

The effects of rainstorms on soybean aphids, *Aphis glycines* (Matsumura)

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Caitlin Colleen Krueger

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Ian V. MacRae, Ken Ostlie

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## **Dedication**

This thesis is dedicated to my loving parents and brother who never stopped believing that counting aphids was a solid career move.

## Abstract

Soybean aphid, *Aphis glycines* (Matsumura), is a significant economic pest of soybean in North America. Three experiments were conducted to determine the influence of abiotic factors on aphid populations and distribution on soybean plant canopies. Aphids were exposed to rainfall simulations in field and in greenhouse settings while resident populations were observed before and after rainstorm events. *Aphis glycines* population changes were influenced most greatly by sample period and a sample period\*treatment interaction during in-field rainfall simulation. Shifts in population distribution were significantly influenced by sample period and interactions between sample period and location of the plot, canopy level and treatment. Visual comparisons before and after trials indicated that small dips in population and random shifts in canopy population distribution quickly returned to original levels after the application of rainfall. An in-field aphid observational study indicated that some abiotic factors associated with rainstorm events are conditionally significant. Average wind speed, the interaction between maximum observed wind speed and rainfall, average aphid starting population and location/soil type of the observed field significantly affected aphid population. Sample period and pre-count averages were found to be significant to aphid distribution on the plant canopy.

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## **Section 1**

**Soybean aphid biology, history, and economics, and the history of research  
on rainstorm effects on insect populations.**

## **The Introduction of Soybean Aphids to North America**

### **Life cycle, host cycle, host range:**

Soybean aphid is an economically important pest of commercial soybean with a large geographic distribution. Native to Asia, SBA was first detected in North America in Wisconsin in July of 2000 (Hill *et al.* 2001, Ragsdale *et al.* 2004). In the decade since, SBA has spread to more than 30 states and 3 Canadian provinces (Ragsdale *et al.* 2011). SBA has substantially increased production costs with the advent of scouting and chemical application (Johnson *et al.* 2009, Song and Swinton 2009, Ragsdale *et al.* 2011). SBA populations can reduce plant yield upwards of 50% in untreated sites (Wang *et al.* 1994, Wang *et al.* 1996). Yield reduction comes from the removal of photosynthates through aphid feeding (Macedo *et al.* 2003, Diaz-montano *et al.* 2007, Ostlie 2011) and from the spread of aphid vectored diseases (Hill *et al.* 2001, Clark and Perry 2002, Wang and Ghabrial 2002).

The original source and means of the SBA introduction remains unknown. Soybean aphid is native to eastern Asia but it also found in a number of countries that cultivate soybean. Possible source countries include Australia, China, Korea, Japan, and the Philippines (Heimpel *et al.* 2004, Venette and Ragsdale 2004) and parts of Africa (Hirano *et al.* 1996). A recent comparison of the genetic diversity of South Korean and North American populations indicates that South Korea was not the original source of North American SBA populations (Ragsdale *et al.* 2011).

The rapid and widespread dispersal of SBA within North America is facilitated by the abundance of preferred primary (*Rhamnus* spp.) and secondary hosts (*Glycine max* L.) (Ragsdale *et al.* 2004, Venette and Ragsdale 2004, McCornack *et al.* 2005). European buckthorn was planted extensively in wind breaks throughout the Midwest (Heimpel *et al.* 2010, DNR 2011, Elhard 2011, USDA 2012).

Soybean aphid (SBA) is native to Eastern Asia (Heimpel *et al.* 2004). SBA has a heteroecious holocyclic life cycle, reproducing parthenogenically on its secondary host, typically cultivated soybean, *Glycine max*, or wild soybean, *Glycine benth* forma *lanceolate* Makino, in Asia (Wang *et al.* 1962, Ragsdale *et al.* 2004). SBA overwinter as an egg on a primary host, usually buckthorn (genus *Rhamnus* spp.), and, in North America the host is frequently *Rhamnus cathartica*, (Ragsdale *et al.* 2004, Voegtlin *et al.* 2005, Heimpel *et al.* 2010).

The initial spring generation of SBA hatch on buckthorn and develop into apterous fundatrices that produce a generation of wingless females. This second generation then produces alate female morphs that migrate to secondary hosts and produce apterous females (Ragsdale *et al.* 2004). These apterous females parthenogenically produce subsequent generations of wingless female morphs until crowding stress (Johnson 1965, Li-hua and Rui-lu 1993), predation and disease (Roitberg *et al.* 1979), or deteriorating host plant quality stimulate the production of new alates (Sutherland and Mittler 1971, Hodgeson *et al.* 2005).

In fall, a generation of alate females called gynoparae and alate males are produced. Gynoparae migrate to primary hosts and produce a generation of wingless



oviparae females which mate with the migrating males and lay eggs under leaf buds. These eggs overwinter on the primary host and hatch in spring (Ragsdale *et al.* 2004, Zhishan Wu *et al.* 2004, Hodgeson *et al.* 2005, Welsman *et al.* 2007).

In North America, the most prevalent primary host is common buckthorn, *R. cathartica* L., introduced from Europe, now found throughout much of the north central and north eastern United States (Heimpel *et al.* 2010, Kurylo *et al.* 2007, Ragsdale *et al.* 2004). Two native buckthorn species, alderleaf buckthorn *Rhamnus alnifolia* L' Hèr and lanceleaf buckthorn *R. lanceolata* Pursh, can also serve as hosts, but are too few in number and distribution to significantly contribute to yearly outbreaks (Voegtlin *et al.* 2005). Species that normally host SBA in Asia, such as *Rhamnus davurica* Pallas and *Rhamnus japonica* Maximowicz, are potential hosts though uncommon in North America (Voegtlin *et al.* 2004).

