Pan trapping soybean aphids, *Aphis glycines* Matsumura (Hemiptera: Aphididae), using sex pheromones and plant volatiles

By

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Chapter 1. General introduction and literature review

Thesis Organization

This thesis is organized into three chapters. Chapter One contains a general introduction, which includes a literature review on the soybean aphid, *Aphis glycines* Matsumara (Hemiptera: Aphididae), the transmission of viruses by aphids, the biology of soybean aphids, the background of buckthorn in the United States, sex pheromones in soybean aphids, and the use of pan traps. Chapter Two compares pan traps with lure compounds to suction traps and plant counts and is prepared in a format to be submitted for publication in the Journal of Economic Entomology. Chapter Three is a list of general conclusions from this research and acknowledgements. An appendix follows with the results presented in graphs.

Introduction and Literature Review

Aphis glycines biology

The soybean aphid, *Aphis glycines* Matsumara (Hemiptera: Aphididae), is a serious invasive pest of the soybean plant (*Glycine max* L. Merrill). While the soybean aphid is widespread in its native China, it is a recently introduced pest to the United States. It is not known exactly when or how its introduction occurred, but it was first reported from Wisconsin (Alleman et al. 2002). Following

introduction, soybean aphids spread rapidly over ten states in the upper Midwest by 2000 (Ragsdale et al. 2004, Venette and Ragsdale 2004) and to more than 20 Midwestern states and parts of Canada within 4 years (Ragsdale et al. 2004). If colonization occurs, a yield loss as high as 40 to 50% can occur (Pedigo and Rice 2001, Ragsdale et al. 2007). Control of the soybean aphid has become increasingly important due to the value of soybeans and the yield losses associated with failure to control the pest (Johnson et al. 2009). Another concern is the possible vectoring of viruses and diseases such as soybean mosaic virus, alfalfa mosaic virus, soybean dwarf, soybean stunt, potato virus Y, cucumber mosaic virus, and bean yellow mosaic virus (Bottenberg and Irwin 1992, Burrows et al. 2005, Davis 2005, Clark and Perry 2007, Donaldson and Gratton 2007, Wang and Ghabrial 2007, Gildow et al. 2008).

The transmission of viruses by aphids has been well documented with many aphid species on a variety plant species. The transmission by soybean aphids has been well documented in laboratory settings, and also documented in field studies. Lab studies have been able to show that soybean aphids are competent vectors of Soybean mosaic virus, cucumber mosaic virus, and potato virus Y (Davis 2005, Clark and Perry 2007, Wang and Ghabrial 2007, Gildow et al. 2008). Soybean aphids are not an efficient vector of soybean mosaic virus in the field when they are feeding for an extended period of time on an individual plant with little movement between plants. Transmission of soybean mosaic virus when soybean aphids feed for a short period of time is highly efficient in both field and laboratory settings (Clark and Perry 2007). Soybean aphids have also

been evaluated as a possible vector of cucumber mosaic virus in snap bean fields (Gildow et al. 2008). Gildow et al. (2008) noted there were high numbers of soybean aphids flying through snap bean fields in the fall which coincided with an increase in incidence of cucumber mosaic virus in snap beans. While soybean aphids were found to be a possible vector of cucumber mosaic virus, it was hypothesized that they are not vectoring the disease, as they do not spend extended time in snap bean fields and are not very competent vectors. Soybean aphids have been shown to be a vector of potato virus Y (Davis 2005). Davis (2005) showed the ability of the soybean aphids to transmit potato virus Y ranged from 14% to 75% in lab studies. While this has not been observed in the field, it is a possible danger. If soybean aphids evolve to be more efficient vectors in the field, advanced monitoring of the soybean aphid will be required, and testing of seed potatoes for virus will need to be increased. This threat may change if in the future soybean aphids are found to be vectoring diseases in the field.

The soybean aphid has a typical holocyclic life on two non-native plant species, with its primary winter host being *Rhamnus cathartica* (common buckthorn), an invasive plant from Europe and its summer host soybeans (*Glycine max* L. Merrill), a cultivated non-native plant from China (Ragsdale et al. 2004, Voegtlin et al. 2005, Bahlai et al. 2009). The soybean aphid overwinters as an egg laid near the bud scale on common buckthorn or on other species of buckthorn (Ragsdale et al. 2004, Wu et al. 2004, Voegtlin et al. 2005, Bahlai et al. 2009). From this egg a female nymph hatches and eventually produces offspring parthenogenetically. Three to four generations are typically produced

while on buckthorn, and from these generations both apterous and alate females are produced (Ragsdale et al. 2004). These alate females are responsible for the initial colonization of soybean fields, which typically happens between early and mid-June. Once on the soybean plant, they can produce approximately 15 generations of alate and apterous morphs and become the main cause of yield loss and injury (Onstad et al. 2009). Controlling these mid- to late-summer soybean aphids can cost millions of dollars in chemical controls, but is important to minimize yield loss. In the fall, aphids on soybeans produce male and female alates that migrate back to buckthorn where they undergo sexual reproduction, and eggs are laid for overwintering (Heimpel et al. 2004, Voegtlin et al. 2005).

The common buckthorn is considered native to Europe and/or Asia (Voegtlin et al. 2004a) and is an introduced species in the United States. The common buckthorn, *R. cathartica*, has been shown in previous studies to be the preferred host of soybean aphids (Yoo et al. 2005); However, other *Rhamnus* spp. may also be acceptable. *Rhamnus alnifolia* (alderleaf buckthorn) is a native buckthorn found within the Midwest states including Iowa, Illinois, Wisconsin, Minnesota, and others. While the alderleaf buckthorn is native and listed as endangered in Illinois and Tennessee, it is also listed along with other buckthorns (except *Rhamnus frangula*) in Iowa as a primary noxious weed (USDA 2010).

The presence of soybean aphids prior to late June or early July on soybean plants is not well documented. While soybean aphids have been found on buckthorn species in March and April (Ragsdale et al. 2004, Voegtlin et al.

2004a, Voegtlin et al. 2005), no soybean plants would be available at that time for alate soybean aphids to colonize in Iowa. Pan traps located in Boone County in 2009 were able to catch alate aphids flying within a soybean field the week prior to the June 4th collection date. This is believed to be one of the earliest documented catches of soybean aphids in soybean fields, with the earliest being June 3rd in 2003 (Ragsdale et al. 2004).

Soybean aphids are known to produce sex pheromones. Zhu et al. (2006) showed that soybean aphids, gynoparae and males, responded on an electroantennogram to two sex pheromones; (4a*S,*7*S,*7a*R*)-nepetalactone (Z,E-nepetalactone) along with (1*R,*4a*S,*7*S,*7a*R*)-nepetalactol (Z,E-nepetalactol), or one of these two compounds alone. These tests of pheromone selectivity were performed on male and gynoparae soybean aphids. While it is not understood if the receptors are present and active in the spring and summer migrants that we trapped, it is possible that they are present. Based on a private communication with Dr. Zhu, we know that spring migrants have been tested with an electroantennogram and that they had a positive response to both sex pheromones.

The economic impact of soybean aphids can be seen both in yield reductions and in the cost of insecticides and application. Scouting for aphids is a tedious and time-consuming practice that needs to be conducted to allow appropriate decisions about insecticide application to be made prior to the aphid population reaching the economic threshold. The established economic threshold for soybean aphids is 250 aphids per soybean plant prior to the R6 plant stage

(and increasing in numbers). This gives the grower a three to five-day time period prior to reaching the economic injury level of 600 aphids per plant (Ragsdale et al. 2007). The economic injury level (EIL) changes as the price and yield of soybeans and insecticide application costs increase or decrease. Scouting can be done either via speed scouting (Hodgson et al. 2007) or *in-situ* scouting. *In-situ* scouting requires the whole plant to be counted, including the stem and pods. Speed scouting takes a binomial approach, where aphids are counted on a trifoliate and the number recorded on a data sheet. If the number of pluses (aphid count per trifoliate >40) on a set number of plants arbitrarily selected in the field falls within the range for treating on two separate occasions, an application of insecticide is economically beneficial.

The use of pan traps as a sampling method for aphid migration has been well documented (Medler and Ghosh 1968, Halbert et al. 1986, Boiteau 1990, Webb et al. 1994, Burrows et al. 2005, Hodgson et al. 2005). Halbert et al. (1986) showed during a study preformed in Nanjing, China that pan traps, both yellow and green, are able to catch *Aphis glycines* (note: data was pooled with *Aphis citricola*). However, it was estimated that only 4 or 5% were *Aphis glycines*. From that study no difference was seen between the yellow or green traps. Webb et al. (1994) showed that *Aphis* spp. were more attracted to yellow pan traps than to green pan traps in watermelon fields. *Aphis* spp. were over 80% of the species composition within yellow pan traps, while they consisted of less than 20% of the population in green pan traps. Burrows et al. and Hodgson et al. (2005) both observed the presence of *Aphis glycines* in green pan traps.

Alate aphids are much harder to identify than the apterous aphids counted on the plant. Alate soybean aphids can be quite difficult to differentiate from other possible species found on soybeans, with the best guide for the identification of alate soybean aphids being “A guide to separating *Aphis glycines* Matsumura and morphologically similar species that share its hosts” (Voegtlin et al. 2004b). Key characteristics that were used to identify the alate soybean aphid were a combination of characteristics: pale to dusky coxal color, pale to dark distal 1/3 of the femora and pale caudae.

