006. Soybean Inoculation: Applying the Facts to Your Field. University of Nebraska-Lincoln NebGuide G1622.

Albrecht, S. L., J. M. Bennett, and K. J. Boote. 1984. Relationship of nitrogenase activity to plant water stress in field grown soybeans. Field Crops Research 8:61-71.

Arora, A., R. K. Sairam and G. C. Srivastava. 2002. Oxidative stress and antioxidative system in plants. Current Science 82(10):1227-1238.

Ash, M., J. Livesey, and E. Dohlman. 2006. Soybean Backgrounder. Electronic Outlook Report from the Economic Research Service, USDA. Retrieved Nov 10, 2006 from http://www.ers.usda.gov/publications/OCS/apr06/OCS200601/OCS200601.pdf

Bacanamwo, M. and L. Purcell. 1999. Soybean dry matter and N accumulation responses to flooding stress, N sources and hypoxia. Journal of Experimental Botany 50(334):689-696.

Balatti, P.A. and S.G. Pueppke. 1992. Identification of North American soybean lines that form nitrogen-fixing nodules with *Rhizobium fredii* USDA257. Canadian Journal of Plant Science 72(1):49-55.

Barbour, W.M., D. R. Hattermann, and G. Stacey. 1991. Chemotaxis of *Bradyrhizobium japonicum* to soybean exudates. Applied and Environmental Microbiology 57(9) 2635-2639.

Bauer, W.D. and B. G. Turgeon. 1982. Early events in the infection of soybean by *Rhizobium japonicum*. Time course and cytology of the initial infection process. Canadian Journal of Botany 60(2):152-161.

Bashor, C. J. and D. A. Dalton. 1999. Effects of exogenous application and stem infusion of ascorbate on soybean (*Glycine max*) root nodules. New Phytology 142:19-26.

Becana, M., D. A. Dalton, J. F. Moran, I. Iturbe-Ormaetxe, M. A. Matamoros, and M. C. Rubio. 2000. Reactive oxygen species and antioxidants in legume nodules. Physiologia Plantarum 109:372–381.

Berglund, D. 1997. Dry Bean Production Guide. NDSU Extension Service Publication A-1133.

Beuerlein, J. 2005. 2005 Ohio Inoculation Study. Ohio State University Agronomic Crops Network. Retrieved Dec 5, 2006 from http://agcrops.osu.edu/soybean/documents/2005SoyInoculationReport.pdf

Beuerlein, J. 2004. 2004 Ohio Inoculation Study. Ohio State University Agronomic Crops Netwok. Retrieved Dec 5, 2006 from http://agcrops.osu.edu/research/2004/04inoc%20report11.pdf

Brewin, N. J. 1991. Development of the legume root nodule. Annual Reviews of Cell Biology 7:191-226.

Broughton, W. J., S. Jabbouri, and X. Perret. 2000. Keys to symbiotic harmony. Journal of Bacteriology 182(20):5641-5652.

Caetano-Anolles, G., A. Lagares, and G. Favelukes. 1989. Adsorption of *Rhizobium meliloti* to alfalfa roots: Dependence on divalent cations and pH. Plant and Soil 117(1):67-74.

Callaham, D. A. and J. G. Torrey. 1981. The structural basis for infection of root hairs of *Trifolium repens* by Rhizobium. Canadian Journal of Botany 59(9):1647-1664.

Catangui, M., B. Carsrud, R. Krantz, D. Mills, and R. Berg. 2003. Seed treatments for soybean aphid control. Southeast South Dakota Experiment Farm 43th Annual Progress Report:87-89.

Carlson, J. B. and N. R. Lersten, 1987. Reproductive morphology. *In*: J. R. Wilcox (ed.) Soybeans: Improvement, Production, and Uses; Second Edition:95-134 American Society of Agronomy. Madison, Wisconsin.

Conley, S. and E. Christmas. 2005. Utilizing Inoculants in a Corn-Soybean Rotation. Purdue University Cooperative Extension Bulletin SPS-100-W.

Cregan, P. B., H. H. Keyser, and M. J. Sadowsky. 1989. Host plant effects on nodulation and competitiveness of the *Bradyrhizobium japonicum* serotype strains constituting serocluster 123. Applied Environmental Microbiology 55(10): 2532-2536.

Cullen, E. M., J. Gaska, and B. Jensen. 2004. Bean leaf beetle control with seed treatments. 2004 Wisconsin Fertilizer, Aglime, & Pest Management Conference Proceedings.

Day, D. A. and L. Copeland. 1991. Carbon metabolism and compartmentation in nitrogen-fixing legume nodules. Plant Physiology and Biochemistry 29(2):185-201.

D'Haeze, W. and M. Holsters. 2002. Nod factor structures, responses, and perception during initiation of nodule development. Glycobiology, 12(6):79-105.

Daniels, W. L., and C. E. Zipper. 1999. Creation and management of productive mine soils. Soybean Facts Winter 1997. Virginia Cooperative Extension Publication Number 460-121.

Dart, P., and J. Day. 1971. Effects of incubation temperature and oxygen tension on nitrogenase activity of legume root nodules. Plant and Soil 35:167–184.

Dazzo, F. B., G. L. Truchet, J. E. Sherwood, E. M. Hrabak, M. Abe, and S. H. Pankratz. 1984. Specific phases of root hair attachment in the rhizobium trifolii-clover symbiosis. Applied and Environmental Microbiology 48(6):1140-1150.

DiFonzo, C. 2003. August Pest Update: Soybean Aphid. Michigan State University Extension Field Crop Advisory Team Alert 18(15).

DiFonzo, C. 2005. Soybean aphids still a problem. Michigan State University Extension Field Crop Advisory Team Alert 20(15).

Dill, G. M. 2005. Glyphosate-resistant crops: history, status and future. Pest Management Science 61(3):219-224.

Dobbins, C. and A. Miller. 2006 Purdue Crop Cost and Return Guide. Purdue University Cooperative Extension Bulletin 166-W.

Dowdle, S. F. and B. B. Bohlool. 1985. Predominance of fast-growing *Rhizobium japonicum* in a soybean field in the People's Republic of China. Applied Environmental Microbiology 50(5):1171-1176.

Duong, T. P., C. N. Diep, N. T. Khiem, N. H. Hiep, N. V. Toi, N. V. Lich and T. K. L. Nhan. 1984. Rhizobium inoculant for soybean [*Glycine max* (L.) Merrill] in Mekong Delta. Plant and Soil 79(2):235-240.

Elliott, M. 1977. Synthetic pyrethroids. *In* M. Elliot (ed.) Synthetic Pyrethroids. ACS Symposium Series 42:1–28. Washington, DC: American Chemical Society.

Evans J., J. Seidel, G. E. O'Connor, J. Watt, and M. Sutherland. 1991. Using omethoate insecticide and legume inoculant on seed. Australian Journal of Experimental Agriculture 31:71-76.

Filho, A. B., J. E. M. Almeida, and C. Lamas. 2001. Effect of thiamethoxam on entomopathogenic microorganisms. Neotropical Entomology 30(3):437-447.

Gallon, J. R., and A. E. Chaplin. 1987. An introduction to nitrogen fixation. Cassell Education Limited, London.

Graham, P. H. 1992. Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Canadian Journal of Microbiology 38: 475–484.

Ham, G. E. 1980. Interaction of *Glycine max* and *Rhizobium japonicum*. *In*: R. J. Summerfield and A. H. Bunting (eds). Advances in Legume Science:298-296. Royal Botanical Gardens, Kew, U.K.

Hanway J. J. and C. R. Weber. 1971. N, P, and K percentages in soybean [Glycine *max* (L.) Memll] plant parts. Agronomy Journal 63:286-290

Hanks, J. F., K. Schubert, and N. E. Tolbert. 1983. Isolation and characterization of infected and uninfected cells from soybean nodules. Plant Physiology 71. 869-873.

Heron, D. S., and S.G. Pueppke. 1984. Mode of infection, nodulation specificity, and indigenous plasmids of 11 fast-growing *Rhizobium japonicum* strains. Journal of Bacteriology. 160:1061-1066.

Ho, S.C., J. L. Wang, M. Schindler and J. T. Loh. 1994. Carbohydrate binding activities of *Bradyrhizobium japonicum*. III. Lectin expression, bacterial binding, and nodulation efficiency. The Plant Journal 5(6):873-884.

Hopkins, J. D. and A. J. Mueller. 1984. Effect of bean pod mottle virus on soybean yield. Journal of Economic Entomology 77:943–947.

Hume D J and D. H. Blair. 1992 Effect of numbers of *Bradyrhizobium japonicum* applied in commercial inoculants on soybean seed yield in Ontario. Canadian Journal of Microbiology 38, 588–593.

Hymowitz, T. 1990. Soybeans: The Success Story. *In:* Janick and James Simon (eds.). Advances in New Crops:159-163. Timber Press, Portland, OR.

Insamde, J, and B. Touraine. 1994. N Demand and the Regulation of Nitrate Uptake. Plant Physiology 105: 3-7.

Jones, F. R. and W. B. Tisdale. 1921. Effect of soil temperature upon the development of nodules on the roots of certain legumes. Journal of Agricultural Research 22:17-37.

Kavroulakis, N., E. Flemetakis, G. Aivalakis, and P. Katinakis. 2000. Carbon metabolism in developing soybean root nodules: the role of carbonic anhydrase. Molecular-Plant Microbe Interactions 13(1):14-22.

Keyser, H. H., B. B. Bohlool, T. S. Hu, and D. F. Weber. 1982. Fast growing rhizobia isolated from root nodules of soybean. Science 215(4540):1631-1632.

Kiddle, G., G. M. Pastori, B. Bernard, C. Pignocchi, J. Antoniw, P. J. Verrier, and C. H. Foyer. 2003. Effects of leaf ascorbate content on defense and photosynthesis gene expression in *Arabidopsis thaliana*. Antioxidants and Redox Signalling 5:23–32.

Kleweno, D. and V. Matthews. Michigan Agricultural Statistical 2004-05. 2004 Michigan Department of Agriculture Annual Report.

Klucas, R. F. 1974. Studies on soybean nodule senescence. Plant Physiology 54:612-616.

Krell, R. K., L. P. Pedigo, J. H. Hill, and M. E. Rice. 2004. Bean leaf beetle (*Coleoptera: Chrysomelidae*) management for reduction of bean pod mottle virus. Journal of Economic Entomology 97(2):192–202.

Kuykendall, L. D. and W. J. Hunter. 1991. Enhancement of nitrogen fixation with *Bradjirhizobium japonicum* mutants. US patent 5,021,076.

Lawn, R. J. and W. A. Brun. 1974. Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulation. Crop Science 14:11-16.

Lersten, N. R. and J. B. Carlson. 1987. Reproductive morphology. *In*: J. R. Wilcox (ed.) Soybeans: Improvement, Production, and Uses; Second Edition. American Society of Agronomy. Madison, Wisconsin:95-134.

Lin, S. C., B. R. Funke, and J. T. Schulz. 1972. Effects of some organophosphate and carbamate insecticides on nitrification and legume growth. Plant and Soil 37(3):489-496.

Lindemann, W. C. and C. R. Glover. 2003. Nitrogen fixation by legumes. New Mexico State University Cooperative Extension Service Guide A-129.

Lynch, D. H., and L. Smith. 1993. Soybean (*Glycine max*) modulation and N_2-fixation as affected by exposure to a low root-zone temperature. Physiologia Plantarum 88(2):212-220.

Mallik M. A. B. and K. Tesfai. 1985. Pesticidal effect on soybean-rhizobia symbiosis. Plant and Soil 85(1):33-41.

Martyniuk, S., J. Oron, M. Martyniuk, and A. Wozniakowska. 2002. Effects of interactions between chemical seed dressings and *Bradyrhizobium japonicum* on soybean seeds. Archives of Agronomy and Soil Science 48(4):305-310.

Matsuda, K., S. D. Buckingham, D. Kleier, J. J. Rauh, M. Grauso, and D. B. Sattelle. 2001. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends in Pharmacological Sciences 22(11):573-580.

McClure, P. R. and D. W. Israel, 1979. Transport of nitrogen in the xylem of soybean plants. Plant Physiology 64:411-416.

McCornack, B. P., and D. W. Ragsdale. 2006. Efficacy of thiamethoxam to suppress soybean aphid populations in Minnesota soybean. Online. Crop Management doi:10.1094/CM-2006-0915-01-RS.

Mylona, P., K. Powlowski, and T. Bisseling. 1995. Symbiotic nitrogen fixation. The Plant Cell 7:869-885.

Newell, C. A. and T. Hymowitz. 1980. A taxonomic revision in the genus *Glycine* subgenus *Glycine* (*Leguminosae*). Brittonia 32(1):63-69.

Ottow, J . C. G. 1975. Ecology, physiology, and genetics of fimbriae and pili. Annual Review of Microbiology 29:79-108.

Pedersen, P. 2003. Soybean Seed Inoculation. Integrated Crop Management Newsletter, Iowa State University Extension. Issue IC-490(5):12-13.

Pedersen, P. and B. Lang. 2006. Use of insecticide seed treatments for managing soybean aphids. Integrated Crop Management Newsletter, Iowa State University Extension. Issue IC-496(1):39-40.

Potter, B. 2003. Seed applied insecticide control of the soybean aphid (*Aphis glycines*) and bean beetle (*Cerotoma trifurcata*). University of Minnesota Southwest Research and Outreach Center (Online). Retrieved Nov 8, 2006 from http://swroc.coafes.umn.edu/SWMNPEST/swmnpest.htm

Puppo, A., K. Groten, F. Bastian, R. Carzaniga, M. Soussi, M. M. Lucas, M. Rosario de Felipe, J. Harrison, H. Vanacker, and C. H. Foyer. 2005. Legume nodule senescence: roles for redox and hormone signalling in the orchestration of the natural aging process New Phytology 165(3): 683–701.

Rice, M. 2004. Seed treatments labeled for corn and soybeans. Integrated Crop Management Newsletter, Iowa State University Extension. Issue IC-494(2):12-13.

Ross, E. J. H., S. B. Kramer, and D. A. Dalton. 1999. Effectiveness of ascorbate and ascorbate peroxidase in promoting nitrogen fixation in model systems. Phytochemistry 52:1203–1210.

Ross, J. P. 1969. Effect of time and sequence of inoculation of soybeans with soybean mosaic and bean pod mottle viruses on yields and seed characters. Phytopathology 59:1404–1408.

SAS Institute, Inc. SAS 9.1.2 Users Guide. Cary, NC: SAS Institute, Inc. 2004.

Schmitt, V. L. and K. VanDee. 2005. Effects of Cruiser seed treatment for bean leaf beetle and soybean aphid management on soybean yield and on incidence of viral disease in Eastern Iowa. Iowa State University Research Farm Annual Reports (Online). Retrieved Nov 8, 2006 from http://agcrops.osu.edu/research/2004/04inoc%20report11.pdf

Schulze J., H. Beschow, E. Adgo, and W. Merbach. 2000. Efficiency of N2 fixation in Vicia faba L. in combination with different *Rhizobium leguminosarum* strains. Journal of Plant Nutrition and Soil Science 163:367–373.