*Rhamnus cathartica* was brought to the US from Europe in the 1800's and served as a popular hedge and windbreak plant. Official sales of common buckthorn ceased in Minnesota in the 1930's, but continued to be grown and sold extensively through the 1950's and 60's (Elhard 2011b). It is now illegal to sell or transport buckthorn in Minnesota (DNR 2011) and is classified as a noxious/prohibited weed in the following states, Minnesota, Connecticut, Iowa, Massachusetts, New Hampshire, and Vermont (Elhard 2011a) .

In North America and Asia, cultivated soybean, *Glycine max* L., serves as the secondary host for soybean aphid populations through much of Asia and North America (Heimpel *et al.* 2004). In Asia, wild soybean, *Glycine Benth f. lanceolata* Makino, also

acts as a secondary host (Wang *et al.* 1962). There are unconfirmed reports that other plants may serve as secondary hosts for SBA populations in North America. Among them are crimson clover, *Trifolium incarnatum* L., red clover, *Trifolium pratense* L. (Alleman *et al.* 2002), and violet prairie clover, *Dalea purpurea* Vent. (Ragsdale *et al.* 2004).

### **Soybean Aphid Host-Plant Damage and Management:**

Soybean aphid infestations can become severely damaging economically, and have been reported to reduce crop yields by 50-70% (He *et al.* 1991, Ostlie 2011). SBA damages soybean crops directly through the disruption of photosynthesis by feeding (Macedo *et al.* 2003, Ragsdale *et al.* 2006b, Diaz-montano *et al.* 2007) and indirectly by vectoring viruses (Berg *et al.* 1997, Hill *et al.* 2001, Alleman *et al.* 2002). Feeding and viral introduction may reduce plant growth and oil production in addition to yield (Sun *et al.* 1991, Wang *et al.* 1996, Beckendorf *et al.* 2008, Ostlie 2011). SBA infestations also increase the risk of sooty mold outbreaks by exuding large quantities of honeydew as a byproduct of feeding (Krupke *et al.* 2005).

Direct feeding injury is caused by the removal of plant sap from phloem tissues by aphid stylets during feeding (Macedo *et al.* 2003, Diaz-montano *et al.* 2007). Plant energy intended for plant growth and seed production is diverted to aphid growth and reproduction (Ragsdale *et al.* 2006a). Heavy infestations can lead to stunting and reduced canopy fill due to the removal of photosynthate, a reduction in the number of soybean pods produced, reduced seed size, a decline in seed oil, and seed quality (Wang *et al.* 1994, Krupke *et al.* 2005b, Ostlie 2011).

In soybean crops, SBA may vector Soybean Mosaic Virus (SMV), a non-persistent virus distributed by soybean aphids worldwide (Alleman *et al.* 2002, Wang and Ghabrial 2002), which can lead to plant dwarfing and shriveled or wrinkled leaves (Luo *et al.* 1991), reduced yield (Ren *et al.* 1997), as well as reducing seed germination (Ostlie 2011). SBA is an effective vector of the virus (Hill *et al.* 2001) but outbreaks of SBA vectored SMV in North America have yet to occur (Ragsdale *et al.* 2011). Soybean aphid can also transmit Soybean Dwarf, Bean Yellow Mosaic Virus, and Soybean Stunt in commercial soybean systems (Berg *et al.* 1997a).

Soybean aphid is known to vector viruses in potato and tobacco crop systems on which they may land and probe but do not feed (Heimpel *et al.* 2004). SBA does not colonize potato but may probe potato plants (Heimpel *et al.* 2004). SBA has been shown to be capable of transmitting Potato Virus Y (PVY), greatly decreasing seed crop values if PVY levels exceed 1-3% per seed lot (Gray *et al.* 2010). While it is not an efficient vector of the virus, its abundant numbers greatly increase the risk of viral transmission (Davis *et al.* 2005, Mello *et al.* 2011). SBA may also transmit Potato Leafroll Virus (PLRV) at low efficiency (Davis and Radcliffe 2008).

Soybean aphid has also been found to transmit Cucumber Mosaic Virus (CMV) at high efficacy in snap beans (Alleman *et al.* 2002) and are vectors of Bean Yellow Mosaic Virus and Tobacco Ringspot Virus (Clark and Perry 2002). SBA may also vector Alfalfa mosaic virus, though with low efficacy (Hill *et al.* 2001). SBA are not proficient at transferring a number of the viruses they may host, however, they still present high virus

risk to crops because they have a high rate of fecundity, short generation times, and their ability to generate alate aphids for greater dispersal (Davis *et al.* 2005) .

Honeydew produced by the aphids can coat soybean leaves and lead to sooty mold outbreaks, further reducing soybean yield (Krupke *et al.* 2005a). In Minnesota, yield losses exceeding 50% have been observed on heavily infested plants (Ostlie 2011). During outbreak years, losses can occur even in areas where SBA is normally well controlled by natural enemies; in China yield losses during these years may reach 50-70% (He *et al.* 1991).

Before the arrival of soybean aphid in North America, treatment of commercial soybean with insecticides was rare (Costamagna and Landis 2006) and almost no scouting (2%) of soybean fields for pest arthropods (Ragsdale *et al.* 2011). Today, control of soybean aphid populations in commercial soybean relies on pesticide applications of foliar organophosphates, pyrethroids, and neonicotinoids (Johnson *et al.* 2009, Song and Swinton 2009, Chandrasena *et al.* 2011a, Hodgson *et al.* 2012).

The presence of SBA has led to a substantial increase in field scouting as soybean aphid populations vary by season and field, and is used to determine if SBA populations have reached sufficient levels to necessitate insecticide application (Hodgson *et al.* 2007, Ragsdale *et al.* 2007, Song and Swinton 2009). Before the arrival of SBA, pesticide applications in soybean fields were uncommon, with less than 1% of soybean fields chemically treated for insect pests (Song and Swinton 2009). SBA treatment with insecticides increased production costs by 5-24 dollars per acre in 2005, and in 2009 scouting costs were estimated at \$2/acre (Ragsdale *et al.* 2006, Song and Swinton 2009).