While pan traps are able to catch soybean aphids, they are not the only means of catching alate soybean aphids. Suction traps are also used in catching aphids. Suction traps are made from 6-m (20-ft) tall PVC tubing with a top diameter of 30.5-cm and a bottom diameter of 38-cm that contains a fan in the base which draws in aphids and other small insects flying over the opening. The fan draws insects into an alcohol field jar in the base of the PVC tubing. A network of these suction traps is located in the north-central states. These 42 suction traps are distributed across 10 states and are operated by a consortium of researchers (the North Central Integrated Pest Management Center). Contents from suction traps are collected weekly from May to September and sent to Dr. Dave Voegtlin, at the Institute of Natural Resource Sustainability in Champaign, IL, for identification.

Objectives

In the summer of 2008 and 2009 I studied the soybean aphid, *Aphis glycines*, and how they respond to sex pheromone lures and plant volatile lures placed over pan traps located in fields of soybean, *Glycine max* (L.).

Chapter two objectives:

1) **Can using pan traps with sex pheromones or plant volatile lures be used to predict outbreaks?**
2) **Is there a difference between using soybean aphid sex pheromones or a soybean volatile?**

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Chapter 2. Pan trapping soybean aphids and how it can relate to an outbreak

A paper to be submitted to the Journal of Economic Entomology.

Nicholas S. Behrens and Joel R. Coats

Abstract

Since it's introduction into the United States in the past ten years, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), has been a damaging pest to soybean (*Glycine max* (L.) Merr.). Predicting their arrival in a soybean field on a year-by-year basis has been difficult as little is known as to what will cause an economic outbreak. During both 2008 and 2009, fields around central and north central Iowa experienced pockets of high soybean aphid populations. Yellow pan traps were placed in fields after planting along with lures which contained sex pheromones of the soybean aphid in 2009 or a plant volatile of the soybean plant and sex pheromones in 2008. Pan trap contents were collected weekly, and plant counts were also made. Aphids were identified, and soybean aphids were counted to determine if one chemical lure was more attractive to spring migrants than the others. In both years numbers of soybean aphids collected in pans with lures were not significantly different from each other or each other. It was observed that soybean aphids were present in soybean fields as early as June 23rd in 2008 and June 4th in 2009, and those populations do not always predict an outbreak.

Keywords: soybean aphid, pan trap, sex pheromone, plant volatiles

Introduction

The soybean aphid, *Aphis glycines* Matsumara, (Hemiptera: Aphididae) is a serious invasive pest of the soybean plant (*Glycine max* L. Merrill). While the soybean aphid is widespread in its native China, it is a recently introduced pest to the United States. It is not known exactly when or how its introduction occurred, but it was first reported from Wisconsin (Alleman et al. 2002). Following introduction, soybean aphids spread rapidly over ten states in the upper Midwest by 2000 (Ragsdale et al. 2004, Venette and Ragsdale 2004) and to more than 20 Midwestern states and parts of Canada within 4 years (Ragsdale et al. 2004). If colonization occurs, a yield loss in excess of 40 to 50% can occur (Pedigo and Rice 2001, Ragsdale et al. 2007). Control of the soybean aphid has become increasingly important due to the value of soybeans and the yield losses associated with failure to control the pest (Johnson et al. 2009). Another concern is the possible vectoring of viruses and diseases such as soybean mosaic virus, alfalfa mosaic virus, soybean dwarf virus, soybean stunt virus, and bean yellow mosaic virus (Bottenberg and Irwin 1992, Burrows et al. 2005, Clark and Perry 2007, Donaldson and Gratton 2007).

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et al. 2005, Bahlai et al. 2009). The soybean aphid overwinters as an egg laid near the bud scale on common buckthorn or on other species of buckthorn (Ragsdale et al. 2004, Wu et al. 2004, Voegtlin et al. 2005, Bahlai et al. 2009). From this egg a female nymph hatches and eventually produces offspring parthenogenetically. Three to four generations are typically produced while on buckthorn, and from these generations both apterous and alate females are produced (Ragsdale et al. 2004). The alate females are responsible for the initial colonization of soybean fields, which happens typically between late-May and mid-June. Once on the soybean plant they can produce around 15 generations of alate and apterous morphs and become the main cause of yield loss and injury (Onstad et al. 2009). Controlling these mid- to late-summer soybean aphids can cost millions of dollars in chemical controls, but is important to minimize yield loss. In the fall, aphids on soybeans produce male and female alates that migrate back to buckthorn. where they undergo sexual reproduction, and eggs are laid for overwintering (Heimpel et al. 2004, Voegtlin et al. 2005).

The economic impact of soybean aphids can be seen both in yield reductions and in the cost of insecticides and application. Scouting for aphids is a tedious and time-consuming practice that needs to be conducted to allow appropriate decisions about insecticide application to be made prior to the aphids reaching the economic threshold. The established economic threshold for soybean aphids is 250 aphids per soybean plant prior to the R6 plant stage (and increasing in numbers). This gives the grower a three to five-day time prior to reaching the economic injury level of 600 aphids per plant (Ragsdale et al. 2007).

The economic injury level (EIL) changes as the prices, yield of soybeans and insecticide application costs increase or decrease. Ragsdale et al. (2009) based the EIL of 600 aphids per plant on the assumptions of $24.51/ha for control, with a market value of the soybeans at $238.83/1000 kg, and a yield of 4,040 kg/ha. Scouting can be done either via speed scouting (Hodgson et al. 2007) or *in-situ* scouting. *In-situ* scouting requires the whole plant to be counted, including the stem and pods. Speed scouting takes a binomial approach, where aphids are counted on a trifoliate and the number recorded on a data sheet. If the number of pluses (aphid count per trifoliate >40) on a set number of plants arbitrarily selected in the field falls within the range for treating on two separate occasions, an application of insecticide is economically beneficial.

Soybean aphids are known to produce sex pheromones. Zhu et al. (2006) showed that soybean aphids, gynoparae and males, responded on an electroantennogram to two sex pheromones; (4a*S,*7*S,*7a*R*)-nepetalactone (Z,E-nepetalactone) along with (1*R,*4a*S,*7*S,*7a*R*)-nepetalactol (Z,E-nepetalactol), or one of these two compounds alone. These tests of pheromone selectivity were performed on male and gynoparae soybean aphids. While it is not understood if the receptors are present and active in the spring and summer migrants that we trapped, it is possible that they are present. Based on a private communication with Dr. Zhu, we know that spring migrants have been tested with an electroantennogram and that they had a positive response to both sex pheromones.

The use of pan traps as a sampling method for aphid migration has been well documented (Medler and Ghosh 1968, Halbert et al. 1986, Boiteau 1990, Webb et al. 1994, Burrows et al. 2005, Hodgson et al. 2005). Halbert et al. (1986) showed during a study preformed in Nanjing, China that pan traps, both yellow and green, are able to catch *Aphis glycines* (note: data was pooled with *Aphis citricola*). However, it was estimated that only 4 or 5% were *Aphis glycines*. From that study no difference was seen between the yellow or green traps. Webb et al. (1994) showed that *Aphis* spp. were more attracted to yellow pan traps than to green pan traps in watermelon fields. *Aphis* spp. were over 80% of the species composition within yellow pan traps, while they consisted of less than 20% of the population in green pan traps. Burrows et al. and Hodgson et al. (2005) both observed the presence of *Aphis glycines* in green pan traps.

We selected the soybean aphid sex pheromones as potential lures based on work done by (Zhu et al. 2006) which found that they have a stimulatory effect on soybean aphids which were attached to an electroantennogram. The other lures (catnip oil, benzaldehyde, catnip oil/ benzaldehyde mixture), were used to evaluate if the use of non-Z,E-nepetalactone/Z,E-nepetalactol products may work as soybean aphid attractants. The interest in these products over Z,E-nepetalactone/Z,E-nepetalactol was due to catnip oil containing significant amounts of the Z,E-nepetalactone/Z,E-nepetalactol in their makeup and being much less expensive and readily available; benzaldehyde is a soybean plant volatile compound and is also inexpensive (Liu et al. 1989).

The goals of the project were to discover if there was a difference in attractiveness to soybean aphids between sex pheromone lure compounds and if pan traps with sex pheromone lures were able to predict outbreaks of soybean aphids.

Materials and Methods

To accomplish the objectives, three Iowa locations were selected in 2008 and four locations in 2009. Since its introduction in 2000, the soybean aphid has appeared to show a cyclical pattern for outbreaks (Ragsdale et al. 2004, Venette and Ragsdale 2004). All sites were commercial soybean fields (5 to 55 acres) located within 90 km of Ames, IA. All selected fields had infestations of *Aphis glycines* in previous years. Field sizes in 2008 ranged from 11 acres to 55 acres and from 5 acres to 40 acres in 2009. Only a portion of the field was utilized when the size of the field was over 10 acres. Two fields in Humboldt County were selected in 2008 initially based on their proximity to one another and being owned by the same land owner. The other field in 2008 was chosen due to it's proximity to Ames, IA, in Boone County. In 2009, we again had fields in Humboldt and Boone counties, and added fields in Hamilton and Story counties. Areas surrounding the fields were not checked for the presence or absence of buckthorn.