Schulze, J. 2003. Source-sink manipulations suggest an N-feedback mechanism for the drop in N2 fixation during pod-filling in pea and broad bean. Journal of Plant Physiology 160:531-537

Sekar, T. and A. Balasubramanian. 1979. Influence of a carbamate pesticide on growth, respiration (^{14}C)-carbon metabolism and symbiosis of a *Rhizobium* sp. Plant and Soil 51(3):355-361.

Seneviratne, G., L. H. J. Van Holm and E. M. H. G. S. Ekanayake. 2000. Agronomic benefits of rhizobial inoculant use over nitrogen fertilizer application in tropical soybean. Field Crop Research 68(3):199-203.

Shah, V. K. and W. J. Brill. Isolation of an iron-molybdenum cofactor from nitrogenase. Proceedings of the National Academy of Science 74(8):3249-3253.

Singh J. and D. Singh. 2005. Bacterial, azotobacter, actinomycetes, and fungal population in soil after diazinon, imidacloprid, and lindane treatments in groundnut (*Arachis hypogaea* L.) fields. Journal of Environmental Science and Health Part B 40(5):785–800.

Smit, G., S. Saskia, B. J. J. Lugtenberg, J. W. Kijne. 1992. Molecular mechanisms of attachment of *Rhizobium* bacteria to plant roots. Molecular Microbiology 6(20):2897–2903.

Smith, S., R. M. Osborn, J. W. Kosanke. 2004. Methods and compositions providing agronomically beneficial effects in legumes and non-legumes. United States Patent Application 20050187107.

Spaink, H. P. 1995. The molecular basis of infection and nodulation by rhizobia: the ins and outs of sympathogenesis. Annual Reviews of Phytopathology 33:345-68.

Streeter, J. G. 1972. Nitrogen nutrition of field grown soybean plants. I. Seasonal variations in oil, nitrogen and nitrogen composition of stem exudate. Agronomy Journal 64: 311-314.

Suszkiw, J. 1992. Farmers adopt ARS-improved soybean bacterium. Agricultural Research Magazine 50(5).

Tate, R. L. 1995. Soil Microbiology. Wiley and Sons, Inc. New York, New York.

Taylor, R. W., M. L. Williams, and K. R. Sistani. 1991. N_2 fixation by soybean-*Bradyrhizobium* combinations under acidity, low P and high Al stresses. Plant and Soil 131(2):293-300.

Thibodeau, P. S. and E. G. Jaworski. 1975. Patterns of nitrogen utilization in the soybean. Planta 127(2):133-147.

Tien, H. H., T. M. Hien, M. T. Son and D. Herridge. 2002. Rhizobial Inoculation and N2 Fixation of Soybean and Mungbean in the Eastern Region of South Vietnam. *In* D. Herridge (ed.) Inoculants and Nitrogen Fixation of Legumes in Vietnam: ACIAR Proceedings 109e.

Tomizawa, M. and J. E. Casida. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annual Review of Pharmacology and Toxicology 45:247-268

United States Department of Agriculture, National Agricultural Statistics Service. 2006. Crop Production, 2005 Summary.

Uren, N. C. 2000. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. *In*: R. Pinton, Z. Varanini and P. Nannipieri (eds.) The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface: 19–40. Marcel Dekker, Inc, New York.

Vesper, S. J. and T. V. Bhuvaneswari. 1988. Nodulation of soybean roots by an isolate of *Bradyrhizobium japonicum* with reduced firm attachment capability. Archives of Microbiology 150(1):15-19.

Vitosh, M. L. 1997. Soybean Inoculation in Michigan. Soybean Facts Winter 1997. Michigan State University Extention Facts Sheet 9708.

Weaver R. W. and R. L. Frederick L. 1972. Effect of inoculum size on nodulation of *Glycine max* (L.) Merril, variety Ford. Agronomy Journal 64, 597–599.

Williams, P. M. and M. S. de Mollorca. 1984. Effect of osmotically induced leaf moisture stress on nodulation and nitrogenase activity of *Glycine max*. Plant and Soil 80(2):267-283.

Williamson, A. J. P. and A. Diatloff. 1975. Effects of supplementary nitrogen fertilizer on nodulation, yield and seed characteristics of soybean (Glycine max) on the Darling Downs. Australian Journal of Experimental Agriculture and Animal Husbandry 15: 694-699.

Worrall, V. S. and R. J. Roughly. 1976. The effect of moisture stress on infection of *Trifolium subterraneum* L. by *Rhizobium trifolii* Dang. Journal of Experimental Botany 27(6):1233-1241.

Xavier, I. J., G. Holloway, and M. Leggett. 2004. Development of rhizobial inoculant formulations. Online. Crop Management doi:10.1094/CM-2004-0301-06-RV.

Yamamoto, I. and J. E. Casida (eds). 1999. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. Tokyo: Springer-Verlag. 300 pp.

Zahran, H. H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Reviews 63(4):, 968-989.

Zhang, F. and D. L. Smith. 1994. Effects of low root zone temperatures on the early stages of symbiosis establishment between soybean [*Glycine max* (L.) Merr.] and *Bradyrhizobium japonicum*. Journal of Experimental Botany 45(10):1467-1473.

CHAPTER 1

EFFECT OF SOYBEAN SEED INOCULANTS AND FUNGICIDAL SEED TREATMENTS ON SOYBEAN YIELD IN MICHIGAN

Abstract

Several new bacterial inoculant products have been introduced for use as a soybean (*Glycine max* (L.) Merr.) seed treatment. The advantages of these commercial soybean inoculants over those used in the past may include higher *B. japonicum* concentrations, bulk seed applications, additives that extend the life of *B. japonicum* on the seed, and inclusion of nod factor molecules within the inoculant carrier. A three year study over 16 site-years determined whether soybean seed inoculation was a worthwhile practice on fields that were in a soybean rotation, if yield or growth differences occurred between soybean treated with commercial inoculant products, and whether use of Apron Maxx RTA fungicide resulted in yield benefits or interacted with commercial inoculants. Eight commercial inoculants were tested both with and without fungicide treatment in a split-plot randomized complete block configuration. Use of soybean inoculant raised soybean yield in 6 of 14 site-years that had been in soybean rotation. Differences between inoculant products were observed at only at first-time soybean sites. Apron Maxx RTA seed treatment improved yield on 3 of 16 site-years, and only interacted with inoculant on the two first-time soybean sites.

Introduction

Soybean (*Glycine max* (L.) Merr.) is the most economically important oilseed crop grown in the United States, constituting 90% of U.S. annual oilseed production (Ash et. al. 2006). Soybean, unlike some legumes, has the ability to derive most of its nitrogen requirements from symbiotic nitrogen fixation, and high yielding soybean depends on adequate biological nitrogen fixation (Duong et. al. 1984). Up to 280 kg ha^{-1} of N can be fixed through symbiotic nitrogen fixation in soybean accounting for about 70% of total plant nitrogen requirement (Lindemann and Glover 2003, Tien et. al. 2002). The reason soybean is capable of fixing a large source of nitrogen is the efficiency of the symbiosis between plant root and *Bradyrhizobium japonicum*, the bacteria species specific to soybean nitrogen fixation in American cultivars (Balatti and Pueppke, 1992).

Inoculation of soybean seed or soil around the seed with *B. japonicum* is commonly practiced to ensure biological nitrogen fixation will take place. In fields where soybean is cropped for the first time, inoculation can increase yields dramatically, by 50% or more (Senviratne et. al. 2000, Duong et. al. 1984). Once a field has been cropped by soybean and inoculated with *B. japonicum*, a soil borne population of *B. japonicum* will reside in that field in following years, and future inoculations will not likely have as great an effect on soybean yield as the initial inoculation.

Some farmers and researchers find inoculation of soybean seed by *B. japonicum* for each planting (successive inoculation) to be a worthwhile practice, as there is evidence suggesting that successive soybean inoculation can increase soybean yield (Beuerlein 2005, Conley and Christmas 2006). However, a number of trials saw no yield increase from successive soybean inoculation (Abendroth 2006, Vitosh 1997, Pedersen

2003). Soil factors such as pH, soil water content, organic matter, and soil texture impact *B. japonicum* populations (Albrect et. al. 1999, Graham 1992, Bacanamwo and Purcell 1999, Abendroth 2006). Successive inoculation of soybean seed may overcome these environmentally mediated reductions of soil borne *B. japonicum* populations by improving soybean root colonization.

Soybean seed inoculant is available in three forms, peat based, liquid, and granular. Historically, peat based powdered inoculants are the most popular commercial form. Liquid inoculants are either aqueous, oil, or polymer based, and have become more popular in recent years because of their ease of use (Xavier et. al. 2004). Soybean inoculants are inexpensive and becoming more convenient, with new bulk seed applications and bacterial life extenders allowing inoculant to live on stored seed for much longer periods while maintaining effectiveness.

Newer products claim higher efficacies than past products for various reasons, such as the use of multiple strains of *B. japonicum*, which are intended to be more competitive nodulators in different environments. Not all strains of *B. japonicum* are equally effective in nodulating soybean roots. Indigenous *B. japonicum* strains belonging to the serogroup USDA 123 are very competitive nodulators, but much less effective at nitrogen fixation than those found used in soybean inoculants such as USDA 110 (Cregan et. al. 1989). Concentrations of 2 billion or more bacteria per gram are now common in many liquid products, allowing for lower application rates and increased efficiency of inoculant use. Genetic advances have also led to improvements in soybean inoculant technology, such as the development Ta11Nod+ from mutagenesis of the common I-110 *B. japonicum* strain (Kuykendall and Hunter 1991). Inoculant manufacturers have applied knowledge of the biochemistry involved in symbiotic nitrogen fixation by incorporating lipochitooligosaccharide nod factors produced by *B. japonicum* into their product. These signal molecules elicit responses such as root hair curling, infection thread formation and nodule formation (Smith et. al. 2004).

This study was performed from 2003 to 2005 over 16 site-years. The objectives of this study was to: 1) determine whether soybean seed inoculation was a worthwhile practice on fields that were in a soybean rotation, 2) if yield or growth differences occurred between soybean treated with commercial inoculant products, and 3) whether use of Apron Maxx RTA fungicide resulted in yield benefits or interacted with commercial inoculants.

Materials and Methods

Field Study

In 2003 and 2004, six field sites were chosen for the soybean inoculant trials, conducted at sites adjacent to the Michigan Soybean Performance Trials throughout the Lower Peninsula of Michigan. In 2003, trials were performed in Montmorency, Grand Traverse, Saginaw, Ingham, Hillsdale, and Lenawee Counties. In 2004, trials were performed in Montmorency, Grand Traverse, Sanilac, Ingham, Hillsdale, and Lenawee Counties. In 2005, four field sites were used, including Montmorency, Sanilac, Ingham, and Hillsdale Counties. Sites at Hillsdale and Saginaw in 2003 and Lenawee in 2004 had a previous crop of wheat. All other sites had a previous crop of corn. All sites had a recent history of soybean production except Montmorency County sites in 2004 and 2005.

Commercial inoculants selected for use in this trial included: Cell-Tech (2003-2005, EMD Crop BioScience Inc.), Nod+ (2003-2005, Becker Underwood Inc.), SowFast (2003-2005, Loveland Products Inc.), NitroFix Liquid (2003-2005, Trace Chemicals LLC), PulseR HP (2003-2005, Agribiotics Inc.), HiStick2 (2003-2004, Becker Underwood), HiStick N/T (2005 Becker Underwood Inc.) , Apex Extra (2004-2005, Agribiotics Inc.) and Optimize (2005, EMD Crop BioScience Inc.). Hi-Stick, SowFast, and PulseR HP are peat powder based inoculants. Cell-Tech, Nod+, NitroFix Liquid, Apex Extra and Optimize are liquid products. Inoculant treatments were tested alone and in combination with Apron Maxx RTA (mefenoxam and fludioxonil) fungicide seed treatment, to determine whether the fungicide treatment changed the efficacy of the inoculant. Soybean seed was treated with Apron Maxx RTA fungicide at the labeled rate of 162 ml /50 kg seed in the week prior to soybean planting.

Each site was established as a randomized complete block design with split plots acting as treatment and control. Each site had 6 complete blocks consisting of all inoculant treatments used twice per block, once in a whole plot without fungicide and once with Apron Maxx RTA. Each 12-row whole plot was divided into 2 subplots, 6 rows treated with one of the commercial inoculants, and six rows not treated. Rows were 38 cm in width. Using this design, split plots could be compared throughout the field to reduce the variance between treatments and controls and increase the reliability of smaller observed yield differences. Each plot was 6.07 m length as planted. Alleys measuring 1.82 m were cut in between plots during the growing season, making the plots 4.24 m in length for harvest.

Spring nitrogen applications were not made to sites except for at Grand Traverse County in 2003 and 2004. A 190 kg ha^{-1} application of 22-11-22 was broadcast on these sites in the spring prior to planting (Table 1.1). This application contained 39 kg ha^{-1} total N. It is not uncommon for soybean farmers to use some type of low rate of early season nitrogen fertilizer on sandy soils where low organic matter is present to provide soybean plants an early nitrogen source until nodules are established (Lindemann and Glover 2003). The Grand Traverse sites had both a high sand content and low organic matter content (Table 1.2), which is common for farmers in northwest lower Michigan.

Many studies have shown that high levels of N fertilizer inputs inhibit soybean nodulation and nitrogen fixation. However, it is unlikely that the low pre-plant nitrogen application made at the Grand Traverse sites significantly impaired nodulation. A series of experiments performed in Australia tested the effects of different fertilizer rates and timings on soybean nodulation. An application of 34 kg ha^{-1} of N at planting had no effect on the number of nodules per plant. Early nodule diameters were reduced where fertilizer was applied, but by four weeks after flowering, no difference in nodule size was observed (Williamson and Diatloff 1975).

Inoculants were applied to seed using one gallon resealable plastic canisters, to which 0.91 kg. of soybean seed and the labeled rate of inoculant was added. The

Table 1.1: Soybean inoculant field trial site information.

County	Nearest Town	Soil Class	Planting	Harvest	Previous Crop	Fertilizer
2003						
Grand Traverse	Buckley	Karlin Sandy Loam	9-May	8-Oct	Corn	170# 22-11-22
Hillsdale	Reading	Blount Silt Loam	27-May	18-Oct	Wheat	150# 0-0-60
Ingham	Mason	Capac/Marlette/Colwood	22-May	17-Oct	Corn	NONE
Lenawee	Britton	Brookston Clay Loam	19-May	13-Oct	Corn	250# 0-0-60
Montmorency	Hillman	Bergland Clay Loam	9-May	20-Oct	Corn	170# 0-14-42
Saginaw	Frankentrost	Parkhill Clay Loam	21-May	6-Oct	Wheat	NONE
2004						
Grand Traverse	Buckley	Karlin Sandy Loam	16-May	7-Oct	Corn	140# 22-11-22
Hillsdale	Reading	Blount Silt Loam	30-Apr	6-Oct	Corn	NONE
Ingham	Mason	Capac Loam	4-Jun	24-Oct	Corn	150# 0-0-60
Lenawee	Britton	Brookston Clay Loam	29-May	26-Oct	Wheat	300# 0-0-60
Montmorency	Hillman	Bergland Clay Loam	19-May	1-Nov	Corn	NONE
Sanilac	Sandusky	Parkhill Clay Loam	2-Jun	28-Oct	Corn	GPS 0-0-60
2005						
Hillsdale	Reading	Blount Silt Loam	3-May	30-Sep	Corn	NONE
Ingham	Mason	Capac Loam	7-May	10-Oct	Corn	150# 0-0-60
Montmorency	Hillman	Bergland Clay Loam	12-May	11-Oct	Corn	NONE
Sanilac	Sandusky	Parkhill Clay Loam	11-May	5-Oct	Corn	NONE

Table 1.2: Soil test results by site-year.