To assist growers in tracking SBA movement through soybean growing areas, a multistate trapping program known as Aphid Alert has been implemented to track the northern migration of a myriad of pest aphid species using a suction trap network. In 2001 Aphid Alert reported the spread of the recently discovered SBA populations (Ragsdale 2001). These reports did not track SBA movement through the suction trapping network as they were rarely found in the traps (Radcliffe and Ragsdale 2003), rather, this information was obtained through general scouting in Minnesota and surrounding states. Periodic updates on SBA research and management advice were occasionally included (Radcliffe *et al.* 2008).

To better manage the timing and frequency of insecticide application, an economic threshold (ET) of 250 aphids per plant, with greater than 80% of plants being infested, and an economic injury level (EIL) of 674 aphids per plant were established for SBA in 2007 (Ragsdale *et al.* 2007). The establishment of a formal ET and EIL was designed to provide a 7 day window from ET to EIL in which growers could apply a foliar insecticide to infested crops before aphid populations reached economically damaging levels. This system was designed with the intention of preventing unnecessary pesticide applications, this reduces grower costs and conserves natural enemy populations (Ragsdale *et al.* 2007). As of 2011 there had been no documented cases of SBA resistance to commonly used pesticides in North America, but multiple exposures to the same insecticide, and frequent use of pesticides increase the risk of development of resistance (Chandrasena *et al.* 2011b, Hodgson *et al.* 2012).

Recently, new soybean varieties with resistance to SBA infestation have been developed to assist in SBA management. Soybean utilizes two types of resistance against soybean aphid, antibiosis (reduces SBA survival and fecundity), and antixenosis (makes plants unattractive or actively repels SBA). Antibiosis is the ability of a resistant host plant to interfere with the feeding, development, and reproduction of the pest insect (Kim *et al.* 2008). Antixenosis is the development of traits that make the plant unattractive or repellent to a pest, and results in a non-preference for the affected plants (Zhang *et al.* 2010).

Five genes have been discovered with SBA resistant properties (Tilmon *et al.* 2011). They include Rag1 (resistance to *Aphis glycines*), discovered in the soybean cultivar ‘Dowling’ (a dominant gene which controls antibiosis) (Li *et al.* 2004, Hill *et al.* 2006), Rag2 (a second dominant gene which also controls antibiosis but is independent of Rag1) (Mian *et al.* 2008), Rag3 and Rag4 (recessive genes, with Rag3 possibly utilizing antixenosis) (Zhang *et al.* 2010). Most recently, Rag5 was discovered which may use antixenosis properties (Jun *et al.* 2012).

There are populations of SBA in North America that have displayed biotypes that may colonize plants with these resistance genes. At least one population of SBA never exposed to plant resistance in North America displayed immunity to the novel resistant gene Rag1 several years before it became commercially available (Kim *et al.* 2008). A recently discovered aphid isolate readily colonized plants with both Rag1 and Rag2 resistance genes before the seed became commercially available (Hill *et al.* 2010).

Plant resistance, conveyed through improved genetics, may offer an economical and environmentally friendly alternative to costly pesticide applications, but development of resistant soybean lines takes time. Few investigations into the genetic basis for soybean resistance to SBA were performed before the introduction of SBA to North America (Kim *et al.* 2008), and initially, no resistant varieties were commercially available. The first commercially available soybean with resistant genes were marketed in 2009 (Tilmon *et al.* 2011), nearly a decade after the initial detection of SBA.

Despite the inclusion of new management options to the grower, soybean aphid management remains a challenge. Soybean aphid populations can increase rapidly; aphid mothers are born pregnant and individual aphids can produce about 9.5 offspring daily. Offspring mature in 3-7 days and populations can double every 2-5 days under ideal conditions (Ostlie 2011). Laboratory research has concluded that optimal conditions for SBA reproduction require temperatures of 22-25°C and a relative humidity below 78% (Wang *et al.* 1962).

Alate soybean aphids are proficient fliers, with alate aphids less than 24 hours old able to maintain active flight for nearly four hours and cover distances in excess of 4 km (Zhang *et al.* 2008). The rapid spread of SBA populations between states may be the result of aphid dispersal by low-level jetstreams (LLJ) (Zhu *et al.* 2006). It is believed that aphids emigrating via atmospheric dispersal maintain neutral buoyancy through flight when caught in updrafts, and cease producing lift in downdrafts. These physical interactions give aphids some degree of control over their dispersal (Reynolds and Reynolds 2009). Many species of aphid, including SBA, ride LLJ to northern growing

regions, aphids are then deposited on new fields by precipitation events (Ragsdale *et al.* 2004, Zhu *et al.* 2006). In areas where winter temperatures may prevent successful SBA overwintering, such as Northern Minnesota and parts of Wisconsin, new aphids may be reintroduced each year by these migration events (McCornack *et al.* 2005).

**Predators, parasitoids, and disease:**

In North America a number of generalist predator groups target SBA. These include Diptera such as syrphid larva (Diptera: Syrphida), lacewing larva (Neuroptera: Chrysopidae), true bugs including nabids (Hemiptera: Nabidae) (Costamagna *et al.* 2007), and carabid beetles (Coleoptera: Carabidae) including *Clavina impressifrons* Le Conte and *Elaphropus anceps* Le Conte (Rutledge *et al.* 2004). Two groups of generalist predators, the minute pirate bug *Orius insidiosus* Say (Rutledge and O'Neil 2005), and lady beetles (Coleoptera: Coccinellidae) (Berg *et al.* 1997a, Fox *et al.* 2004, Koch 2003), are known to be effective at reducing soybean aphid populations. Coccinellidae observed eating SBA include the multicolored Asian lady beetle *Harmonia axyridis* (Koch 2003) the seven-spotted lady beetle *Coccinella septempunctata* (L.) (Zhu and Park 2005), the fourteen spotted lady beetle *Propylea quatuordecimpunctata* (L.), and the pink spotted lady beetle *Coleomegilla maculata lengi* (Timberlake) (Mignault *et al.* 2006).