Fields were planted with either 38 or 76-cm rows in 2008 and 38-cmm rows in 2009, based on the equipment used by the farmer. In 2008 soybean planting was delayed until mid-June due to heavy spring rains and the subsequent long delay in corn planting. During 2009 fields were planted in mid-

May or early June. Pan traps were installed on June 16th for both fields in Humboldt County and June 17th in Boone County after plants were at V2 stage in 2008. In 2009 pan traps were installed prior to VC stage in Boone County on May 30th, VC stage in Humboldt Country on June 4th, at the VC stage in Hamilton County on June 11th, and prior to V2 stage in Story County on June 23rd. The field layout was a randomized complete block design with three replicates per treatment in 2008. In 2009 the field in Story County had three replicates per field while the other fields had four replicates. Two treatment sets were used in 2008; one consisted of three replicates of Z,E-nepetalactone, a mixture of Z,E-nepetalactone/Z,E-nepetalactol (65:35), and a solvent "control" of di-ethyl ether; this was also used in 2009 with either four or three replicates of the sex pheromones. Note that no empty vial was used, as our solvent, di-ethyl ether, evaporated extremely rapidly. In 2008 our other treatment included the two sex pheromone products and control, along with benzaldehyde, benzaldehyde/Z,E-nepetalactone, catnip oil, catnip oil/benzaldehyde.

Prepared vials containing the pheromone or plant volatiles in 2008 were provided by MSTRS Technologies, Inc. (Ames, IA), and the pure pheromones were provided in 2009. In 2008 the pheromone or plant volatile was mixed with the solvent and kept in a -20° C freezer until they were transported to the Pesticide Toxicology Laboratory at Iowa State University. Then they were placed in a -76° C freezer for storage over the weekend. Lures were made on the day prior to deploying them in the field in 2009. A stock solution was first made with 120 mg of Z,E-nepetalactone and for the mixture product 78 mg of Z,E-

nepetalactone and 42 mg of Z,E-nepetalactone (65:35) was weighed. The products were mixed with 600 µl of di-ethyl ether in the vial that was used to weigh the product. This stock solution was enough to make 12 lures; each vial received 50 µl of the solution. Vials were made from amber-colored glass and had a 4-mm interior and 8-mm exterior diameter with a length of 4.1 cm. Vials had a small plastic cap that had a 3-mm hole drilled in the lid. This helped to provide a more time-released dispersion.

Stakes were made of 1.9 – 2.5-cm hardwood purchased at a local hardware store, and approximately 1.2 meters tall, which left approximately 0.9 to 1.0 meter exposed after placement in the ground. Holes were drilled approximately every 15 cm with a 0.8-cm drill bit. Stakes were painted with Valspar gloss yellow (color #64004) spray paint to allow the stakes to be seen in the field by spray applicators and for collection purposes. Stakes were driven into the ground with a rubber mallet.

Pan traps were made from plastic hardware drawers (14 x 11 x 6 cm) purchased at Lowes. Hardware drawers had a 0.6-cm hole drilled in the back 1.9 cm from the top to allow mounting to the stake. Three 0.3-cm holes were drilled, one on each side and one on the front. Traps were sanded to remove plastic burs from drilling. After sanding, traps were painted yellow (color #68108) with Valspar plastic paint (Valspar, Minneapolis, MN). The 0.3-cm holes were then covered with a fine mesh screen and glued into place. This mesh was used to allow excess rain water and the propylene glycol mixture to run out while retaining aphids inside the trap. Pan traps were filled with 100 to 130 ml of

propylene glycol:water (1:1). The traps were attached to the poles with 6.4-mm wide by 5-cm long bolts and wing nuts. A washer was placed on the inside of the trap to help add support to the plastic.

Vials were attached to the stake with a 14-gauge wire that was twisted around the vial and the pole. The wire allowed us to change vials in and out efficiently on a weekly basis and also allowed us to raise the vials as the pan traps were raised. Vials were positioned approximately 2 cm from the back of the pole, over the propylene glycol:water mixture.

Pan traps were located at approximately 46-m (±3 m) intervals in the fields. Traps were located approximately 15 m from the field edges to help minimize any possible edge effects. At each pan trap location, five arbitrarily selected plants within 1.5 m of the stake were counted for aphids, and the number was recorded along with the plant stage. Aphids were counted in-situ on the leaves and stems of the plant, and, when applicable, on the pods.

Aphids were identified under a dissecting stereomicroscope. Aphids were removed from the vial and placed into a small glass dish with ethanol. Soybean aphids were identified using the key published by Voegtlin et al. (2004) and by the presence of the pale to dusky coxal color, pale to dark distal 1/3 of the femora and pale cauda. Counts of soybean aphids were recorded on a data sheet with the trap letter and date, and the data entered into Excel® (Microsoft). Other aphids were found in the trap, but were not identified or enumerated. Aphids were removed from the glass dish with a disposable pipette and placed back into the vial with the location tag and 70% ethanol.

The pan trap counts of soybean aphids were log(x+1) transformed to provide a more normally distributed data set. These log transformed values were used in a Proc Mixed model (SAS 9.2, SAS Institute). Our model was set-up with the log(x+1) transformed values being equal to our main effects. Means and standard errors of the means reported were calculated from the lsmeans statement.

Results

During the study pan traps were deployed for up to 8 weeks in 2008 and up to 12 weeks in 2009 with insects collected from them weekly. During 2008, deployment of pan traps was delayed due to the heavy rains and subsequent flooding, while 2009 had a more normal planting window occurring in mid to late-May, with one field in 2009 being planted in mid-June. Pan trap collections and plant counts were omitted for one week during each year due to heavy rain and the possible destruction of the plants. The dates skipped due to rain were July 21st through 25th in 2008 and June 15th through 19th in 2009.

During 2008 and 2009 all fields had aphid pressure, with four out of seven fields receiving insecticide applications. In 2008 and 2009 alate aphids were found in June pan trap samples prior to seeing aphids on plants. Soybean aphids were first seen on plants in early to mid-July each year and continued to climb until an application of insecticide or until the last week of monitoring. Boone field in 2008 and both Curtiss and Sorenson fields in 2009 were not treated with insecticides, while the other fields were treated on July 28 for Humboldt fields in 2008, and July 31st for Humboldt field and August 13 for Stanhope field in 2009.

Peak aphid numbers were observed during the last week of July in the Humboldt fields and the third week of August for Boone field during 2008. Peak aphid levels during 2009 were similar to 2008 with Humboldt again having peak levels during the first week of August, Stanhope field during the second week of August, and during the third week of August in both Curtiss and Sorenson fields.

The means (±SEM) for the two fields in 2008 that evaluated only the sex pheromones are in Table 3. The third field that included the sex pheromone and plant volatile was not included due to the possible concern of interaction. Data is included prior to the application of insecticides in Humboldt County. Table 4 includes the data prior to application of insecticides in Humboldt County and after all fields had been planted. Table 5 (2008) and 6 (2009) report the ANOVA table used in the analysis, and show that no significant difference was seen between lures. The only two-way interaction that was significant was field*date ($p=0.0082$ (2008), $p=0.0002$ (2009)), while no other two or three-way interactions were significant. Both field and date were also significant ($p=0.0145$, $p=0.016$ (2008), $p<.0001$, $p<.0001$ (2009) respectively). No significant difference was seen between the means of the sex pheromone lures by week.

A regression analysis was preformed, and the line equations and R^2 values are provided in Table 7. No clear trend was observed in the slope of the lines by field during either year. The slopes of the lines ranged from 0.2853 to 1.5607 while the R^2 values ranged from 0.0371 to 0.724.

Discussion

During the 2008 field season soybean aphid flights were observed as early as June 23rd at all locations, and as early as June 4th in Boone county in 2009, due to having the earliest installation of pan traps since that field was planted first. There was not an initial week without one trap having soybean aphids in either 2008 or 2009, which was unexpected. It may have been beneficial to have the traps out prior to planting, but it would have impacted the farmers' ability to plant and was thus not practical. This raises the question about how early soybean aphids migrate from buckthorn to soybeans. In both 2008 and 2009 a similar pattern was seen with an average of 0.1 to 2 soybean aphids collected per pan trap per week from the initial setup date. It is not known if the low numbers of aphids seen in the pan traps during June will always indicate an outbreak, because each year there were fields that reached thresholds that required spraying and fields that did not, and both types had low populations of aphids caught in the pan traps early in the season.

While it is suspected that early season aphids have the same receptors that have been shown in previous research by Zhu et al. (2005), the current research may suggest that these receptors are not present. If the receptors are not present or not active, the use of sex pheromones may not be advantageous and only increase the cost of using pan traps for monitoring aphids. While there was no statistical difference between the lures when considering all of the dates in a field, there was a difference between lures when the data is pooled for 2009 on August 3rd with the sex pheromone lures.

Some pan traps were destroyed during the application of herbicides and insecticides. In 2009 some degradation of the pan traps was noted, which was noticed when they were moved up a level and found the next week with a crack, possibly due to over tightening and/or brittle plastic. This was not seen in 2008 and also not seen with new pan traps used in 2009.

Future studies could evaluate the comparison of aphid populations detected in pan traps near, (within ~10 m) suction traps. The pan traps could be set at ≤ 1 m or at various elevations up to the height of suction traps. It may also be beneficial to deploy pan traps earlier in the growing season. If traps are deployed prior to soybean planting and found to contain aphids, it could provide more information about the soybean aphid's biology and what happens from when the eggs hatch until they are found in the soybean fields. It also may help identify a possible intermediate host, if one exists. It would also be beneficial to know if the spring migrants possess the receptors shown to be present in both the gynoparae and male aphids.

The use of pan traps may work well for the early detection, however, it may not be able to provide the grower with a good prediction of a forthcoming outbreak occurring. It instead may assist in aiding when fields would need scouting. While pan traps may require more time and expertise than plant counts, it may be worth the extra effort to know when aphids are flying into fields. The value of pan traps to may only be recognized by researchers as the costs associated with using them can be high due to the cost of a microscope , the sorting of the trap samples for aphids and the number of replications needed.