Site	Clay	Silt	Sand	Type	O.M.	CEC	pH	P	K	Mg	Ca
2003											
Grand Traverse	2.2	13.6	84.2	Loamy Sand	1.4	5.3	6.1	175	116	73	643
Hillsdale	11.4	24.2	64.4	Sandy Loam	2.9	7.1	6.4	20	75	148	1126
Ingham	7.4	29.2	63.4	Sandy Loam	1.9	6.1	5.2	35	172	99	729
Lenawee	35.4	40.2	24.4	Clay Loam	3.2	14.6	6.2	62	199	261	2147
Montmorency	17.8	35.8	46.4	Loam	2.3	12.8	7.9	18	116	272	2046
2004											
Grand Traverse	2.2	13.6	84.2	Loamy Sand	1.4	5.3	6.1	175	116	73	643
Hillsdale	15.4	20.2	64.4	Sandy Loam	2.4	9.6	6.1	26	117	221	1243
Ingham	8.8	26.8	64.4	Sandy Loam	1.5	4.6	5.7	43	126	109	664
Lenawee	31.4	48.2	20.4	Clay Loam	4.3	17.4	6.4	71	205	397	2474
Montmorency	15.4	32.2	52.4	Sandy Loam	2.4	10.5	7.8	23	86	277	1602
Sanilac	11.8	31.8	56.4	Sandy Loam	2.2	5.9	6.1	67	171	141	849
2005											
Hillsdale	11.4	25.2	63.4	Sandy Loam	3.1	10.6	6.3	25	126	248	1636
Ingham	7.4	29.2	63.4	Sandy Loam	1.9	6.1	5.2	35	172	99	729
Montmorency	37.4	30.2	32.4	Clay Loam	3.4	18.8	7.9	28	204	430	2948
Sanilac	21.4	39.2	39.4	Loam	2.6	8.8	6.6	75	174	302	115

Table 1.3: Inoculant and fungicide treatment ratcs.

Treatment	Product	Rate
1.	Cell-Tech	136 ml/50 kg seed
2.	PulseR HP	118 g/50 kg seed
3.	SowFast	124 g/50 kg seed
4.	Nod+	136 ml/50 kg seed
5.	HiStick2/NT	87 g /50 kg seed
6.	NitroFix Liquid	162 ml/50 kg seed
7.	Apex Extra	136 ml/50 kg seed
8.	Optimize (110 ml inoculant + 26 ml extender)	136 ml/50 kg seed
Fungicide	Apron Maxx RTA	162 ml/50 kg seed

canister for each treatment was then tumbled for several minutes on a mechanical tumbling machine used for seed treatment, this ensured a uniform inoculant application. After the inoculant was applied, the seed was packaged into small coin envelopes. Approximately 670 seeds were packaged for planting in each plot, to obtain a seeding rate of 445,000 seeds ha^{-1}. Plots were planted using a 6 row, 2.28 m plot cone planter (Almaco Inc., Nevada, IA). Two seed dump funnels fed the seed into the planting units. This planter was split so that rows 1-3 on the planter were fed by a left seed dump funnel, and rows 4-6 were fed by a right seed dump funnel. Each seed dump was fed with separate envelopes, therefore, each subplot required two envelopes containing approximately 335 seeds

The seed was packaged and planted in a manner as to avoid contamination of non-inoculated plots with residual inoculum. To accomplish this, the planter's fourth, fifth, and sixth row units were reserved for inoculant treatments, while the first, second, and third row units were reserved for control subplots that never received inoculated seed. The planter would plant three rows of two subplots at a time. As the planter made an away pass, the final three rows of the control subplot from the previous whole plot, and the first three rows of the inoculated subplot from a new whole plot were planted. As the planter made a return pass, the final three rows of the inoculated subplot was planted along with the first three rows of the new control subplot. All sites were planted in a conventionally prepared seedbed. Plot yields were taken using an Almaco plot combine (Almaco Inc., Nevada, IA). This combine harvested the middle four rows of each six row subplot in the field. Total area harvested for each plot was 6.45 m^2. An Almaco plot weigh bucket controlled by a Harvestmaster control on the combine allowed for seed yield and moisture to be measured as plots were harvested (Almaco Inc., Nevada, IA and Juniper Systems, Logan UT).

Yield data analysis was conducted separately for each location by year. Whole plots were used as an error term to test the effect of fungicide, and split plots were used to test the effect of inoculant. Mean square for residuals was used to test the effect of interaction between fungicide and inoculant. The main model analysis was performed in PROC MIXED (The SAS Institute, 2007). At sites where significant inoculant effects were observed, inoculant products were combined and contrasted against untreated plots to determine if a significant inoculant treatment effect occurred in each site-year. All pairwise comparisons between the inoculant products were also performed with yield data combined across sites for each year. At sites where significant fungicide-inoculant interactions were observed, the main inoculant effect was sliced between fungicide treated plots and plots with no fungicide treatment.

Harvest samples (approx. 0.5 kg.) were taken from Lenawee in 2004, Hillsdale in 2005, and Montmorency in 2004 and 2005. These samples were analyzed for protein and oil content using a Foss NIRSYSTEM 6500 near-infrared spectroscopy instrument (Foss North America, Eden Prairie, MN). Data analysis for protein and oil content proceeded similarly to that of yield data, though analysis was only of inoculant treatments combined.

Greenhouse Study

To observe growth effects of inoculant products in a controlled environment, a greenhouse study was performed with several inoculant products. Soybean nodulation was of particular interest in this study, as nodule data in field studies is difficult to obtain and typically highly variable. The experiment was a completely randomized design, with 10 pots of 7 different inoculant products: Hi-Stick N/T, Cell-Tech, Nod+, Sow Fast, PulseR HP, Apex Extra, and Optimize, with an untreated control. Cell-Tech inoculant was also tested with Apron Maxx RTA fungicide to determine whether there was a detrimental effect by the fungicide on soybean nodulation.

Inoculants and fungicide treatments were applied to seed using one gallon resealable plastic canisters, to which 0.91 kg of soybean seed and the labeled rate of inoculant or fungicide was added. Pesticide seed treatments were applied the day prior to inoculation and planting to allow the chemical to dry on the seed. The canister for each treatment was then tumbled for several minutes on a mechanical tumbling machine used for seed treatment; this ensured a uniform seed treatment application. Inoculants were applied during the day of planting. Five treated soybean seeds (DF8251RR variety) were planted approximately 2.5 cm deep into 15 cm diameter clay pots filled with BACTTO high porosity planting mixture (Michigan Peat Company, Houston, TX). First emergence occurred four days after planting. Untreated seed was planted first, to avoid contamination with inoculum, and rubber gloves were changed between seed treatments to avoid contamination between treatments. Plants were grown using a 16/8 light/dark day length cycle. The greenhouse was lit with S51 400 watt high pressure sodium lamps (Lithonia Lighting, Conyers, GA), and the average greenhouse temperature was 25°C. Thrips were controlled weekly with an application of Conserve (spinosad, Dow Agrosciences, Indianapolis, IN) at 0.46 ml/liter. At 14 DAP, pots were thinned to 1 bean plant per pot. Pots were re-randomized every two weeks during the study.

SPAD meter readings were taken every 7 days between 14 and 35 DAP. Plant heights were also taken every 7 days between 21 and 35 DAP. At 49 DAP, plant shoot material was harvested. Leaf area meter readings were taken, as well as fresh biomass and dry biomass. Finally, nodule counts were conducted at 50 DAP. Root masses were removed from pots and gently washed in root washing sinks. Nodules were then counted from the exposed root system.

This experiment was run twice. The first run was planted on February 15, 2006. Plants were harvested on April 7, 2006 and nodule counts were performed on April 7 and 8, 2006. The second run was planted on December 1, 2006. Plants were harvested on January 19, 2007 and nodule counts were performed on January 19 and 20, 2007. Data was analyzed as a single factor completely randomized design in PROC MIXED (The SAS Institute, 2007). If the main effect was significant, all paired comparisons were performed between treatments.

Results and Discussion

Climate

In 2003, precipitation was above average at all sites in May. However, rainfall patterns through the state were quite variable later in the growing season. Ingham County (E. Lansing) had a rainfall deficit in each month between June and September. Montmorency County (Hawks) experienced a rainfall deficit each month except July. Lenawee County experienced rainfall surpluses in July, August and September, with August nearly 10 cm above average. Other sites experienced normal precipitation in 2003. The 2004 growing season began with a remarkably wet spring, with widespread record and near-record May rainfall throughout the state. July through August had nearer to normal precipitation throughout the state, although Lenawee County continued to have high precipitation through June. September was very dry throughout the state, with no site receiving more than 3.12 cm of rainfall. The 2005 growing season began very dry, with every site experiencing a rainfall deficit for the month of May. June and July were close to average overall, while Ingham County became very dry in August and September. September was again very dry throughout the state in 2005 (Table 1.4).

In 2003, Ingham and Saginaw Counties saw temperatures below normal in May and June, but above normal in August. Other sites had near normal temperatures throughout the year, with the exception of August being above normal. In 2004, Michigan experienced a cool July and August, as temperatures were over 1°C below normal at every field site for both months, with the exception of Lenawee County. September temperatures were above normal however, and actually exceeded August averages in Northern Michigan. In 2005, above average temperatures were observed at every site in every month of the growing season except May. Average temperatures were at least 2.3°C above normal at every site during the month of June (Table 1.5)

Table 1.4: Precipitation by month(cm), field Sites 2003-2005. Long term means (1970-2000) included for comparsion.

		May	June	July	August	September
Grand Traverse	2003	7.59	5.41	10.31	11.43	7.31
(Kingsley)	2004	16.51	6.17	4.21	5.56	2.08
	30 yr.	5.84	8.12	6.60	7.37	10.16
Hillsdale	2003	14.98	5.63	6.83	16.71	14.55
(Coldwater)	2004	15.16	11.45	22.55	5.61	3.20
	2005	6.10	7.95	11.02	4.98	7.49
	30 yr.	9.62	9.44	9.65	10.01	9.42
Ingham	2003	10.36	3.73	3.58	4.62	6.55
(E. Lansing)	2004	20.50	8.92	10.16	8.71	2.67
	2005	3.33	10.87	8.71	1.63	7.67
	30 yr.	6.90	8.07	7.56	8.74	9.22
Lenawee	2003	12.52	5.13	9.49	18.80	13.03
(Tecumseh)	2004	15.04	15.11	8.61	6.01	2.15
	30 yr.	8.38	8.89	8.13	8.89	8.38
Montmorency	2003	5.92	5.13	8.18	3.99	5.99
(Hawks)	2004	12.19	5.59	11.48	6.12	3.12
	2005	1.91	8.20	6.30	13.44	4.50
	30 yr.	7.36	6.85	7.62	9.65	8.89
Saginaw	2003	10.79	6.88	8.61	4.24	5.33
(Saginaw)	30 yr.	7.34	8.61	7.46	8.51	10.80
Sanilac	2004	16.08	8.51	12.67	2.97	1.96
(Sandusky)	2005	4.98	7.04	10.19	6.60	5.08
	30 yr.	6.78	7.61	6.73	7.37	10.24

Table 1.5: Average temperature by month(°C), field Sites 2003-2005. Long term means (1970-2000) included for comparsion.

		May	June	July	August	September
Grand Traverse	2003	11.7	17.3	20.0	21.1	16.3
(Kingsley)	2004	12.3	17.5	19.3	17.8	18.6
	30 yr.	12.2	17.8	20.6	19.4	15.6
Hillsdale	2003	12.8	18.4	21.4	21.9	16.1
(Hillsdale)	2004	16.1	18.9	20.6	18.9	18.9
	2005	12.7	22.4	22.4	21.1	18.4
	30 yr.	14.1	19.3	21.5	20.3	16.1
Ingham	2003	12.6	18.1	21.0	21.1	15.7
(E. Lansing)	2004	14.6	18.1	20.1	18.4	17.6
	2005	12.3	21.9	22.0	21.8	18.3
	30 yr.	14.0	19.2	21.4	20.3	16.1
Lenawee	2003	14.3	19.1	22.3	22.4	16.6
(Tecumseh)	2004	16.4	19.9	21.6	19.6	18.9
	30 yr.	14.4	19.4	21.7	20.6	16.7
Montmorency	2003	10.5	16.3	19.2	19.6	14.1
(Hawks)	2004	10.3	15.9	18.3	16.4	16.8
	2005	10.6	19.8	20.7	18.9	16.6
	30 yr.	10.6	16.1	19.4	17.8	13.9
Presque Isle	2006	13.2	18.7	23.0	20.0	14.9
(Rogers City)	30 yr.	11.1	16.4	19.6	18.6	14.3
Saginaw	2003	13.1	18.3	21.5	22.3	17.1
(Saginaw)	30 yr.	15.4	20.4	22.8	21.5	17.3
Sanilac	2004	12.4	16.9	19.4	18.2	17.6
(Sandusky)	2005	11.8	21.2	21.6	20.9	18.1
	30 yr.	13.5	18.5	21.2	20.1	15.8

Inoculant Yield Effects

Overall, for the 14 site-years on which inoculant trials were performed on soil with a history of soybean production, the average yield difference between inoculated and uninoculated plots was 85.58 kg ha^{-1}. This result is consistent with results in Purdue University trials, but somewhat less than results from Ohio State University trials (Conley and Christmas 2006, Beuerlein 2005). Of these 14 site-years, 6 sites showed significant main inoculant effects (Table 1.6).

In 2003, inoculated plots at Hillsdale, Saginaw, and Montmorency County sites showed significant yield increases of 194 kg ha^{-1} (6.3%), 156 kg ha^{-1} (8.3%), and 185 kg ha^{-1} (5.5%) respectively. Sites in Lenawee, Ingham, and Grand Traverse Counties showed no significant yield differences due to inoculation.

In 2004, the site at Ingham County showed a significant yield increase in inoculated plots of 231 kg ha^{-1} (6.9%) Inoculated plots in Lenawee County showed a significant yield decrease of 281 kg ha^{-1}(5.9%). However, this was the only site where a significant decrease in yield was observed in inoculated plots. Sites in Hillsdale, Grand Traverse, and Sanilac Counties showed no significant yield differences due to inoculant (Table 1.7).

Table 1.6: Summary of combined inoculants effects, ANOVA for grain yield by site-year.