*Orius insidiosus* is known to suppress soybean aphid population growth when populations are small and aphid distribution is relatively clustered (Rutledge and O'Neil 2005). However, the presence of alternative prey species, such as the soybean thrips



*Neohydatothrips variabilis* (Beach), results in fewer attacks on soybean aphids regardless of prey density (Butler and O'Neil 2008).

In Asia, SBA is attacked by a large suite of predators and parasitoids (Rutledge *et al.* 2004). In North America however, native parasitoids began targeting SBA within the first few years after introduction. A 2007 survey of parasitoids in Michigan found 6 species of parasitoids from Hymenoptera: Braconidae and Hymenoptera: Aphelinidae (Kaiser *et al.* 2007). Another survey in New York found 3 parasitoids targeting SBA, *Aphidius sp.* and two *Praon sp.* (Nielsen and Hajek 2005). *Aphelinus certus* Yasnosh, a parasitoid native to Asia has been accidentally introduced into North America has also been observed to attack SBA in the field (Frewin *et al.* 2010, Heimpel *et al.* 2010).

To better manage the SBA invasion, research is underway to identify possible biological controls for potential introduction. To date, several species of hymenopteran parasitoids known to target soybean aphid in Asia have been screened for introduction suitability in North America. One of these *Binodoxys communis* Gahan, was approved for release in 2008, though research is still in progress to determine an optimum release method for maximum establishment and aphid suppression (Hogg *et al.* 2009, Wyckhuys *et al.* 2009, Heimpel and Asplen 2011). In addition the parasitoids *A. albipodus* Hayat & Fatima and *L. gracilis* Förster are now available for release against SBA populations (Heimpel *et al.* 2004).

Another imported parasitoid *Aphelinus albipodus* Hayat and Fatima may be suitable for future soybean aphid control. *Aphelinus albipodus* was imported from China in 1992 to control Russian wheat aphid *Diuraphis noxia* Mordvilko. University of

Wisconsin researchers have released populations into North America in 2002 and 2003 with the goal of establishing a sustained population targeting SBA (Wisconsin 2009).

A number of parasitoids already established in North America have been found to attack soybean aphid; these include parasitoids from families Aphelinidae and Braconidae (Noma and Brewer 2008). Among these are *Lysephlebus testaceipes* Cresson, *A. albipodus* (Wisconsin 2009), and *Aphelinus certus* Yasnosh (Frewin *et al.* 2010). In subsequent years, several researchers have noted more predators feeding on SBA (Wisconsin 2009, Initiative 2012) and forming a potential natural enemy complex which may naturally suppress aphid outbreaks. In North America, native species such as *L. testaceipes* may have increased in number in response to SBA invasion and spread (Hogg *et al.* 2009).

Pathogens are known to reduce SBA populations; the fungal pathogen Entomophthorales targets aphid pests in cropping systems (Koch and Ragsdale 2011). In Minnesota soybean systems the most common pathogen infecting SBA is *Pandora neoaphidis* (Ragsdale and Koch 2008). Pathogen outbreaks are established after rainfall, which create the humidity necessary to establishing *Entomophthora* fungi. Rainfall totals appear to be less important than the frequency and distribution of storms (Shands *et al.* 1963, Dean and Wilding 1971, Voronina 1971, Wilding 1975).

### **Rainstorm Events Impact on Insect Population Mortality:**

One of the underexplored topics in modern entomology is the effect of abiotic mortality factors associated with rainstorm events on insect populations. While the indirect results of rainstorms on insects, such as the onset of epizootics, are well studied,

the direct effects of abiotic factors such as wind and rain are little understood. Among the publications that exist on the effect of rainstorm events on insect populations, most propose the primary responsible factor to be rain, wind, soil, or some combination of the three factors.

Rainfall is known to initiate changes in insect foraging and reproductive behavior. Rainstorms were associated with reduced mating activity in the mosquito *Anopheles funestus* Giles, and instigated changes in the overall condition of female ovaries and follicles (Charlwood and Braganca 2012). In addition a study found that cold fronts sometimes associated with rainstorm events reduced *A. Funestus* numbers collected at light traps (Charlwood and Braganca 2012). Simulated rain and wind halted foraging behavior and significantly curbed foraging and oviposition in the aphid parasitoid *Aphidius rosae* (Fink and Völkl 1995).

Rainstorms can also interfere with or alter the course of insect migrations through changing winds (Burt and Pedgley 1997). Windstorms have been observed to increase the risk of drowning when crossing bodies of water during monarch butterfly migrations (Howard and Davis 2012). Rainfall may also deposit insects migrating by low level jet streams, which often produce rainfall events on their northern edges (Zhu *et al.* 2006).

Many publications report seeing population declines/increases in insect mortality after rainstorm events (Dean and Wilding 1971, Jones 1979, Ba-Angood and Stewart 1980a, Walker *et al.* 1984, Moran and Hoffmann 1987, Moran *et al.* 1987). Rainstorms can impact insect communities in addition to individual species, a study applying simulated rainfall on the ant *Tapinoma sessile* Say tending the black citrus aphid

*Toxoptera aurantii* Boyer de Fonscolombe found that *T. sessile*, and the aphids they tended, experienced heightened mortality and reduced ant foraging in relation to heavy rain events (Powell *et al.* 2009).

Mortality is often observed when the rainstorm occurs in locations or during periods when rain is rare or uncommon (Moran and Hoffmann 1987, Moore 1989). Many reports note population drops for small arthropods, including aphids, after rainstorm events, both simulated and natural (Hughes 1963, Maelzer 1977, Jones 1979, Moran and Hoffmann 1987, Moran *et al.* 1987). Over 50% of mortality of the eggs and larvae of the thistle head weevil *Rhinocyllus conicus* Froelich was attributed to wind and rain events acting on thistle patches unprotected by trees and sheltering terrain (Surles *et al.* 1975).