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Tables

Table 1. List of fields and Counties along with the treatments used in the fields and the number of replications per field for 2008.

County "Field"	Treatments	# of reps
Boone "Boone"	Z,E-Nepetalactone	3
	Z,E-Nepetalactone & -lactol	3
	Di-ethyl ether	3
Humboldt "11a"	Z,E-Nepetalactone	3
	Z,E-Nepetalactone & -lactol	3
	Di-ethyl ether	3
Humboldt "55a"	Z,E-Nepetalactone	3
	Z,E-Nepetalactone & -lactol	3
	Di-ethyl ether	3
	Benzaldehyde	3
	Catnip oil	3
	Z,E-Nepetalactone & Benzaldehyde	3
	Benzaldehyde & catnip oil	3

Table 2. List of fields and Counties along with the treatments used in the fields and the number of replications per field for 2009.

County "Field"	Treatments	# of reps
Humboldt	Z,E-Nepetalactone	4
"Humboldt"	Z,E-Nepetalactone & -lactol	4
	Di-ethyl ether	4
Hamilton	Z,E-Nepetalactone	4
"Stanhope"	Z,E-Nepetalactone & -lactol	4
	Di-ethyl ether	4
Boone	Z,E-Nepetalactone	4
"Sorenson"	Z,E-Nepetalactone & -lactol	4
	Di-ethyl ether	4
Story	Z,E-Nepetalactone	3
"Curtiss"	Z,E-Nepetalactone & -lactol	3
	Di-ethyl ether	3

Table 3. Mean number of soybean aphids caught in yellow pan traps (±SEM) by week in 2008 for two fields combined by lure compound.

	June 23 2008	June 30 2008	July 7 2008	July 14 2008	July 28 2008
Z,E-Nepetalactone	0.297(±0.25)	0.000(±0.25)	0.116(±0.25)	0.527(±0.29)	1.290(±0.25)
Z,E-Nepetalactone & -lactol	0.567(±0.25)	0.000(±0.25)	0.4142(±0.25)	0.530(±0.25)	0.933(±0.25)
Di-ethyl ether	0.116(±0.25)	0.499(±0.25)	0.530(±0.25)	0.482(±0.29)	0.461(±0.25)

Table 4. Mean number of soybean aphids caught in yellow pan traps (±SEM) by week in 2009 for two fields combined by lure compound.

	June 29 2009	July 6 2009	July 13 2009	July 20 2009	July 27 2009
Z,E-Nepetalactone	0.140(±0.14)	0.378(±0.14)	0.607(±0.14)	1.048(±0.14)	1.893(±0.14)
Z,E-Nepetalactone & -lactol	0.460(±0.13)	0.214(±0.13)	0.764(±0.14)	1.209(±0.13)	1.724(±0.13)
Di-ethyl ether	0.323(±0.14)	0.448(±0.15)	0.282(±0.14)	1.209(±0.13)	1.714(±0.13)

Table 5. ANOVA table for 2008 including the F value and p-values.

Source	df	F value	p-value
field	1	10.42	0.0145
lure	2	0.09	0.9154
date	4	3.41	0.0160
field*row	4	0.72	0.6058
field*date	4	2.8	0.0366
lure*date	8	1.16	0.3458
field*lure	2	0.23	0.7994
trap*lure*date	8	0.72	0.6687

Table 6. ANOVA table for 2009 including the F value and p-values.

Source	df	F value	p-value
field	3	14.2	<.0001
lure	2	0.4	0.6724
date	4	72.4	<.0001
field*row	12	0.7	0.7335
field*date	12	3.46	0.0002
lure*date	8	1.64	0.1203
field*lure	6	0.27	0.9439
trap*lure*date	24	1.27	0.2010

Table 7. Linear equations for each field plotted by the log(pan trap count+1) vs log(plant count +1).

Field	Formula	R^2
11a	y=0.2853x+0.2785	0.0371
Boone	y=1.2807x+0.1918	0.724
Humboldt	y=0.672x+0.1691	0.2401
Sorenson	y=0.9062x-0.0183	0.383
Stanhope	y=0.4485x+0.1138	0.186
Curtiss	y=1.5607x+0.1251	0.6469

Chapter 3. General conclusions

Chapter two

- Soybean aphids move into soybean fields soon after planting, but may be at such low numbers it is virtually impossible to detect them using plant counts or suction traps.
- The arrival of soybean aphids in June may not always forecast that an outbreak will occur, as we had three fields that did not have populations of 250 aphids per plant prior to R6 yet had aphids in pan traps in June. Further studies on the possible relationship between early season collections and subsequent outbreaks will be necessary.
- The use of the soybean aphid sex pheromones may not be as useful in monitoring spring flights as it may be for late season flights of gynopare and male aphids returning to buckthorn.
- The use of a soybean plant volatiles may not be attractive to aphids in a field setting due to the plants giving off their own volatiles.
- After the application of insecticides it was typical to see an increase in soybean aphids caught in pan traps, which maybe the result of emigration, as after this flight an increase is seen in apterous aphids on the plant.
- The number of soybean aphids caught in pan traps varied greatly from year to year within the same region. In 2008 between 300 and 400 aphids were collected on average in pan traps during the end of July, beginning

of August, while in 2009 the average was never over 200 in any study site during the same time period.

What does this mean?

We now have evidence that aphids are flying into soybeans very soon after they are planted. We also have evidence that aphids do fly at a low elevation in the spring, as soybean aphids were caught in pan traps (at <1 m) and not in suction traps (at 6 m). It could be that suction traps do not cover a large enough area to be sensitive to low numbers of soybean aphids, while 9 to 21 pan traps do, or it could be that soybean aphids while looking for soybean fields actively fly low trying searching for chemical cues. Our data suggests that soybean aphids do not respond to sex pheromone chemicals in the spring and mid-summer. It also suggests that they do not respond to soybean plant volatiles, which may be due to the over-saturation of those compounds in the air in soybean fields. The use of the sex pheromones may be more appropriate in the fall due larger populations and the lack of volatiles given off in the soybean field as the plants are entering senescence. All fields had soybean aphids collected in the early season, but not all fields experienced outbreaks; however most did or were close to the 250 aphid threshold. It would be helpful to know if in years or fields in which soybean aphids did not reach 50% of economic levels on plants, the small amount of aphids caught early in the year. It would also be helpful to know if the spring migrants showed an EAG response when exposed to the sex

pheromone chemicals. We know that the sex pheromones were volatile, but the range and seasonality of the sex pheromones are unknowns.

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My committee has helpful in providing guidance and new ideas during meetings. Dr. Tylka's experience with soybean cyst nematodes has allowed me to have a different perspective on not only thinking of the above ground pests, but also the below ground pests. Thanks to Dr. O'Neal for adding to this project with new ideas and for also being available for questions when needed, and to ask the questions that needed to be asked during practice talks.

It has been an adventurous time doing research in soybeans. It was a very different experience than working in corn. While you have a breeze in the soybean fields, you do not have the shade as you do in corn. I also thank the

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Appendix

Figure legends

Figure 1a-d. Mean number of soybean aphids caught in pan trap (±SEM) on the left Y-axis and plant counts averaged over the entire field on the right Y-axis, with date on the X-axis. All graphs are for 2008. a) Humboldt County, field 11a, b) Humboldt County, field 11a June 23 to July 28, c) Boone County, field Boone, d) Boone County, field Boone June 24 to July 29.

Figure 2a-f. Mean number of soybean aphids caught in pan trap (±SEM) on the left Y-axis and plant counts averaged over the entire field on the right Y-axis, with date on the X-axis. All graphs are for 2009 a) Humboldt County, field Humboldt, b) Humboldt County, field Humboldt June 12 to July 31, c) Hamilton County, field Stanhope June 23 to July 28, d) Boone County, field Sorenson June 4 to July 30, e) Story County, field Curtiss, f) Story County, field Curtiss June 30 to July 28.