	P-values from ANOVA		
	2003	2004	2005
Grand Traverse	0.0830	0.0905	------
Hillsdale	0.0002	0.3790	<0.0001
Ingham	0.6417	0.0015	0.5275
Lenawee	0.3355	<0.0001	------
Montmorency	0.0001	<0.0001*	<0.0001*
Saginaw	0.0005	----------	------
Sanilac	---------	0.5027	0.0008

*virgin soybean ground

Researchers in other states have observed rare cases where soybean inoculation led to decreases in yield. A trial at the Throckmorton-Purdue Agricultural Center in 2005 also showed a yield decrease as a result of soybean inoculation (Conley, unpublished data). Both sites had very high trial yield averages. Untreated plots at the 2004 Lenawee site averaged 4946 kg ha^{-1}, and untreated plots at the 2005 Throckmorton site averaged 4804 kg ha^{-1}.

Lenawee 2004 and Ingham 2005, the two site years in the trial that had the most numerically negative yield response to inoculant, had only 2.64 and 1.54 cm of rainfall in the 30-60 day preharvest period when soybean would be in pod filling stages. At the site of the 2005 Purdue study, only .01 inches of rain was accumulated during a 24 day period between August 21 and September 13. This long period with lack of rainfall seems to fit a trend of negative inoculant results in years where a rainfall deficit occurs during podfill stages.

When rainfall in the 30-60 day preharvest period was plotted against combined inoculant yield differences by site-year in this study, a moderate correlation existed when a quadratic curve was fitted to the data (R^2=.403). The model used for this curve was $y= -2.33x^2 + 12.51x - 10.08$, with x equal to rainfall in the 30 to 60 day preharvest period and y equal to the percent yield difference between inoculated and uninoculated plots by site-year. Analysis of this model in PROC REG (The SAS Institute, 2007) resulted in a Pr>F value of 0.0582. While the model was not significant at the 95% confidence level, this may be an interesting trend worthy of more evaluation.

Lawn and Brun observed that as soybean pod fill begins, N fixation by nodules peaks and then rapidly decreases. Plant depodding allowed nodule activity to be maintained longer, while plant defoliation caused N fixation in nodules to decline earlier (Lawn and Brun 1974). Others have found N fixation to peak later than early pod fill. (Thibodeau and Jaworski 1975, Abendroth and Elmore 2006).

One hypothesis to explain nodule death may be that the plant-nodule relationship is a source-sink relationship, and that as photosynthate is supplied to new seeds, it is diverted from feeding nodules, particularly if resources are limited under stressful conditions. If this source-sink relationship exists and photosynthate is competed over between developing fruit and nodules particularly during a water-stressed period, this may explain potential yield losses under extended dry periods during pod fill. If inoculated plots had superior nodulation, they may compete with developing fruit for photosynthate from leaves, at least during early pod fill. While indirect evidence supporting the source-sink hypothesis has been observed (Lawn and Brun 1974, Schulze 2003), this hypothesis has not been supported with direct evidence relating carbon assimilate competition by nodules and nitrogen fixation (Schulze et. al. 2000, Puppo et. al. 2005). There has also been speculation that enhanced early season growth from inoculants in high yielding beans could create a large plant vegetative sink, and if rainfall is lacking late in the growing season, this could cause a negative yield response.

In 2005, Hillsdale and Sanilac County sites showed significant yield increases. Inoculated plots in Hillsdale County showed a yield increase of 358 kg ha^{-1}, or 9.0%. Inoculated plots in Sanilac County showed a yield increase of 273 kg ha^{-1}, or 8.4%. Ingham County plots showed no significant yield difference (Table 1.7).

Montmorency County plots in 2004 and 2005 were conducted on sites that did not have a history of soybean production. As a result, yield increases were significantly higher than other locations. Inoculated plots in 2004 yielded 1433 kg ha^{-1} higher than non-inoculated plots (82.2%). In 2005, inoculated plots yielded 551 kg ha^{-1} higher than non-inoculated plots (29.9%, Table 1.7).

Table 1.7: Inoculant grain yield effects, by site-year (kg ha^{-1}, treatments combined).

	2003	2004	2005
Grand Traverse			
Inoculated	2223	1305	
Untreated	2253	1150	
	-30	155	
Hillsdale			
Inoculated	3262	3119	4348
Untreated	3067	3053	3990
	195*	67	358*
Ingham			
Inoculated	2207	3587	3149
Untreated	2151	3356	3287
	56	231*	-138
Lenawee			
Inoculated	2610	4666	
Untreated	2639	4947	
	-29	-281***	
Montmorency			
Inoculated	3544	3176	2412
Untreated	3359	1743	1861
	185*	1433* **	551* **
Saginaw			
Inoculated	2023		
Untreated	1867		
	156*		
Sanilac			
Inoculated		3942	3524
Untreated		3927	3250
		15	274*

***significant yield increase at P<0.05**
****virgin soybean ground**
*****significant yield decrease at P<0.05**

Inoculant Brand Yield Effects

During certain site-years, treatment with particular inoculant brands produced significantly higher soybean grain yield than other brands, but averaged across site-years, no significant yield advantage was observed for any inoculant brand over another (Table 1.8). However, when the two virgin soybean sites, Montmorency 2004 and 2005, are combined and analyzed, some interesting separation does occur. While six of the seven brands are statistically similar, three of the four brands that also have a "b" grouping are peat based powders: HiStick2/NT, PulseR, and SowFast (Table 1.9). When mean inoculant treatment yield are combined and contrasted based on type of carrier, there is a significant yield advantage from liquid inoculants at these sites with no previous soybean production (Table 1.10).

Most research regarding inoculant carrier materials found that in soybean, liquid and peat based carriers provide similar yield effects. In the series of trials performed by Ohio State, no significant difference between peat based and liquid formulations were observed, although their trials were performed exclusively on rotational soybean ground (Beuerlein 2004, 2005). The differences observed between liquid and peat based inoculants on first-time soybean fields were interesting and unexpected. Trials on first-time soybean fields in Ontario found yields to be similar between peat based and liquid inoculant treatments (Bohner 2002). There may be a coverage advantage using liquid inoculants compared to peat-based inoculant. Whereas seed adhesion of liquid inoculants is excellent, some percentage of peat inoculant will fail to adhere to seed or can fall off during seed transport and seed movement within the planter and fall to the bottom of the hopper (Smith 1992). A trial performed in Australia found that a water slurry application

Table 1.8: Inoculant treatment grain yield averages, by year (kg ha^{-1}) sites combined, excluding Montmorency '04-'05).

Inoculant	2003	2004	2005
Cell Tech	2685a	3281a	3543a
PulseR	2656a	3326a	3794a
SowFast	2616a	3294a	3541a
Nod+	2674a	3312a	3749a
HiStick2/NT	2656a	3346a	3630a
NitroFix Liquid	2616a	3372a	3729a
Apex Extra		3450a	3698a
Optimize			3656a

Table 1.9: Inoculant treatment grain yield averages, Montmorency 2004-2005 (kg ha^{-1}).

Inoculant	yield	carrier
NitroFix Liquid	3206a	liquid
Cell Tech	3084a	liquid
Nod+	3056a	liquid
Apex Extra	2922ab	liquid
PulseR	2889ab	peat
SowFast	2796ab	peat
HiStick2/NT	2442b	peat

Table 1.10: Contrast of grain yield means for liquid and peat based inoculants, Montmorency 2004-2005 (kg ha^{-1}, no fungicide plots only).

	Yield	Pr >t
Liquid Carrier Inoculants	3073	
Peat Carrier Inoculants	2709	
	364	0.0193

of inoculant did increase yields over a dry inoculant form (Brockwell et. al. 1988). However, most peat based inoculants used today include sticking agents to minimize these problems, and seed coverage was usually observed to be adequate during planting of seed treated with the peat based products.

Overall results in these trials were similar to those performed in Ohio and Indiana, in that certain site-years had obvious responses to soybean inoculation, while others showed no response. In conclusion, it appears that in the long term, inoculation of soybean seed is an economically viable practice, though not necessarily of a large magnitude. It also appears that there are not significant differences in the effectiveness of any particular commercial brand of inoculant except when soybeans have not been grown in the past, where it may be advantageous to use a liquid inoculant. There may also be a trend for reduction in inoculant effectiveness or a potential for negative yield effects when extremely dry conditions occur during pod fill.

Fungicide Yield Effects

Significant fungicide main effects were observed at five of sixteen site-years (Table 1.11). In cases where an inoculant-fungicide interaction was observed in the model or there was a significant negative effect of fungicide within a contrast at a particular site, the fungicide treatment was sliced between inoculated and uninoculated plots to observe whether the inclusion of the fungicide treatment interacted with inoculant. In cases where the main effect of the fungicide produced a yield increase, interactions between fungicide and inoculant were not observed. Saginaw saw a yield increase of 105 kg ha^{-1} in 2003. Hillsdale saw yield increases in both 2004 (260 kg ha^{-1}) and 2005 (102 kg ha^{-1}). However, significant effects were observed at Montmorency in 2004 and 2005; and these effects produced yield decreases. These are the two sites which had not previously had a soybean crop (Table 1.12).

Table 1.11: Summary of ANOVA for fungicide main effects on grain yield by site-year.

	P-values from ANOVA		
	2003	2004	2005
Grand Traverse	0.2600	0.5914	------
Hillsdale	0.7541	0.0174	0.0205
Ingham	0.7505	0.1225	0.4047
Lenawee	0.4176	0.2559	------
Montmorency	0.0695	<0.0001* **	0.0425* **
Saginaw	0.0090	------	------
Sanilac	------	0.9321	0.6921

*new soybean ground
** significant yield decrease, other significant effects were increases

Table 1.12: Fungicide yield effects, by site-year (kg ha^{-1}).

	2003	2004	2005
Grand Traverse			
Apron Maxx	2257	1288	
Untreated	2196	1258	
	61	-33	
Hillsdale			
Apron Maxx	3228	3247	4365
Untreated	3240	2974	4252
	-12	273*	113*
Ingham			
Apron Maxx	2185	3518	3206
Untreated	2205	3601	3148
	-20	-83	58
Lenawee			
Apron Maxx	2646	4729	
Untreated	2581	4676	
	65	53	
Montmorency			
Apron Maxx	3555	2803	2297
Untreated	3479	3190	2412
	76	-387*	-115*
Saginaw			
Apron Maxx	2053		
Untreated	1948		
	105*		
Sanilac			
Apron Maxx		4063.7	3416
Untreated		4058.8	3445
***significant at P<0.05**		4.9	-29

Slicing effects between inoculated and uninoculated subplots were highly significant at Montmorency in 2004. There was no significant yield difference between uninoculated subplots with or without Apron Maxx RTA. However, treatment of inoculated subplots by Apron Maxx RTA resulted in a 504.4 kg ha^{-1} decrease in yield compared to inoculated subplots with no fungicide. Slicing was also significant at Montmorency in 2005, where there was again no yield difference in uninoculated soybean with or without Apron Maxx RTA, but a significant yield decrease of 134 kg ha^{-1} when inoculants were used with Apron Maxx RTA treatment. Thus, the lack of main fungicide effects at these two sites was masked by these inoculant-fungicide interactions during these two site-years (Table 1.13).

Interestingly, when inoculant brands were compared based on carrier in the presence of Apron Maxx RTA, the significance of the difference between liquid and peat products is lost. Also, when inoculant-fungicide interaction effects are sliced based on the inoculant carrier used, the interaction remains significant for liquid products, but is not significant when peat-based products are used. When the differences between inoculant-fungicide interactions are contrasted, there is a significant difference between the interaction caused by Apron Maxx RTA to liquid inoculants and Apron Maxx RTA to peat based inoculants. These results indicate that while liquid inoculants may be more effective on virgin soil, these advantages may be mitigated when Apron Maxx RTA is used as a seed treatment (Table 1.14).

Researchers have commented on the possibility that the particle matrix associated with peat based inoculants provide some protection against environmental stresses, particularly lack of moisture and resulting bacterial desiccation (Zdor and Pueppke, 1990,

Table 1.13: Inoculant-fungicide interaction slicing effects at Montmorency, 2004-2005.

	Montmorency 2004	
	Yield	Pr>F
Apron Maxx RTA	1799	
Untreated	1687	
	112	0.2943
Apron Maxx RTA + Inoculant	3241	
Inoculant Only	3746	
	-505	<0.0001
	Montmorency 2005	
	Yield	Pr>F
Apron Maxx RTA	1855	
Untreated	1858	
	-3	0.9054
Apron Maxx RTA + Inoculant	2345	
Inoculant Only	2479	
	-134	0.0377

Kyei-Boahen et al. 2002, Bashan 1998, Smith 1992). It is plausible that peat carriers could provide a physical barrier against fungicide-bacteria interaction in the same manner.

The Apron Maxx formulation used in this study (Apron Maxx RTA) is the most common of two Apron Maxx formulations available for use on soybean today. The second formulation is Apron Maxx RFC (Rhizobium Friendly Concentrate), which was introduced in 2005 (Syngenta, Greensboro, NC) for use and co-application with liquid inoculants. This formulation has a higher concentration of active ingredients (mefenoxam and fludioxonil), and is applied at a 48.6 ml/50 kg of seed rate, rather than

Table 1.14: Inoculant-fungicide interaction slicing effects at Montmorency, 2004-2005 (grain yield, kg ha^{-1}).

	Yield	Pr>F
Peat Based Inoculants (No Fungicide)	2709	
Peat Based Inoculants (With Apron Maxx RTA)	2558	
	151	0.1367
Liquid Inoculants (No Fungicide)	3074	
Liquid Inoculants (With Apron Maxx RTA)	2677	
	397	0.0030
Liquid Inoculant/Apron Maxx Difference	397	
Peat Based Inoculant/Apron Maxx Difference	151	
	246	0.0420
Peat Based Inoculants (With Apron Maxx RTA)	2558	
Liquid Inoculants (With Apron Maxx RTA)	2677	
	-119	0.1874

162 ml/50 kg rate used with Apron Maxx RFC. Active ingredient rates are the same for both products, but less total product is used in application of Apron Maxx RFC to seed.

While Apron Maxx RTA did not interact at the six rotational sites that were responsive to inoculants, clear evidence of interaction between Apron Maxx RTA and inoculants was observed at sites with no history of soybean production, where inoculant effects were more pronounced. Use of Apron Maxx RFC may be an option for farmers producing soybean at such sites.

Overall, Apron Maxx RTA did not provide widespread yield increases during this study. Yields were increased at three of sixteen site-years throughout the span of the trial. The largest increase was 272 kg ha^{-1} at Hillsdale in 2004. This yield increase could be explained by climatic conditions experienced that year, as Hillsdale was planted on April 30, which preceded the record setting rainfall pattern experience in May throughout

Central and Southern Michigan. This may have caused more early soil-borne disease pressure. Apron Maxx RTA also increased yield at Hillsdale in 2005, which was the earliest planted site and had temperatures that were well below normal throughout the month of May. Early stands were somewhat reduced overall at Hillsdale in 2004, but no fungicide treatment effects were observed on plant stands in 2004 or 2005 other than at Lenawee (Table 1.15).

Table 1.15: Fungicide plant stand effects by site (plants ha^{-1}).