Insect eggs and juvenile insects are frequently observed to be vulnerable to rain (Shade *et al.* 1969, Moran and Hoffmann 1987, Paaijmans *et al.* 2007, Montoya *et al.* 2008, Extension 2012). Rains have been observed to wash young insects and eggs off of host plants (Wood 1965, Moore 1989). One study attributed over half the observed mortality of the eggs and larvae of the thistle head weevil *Rhinocyllus conicus* Froelich to rainstorm and wind events on host thistles (Surles *et al.* 1975). Another study observed that larval *Anopheles gambiae* Giles SS. were washed from their puddles as a result of rainstorms. During precipitation events, larvae may be flushed out of these pools by precipitation, and on the nights where rainfall occurred larval mortality increased upwards of 6.9% (Paaijmans *et al.* 2007). Two studies of rainfall on colonial scale insects observed that the waxy coating over colonial scale insects was deteriorated by rain;

younger colonies were more likely to be washed away by this phenomena (Moran and Hoffmann 1987, Moran *et al.* 1987).

The ability of precipitation events to kill insects has not gone unnoticed in the agricultural world. Flooding and irrigation have been proposed as possible means of pest insect control (Vincent *et al.* 2003). Agricultural publications often report reductions in insect populations; one Cornell University Extension report found significant mortality for juvenile potato leafhoppers in potato and thrips in onion and cabbage as a result of wind-driven rain (Extension 2012).

Soybean aphids are have been observed to display population declines as the result of rainstorm events (Ragsdale 2001, Ostlie 2011), and there are observations of substantial population declines in the wake of rains. Field observation of a population drop of 60% has been made after a 1 inch rainfall (Ragsdale 2001). Rainfall may even have an effect on its within-plant distribution (McCornack *et al.* 2008).

In aphid studies, aphid crowding and reductions in host quality appear to encourage aphid movement within or between host plants (Hodgson 1991). The impact of rain events may be mitigated by the presence of a closed and mature plant canopy. Closed canopies damaged by storms are thought to be partially responsible for lower numbers of *Allothrombium mitchelli* Davis on *Fagus grandifolia* Ehrhart (Wiggins *et al.* 2001).

Other aphid species have been observed to display population declines as a result of rainfall. A study on cereal aphids in Quebec noted a population decline during mid-July of 1979 after two consecutive rainstorms dropped 174mm of rain, resulting in samples with dead and “waterlogged” aphids (Ba-Angood and Stewart 1980). Another

study on the aphid epizootic fungus *Entomophthora aphidis* noted a 65% population decline in the host cereal aphid *Metopolophium dirhodum* after “a very heavy rain” (Dean and Wilding 1971).

### **Wind as a Significant Factor in Insect Population Mortality:**

Rain alone seems to have less impact than rain events with wind, especially high winds. A study on potato aphid *Macrosiphum euphorbiae* Thomas on tomato systems found rainfall associated with high winds to be a major contributing factor to mortality in the field (Walker *et al.* 1984). A five year study on the abundance of cereal aphids revealed that aphid populations dropped after heavy rainfalls; Jones (1979) hypothesized that heavy rains washed aphids off of leaves and stems and that strong winds dislodged aphid nymphs, noting that periods of strong wind coincided, in part, with slower population growth.

Direct death may result from insects being thrown from the plant, or from the deterioration of the plant parts they inhabit if they become detached from the plant (Williams and Whitham 1986). Indirect chances of mortality may result when aphids knocked from the plant risk attack by ground predators (Losey and Denno 1998a), or from the relatively low probability of finding a suitable replacement host if migrating to a new location (Ward *et al.* 1998). Debris, such as sand, powered by windstorms had been observed to bury alive migrating monarch butterflies; these storms also increased the risk of mortality by drowning for monarchs crossing over bodies of water (Howard and Davis 2012).

Aphid population decrease in the wake of wind storms, both simulated and in-field have been observed by researchers (Jones 1979, Narayandas and Alyokhin 2006). Mann *et al.* (1995) found aphid spp. aphids were most frequently dislodged or lost from a plant when exposed to artificial wind gusts .5 to 5 meters per second, while light gusts and constant wind had little effect. Wind gusts (gusts greater than 52 km/hr) have also been observed dislodging both alate and apterous aphids from broccoli (Trumble 1982). A study on Russian wheat aphid isolated one treatment group from the negative abiotic effects of wind and rain, and found it to be partially responsible for increased aphid densities (Lee *et al.* 2005).

Aphid parasitoids have been observed to have greatly reduced foraging and oviposition during adverse weather conditions (wind and rain). The aphid parasitoid *A. rosae* has been observed to cease foraging during rain, and severely reduce foraging during times of wind (Fink and Völkl 1995, Weisser *et al.* 1997).

#### **Soil as a Significant Factor in Insect Population Mortality:**

Soil is the third factor observed to influence insect mortality during rainstorm events; of the three factors the importance of soil is the least studied in the literature. Soil dwelling juvenile insects are understood to be at greater risk for mortality in rain-shifted soil. The pink bollworm, which pupates between cracks or clumps in the soil surface, experiences high mortality when rainfall hits soil unsheltered by a plant canopy and are buried by wet soil. A study using a no-shelter rain simulation on bollworm larvae indicated that nearly 95% of pupae were sealed by simulated rainfalls of less than an inch

(Fye 1973). A study of fruit fly emergence from soil found that soils saturated by rain reduced successful emergence of adults (Montoya *et al.* 2008).

Soil has been observed to contribute to aphid mortality during rainstorm events. Potato aphids washed off plants by rainstorm events have been observed to become trapped in the mud and water around the base of plants leading to dead waterlogged aphids (Walker *et al.* 1984).

The potential for rain to cause erosion, or rain erosivity (Salles and Poesen 2000), is thought to be influenced by three factors which include resistance, protection, and energy. Energy encompasses wind and rainfall, as well as runoff; all have the potential to erode soil (Morgan 2005). Soil erosion may happen as a raindrop impacts on a soil surfaces launching part of this soil away from the ground. This phenomenon, called soil splash, results when a raindrop impacts a soil surface with enough kinetic force to dislodge soil particles (Al-Durrah and Bradford 1982b, Durrah and Bradford 1982a, Salles and Poesen 2000, Legue´dois *et al.* 2005, 2008).