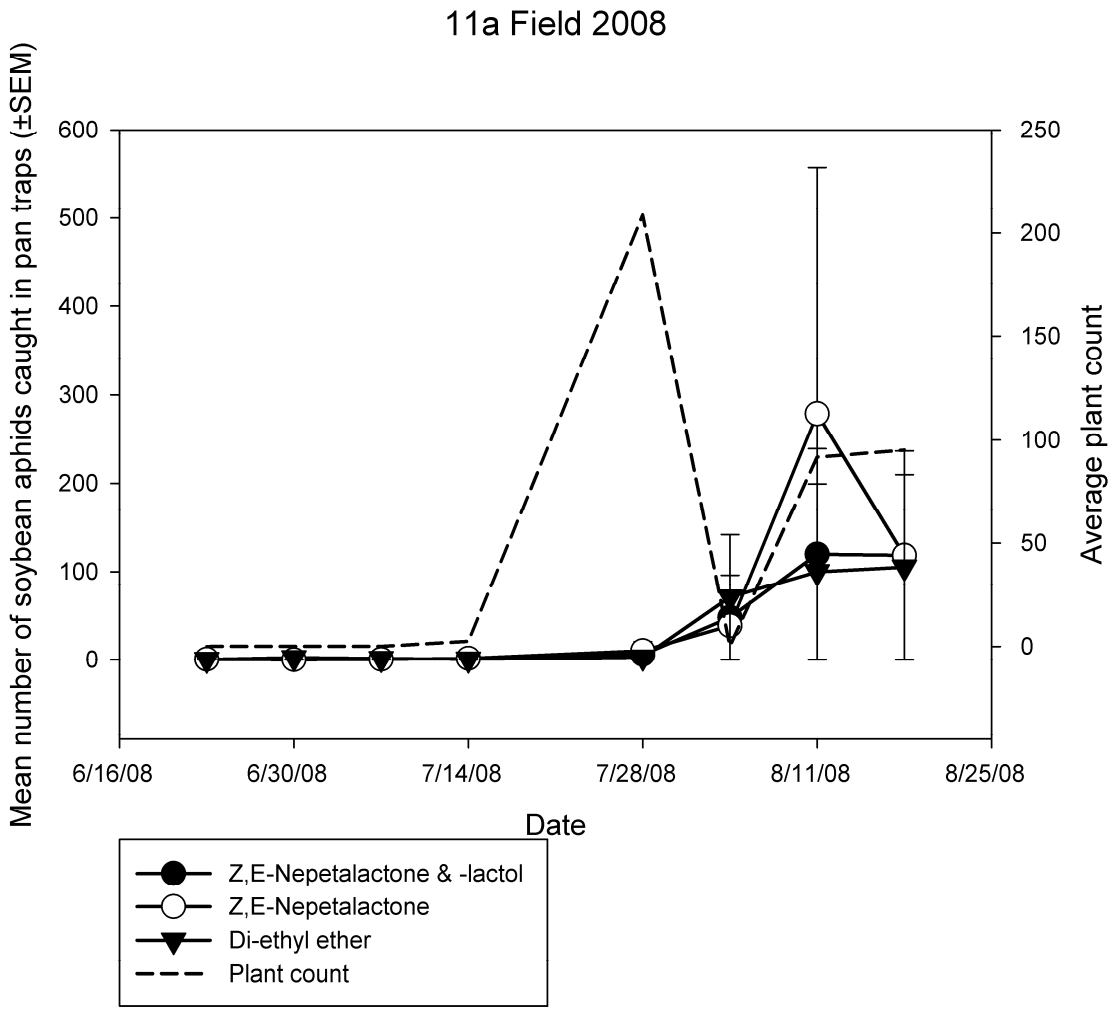
11a Field 2008
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
600
500
400
300
200
100
0
250
200
150
100
50
0
6/16/08
6/30/08
7/14/08
7/28/08
8/11/08
8/25/08
Date
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant count

Figure 1a.

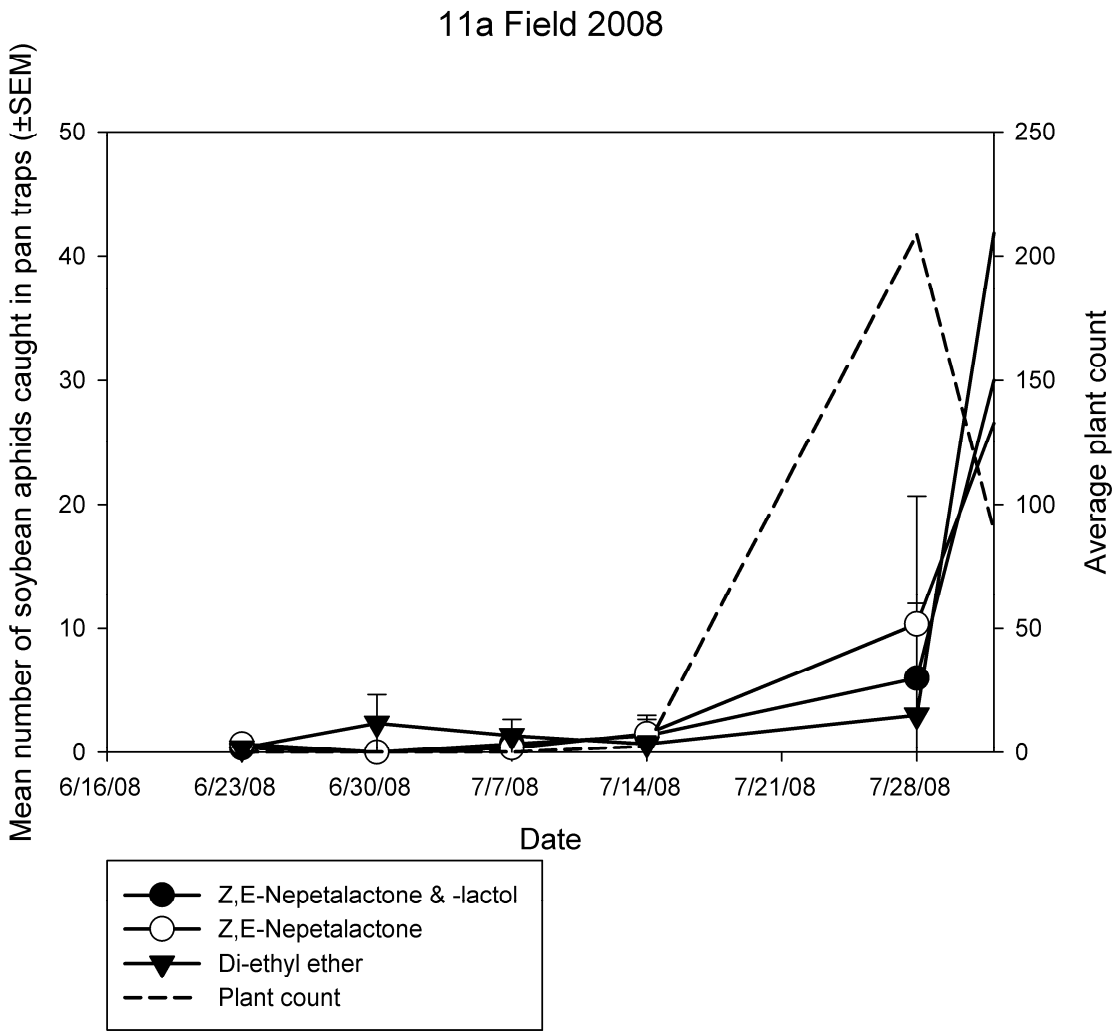
11a Field 2008
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
50
40
30
20
10
0
250
200
150
100
50
0
6/16/08
6/23/08
6/30/08
7/7/08
7/14/08
7/21/08
7/28/08
Date
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant count

Figure 1b.

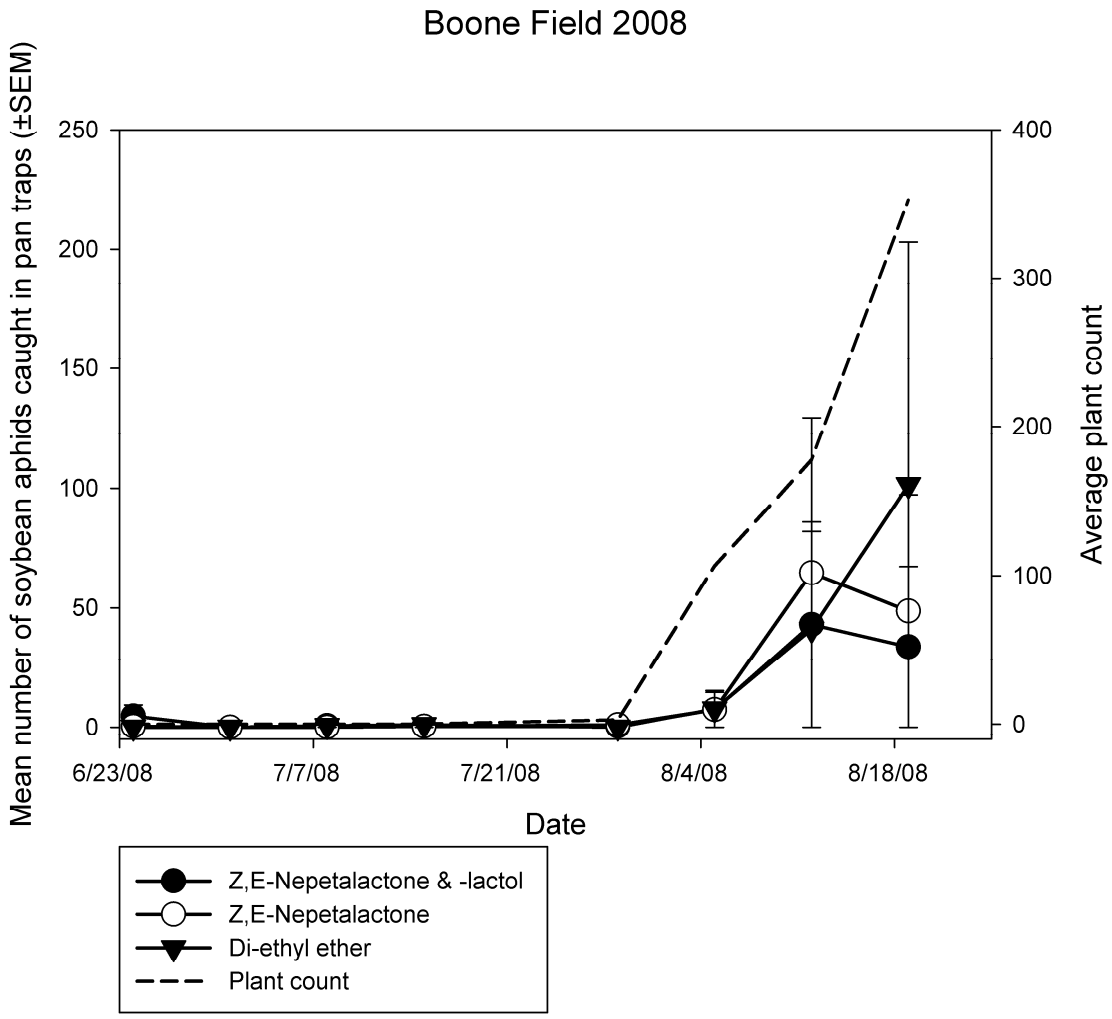
Boone Field 2008
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
250
200
150
100
50
0
400
300
200
100
0
6/23/08
7/7/08
7/21/08
8/4/08
8/18/08
Date
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant count

Figure 1c.