	G.T.	Hillsdale	Ingham	Lenawee	Mont.	Sanilac	Avg.
2004							
Apron Maxx	400695	356990	416052	380101	403422	410949	394717
No Fungicide	402921	368223	407727	359147	409521	395516	390479
	-2226	-11223	8325	20954*	-6099	15433	4238
2005							
Apron Maxx		304036	357057		240460	247994	286801
No Fungicide		301096	362599		246971	247779	289361
		2940	-5542		-6511	215	-2560

***significant at P<0.05**

Grain Protein and Oil Effects

Inoculation had no effect on grain protein content at Lenawee in 2004 or Hillsdale in 2005. Significant differences were found at Montmorency in both 2004 and 2005, the two first-time soybean sites in this study. Grain protein content was 11.8% and 8.17% higher in soybean harvested from inoculated plots at Montmorency in 2004 and 2005 (Table 1.16). An inoculant-fungicide interaction was observed at Montmorency in 2004, as among inoculated subplots protein levels were reduced by 3% where fungicide seed treatment was used in the whole plot (Table 1.17). This interaction was not observed at Montmorency in 2005.

Similar results were observed for grain oil content analysis. Lenawee 2004 and Hillsdale 2005 sites saw no significant effect on grain oil content by inoculant. However, both Montmorency sites saw significant increases in oil content in soybean from inoculated subplots. Grain oil content was 7.0% and 3.3% higher in soybean harvested from inoculated plots at Montmorency in 2004 and 2005 (Table 1.16). Again, an interaction between inoculant and fungicide was observed, as inoculated subplot grain oil levels were increased by 3% where fungicide seed treatment was used in the whole plot (Table 1.17). This interaction was not observed at Montmorency in 2005.

There is little question that yield differences observed at Montmorency in 2004 and 2005 correlated with the protein and oil content of each treatment. Yield was positively correlated with grain protein content of subplots at both sites in 2004 (R^2=0.53) and in 2005 (R^2= 0.63). Yield was negatively correlated with grain oil content with an R^2 value of 0.63 in both 2004 and 2005.

Table 1.16: Inoculant effects on soybean grain protein and oil content.

	Protein (%)			Oil (%)		
	Inoc.	No Inoc.	Pr<t	Inoc.	No Inoc.	Pr<t
Lenawee 2004	42.29	41.86		20.72	20.76	
Montmorency 2004	37.75	33.76	<0.0001	21.49	23.00	<0.0001
Hillsdale 2005	42.48	42.58		20.83	20.87	
Montmorency 2005	33.36	30.84	<0.0001	23.27	24.03	<0.0001

Table 1.17: Inoculant-fungicide interaction effects on soybean grain protein and oil content, Montmorency 2004.

	Protein (%)	Pr<t	Oil (%)	Pr<t
Inoculated Only	38.30		21.27	
Inoculant + Fungicide	37.20	0.0004	21.71	0.0017

Greenhouse Results

Inoculant brand had a significant main effect on nodule count in both greenhouse runs. In the first run, average nodule counts ranged between 17 and 67 among inoculant treatments, and only 6 for uninoculated plants. In the second run of the greenhouse trial, average nodule counts ranged between 58 and 114 among inoculant treatments, and only 23 for uninoculated plants. Apex Extra and PulseR HP were in the "high nodule count" group for means separation during both runs. Cell Tech was not significantly different from Cell Tech + Apron Maxx in the first run, but averaged 47 nodules less per plant in the second run of the experiment (Table 1.18).

Inoculant brand or carrier type was not a significant factor for determining leaf area index readings, whole plant fresh weight, or whole plant dry weight for either run. Inoculant brand was also not a significant factor in SPAD meter readings or plant height at any measurement timing. Uninoculated soybean likely performed better in the greenhouse than the field due to the high organic matter peat potting mix used in the greenhouse study, which may have provided more available N to compensate for the lack of nodulation.

Table 1.18-Average nodule counts by inoculants treatment (50 DAP), greenhouse study.

	Run 1		Run 2
PulseR HP	67 a	SowFast	114 a
Apex Extra	66 a	Apex Extra	107 ab
Cell Tech + Apron Maxx	48 ab	Cell Tech	106 ab
Cell Tech	39 b	PulseR HP	102 ab
Nod+	37 b	Optimize	88 abc
SowFast	34 bc	HiStick N/T	83 abc
Optimize	30 bc	Nod +	69 bc
HiStick N/T	17 cd	Cell Tech + Apron Maxx	58 cd
Untreated	6 d	Untreated	23 d

Conclusions

Soybean seed inoculants increased soybean grain yield on 6 of 14 site-years which had been in a soybean rotation. The average yield increase as a result of soybean seed inoculation at these 14 site-years was 85.58 kg ha^{-1}. With inoculant costs of $7-$10 ha^{-1}, soybean inoculation appears to be a profitable practice in the long run, if not on an annual basis. Soybean inoculant appeared to benefit grain yield most often when normal precipitation levels were observed during the pod fill period.

No consistant advantage between inoculant products was observed on soils that had been in soybean rotation, though on soil new to soybean production, liquid inoculants provided larger yield increases. Inoculant brand effects on soybean nodulation were not consistently observed in greenhouse studies.

Use of mefenoxam and fludioxonil as a fungicidal seed treatment did not increase soybean yields on a wide-spread basis. Fungicide-inoculant interactions were not observed on soils in soybean rotation, but were observed on soils new to soybean production. This interaction was observed in grain yields, protein content of grain, and oil content of grain.

References

Albrecht, S. L., J. M. Bennett, and K. J. Boote. 1984. Relationship of nitrogenase activity to plant water stress in field grown soybeans. Field Crops Research 8:61-71.

Ash, M., J. Livesey, and E. Dohlman. 2006. Soybean Backgrounder. Electronic Outlook Report from the Economic Research Service, USDA. Retrieved Nov 10, 2006 from http://www.ers.usda.gov/publications/OCS/apr06/OCS200601/OCS200601.pdf

Bacanamwo, M. and L. Purcell. 1999. Soybean dry matter and N accumulation responses to flooding stress, N sources and hypoxia. Journal of Experimental Botany 50(334):689-696.

Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnology Advances 16(4):729-770.

Beuerlein, J. 2005. 2005 Ohio Inoculation Study. Ohio State University Agronomic Crops Network. Retrieved Dec 5, 2006 from http://agcrops.osu.edu/soybean/documents/2005SoyInoculationReport.pdf

Beuerlein, J. 2004. 2004 Ohio Inoculation Study. Ohio State University Agronomic Crops Netwok. Retrieved Dec 5, 2006 from http://agcrops.osu.edu/research/2004/04inoc%20report11.pdf

Bohner, H. 2002. Head-to-Head Comparison of Soybean Inoculants in 2002. Ontario Ministry of Food, Agriculture, and Rural Affairs (Online). Retrieved February 18, 2007 from http://www.omafra.gov.on.ca/english/crops/field/news/croptalk/2003/ct_0103a8. Htm

Brockwell J, D. F. Herridge, L. J. Morthorpe, and R. J. Roughley. 1988. Numerical effects of *Rhizobium* population on legume symbiosis. *In* D. P. Beck and L. A. Materon (eds.) Nitrogen Fixation by Legumes in Mediterranean Agriculture. 179–193. ICARDA.

Conley, S. and E. Christmas. 2005. Utilizing Inoculants in a Corn-Soybean Rotation. Purdue University Cooperative Extension Bulletin SPS-100-W.

Cregan, P. B., H. H. Keyser, and M. J. Sadowsky. 1989. Host plant effects on nodulation and competitiveness of the *Bradyrhizobium japonicum* serotype strains constituting serocluster 123. Applied Environmental Microbiology 55(10): 2532-2536.

Duong, T. P., C. N. Diep, N. T. Khiem, N. H. Hiep, N. V. Toi, N. V. Lich and T. K. L. Nhan. 1984. Rhizobium inoculant for soybean [*Glycine max* (L.) Merrill] in Mekong Delta. Plant and Soil 79(2):235-240.

Graham, P. H. 1992. Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Canadian Journal of Microbiology 38: 475–484.

Kuykendall, L. D. and W. J. Hunter. 1991. Enhancement of nitrogen fixation with *Bradjirhizobium japonicum* mutants. US patent 5,021,076.

Kyei-Boahen, S., A. E. Slinkard, and F. L. Walley. 2002. Evaluation of rhizobial inoculation methods for chickpea. Agronomy Journal 94:851-859.

Lawn, R. J. and W. A. Brun. 1974. Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulation. Crop Science 14:11-16.

Lindemann, W. C. and C. R. Glover. 2003. Nitrogen fixation by legumes. New Mexico State University Cooperative Extension Service Guide A-129.

Pedersen, P. 2003. Soybean Seed Inoculation. Integrated Crop Management Newsletter, Iowa State University Extension. Issue IC-490(5):12-13.

Puppo, A., K. Groten, F. Bastian, R. Carzaniga, M. Soussi, M. M. Lucas, M. Rosario de Felipe, J. Harrison, H. Vanacker, and C. H. Foyer. 2005. Legume nodule senescence: roles for redox and hormone signalling in the orchestration of the natural aging process New Phytology 165(3): 683–701.

Schulze J., H. Beschow, E. Adgo, and W. Merbach. 2000. Efficiency of N2 fixation in Vicia faba L. in combination with different *Rhizobium leguminosarum* strains. Journal of Plant Nutrition and Soil Science 163:367–373.

Schulze, J. 2003. Source-sink manipulations suggest an N-feedback mechanism for the drop in N2 fixation during pod-filling in pea and broad bean. Journal of Plant Physiology 160:531-537

Seneviratne, G., L. H. J. Van Holm and E. M. H. G. S. Ekanayake. 2000. Agronomic benefits of rhizobial inoculant use over nitrogen fertilizer application in tropical soybean. Field Crop Research 68(3):199-203.

Smith, R. S. 1992. Legume inoculant formulation and application. Canadian Journal of Microbiology 38, 485-492.

Smith, S., R. M. Osborn, J. W. Kosanke. 2004. Methods and compositions providing agronomically beneficial effects in legumes and non-legumes. United States Patent Application 20050187107.

Thibodeau, P. S. and E. G. Jaworski. 1975. Patterns of nitrogen utilization in the soybean. Planta 127(2):133-147.

Tien, H. H., T. M. Hien, M. T. Son and D. Herridge. 2002. Rhizobial Inoculation and N2 Fixation of Soybean and Mungbean in the Eastern Region of South Vietnam. *In* D. Herridge (ed.) Inoculants and Nitrogen Fixation of Legumes in Vietnam: ACIAR Proceedings 109e.

Vitosh, M. L. 1997. Soybean Inoculation in Michigan. Soybean Facts Winter 1997. Michigan State University Extention Facts Sheet 9708.

Xavier, I. J., G. Holloway, and M. Leggett. 2004. Development of rhizobial inoculant formulations. Online. Crop Management doi:10.1094/CM-2004-0301-06-RV.

Zdor, R. E. and S. G. Pueppke. 1990. Competition for nodulation of soybean by *Bradyrhizobium japonicum* 123 and 138 in soil containing indigenous rhizobia. Soil Biology and Biochemistry 22(5):607-613.

CHAPTER 2

EFFECT OF NEONICTINOID SEED TREATMENTS ON SOYBEAN GROWTH AND YIELD IN MICHIGAN

Abstract

Two recently registered neonicotinoid seed treatments, thiamethoxam and imidacloprid provide early season protection against several different insect pests, including soybean aphid and bean leaf beetle. These seed treatments may also reduce seedling mortality, increase plant height, and improve root, stem, and leaf development. This research determined whether grain yield and plant health effects were observed as a result of neonicotinoid seed treatments, as well the effectiveness and duration of soybean aphid control. Positive neonicotinoid effects on grain yield were mainly limited to 2005, a year in which significant soybean aphid pressure was observed in Michigan. Both thiamethoxam and imidacloprid provided significant yield increases at two of four field sites in 2005. Significant soybean aphid population suppression occurred through R2 in 2005, but was not significant after R2. Improvement of plant health indicators such as plant height, SPAD chlorophyll index, and stand counts from neonicotinoid seed treatments was not observed on a widespread basis.

Introduction

Neonicotinoids are the most recent class of insecticides introduced for crop protection. Neonicotinoids are systemic insecticides, absorbed by plants from foliar or soil application, or seed treatment. Neonicotinoids have high water solubility, an important property of systemic insecticides (Tomizawa and Casida 2005). Neonicotinoids act upon the nervous system by depolarizing giant interneurones at nicotinic acetylcholine receptors, leading to paralysis and death of the insect (Matsuda et. al. 2001, Rice 2004). Neonicotinoids preferentially bind to insect acetylcholine receptors rather than vertebrate acetylcholine receptors, making them less toxic to humans than other systemic insecticides (Yamamoto and Casida 1999, Tomizawa and Casida 2005, Elliott 1977). This favorable property makes the neonicotinoids popular options for insect control in many crops.

Since 2004, two new neonicotinoid seed treatments for soybean have been introduced. Thiamethoxam (Cruiser, Syngenta, Greensboro, NC), and imidacloprid (Gaucho, Bayer Crop Science, Research Triangle Park, NC) have been approved by the EPA for use as a soybean seed treatment. These chemicals provide early season control or suppression of insects such as soybean aphid, bean leaf beetle, leafhoppers, thrips, Mexican bean beetle, and wireworms. The product manufacturers advertise crop benefits such as increased plant density, plant height, root, stem and leaf development, as well as increased yields.

Bean leaf beetles defoliate leaves and transmit bean pod mottle virus (BPMV), which can significantly reduce soybean yield and quality, particularly when damage occurs early in the season (Krell et. al. 2004). Thiamethoxam or imidacloprid seed treatment may be ideal to control early season bean leaf beetle infestation and bean pod mottle virus transmission. Trials conducted in Wisconsin, Iowa, and Minnesota have tested neonicotinoid control of bean leaf beetle feeding. These trials showed that neonicotinoid seed treatments significantly reduce bean leaf beetle feeding for first generation infestations (Cullen et. al. 2004a, Schmitt and VanDee 2005, Potter 2002).

Soybean aphid infestations usually occur later into the growing season than bean leaf beetle. Soybean aphid pressure usually increases to its highest level in late July or early August in Michigan (DiFonzo 2003, 2005a), which poses a challenge for neonicotinoid seed treatments. The insecticide must persist longer and maintain potency in a larger soybean plant to provide aphid control late in the growing season. Thiamethoxam has been shown to provide systemic activity and significant SBA mortality for 35 days after planting and a reduction in nymph production up to 49 days after planting (McCornack and Ragsdale 2006). Wisconsin trials performed in 2003 determined the effectiveness of neonicotinoid seed treatments in controlling soybean aphid (Cullen et. al. 2004b). Soybean was planted in these trials on June 9, and throughout the growing season, thiamethoxam and imidacloprid treated soybean had significantly reduced aphid populations, though by mid-August, aphid control appeared to weaken.

A South Dakota study performed in 2003 produced no significant difference between untreated and insecticide treated plots in mean soybean aphids per plot at 67 days after planting. High aphid pressure was observed at this site, with an average of 1,051 aphids per untreated plant, and no resulting yield advantage was observed (Catangui et. al. 2004). Trials performed in Iowa from 2002-2005 determined the effectiveness and duration of soybean aphid control from neonicotinoid seed treatments (Pederson and Lang 2006). In 2003, treated soybean did not reach economic threshold aphid pressure until July 20, compared to June 30 for untreated soybean. In 2005, neonicotinoid seed treatment held soybean aphid pressure under threshold through July 18, about one week longer than untreated soybean. Pederson concluded that seed treatments can control soybean aphid approximately 60 days after planting, which may be useful in some late planting or replant situations (Pederson and Lang 2006).