One study described this phenomena in relation to insects wherein soil splashing caused by rain striking a soil surface has been observed to strike the underside of leaves, encasing insect eggs and causing significant mortality by desiccating the eggs as the soil dried (Shade *et al.* 1969). Soil splashing, or rain splashing off of soil, is also known to introduce soil borne pathogens such as epizootic fungal pathogens which lead to disease outbreak and substantial reductions in populations (Blakeman 1989, Jackson *et al.* 2012). Plant dwelling insect mortality can be the result of insects being washed from a host plant and drowning in mud (Walker *et al.* 1984). Saturated and muddy soil can cause pupal



mortality in fruit flies by saturating soils and decreasing successful adult emergence (Montoya *et al.* 2008)

Soil texture is the primary determining factor in both water retention and soil splash behavior. In agricultural soils, soil texture may be used to calculate the ability of soil to hold water (Saxton *et al.* 1986, Saxton and Rawls 2006). Each soil type possesses a different capacity for holding water based on its component levels of sand, silt, and clay (Extension 1999b, Extension 1999a). Soil splashing is likewise controlled by soil texture, soil types with finer particles (<50  $\mu\text{m}$ ) travel as droplets, soils with coarser particles, upwards of 2000  $\mu\text{m}$  travel as a single mass (Legue´dois *et al.* 2005)

#### **Rainstorm Events Impact on Insect Population Movement:**

Rain, wind, and soil are known to impact insect movement as well as insect survival (Narayandas and Alyokhin 2006). Rainstorm events may alter the path of insect migrations and knock flying insects to ground level or into bodies of water (Burt and Pedgley 1997, Zhu *et al.* 2006, Howard and Davis 2012). Temperature changes associated with rainstorms reduced the movement patterns of *A. funestus* when numbers were tracked using light traps (Charlwood and Braganca 2012). Simulated rainfalls, even simulated drizzles, drastically reduced foraging and oviposition of *Aphidius rosae* Haliday (Fink and Völkl 1995).

Wind has been observed to prompt or hinder normal movement patterns in insect movement. A study of the potato aphid *Macrosiphum euphorbiae* Thomas found both that wind and rain instigated movement between potato plants, sometimes by walking over soil (Narayandas and Alyokhin 2006). Simulated windstorm events on English

Grain Aphid *Sitobion avenae* F., found wind speeds of .5 to 5 meters per second dislodged a substantial number of aphids from the plant (Mann *et al.* 1995).

Soil has not been directly associated with initiating insect movement, but movement over soil may be hindered by rainstorm events. Wet soils are capable of trapping aphids and preventing them from walking back to host plants if dislodged (Walker *et al.* 1984). Rainstorm events can increase the frequency of on-soil walking between plants (Narayandas and Alyokhin 2006) and exposure to ground predation (Losey and Denno 1998b), causing further deviation from normal movement patterns.

#### **Aphid Host Location Preference and Within-Plant Distribution:**

Aphid species have preferred feeding locations on a given host plant species which populations will concentrate on and disperse to repeatedly as the host plant matures (Harrington and Taylor 1990). A study on the distribution of the aphid *Acyrtosiphon kondoi* Shinji and Kondo on highly and moderately resistant alfalfa in a greenhouse were found in the greatest numbers on the leaf blade trifoliates of aphid resistant plants. *A. kondoi* infestations on susceptible alfalfa plants were found to initially have high concentrations of aphids on the leaf blades, but 8 or more days after the establishment of an infestation the greatest concentrations were found on the stem (Zarrabi *et al.* 2005). A greenhouse study of another alfalfa pest, cowpea aphid *Aphis craccivora* Koch on susceptible alfalfa was found they preferentially feed on the alfalfa intermodal stem (Zarrabi *et al.* 2002).

Soybean aphids are frequently observed in the top trifoliolate in the upper canopy of soybean plants (Berg *et al.* 1997, McCornack *et al.* 2008, Brosius *et al.* 2010). These

populations will redistribute throughout the plant as it matures into reproductive stages (Ragsdale *et al.* 2004). Soybean aphid location within plant canopies has been observed to vary by year (Brosius *et al.* 2010).

### **Aphid Movement:**

Aphids use complex life cycles to migrate to host plants: by flight, if alate or by walking if apterous. This paper will use two terms to describe aphid movement. Migration, as defined by Harrington (1990) defines a movement for which there is no possibility of return (Harrington and Taylor 1990) and dispersal for a movement where return is possible. Alate morphs migrate short distances under their own flight power to reach nearby hosts; these typically produce several offspring before moving on to search for other host plants (Bullock *et al.* 2002). Alates also make long distance migrations by riding low-level Jetstreams (LLJ) (Zhu *et al.* 2006, Zhang *et al.* 2008, Reynolds and Reynolds 2009). Alates migrating on LLJ's can be deposited onto new host plants by precipitation events (Ragsdale *et al.* 2004, Zhu *et al.* 2006).

Long distance migrations using LLJs are advantageous, as they allow aphids to reach host populations that apterous dispersal cannot. This behavior increases the area that aphids may inhabit, and repopulates aphids in areas where seasonal temperatures prevent overwintering (McCornack *et al.* 2005). However, migration by LLJ events come at the cost of a reduction in the rate of success in finding a suitable host (Ward *et al.* 1998). Alate aphid morphs are further limited by a smaller reproductive capacity than their apterous counterparts (Dixon *et al.* 1993).