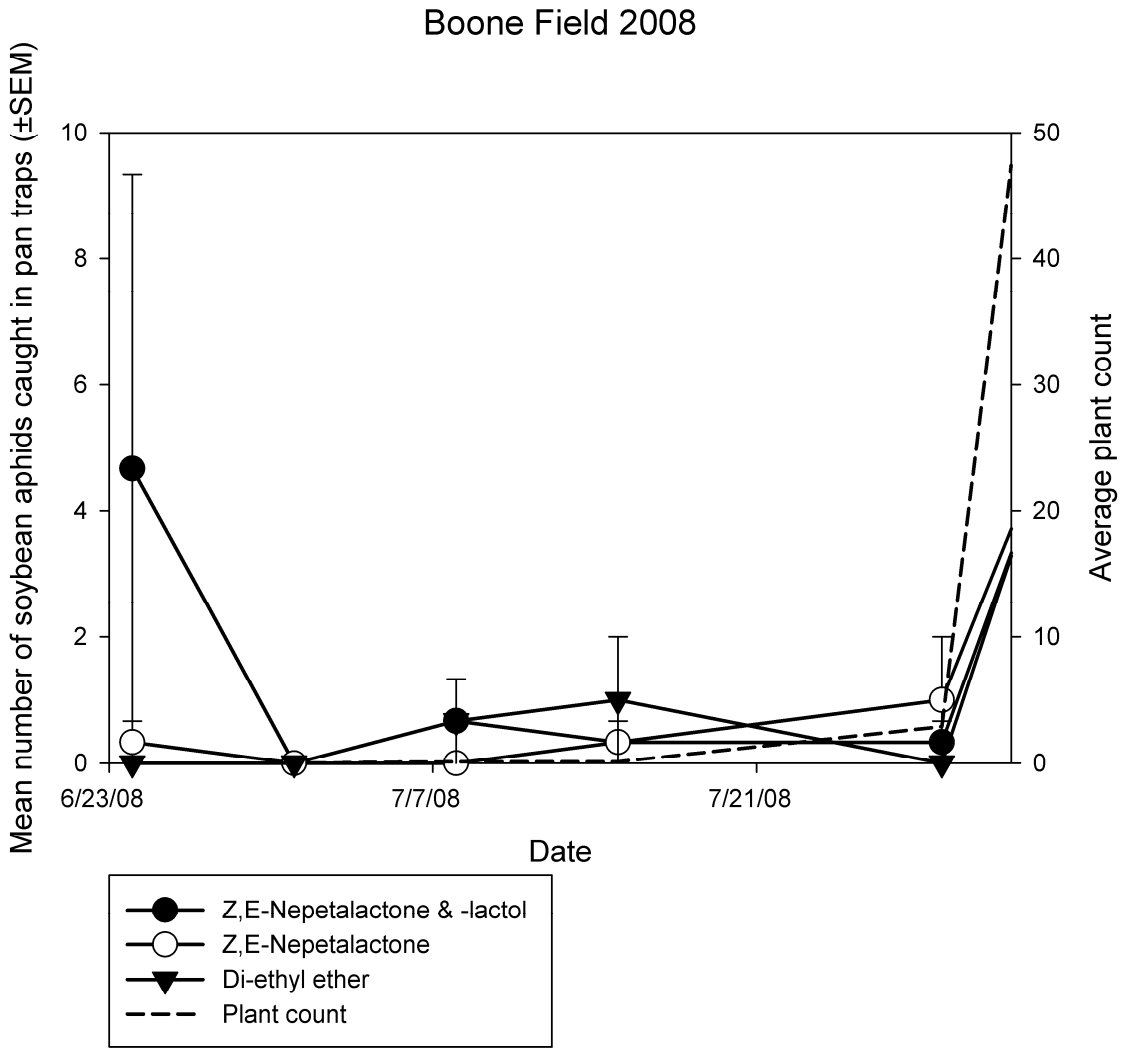
Boone Field 2008
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
10
8
6
4
2
0
50
40
30
20
10
0
6/23/08
7/7/08
7/21/08
Date
Z,E-Nepetalactone & -Iactol
Z,E-Nepetalactone
Di-ethyl ether
Plant count

Figure 1d.

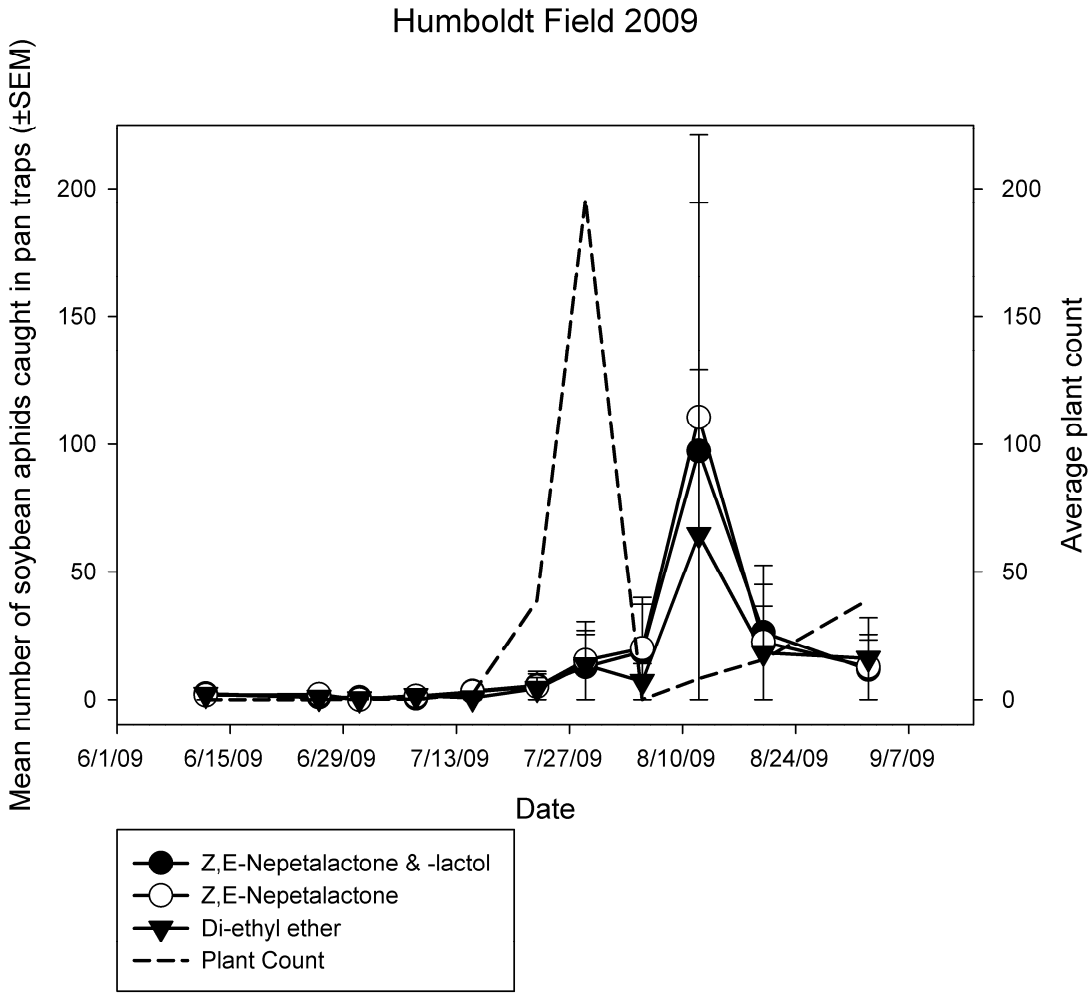
Humboldt Field 2009
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
200
150
100
50
0
6/1/09
6/15/09
6/29/09
7/13/09
7/27/09
8/10/09
8/24/09
9/7/09
Date
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant Count

Figure 2a.