Research has been performed regarding rhizobial interactions with soil insecticides from the carbamate and organophosphate groups. A few of these chemicals reduced rhizobial growth in-vitro or rhizobial survival on seed, but were not detrimental to nodulation or soybean growth (Mallik and Tesfai 1984, Martyniuk et. al. 2002, Evans et. al. 1991, Lin et. al. 1972). There is limited research regarding potential interactions between neonicotinoids and rhizobial bacteria. Thiamethoxam had little toxicity in-vitro to entomopathogenic bacteria (Filho et. al. 2001). Imidacloprid seed treatments had no antagonistic effect on soil bacteria populations (Singh and Singh 2005).

Materials and Methods

Field Study

The experiment took place on farms throughout the Lower Peninsula of Michigan. In 2004, trials were located at six sites: Montmorency, Grand Traverse, Saginaw, Ingham, Hillsdale, and Lenawee Counties, at sites adjacent to the Michigan Soybean Performance Trials. In 2005 and 2006, four field sites were used, including Montmorency County in 2005, Presque Isle County in 2006, and Sanilac, Ingham, and

Hillsdale Counties both years. Lenawee in 2004 and Hillsdale in 2006 had previous crops of wheat. All other sites had previous crops of corn (Table 2.1).

In 2004 and 2005, a randomized complete block design with split plots acting as treatment and control was used. Both thiamethoxam and imidacloprid were used alone, as well as in combination with Cell-Tech Inoculant. A Cell-Tech only subplot treatment tested for insecticide-inoculant yield interaction; thus, a total of 5 different subplot treatments were used. These treatments were used twice in each replicate, once in an untreated whole plot, and once in a whole plot with mefenoxam + fludioxonil treated seed. Therefore, each replication had 10 whole plots. Each whole plot contained twelve 38.1 cm rows, six rows treated with an insecticide and/or inoculant, the other six rows acting as a control. Each plot was 6.1 m in length as planted. Alleys measuring 1.8 m were cut in between ranges during the growing season, making the plots 4.3 m in length for harvest.

In 2006, a standard randomized complete block design was used with whole plots only. Three levels of insecticide treatment, (thiamethoxam, imidacloprid, and none) and two levels of both fungicide (mefenoxam + fludioxonil or none) and inoculant (Cell-Tech and none) were tested in all possible combinations, for a total of twelve whole plots per replication. Whole plots contained six 38.1 cm rows, and plot length was the same as in 2004 and 2005.

Seed was treated with insecticides and fungicides inside a large drum, to which 4.53 kg of seed and the labeled rates of the chemicals were added. The drum was then tumbled for several minutes on a mechanical tumbling machine used for seed treatment,

Table 2.1: Insecticide seed treatment trial site information.

County	Nearest Town	Soil Class	Planting	Harvest	Previous Crop	Fertilizer
2004						
Grand Traverse	Buckley	Karlin Sandy Loam	16-May	7-Oct	Corn	140# 22-11-22
Hillsdale	Reading	Blount Silt Loam	30-Apr	6-Oct	Corn	N/A
Ingham	Mason	Capac Loam	4-Jun	24-Oct	Corn	150# 0-0-60
Lenawee	Britton	Brookston Clay Loam	29-May	26-Oct	Wheat	300# 0-0-60
Montmorency	Hillman	Bergland Clay Loam	19-May	1-Nov	Corn	N/A
Sanilac	Sandusky	Parkhill Clay Loam	2-Jun	28-Oct	Corn	GPS 0-0-60
2005						
Hillsdale	Reading	Blount Silt Loam	3-May	30-Sep	Corn	N/A
Ingham	Mason	Capac Loam	7-May	10-Oct	Corn	150# 0-0-60
Montmorency	Hillman	Bergland Clay Loam	12-May	11-Oct	Corn	N/A
Sanilac	Sandusky	Parkhill Clay Loam	11-May	5-Oct	Corn	N/A
2006						
Hillsdale	Reading	Blount Silt Loam	8-May	9-Oct	Wheat	150# 0-0-60
Ingham	Mason	Capac Loam	5-June	2-Nov	Corn	N/A
Presque Isle	Rogers City	Bergland Clay Loam	24-May	10-Oct	Corn	175# 0-0-60
Sanilac	Sandusky	Parkhill Clay Loam	18-May	16-Oct	Corn	80# 0-0-60

Table 2.2: Soil test results by site-year.

Site 2004	Clay	Silt	Sand	Type	O.M.	CEC	pH	P	K	Mg	Ca
Grand Traverse	2.2	13.6	84.2	Loamy Sand	1.4	5.3	6.1	175	116	73	643
Hillsdale	15.4	20.2	64.4	Sandy Loam	2.4	9.6	6.1	26	117	221	1243
Ingham	8.8	26.8	64.4	Sandy Loam	1.5	4.6	5.7	43	126	109	664
Lenawee	31.4	48.2	20.4	Clay Loam	4.3	17.4	6.4	71	205	397	2474
Montmorency	15.4	32.2	52.4	Sandy Loam	2.4	10.5	7.8	23	86	277	1602
Sanilac	11.8	31.8	56.4	Sandy Loam	2.2	5.9	6.1	67	171	141	849
2005											
Hillsdale	11.4	25.2	63.4	Sandy Loam	3.1	10.6	6.3	25	126	248	1636
Ingham	7.4	29.2	63.4	Sandy Loam	1.9	6.1	5.2	35	172	99	729
Montmorency	37.4	30.2	32.4	Clay Loam	3.4	18.8	7.9	28	204	430	2948
Sanilac	21.4	39.2	39.4	Loam	2.6	8.8	6.6	75	174	302	1159
2006											
Hillsdale	6.4	15.2	78.4	Loamy Sand	2.4	9.3	6.8	34	107	247	1402
Ingham	8.8	26.8	64.4	Sandy Loam	1.5	4.6	5.7	43	126	109	664
Presque Isle	2.7	15.7	82.2	Loamy Sand	1.4	5.8	7.4	88	140	118	889
Sanilac	12.8	30.8	56.4	Sandy Loam	2.3	8.6	6.6	71	206	286	1134

Table 2.3: Pesticide and inoculant seed treatment rates.

Product	Rate
Insecticide	
thiamethoxam (Cruiser 5FS)	83 ml/50 kg seed
imidacloprid (Gaucho 480)	130 ml/50 kg seed
Fungicide	
mefenoxam/ fludioxonil (Apron Maxx RTA)	162 ml/50 kg seed
Inoculant	
Cell-Tech	136 ml/50 kg seed

ensuring uniform application. For inoculated treatments, inoculants were applied to seed several days later using one gallon resealable plastic canisters, to which 0.91 kg of soybean seed and the labeled rate of inoculant was added and then tumbled for several minutes. Approximately 670 seeds were packaged for planting in each plot, to obtain a seeding rate of 445,000 seeds ha^{-1}. Plots were planted using a 6 row, 2.29 m plot cone planter (Almaco Inc., Nevada, IA). Two seed dump funnels fed the seed into the planting units. This planter was split so that rows 1-3 on the planter were fed by a left seed dump funnel, and rows 4-6 were fed by a right seed dump funnel. Each seed dump was fed with separate envelopes, therefore, each subplot required two envelopes containing approximately 335 seeds.

Plot yields were taken using an Almaco plot combine (Almaco Inc., Nevada, IA). This combine harvested the middle four rows of each six row plot in the field. Total area harvested for each plot was 6.45 m^2. An Almaco plot weight bucket controlled by a Harvestmaster control on the combine allowed for seed yield and moisture to be measured as plots were harvested (Almaco Inc., Nevada, IA and Juniper Systems, Logan

UT). Harvest samples (approx. 0.5 kg) were taken from each plot, and analyzed for protein and oil content using a Foss NIRSYSTEM 6500 near-infrared spectroscopy instrument (Foss North America, Eden Prairie, MN).

During the growing season, several observations were taken to observe plant growth. Stand counts were performed approximately 1 month after planting at all sites. Two 0.76 x 1.52 m quadrats were sampled from each plot to determine estimated plant stand. Four plants in plot were measured for plant height. These measurements were taken approximately 6 weeks after planting at each site. At Hillsdale and Ingham sites in 2005, SPAD chlorophyll index readings (Spectrum Technologies, Inc., Plainfield, IL) were taken on each leaflet of a single newly expanded trifoliate leaf of ten random plants in each plot. At harvest, four plants were removed from each plot at two sites in each year, Montmorency and Lenawee in 2004, Montmorency and Hillsdale in 2005, and Sanilac and Hillsdale in 2006. These plants were taken back to the laboratory where plant heights were measured and pods per plant were counted.

The data analysis was conducted separately for each location by year. For yield data in 2004 and 2005, whole plots were used as an error term for fungicide, and split plots were initially used to test the effect of insecticide and inoculant. If the inoculant + insecticide main effect was significant, paired contrasts were performed to determine whether an insecticide-inoculant interaction was present. After these contrasts were performed, inoculated treatments were removed from the data set. Yield and all other plant growth measurements were then analyzed with fungicide as the whole plot term and insecticide as the split plot term. Reported P-values for main insecticide effects are from the analysis of this model. In 2006, data was analyzed as a three-factor RCBD, and reported P-values for main insecticide effects were reported from this model. Paired comparisons among the means were conducted using contrasts when respective factor, interaction or slicing effects were found to be statistically significant at the 0.05 level. The data analysis was conducted in PROC MIXED (The SAS Institute, 2007).

Greenhouse Study

To observe growth effects of neonicotinoid seed treatment products in a controlled environment, a greenhouse study was performed. The experiment was a completely randomized design, with 30 pots planted with soybeans treated with either thiamethoxam or imidacloprid. An untreated check was included as well. All seed was also treated with Cell-Tech inoculant.

Inoculants and pesticide treatments were applied to seed using one gallon resealable plastic canisters, to which 0.91 kg of soybean seed and the labeled rate of insecticide was added. Pesticide seed treatments were applied the day prior to inoculation and planting to allow the chemical to dry on the seed. The canister for each treatment was then tumbled for several minutes on a mechanical tumbling machine used for seed treatment; this ensured a uniform application. Inoculants were applied during the day of planting. Five treated soybean seeds (DF8251RR variety) were planted into 15 cm diameter clay pots filled with BACCTO potting mixture (Michigan Peat Company, Houston, TX) at approximately 1 inch depth. Rubber gloves were changed between chemical treatments to avoid contamination between treatments. First emergence occurred four days after planting. Plants were grown using a 16/8 light/dark day length cycle. The greenhouse was lit with S51 400 watt high pressure sodium lamps (Lithonia

Lighting, Conyers, GA), and the average greenhouse temperature was 25°C. Thrips were controlled weekly with an application of Conserve (spinosad, Dow Agrosciences, Indianapolis, IN) at 0.46 ml/liter. At 14 DAP, pots were thinned to 1 bean plant per pot. Pots were re-randomized every two weeks during the study.

SPAD meter readings were taken every 7 days between 14 and 35 DAP, and plant heights were taken every 7 days between 21 and 35 DAP. At 30, 40 and 50 DAP, the plant shoot material of 10 randomly selected plants was harvested to analyse growth habits over time. Leaf area meter readings were taken, as well as fresh biomass and dry biomass at these 10 day intervals. Finally, nodule counts were conducted 50 DAP. Root masses were removed from clay pots and gently washed in root washing sinks. Nodules were then counted from the exposed root system.

This experiment was conducted two times. The first run was planted on February 15, 2006. Plants were harvested on April 7, 2006 and nodule counts were performed on April 8, 2006. The second run was planted on December 1, 2006. Plants were harvested on January 19, 2007 and nodule counts were performed on January 20, 2007.

Results and Discussion

Climate

The 2004 growing season began with a remarkably wet spring, with widespread record and near-record May rainfall throughout the state. July through August had near normal precipitation throughout the state, though Lenawee County continued to see high precipitation through June. September was very dry throughout the state, with no site receiving more than 3.12 cm of rainfall. The 2005 growing season began very dry, with every site experiencing a rainfall deficit for the month of May. June and July were close to average overall, while Ingham County became very dry in August and September. September was again very dry throughout the state in 2005. Aggregate precipitation in 2006 was near normal throughout Michigan. Ingham and Hillsdale sites experienced above normal rainfall in May, but near normal rainfall the rest of the season. Presque Isle experienced below normal precipitation in June and July, and Sanilac experienced below normal precipitation in August and September (Table 2.4).

In 2004, Michigan experienced an extremely cool July and August, as temperatures were over 1°C below normal at every field site for both months, with the exception of Lenawee County. September temperatures were well above normal however, and actually exceeded August averages in Northern Michigan.
2005 saw above average temperatures at every site in every month of the growing season except May. Average temperatures were at least 2.3°C above normal at every site during the month of June. In 2006, temperatures were near normal at most sites in May, June, and August. Temperatures were 1°C or more above normal at every site in July. Presque Isle experienced temperatures well above normal from May to August. September was cooler than normal at all sites except Presque Isle (Table 2.5)

Insecticide Yield Effects

During the 14 site-years of these insecticide seed treatment trials, main insecticide effects were seen only four times (Table 2.6). A yield increase of 338 kg ha^{-1} was observed in thiamethoxam treated soybean at Ingham in 2004. Significant yield decreases were observed from both insecticides at Lenawee in 2004. Four of six sites in

Table 2.4: Precipitation by month(cm), field Sites 2003-2005. Long term means (1970-2000) included for comparsion.

		May	June	July	August	September
Grand Traverse	2004	16.51	6.17	4.21	5.56	2.08
(Kingsley)	30 yr.	5.84	8.12	6.60	7.37	10.16
Hillsdale	2004	15.16	11.45	22.55	5.61	3.20
(Coldwater)	2005	6.10	7.95	11.02	4.98	7.49
	2006	14.75	8.12	13.08	7.06	10.41
	30 yr.	9.62	9.44	9.65	10.01	9.42
Ingham	2004	20.50	8.92	10.16	8.71	2.67
(E. Lansing)	2005	3.33	10.87	8.71	1.63	7.67
	2006	11.07	7.08	8.03	9.25	7.47
	30 yr.	6.90	8.07	7.56	8.74	9.22
Lenawee	2004	15.04	15.11	8.61	6.01	2.15
(Tecumseh)	30 yr.	8.38	8.89	8.13	8.89	8.38
Montmorency	2004	12.19	5.59	11.48	6.12	3.12
(Hawks)	2005	1.91	8.20	6.30	13.44	4.50
	30 yr.	7.36	6.85	7.62	9.65	8.89
Presque Isle	2006	7.23	3.00	4.90	8.91	9.68
(Rogers City)	30 yr.	6.47	6.78	7.46	9.34	7.51
Sanilac	2004	16.08	8.51	12.67	2.97	1.96
(Sandusky)	2005	4.98	7.04	10.19	6.60	5.08
	2006	6.17	6.70	7.08	5.10	5.25
	30 yr.	6.78	7.61	6.73	7.37	10.24

2004 had no significant effect from insecticidal seed treatments (Table 2.7). Heavy soybean aphid pressure was observed in Michigan during 2005, and main insecticide effects were observed at three of four sites. When contrasts were performed, insecticidal seed treatments had significant effects at two of four sites during this year. Insecticide treatments at Hillsdale in 2005 produced yield increases of 382 and 520 kg ha^{-1} for thiamethoxam and imidacloprid, respectively. Thiamethoxam and imidacloprid treatments at Sanilac at 2005 produced yield increases of 856 and 603 kg ha^{-1}

respectively (Table 2.8). 2006 saw very little soybean aphid pressure, much like 2004. As a result, no sites saw a significant yield effect from insecticide seed treatment in 2006 (Table 2.9).