Alate soybean aphids are known to migrate long distances on LLJ's; LLJ migration repopulates SBA in Northern Minnesota and Canada where cold winter conditions can prevent successful overwintering (McCornack *et al.* 2005). Alates have also been found to make shorter distance migrations under their own flight power, one study found that new alate SBA (less than 24 hours old) can maintain active flight for nearly four hours and travel distances excess of 4 km (Zhang *et al.* 2008). A study of the genetic diversity of soybean aphid populations suggest these short flights from primary to secondary hosts are made early in the season and longer LLJ migrations are made from secondary hosts several generations later (Zhang *et al.* 2008, Michel *et al.* 2009).

Apterous aphids may engage in intraplant dispersal, though this can be considered migration if this dispersal is the result of the abscission of the leaves previously being fed upon (Harrington and Taylor 1990). The dispersal rate of apterous aphids is linked in part to their species and age; usually it is the young adult stages that migrate (Hodgson 1991, Boiteau 1997).

Apterous aphid morphs may migrate using leaf bridges to cross between plants (Williams and Whitham 1986, Whalen and Harmon 2012). Apterous aphids have also been observed to migrate to new host plants by walking on the ground (Harrington and Taylor 1990, Alyokhin and Sewell 2003). Soil walking has been observed to be less preferable to traveling directly from host to host; Alyokhin and Sewell observed this behavior only as the result of being dislodged or removed from the host rather than as active and intentional migration (Alyokhin and Sewell 2003). Aphid species that drop

from plants to escape predators are also forced to walk over soil to return to hosts and are at risk of predation by ground dwelling predators (Losey and Denno 1998b).

Apterous soybean aphids are considered sedentary (Ragsdale *et al.* 2004) and are not observed to move once an adult apterous aphid has settled in a desirable feeding location (I.V. MacRae pers. Comm.). Steady rates of dispersal through a field have been attributed to the movement of apterous aphids onto the plants nearest to those on which they were larviposited (Rhainds *et al.* 2008). Soybean aphids have been observed to depart or avoid resistant soybean plants even if no alternative host is available (Li *et al.* 2004, Diaz-montano *et al.* 2006). Observations during research on soybean aphid have found apterous morphs to readily move off of treated leaf surfaces (I.V. MacRae Unpublished Data). Field researchers have observed ant aided dispersal in the field (Ostlie 2011), but most colonization by SBA throughout soybean fields is attributed to alate morphs (Fox *et al.* 2004).

#### **Biotic Influences on Aphid Population Movement:**

Deteriorating host quality can be initiated by aphid feeding; the act of gall formation by the aphid *Pemphigus betae* Doane on the narrowleaf cottonwood *Populus angustifolia* James promotes leaf abscission. The abscission process is preceded by the removal of recoverable nutrients and chlorophyll by the tree, which can prompt aphids to mature at smaller sizes and migrate to other leaves before leaf drop and near certain mortality (Williams and Whitham 1986). Plant resistance genes may also prompt migration from a host; one study found the presence of resistance genes in host plants can prompt higher inter and intraplant movement of insects (Whalen and Harmon 2012). A

study of cotton aphid in intercropping systems found that aphid location among the various levels of plant canopy was influenced by the plant maturity (recorded as days after planting) (Fernandes *et al.* 2012).

Aphid distribution may be effected by the presence of plant viruses; aphid vectors of some plant viruses preferentially feed on uninfected host plants after virus inoculation (Ingwell *et al.* 2012). A settling bioassay of apterous and alate green peach aphid on potato found that given a choice between leaflets from plants infected with either Potato Virus Y or Potato Leafroll Virus versus plants infected with both viruses, aphids preferentially chose hosts infected with both viruses (Srinivasan and Alvarez 2007).

Biotic factors are known to influence aphid movement and distribution. Known influential biotic factors include the maturity of a plant (McCornack *et al.* 2008), the presence of plant viruses (Donaldson and Gratton 2007), and the presence of predators (Brosius *et al.* 2010). Host plant nutrient levels may also influence distribution, SBA distribution on young plants is concentrated at the youngest trifoliolate where plant nutrients, like nitrogen, concentrate (Ragsdale *et al.* 2004). A study by Whalen and Harmon found that aphid resistance genes appear to prompt movement between leaves or off of vegetative stage soybean plants, while aphids on susceptible plants are more sedentary (Whalen and Harmon 2012).

Within plant distribution of SBA populations has been studied for the purpose of creating efficient counting methods to estimate and manage soybean aphid populations in the field (McCornack *et al.* 2008, Brosius *et al.* 2010). Early studies on the soybean aphid relied on destructive whole plant counts, as with a study to create a viable economic

threshold for SBA control (Ragsdale *et al.* 2007). This technique, while accurate, is extremely time intensive and impractical in a field setting.

A 2008 study focusing on the creation of an efficient node based sampling technique by tracking vertical distribution of SBA on host plants found that aphid density favored the highest (and youngest) node during younger plant stages, but shift to the middle and lower nodes as the plants mature. Populations distribution shifted significantly based on both planting and sample date (McCornack *et al.* 2008). Field observations suggest that soybean aphid distribution is concentrated in protected areas on the lower portion of the plant when faced with abiotic stressors such as rain. Population distributions prior to rainfall may favor the nutrient rich upper canopy, but aphids surviving rainfall events would be those feeding in lower levels of the host-plant canopy (Ragsdale pers comm.).

Another study focusing on which biotic and abiotic facts initiated SBA movement found aphid populations concentrated on the upper canopy in the first year of the study, and the middle canopy then next. Temperature was not found to significantly affect aphid distribution, though temperatures were significantly different between different levels of the canopy. However, biotic influences, like the presence of the minute pirate bug *Orius insidiosus* Say was found to significantly influence SBA distribution among the canopy layers (Brosius *et al.* 2010).

### **Abiotic Influences on Aphid Population Movement:**

Environmental factors such as wind, rain, and temperature may determine aphid distribution within and between plants in both the short and long term (Narayandas and

Alyokhin 2006). Cold fronts associated with rainstorms reduced *A. funestus* numbers collected at light traps (Charlwood and Braganca 2012). Simulated winds of 2 m/s and simulated rainfall (both drizzle and rain showers) were shown to greatly reduce foraging and oviposition in the aphid parasitoid *A. rosae* (Fink and Völkl 1995). Storms alter insect migrations through changing winds (Burt and Pedgley 1997) and rainfall is thought to deposit insects migrating by low level jet streams (Zhu *et al.* 2006). Rainfall may have an effect on soybean aphid within-plant distribution (McCornack *et al.* 2008).