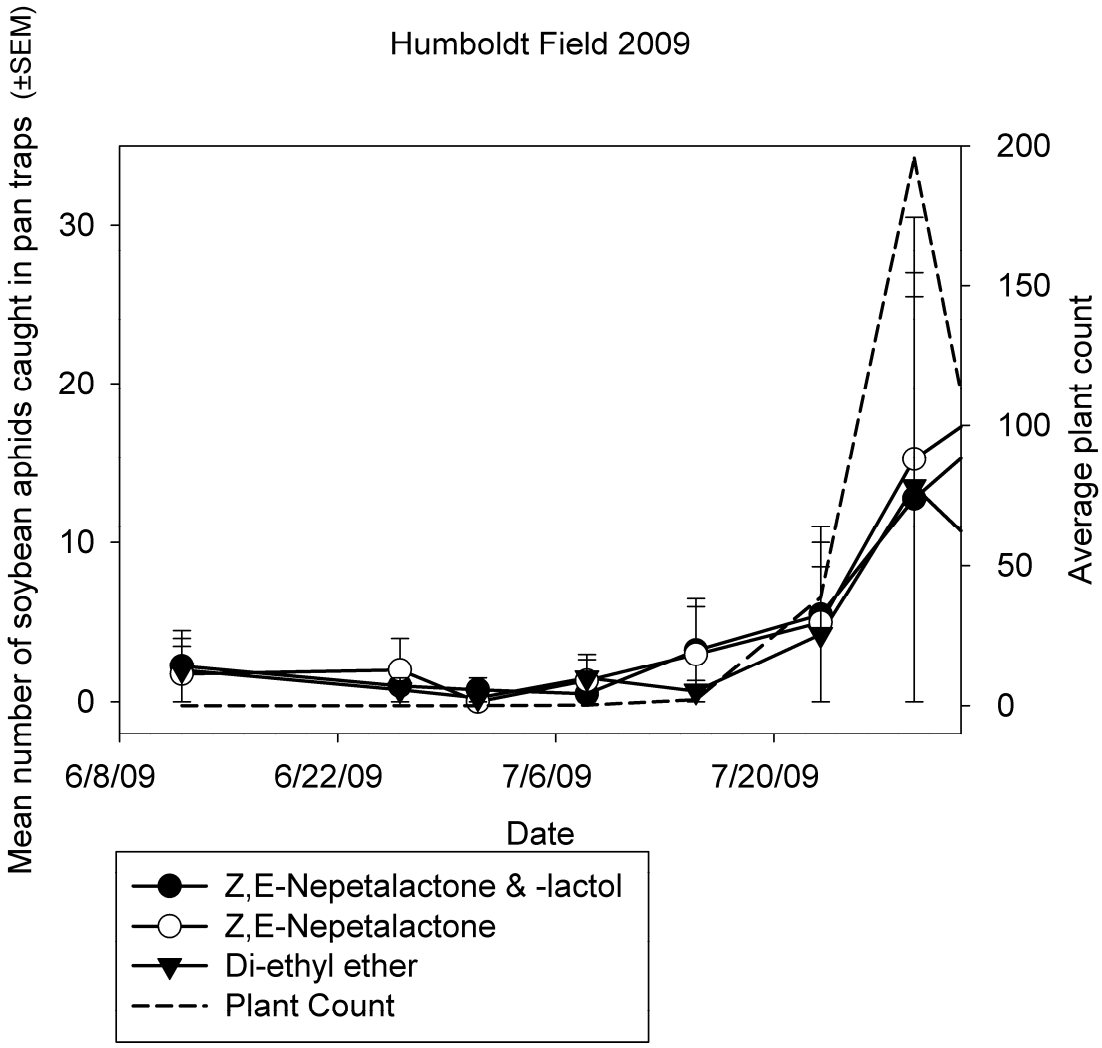
Humboldt Field 2009
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
0
10
20
30
0
50
100
150
200
6/8/09
6/22/09
7/6/09
7/20/09
Date
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant Count

Figure 2b.

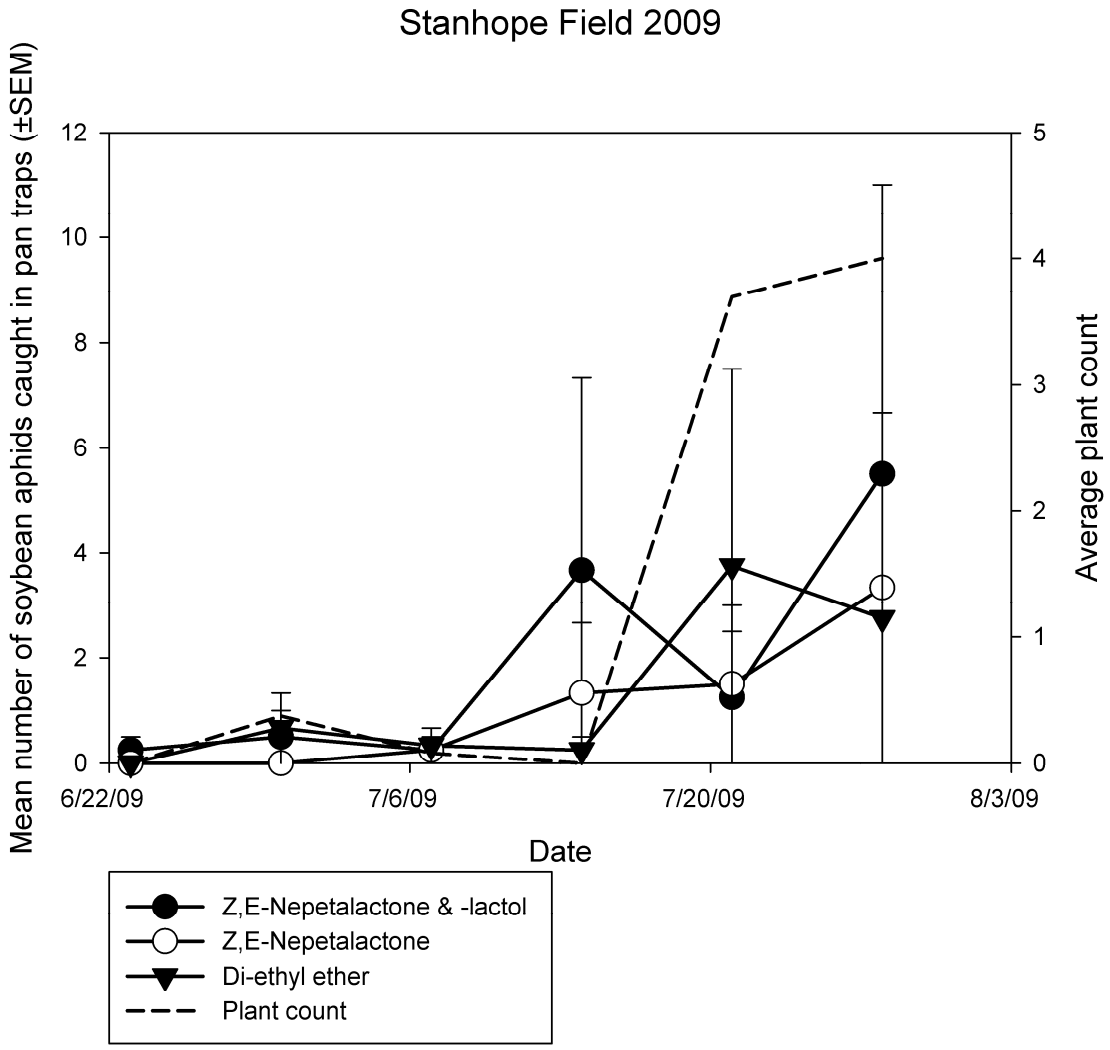
Stanhope Field 2009
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
Date
6/22/09
7/6/09
7/20/09
8/3/09
0
2
4
6
8
10
12
0
1
2
3
4
5
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant count

Figure 2c.

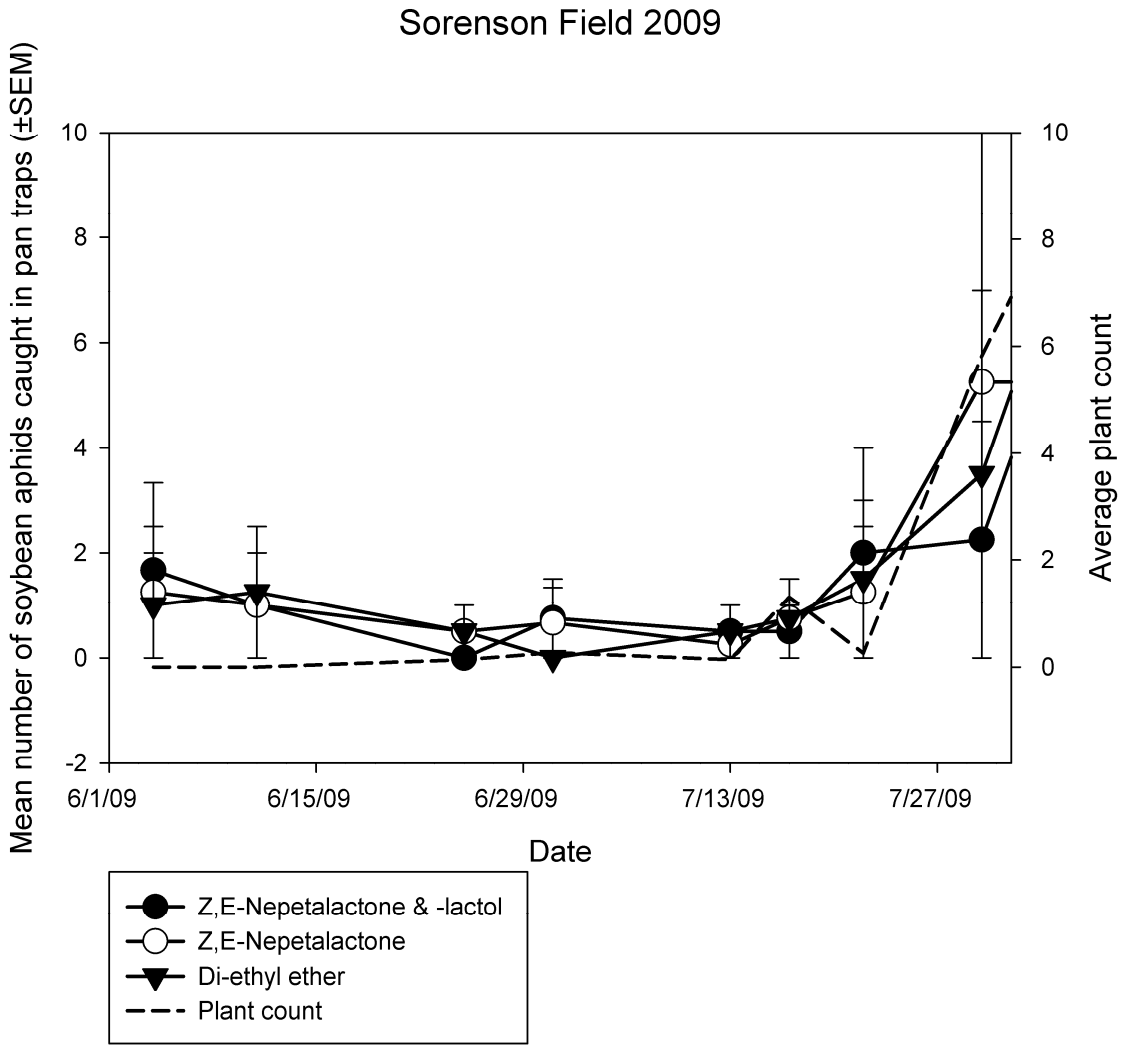
Sorenson Field 2009
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
10
8
6
4
2
0
-2
6/1/09
6/15/09
6/29/09
7/13/09
7/27/09
Date
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant count

Figure 2d.