Table 2.5: Average temperature by month(°C), field Sites 2003-2005. Long term means (1970-2000) included for comparsion.

		May	June	July	August	September
Grand Traverse	2003	11.7	17.3	20.0	21.1	16.3
(Kingsley)	2004	12.3	17.5	19.3	17.8	18.6
	30 yr.	12.2	17.8	20.6	19.4	15.6
Hillsdale	2004	16.1	18.9	20.6	18.9	18.9
(Hillsdale)	2005	12.7	22.4	22.4	21.1	18.4
	2006	14.2	19.2	22.8	20.9	15.3
	30 yr.	14.1	19.3	21.5	20.3	16.1
Ingham	2004	14.6	18.1	20.1	18.4	17.6
(E. Lansing)	2005	12.3	21.9	22.0	21.8	18.3
	2006	14.6	19.1	22.6	20.8	14.8
	30 yr.	14.0	19.2	21.4	20.3	16.1
Lenawee	2004	16.4	19.9	21.6	19.6	18.9
(Tecumseh)	30 yr.	14.4	19.4	21.7	20.6	16.7
Montmorency	2004	10.3	15.9	18.3	16.4	16.8
	2005	10.6	19.8	20.7	18.9	16.6
	30 yr.	10.6	16.1	19.4	17.8	13.9
Presque Isle	2006	13.2	18.7	23.0	20.0	14.9
(Rogers City)	30 yr.	11.1	16.4	19.6	18.6	14.3
Sanilac	2004	12.4	16.9	19.4	18.2	17.6
(Sandusky)	2005	11.8	21.2	21.6	20.9	18.1
	2006	14.4	18.8	22.2	19.8	14.8
	30 yr.	13.5	18.5	21.2	20.1	15.8

Table 2.6: Summary of combined insecticide effects, ANOVA for grain yield by site-year.

	P-values from ANOVA		
	2004	2005	2006
Grand Traverse	0.6807		
Hillsdale	0.4064	0.0008	0.8199
Ingham	0.0261	0.5069	0.5670
Lenawee	0.0111*		
Montmorency	0.4407	0.0478	
Presque Isle			0.1430
Sanilac	0.2439	0.0003	0.1091

* significant yield decrease, other significant effects were increases

Table 2.7: Insecticide grain yield effects, 2004 by site (kg ha^{-1}).

	G.T.	Hillsdale	Ingham	Lenawee	Mont.	Sanilac
thiamethoxam	1188	3182	3727	4721	2137	4150
no insecticide	1074	3069	3389	4863	2109	3991
(Corresponding Subplots)	114	113	338*	-142*	28	159
imidacloprid	1247	3377	3750	4672	1886	3936
no insecticide	1241	3111	3527	4848	1761	4108
(Corresponding Subplots)	6	266	223	-174*	125	-172

***significant at P<0.05**

Table 2.8: Insecticide grain yield effects, 2005 by site (kg ha^{-1}).

	Hillsdale	Ingham	Montmorency	Sanilac
thiamethoxam	4298	3255	1858	3954
no insecticide	3916	3034	1689	3098
(Corresponding Subplots)	382*	221	169	856*
imidacloprid	4265	3104	2056	3818
no insecticide	3745	3133	1933	3215
(Corresponding Subplots)	520*	-29	123	603*

***significant at P<0.05**

Table 2.9: Insecticide grain yield effects, 2006 by site (kg ha^{-1}).

	Hillsdale	Ingham	Montmorency	Sanilac
thiamethoxam	3386	3310	2278	3981
imidacloprid	3323	3233	2104	4118
no insecticide	3356	3189	2227	4010

No significant insecticide effects at any site in 2006

In years when soybean aphid was not a pest problem in Michigan, only one site-year showed a significant positive response from insecticidal seed treatments. Thiamethoxam and imidacloprid had highly significant effects on yields at Hillsdale and Sanilac sites in 2005, indicating that these products have efficacy on soybean aphid early in the season. Only the Sanilac site, which saw severe aphid pressure, was treated with a foliar insecticide to control soybean aphid. By the time insecticide application occurred at this site, significant yield loss had already occurred. Yield contrasts showed no significant difference between thiamethoxam and imidacloprid at sites where insecticide treatment was a significant factor.

Insecticide-Inoculant Interaction

Contrasts were performed on yields at Montmorency sites in 2004 and 2005 for Cell-Tech inoculated soybean between treatments which also had a neonicotinoid seed treatment applied and treatments that did not. These were first-time soybean production sites where use of (Apron Maxx RTA) fungicide had significant interaction effects in the soybean inoculant study. The insecticides alone had no significant impact on yield in either year. The analysis showed that thiamethoxam and imidacloprid did not interact

Table 2.10: Grain yield treatment means for soybean inoculated with Cell-Tech inoculant, Montmorency 2004 and 2005 (kg ha $^{-1}$).

2004		2005	
thiamethoxam	3405	thiamethoxam	2324
imidacloprid	3551	imidacloprid	2417
no insecticide	3541	no insecticide	2353

No significant differences

with soybean inoculant. Inoculated soybean yielded the same at these sites regardless of insecticide seed treatment (Table 2.10).

Few studies found interactions between soil insecticides and bacteria. The limited research on the neonicotinoid effects on bacteria has shown no ill effects on soil-borne bacteria (Singh and Singh 2005).

Soybean Aphid Population Effects

In 2005, neonicotinoid seed treatments significantly effected on soybean aphid populations through R2. Soybean aphid population effects were significant at both Ingham and Hillsdale sites, with aphid populations reduced by over 50% by both treatments at both sites during the earliest count timings. However, aphid pressure was very low at this time, averaging 6 and 10 adult aphids per plant at Ingham and Hillsdale. Insecticide effects on aphid population continued to be significant through late R2, while percent control fell for both treatments in each successive count timing. Late R2 was the last growth stage in which insecticide treatments significantly reduced aphid populations. Aphid populations were reduced an average of 39% (15 vs. 9 adult aphids per plant) and 36% (42 vs. 26 adult aphids per plant) at Hillsdale and Ingham sites at late R2. By the R3 stage at Hillsdale (43 vs. 39 adult aphids per plant) and the R4 stage at Ingham (450 vs. 348 total aphids per plant), there was no significant difference in aphid number between treated and untreated plots (Table 2.11 and 2.12).

These results corroborate observations made by Pedersen and Lang (2006); that some aphid suppression can occur through about 60 days after planting (Pedersen and Lang 2006). This study saw aphid suppression ending after late R2, which occurred at 57 and 69 days after planting at the Ingham and Hillsdale sites, respectively.

Table 2.11: Effects of neonicotinoid seed treatment on soybean aphid pressure, Hillsdale 2005 (average adult aphids/plant)

Count Timing		Thiamethoxam			Imidacloprid		
		Treated	Control	P-value	Treated	Control	P-value
Early R2	(62 DAP)	6	11	0.0007	3	9	0.0004
Late R2	(67 DAP)	12	15	0.2194	5	14	0.0015
Early R3	(72 DAP)	37	45	0.2096	39	41	0.7530

Table 2.12: Effects of neonicotinoid seed treatment on soybean aphid pressure, Ingham 2005 (average aphids/plant)

Count Timing		Thiamethoxam			Imidacloprid		
		Treated	Control	P-value	Treated	Control	P-value
V7[a]	(43 DAP)	2	7	<0.0001	2	5	0.0005
Late R1[a]	(49 DAP)	4	10	0.0022	8	16	<0.0001
Late R2[a]	(57 DAP)	26	39	0.0343	28	45	0.0062
R4[b]	(74 DAP)	388	546	0.2048	308	314	0.9624

[a]-Adult aphids counted
[b]-All aphids counted

Plant Stand Effects

As no pattern of interaction was observed between insecticide, fungicide, or inoculant, mean contrasts were conducted across fungicide and inoculant classes. Neonicotinoid seed treatments had significant effects on plant stands at only 3 of 16 site-years in this study (Table 2.13).

Table 2.13: Summary of insecticide main effects on plant stand, by site-year.

	P-values from ANOVA		
	2004	2005	2006
Grand Traverse	0.0962	---------	---------
Hillsdale	0.0328	0.1722	0.1330
Ingham	0.9276	0.0036	0.3382
Lenawee	0.2061	---------	---------
Montmorency	0.1914	0.9132	---------
Presque Isle	--------	---------	0.6615
Sanilac	0.0207	0.2796	0.1995

At Hillsdale and Sanilac in 2004, neonicotinoids protected soybean stand. At Ingham in 2005, negative effects on soybean stands were observed. (Table 2.14). With sites combined for each year, no significant difference in plant stand was observed between insecticide treated and untreated soybean (Table 2.15).

No pattern of significant stand improvement was observed. The greatest stand improvement at any site-year was 10.4% at Hillsdale in 2004. While statistically significant, this level of stand loss at the plant populations observed would be unlikely to cause significant yield loss (Nafziger 2002). None of the sites that experienced a plant stand difference also experienced a yield effect.

Table 2.14: Split plot contrasts of insecticide plant stand effects, sites with significant main effects (plants ha^{-1}, insecticide treatments combined).

	2004 Hillsdale	2004 Sanilac	2005 Ingham
thiamethoxam	371,883	425,625	331,688
no insecticide	346,410	388,203	372,422
(Corresponding Subplots)	25,473	37,422*	-40,734*
imidacloprid	385,752	404,792	359,903
no insecticide	349,280	401,957	367,325
(Corresponding Subplots)	36,472*	2,835	-7,422

***significant at P<0.05**

Table 2.15: Plant stand means of insecticide treated plots, sites combined for each year (plants ha^{-1}, insecticide treatments combined).

2004 (Split Plots)		2005 (Split Plots)	
thiamethoxam	396,734	thiamethoxam	288,017
no insecticide	392,004	no insecticide	289,766
imidacloprid	398,036	imidacloprid	285,239
no insecticide	389,944	no insecticide	282,438

2006 (Whole Plots)	
thiamethoxam	358,878
imidacloprid	357,625
no insecticide	362,377

No significant effects in any year

A Minnesota study conducted in 2002 found neonicotinoid seed treatments improved soybean stand between 11.4 and 17.1% in early planted soybean (May 2), but not in late planted soybean (May 23, Potter 2002). This study found the best overall effects by the neonicotinoids on stand count was at Hillsdale in 2004. Planting at Hillsdale in 2004 occurred on April 30th, the earliest of any site-year within this trial. This planting was followed by record May rainfall. If these sites experienced delayed emergence, some seedling loss due to early soil-borne insect pressure may have occurred. However, early May plantings in other site-years did not experience increased plant stands.

Plant Height Effects

Data from early season plant height measurements (5-6 weeks after planting) showed little evidence to support the claim of increased plant height from neonicotinoid insecticide treatments. Over 16 site-years, insecticide main effects were significant five times (Table 2.16). Contrasts performed at these 5 site-years showed only two sites where significant plant height increases resulted from insecticide seed treatments (Table 2.17). At Hillsdale in 2004, imidacloprid provided a 1.57 cm plant height increase over untreated plants. However, thiamethoxam did not provide a significant plant height increase at this site. At Ingham in 2005, both thiamethoxam and imidacloprid provided increases in plant height, of 2.28 and 3.28 cm respectively.

Table 2.16: Summary of insecticide main effects on early plant height, by site (P-values from ANOVA).

	2004	2005	2006
Grand Traverse	0.4713	---------	---------
Hillsdale	0.0271	0.4628	0.8202
Ingham	<0.0001	0.0145	0.0077
Lenawee	0.0720	---------	---------
Montmorency	0.0523	0.2010	---------
Presque Isle	--------	---------	0.2926
Sanilac	0.0002	0.1040	0.5771

Table 2.17: Contrasts of insecticide effects on early plant height (cm), site-years with significant positive insecticide effects.

Hillsdale 2004		P-value			P-value
thiamethoxam	30.49		imidacloprid	30.74	
no insecticide	30.00		no insecticide	29.17	
	0.49	0.3428		1.57	0.0031
Ingham 2005					
thiamethoxam	24.79		imidacloprid	25.79	
no insecticide	22.41		no insecticide	22.41	
	2.28	0.0303		3.28	0.0024

When sites are combined over year, not only were insecticide main effects not significant, but treatment means between insecticide treated and no insecticide plots were nearly numerically identical in 2004 and 2005 (Table 2.18). The increased numerical difference in 2006 is attributed to the significant effects seen at the Ingham site alone.

Table 2.18: Treatment means, plant height (cm), by year, sites combined.

2004		2005	
thiamethoxam	21.35	thiamethoxam	19.52
No Insecticide	21.45	no insecticide	19.53
imidacloprid	21.18	imidacloprid	19.40
no insecticide	21.15	no insecticide	18.92

2006	
thiamethoxam	21.26
imidacloprid	21.63
no insecticide	20.72

no significant insecticide effects in any year

Overall, any positive effects of neonicotinoid seed treatments on early season plant height were isolated.

Plant heights at harvest were taken for 6 site-years, at Montmorency and Lenawee in 2004, Montmorency and Hillsdale in 2005, and Sanilac and Hillsdale in 2006. Results for plant height at harvest were similar to those of the early plant heights. Of the six sites that were sampled for plant height at harvest, two had significant main insecticide effects: Montmorency in 2005 and Hillsdale in 2006. At Montmorency in 2005, neither thiamethoxam nor imidacloprid increased plant height compared with the insecticide subplots, though the contrast between thiamethoxam and imidacloprid treatments gave thiamethoxam a 3.38 cm height advantage. At Hillsdale in 2006, imidacloprid treated plants were significantly taller than both thiamethoxam treated plants and no insecticide plants (Table 2.19).

No pattern of significant plant height increase could be attributed to the use of neonicotinoid insecticide seed treatments. Although in certain site-years some differences could be observed, such as at Ingham in 2006, height differences were infrequent and small.

Table 2.19: Significant contrasts of insecticide effects on harvest plant height (cm).

Montmorency 2005		P-value			
thiamethoxam	61.60				
imidacloprid	58.22				
	3.38	0.0246			
Hillsdale 2006					
imidacloprid	92.42		imidacloprid	92.42	
thiamethoxam	88.30		no insecticide	87.95	
	4.12	0.0321		5.07	0.0209

Other Plant Growth Indicators

No site-years showed any significant main effects of insecticide seed treatment on average pods per plant. Annual pod count means were higher in 2006 than 2004 and 2005, consistent with overall grain yield for the specific site (Table 2.20). No significant differences in SPAD chlorophyll indexes were observed at three of four site years in which these measurements were taken. The only site where insecticide main effects were significant was Hillsdale in 2006. Imidacloprid treated soybean plants at Hillsdale in 2006 had a higher mean SPAD index than thiamethoxam treated soybean plants (Table 2.21). This was the only significant insecticide effect observed at any of the four site years where SPAD readings were taken. Two other site-years, Hillsdale 2005 and Ingham 2006 saw both thiamethoxam and imidacloprid have numerically but not significantly higher SPAD chlorophyll indexes. So while a trend for increased SPAD chlorophyll index readings from neonicotinoid seed treatments may exist, significant differences again appear to be isolated.

Table 2.20: Treatment means, pods per plant by year, sites combined.

2004		2005	
thiamethoxam	27.13	thiamethoxam	28.20
no insecticide	28.87	no insecticide	29.37
imidacloprid	29.18	imidacloprid	27.06
no insecticide	30.12	no insecticide	27.96
2006			
thiamethoxam	41.44		
imidacloprid	40.27		
no insecticide	39.76		

no significant insecticide effects in any year

Table 2.21: Insecticide treatment contrasts, mean SPAD chlorophyll index, Hillsdale 2006.

		P-value			P-value
thiamethoxam	46.73		imidacloprid	47.88	
no insecticide	46.96		no insecticide	46.93	
	0.23	0.6174		0.95	0.0524
thiamethoxam	47.88				
imidacloprid	46.73				
	1.15	0.0148			

There has been much speculation about the potential for neonicotinoids to have plant growth "vigor" effects even with negligible insect pressure. Claims have been made and a United States patent has been granted regarding neonicotinoid plant vigor effects (Senn et. al. 2004). A recent press release by Syngenta (December 5, 2006) states that the company has discovered the mechanism by which thiamethoxam produces a vigor effect. This mechanism is purportedly a plant response elicited by the chemical that results in an increase of plant produced proteins that convey stronger plant defenses under stressful conditions. More published research to support these findings would be beneficial.

Greenhouse Trial

Five sets of measurements of plant growth were taken during the greenhouse experiment, plant height, SPAD index readings, leaf area index, and plant dry weight. There was no significant difference in plant height between thiamethoxam-treated, imidacloprid-treated, untreated plants at any measurement timing under greenhouse conditions (Figure 2.1). SPAD chlorophyll indices showed no significant insecticide treatment effect at any timing (Figure 2.2). Leaf area index, fresh plant weight, and dry plant weight were statistically similar with runs 1 and 2 combined. The results of this greenhouse trial corroborate the field observations of negligible to no growth effects from insecticidal seed treatments.

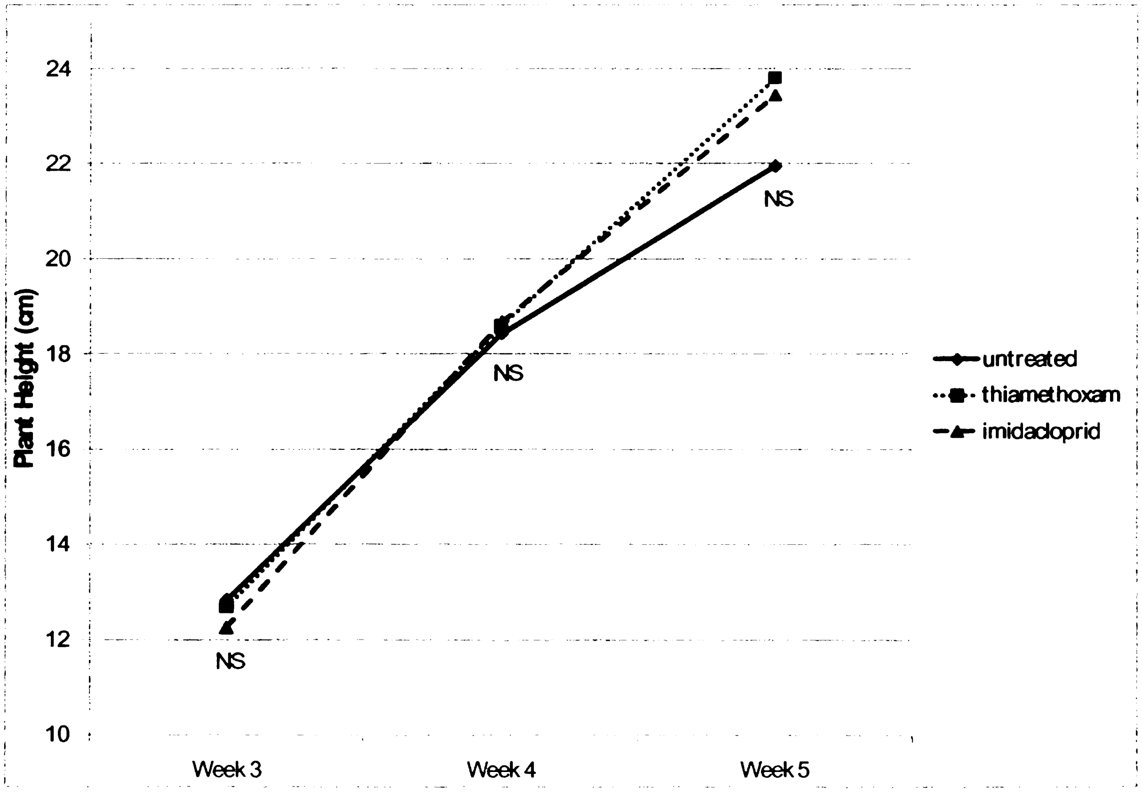

Figure 2.1: Plant height by insecticide treatment and week (cm, runs combined).

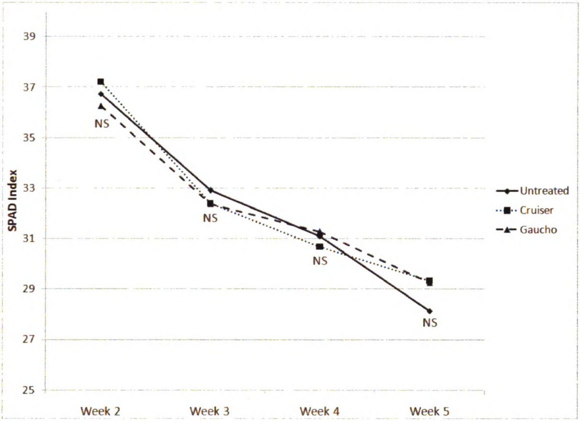

Figure 2.2: Greenhouse SPAD chlorophyll indices by treatment and week (runs combined).

Economic Return from Neonicotinoid Seed Treatment Use

Economic return on use of neonicotinoid seed treatment was calculated by taking the numerical yield difference found in insecticide treated plots (Tables 2.6-2.8), multiplying that difference by the average soybean price received by farmers in each year, and subtracting the cost of the seed treatment. Substantial economic gain was seen at Hillsdale and Sanilac in 2005. However, these gains are offset over the course of this study, as most sites in 2004 and 2006 saw a net loss by using neonicotinoid seed treatments (Table 2.21). Based on the cost of these products, a 130 to 170 kg ha^{-1} yield increase would typically be required to recuperate the cost of seed treatment.

Table 2.21: Economic return on use of neonicotinoid seed treatments

		thiamethoxam		imidacloprid	
		Yield Effect	Net Return*	Yield Effect	Net Return*
Year	Site	(kg ha^{-1})	(\$ ha^{-1})	(kg ha^{-1})	(\$ ha^{-1})
2004	Grand Traverse	114	-15.59	6	-34.32
2004	Hillsdale	11	-15.80	266	20.20
2004	Ingham	338	31.37	233	13.28
2004	Lenawee	-142	-65.35	-174	-72.06
2004	Montmorency	28	-33.62	125	-9.36
2004	Sanilac	159	-6.16	-172	-71.64
2005	Hillsdale	382	40.75	520	73.67
2005	Ingham	221	6.93	-29	-41.67
2005	Montmorency	169	-3.99	123	-9.73
2005	Sanilac	859	140.97	603	91.11
2006	Hillsdale	30	-32.79	-33	-42.96
2006	Ingham	121	-12.43	44	-25.73
2006	Montmorency	51	-28.09	123	-8.06
2006	Sanilac	-29	-45.98	108	-11.42

*Economic Assumptions
2004 Average Price Paid in Michigan, \$0.2097 kg^{-1} (\$5.72/bu.)
2005 Average Price Paid in Michigan, \$0.2101 kg^{-1} (\$5.73/bu.)
2006 Average Price Paid in Michigan, \$0.2237 kg^{-1} (\$6.10/bu.)
(USDA-NASS 2007, Prices do not include LDP and other government payments)

Cost of Cruiser Maxx treatment (thiamethoxam and mefenoxam + fludioxonil), \$39.50/ha. (Cruiser only treatment no longer available)

Cost of Gaucho (imidacloprid) treatment, \$35.58/ha
Determined through consultation with agribusiness

Conclusions

Neonicotinoid seed treatments had a positive yield effect when soybean aphid pressure was high. While aphid populations were suppressed well into the growing season, was lost 40 to 60 days after planting. Efficacy of seed treatments on soybean aphid control and soybean grain yield depends on the time at which soybean aphid begins to colonize soybean in Michigan. In 2004 and 2006, only one site-year saw a positive yield effect from use of neonicotinoid seed treatments. Based upon these results, it is difficult to recommend regular use of neonicotinoid seed treatments unless threshold soil borne insect populations are present around planting time, or major pest infestations are expected within the first two months of the growing season. These experiments were performed in a conventionally tilled system, and farmers may see a benefit to using neonicotinoids seed treatments in a no-till system, where soil-borne insect populations are less disturbed in the spring and soil temperatures rise slowly.

Indicators of crop health such as stand count, plant height, SPAD meter readings, leaf area indices both in the field and in the greenhouse trial did not support claims of improved crop health as an effect of neonicotinoid seed treatments. Only one site year, Ingham in 2006, showed a marked improvement in plant height by both thiamethoxam and imidacloprid. Observed neonicotinoid impacts on soybean plant health appeared to be very isolated in these trials, despite the stressful conditions observed at some sites.

References

Catangui, M., S. Swanson, R. Jons, and R. Berg. 2004. Differential yield increases in soybeans after treatment with insecticides for soybean aphids. Southeast South Dakota Experiment Farm 44th Annual Progress Report:109-112.

Cullen, E. M., J. Gaska, and B. Jensen. 2004. Soybean aphid control with seed treatments. 2004 Wisconsin Fertlizer, Aglime, & Pest Management Conference Proceedings.

Cullen, E. M., J. Gaska, and B. Jensen. 2004. Bean leaf beetle control with seed treatments. 2004 Wisconsin Fertlizer, Aglime, & Pest Management Conference Proceedings.

DiFonzo, C. 2003. August Pest Update: Soybean Aphid. Michigan State University Extension Field Crop Advisory Team Alert 18(15).

DiFonzo, C. 2005. Soybean aphids are early in Michigan and surrounding states. Michigan State University Extension Field Crop Advisory Team Alert 20(8).

DiFonzo, C. 2005. Soybean aphids still a problem. Michigan State University Extension Field Crop Advisory Team Alert 20(15).

Elliott, M. 1977. Synthetic pyrethroids. *In* M. Elliot (ed.) Synthetic Pyrethroids. ACS Symposium Series 42:1–28. Washington, DC: American Chemical Society.

Evans J., J. Seidel, G. E. O'Connor, J. Watt, and M. Sutherland. 1991. Using omethoate insecticide and legume inoculant on seed. Australian Journal of Experimental Agriculture 31:71-76.

Filho, A. B., J. E. M. Almeida, and C. Lamas. 2001. Effect of thiamethoxam on entomopathogenic microorganisms. Neotropical Entomology 30(3):437-447.

Krell, R. K., L. P. Pedigo, J. H. Hill, and M. E. Rice. 2004. Bean leaf beetle (*Coleoptera: Chrysomelidae*) management for reduction of bean pod mottle virus. Journal of Economic Entomology 97(2):192–202.

Mallik M. A. B. and K. Tesfai. 1985. Pesticidal effect on soybean-rhizobia symbiosis. Plant and Soil 85(1):33-41.

Martyniuk, S., J. Oron, M. Martyniuk, and A. Wozniakowska. 2002. Effects of interactions between chemical seed dressings and *Bradyrhizobium japonicum* on soybean seeds. Archives of Agronomy and Soil Science 48(4):305-310.

McCornack, B. P., and D. W. Ragsdale. 2006. Efficacy of thiamethoxam to suppress soybean aphid populations in Minnesota soybean. Online. Crop Management doi:10.1094/CM-2006-0915-01-RS.

Pedersen, P. and B. Lang. 2006. Use of insecticide seed treatments for managing soybean aphids. Integrated Crop Management Newsletter, Iowa State University Extension. Issue IC-496(1):39-40.

Potter, B. 2003. Seed applied insecticide control of the soybean aphid (*Aphis glycines*) and bean beetle (*Cerotoma trifurcata*). University of Minnesota Southwest Research and Outreach Center (Online). Retrieved Nov 8, 2006 from http://swroc.coafes.umn.edu/SWMNPEST/swmnpest.htm

McCornack, B. P., and D. W. Ragsdale. 2006. Efficacy of thiamethoxam to suppress soybean aphid populations in Minnesota soybean. Online. Crop Management doi:10.1094/CM-2006-0915-01-RS.

Rice, M. 2004. Seed treatments labeled for corn and soybeans. Integrated Crop Management Newsletter, Iowa State University Extension. Issue IC-494(2):12-13.

Lin, S. C., B. R. Funke, and J. T. Schulz. 1972. Effects of some organophosphate and carbamate insecticides on nitrification and legume growth. Plant and Soil 37(3):489-496.

Nafziger, E. 2002. Soybeans. *In*: Illinois Agronomy Handbook, 23rd edition. University of Illinois Extension Service, Urbana, IL.

Schmitt, V. L. and K. VanDee. 2005. Effects of Cruiser seed treatment for bean leaf beetle and soybean aphid management on soybean yield and on incidence of viral disease in Eastern Iowa. Iowa State University Research Farm Annual Reports (Online). Retrieved Nov 8, 2006 from http://agcrops.osu.edu/research/2004/04inoc%20report11.pdf

SAS Institute, Inc. SAS 9.1.2 Users Guide. Cary, NC: SAS Institute, Inc. 2004.

Senn, R., D. Hofer, T. Thieme, L. Zang. 2004. Method for improving plant growth. US Patent 6,753,296

Singh, J. and D. Singh. 2005. Bacterial, azotobacter, actinomycetes, and fungal population in soil after diazinon, imidacloprid, and lindane treatments in groundnut (*Arachis hypogaea* L.) fields. Journal of Environmental Science and Health Part B 40(5):785–800.

Tomizawa, M. and J. E. Casida. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annual Review of Pharmacology and Toxicology 45:247-268.

United States Department of Agriculture. National Agricultural Statistics Service. 2007. Crop Values, 2006 Summary.

Yamamoto, I. and J. E. Casida (eds). 1999. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. Tokyo: Springer-Verlag.