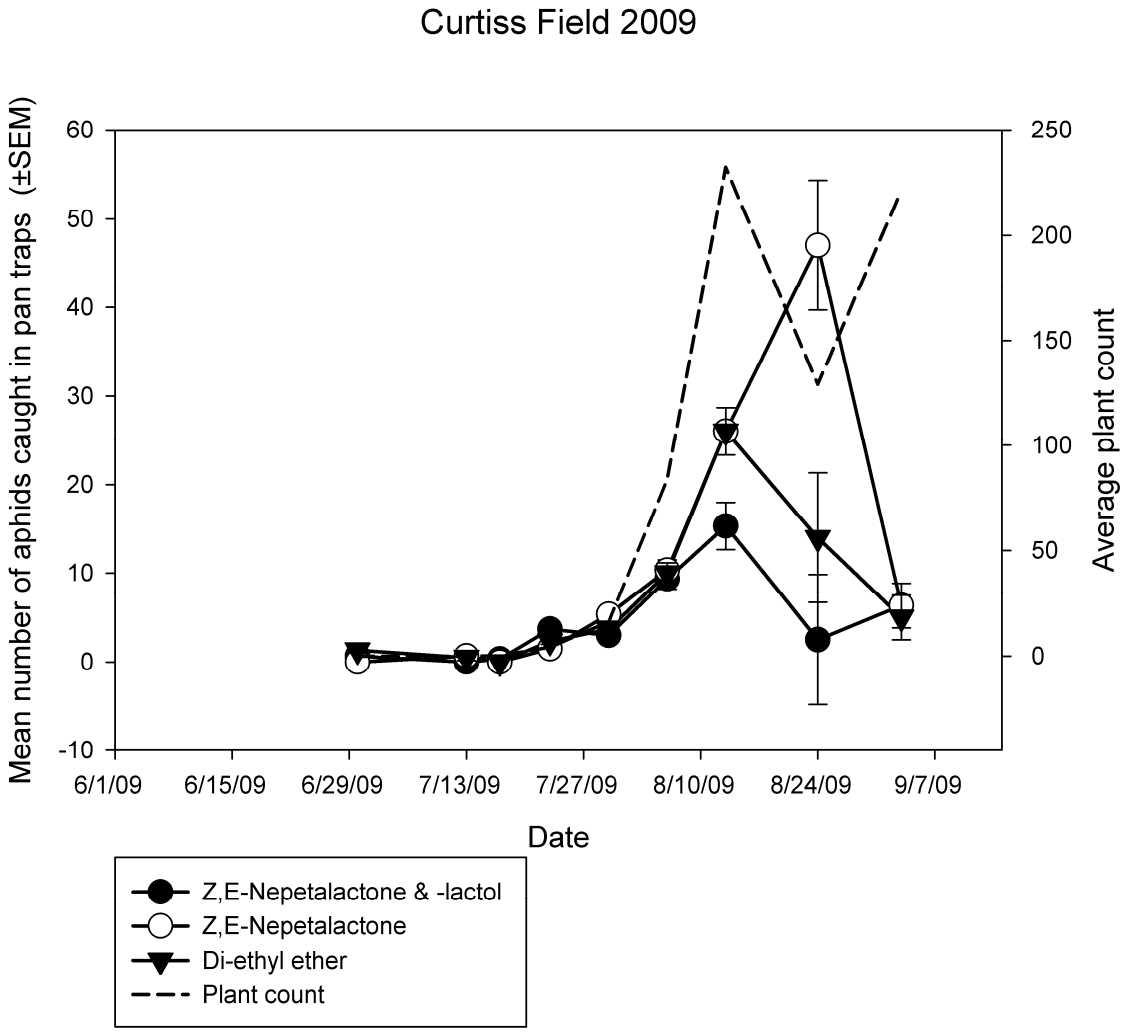
Curtiss Field 2009
Mean number of aphids caught in pan traps (±SEM)
Average plant count
60
50
40
30
20
10
0
-10
250
200
150
100
50
0
6/1/09
6/15/09
6/29/09
7/13/09
7/27/09
8/10/09
8/24/09
9/7/09
Date
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant count

Figure 2e.

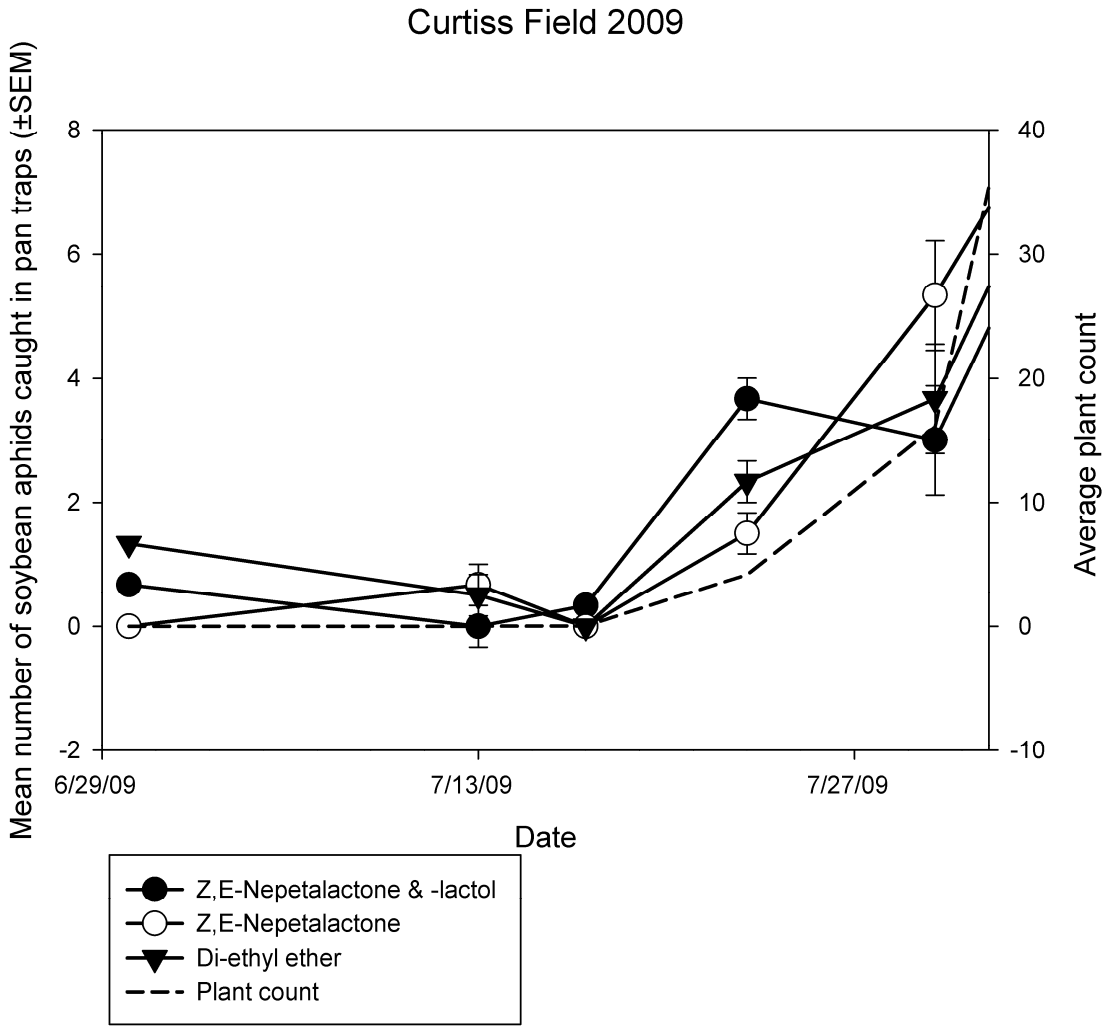
Curtiss Field 2009
Mean number of soybean aphids caught in pan traps (±SEM)
Average plant count
8
6
4
2
0
-2
40
30
20
10
0
-10
6/29/09
7/13/09
7/27/09
Date
Z,E-Nepetalactone & -lactol
Z,E-Nepetalactone
Di-ethyl ether
Plant count

Figure 2f.