Wind may also prompt aphid movement or shifts in population distribution on the host-plant canopy. Field observations have reported aphid population declines in the wake of windstorm events (Dean and Wilding 1971, Jones 1979, Ba-Angood and Stewart 1980, Trumble 1982). Constant winds appear to have little effect, but in a study on the effects of simulated windstorm events on English Grain Aphid *Sitobion avenae* F., the greatest number of aphids lost or dislodged from host plants resulted from wind gusts ranging from .5 to 5 meters per second (Mann *et al.* 1995). A study on the effects of wind and rain on potato aphid *Macrosiphum euphorbiae* Thomas found both factors significantly increase aphid movement between potato plants with and without overlapping canopies (Narayandas and Alyokhin 2006).

The study of abiotic factors on small arthropods is generally done as a series of in-field observations, but use of rain simulators in controlled conditions is not unheard of. The methods for simulating wind and rain to explore their effects on arthropods vary. Wind in many studies is simulated by the use of a box fan, but for rain researchers are split between building dedicated rainfall simulators or relying on modified spray wands



and variable shower heads (Moran and Hoffmann 1987, Moran *et al.* 1987, Fink and Völkl 1995, Narayandas and Alyokhin 2006).

### **Soybean Aphid Within-Plant Distribution:**

Rainstorm events may prompt changes in within-plant distribution in aphids. While individual aphids are unlikely to move (K. Ostlie pers. comm) population concentration in the plant canopy may shift down a plant in response to changing abiotic and biotic conditions (Wang *et al.* 1962, McCornack *et al.* 2008, Brosius *et al.* 2010, Dave Ragsdale pers. comm.). Soybean aphid congregates in a non-random pattern on the new leaves at the upper axis of a soybean plant (Wang *et al.* 1962), however rainstorm events may reduce populations to survivors in the sheltered lower portions of the host-plant (Dave Ragsdale pers. Comm).

Aggregation on new trifoliates has been attributed to distribution of nutrients which concentrate in areas of new growth. Soybean aphids are frequently observed in the upper canopy of soybean plants (Wang *et al.* 1962, Berg *et al.* 1997b, McCornack *et al.* 2008, Brosius *et al.* 2010). As the host-plant grows and matures to reproductive stages, population distribution becomes more concentrated at the middle portion of the canopy and then on to stems, leaves, and pods in later reproductive stages (Ragsdale *et al.* 2004, Zhishan Wu *et al.* 2004).

Soybean aphid location within plant canopies has also been observed to vary by year and the presence of predators (Brosius *et al.* 2010). A recent study in Nebraska on aphid location within the various strata of a soybean canopy found that natural enemy abundance was responsible for differences in distribution. Of those predators observed in

the study, *Orius insidiosus* (Say) was determined to be most responsible for shifts in aphid distribution to lower portions of the plant (Brosius *et al.* 2010).

### **Rainfall Simulation:**

Many studies on the effects of abiotic factors on arthropods are conducted through in-field observations, but a few studies have made use of rainfall simulators. Most of these are fairly simple, making use of spray wands and showerheads, though some build more complex systems. If wind is also applied in these studies, it is usually provided by a box fan (Moran and Hoffmann 1987, Moran *et al.* 1987, Fink and Völkl 1995, Narayandas and Alyokhin 2006).

The primary goal of a rain simulator is to approximate natural rainfall conditions, mimicking the size, intensity, and kinetic energy of droplets. Rainfall simulation is a staple of soil erosion research and has been used in that field for more than 70 years (Al-Durrah and Bradford 1981, Shelton *et al.* 1985). Soil scientists have worked for some time to determine the specific properties of rainfall and design simulators to mimic these properties (Meyer 1979, Blanquies *et al.* 2003).

Mimicking rainfall is a difficult proposition, rain is an extremely variable phenomenon and can produce vastly different raindrop sizes both during and between storms and from region to region (Strangeways 2011, Mason and Andrews 1960, McCool 1979). As an example, raindrops collected in a dish of oil for a single storm varied widely in size, ranging between .5mm to 5 mm (Strangeways 2011). The properties of rain, such as drop size, distribution, intensity (measured as mm of water per hour), and kinetic impact on the ground, can vary significantly between storms (Shelton *et al.* 1985).

Water droplet size, in conjunction with fall height, determines the kinetic impact of rainfall. Droplet size is determined by the size of the particle around which they form, called an aerosol. Water droplets form either by homogenous nucleation, when water vapor molecules collide and form a liquid-phase drop, or by heterogeneous nucleation, when water vapor collects on a particle such as dust or salt (Strangeways 2011). Soil splash is caused by the impact of raindrops on soil; this phenomenon is intensely studied as it is considered the primary responsible factor for water-based erosion and can be described as momentum multiplied by raindrop diameter (Salles and Poesen 2000).

Simulators have been used to study the properties of soil splash, though often with a raindrop tower to analyze the impact of a single drop at a time (Al-Durrah and Bradford 1981, Al-Durrah and Bradford 1982a). Soil texture is the dominant factor in influencing soil splashing and soil water capacity (Saxton *et al.* 1986, Saxton and Rawls 2006). Soil water capacity is important both to soil splash and to determining how quickly a soil drains, which may influence aphid survival and movement after rainstorms. Soil texture and water capacity may be classed by the component levels of sand, silt, and clay in a soil (Extension 1999b, Extension 1999a). Soil types with finer particles (silts and clays) (<50  $\mu\text{m}$ ) travel as droplets, soils with courser particles (soils with more sand) sized upwards of 2000  $\mu\text{m}$  are launched as a single mass (Legue'dois *et al.* 2005).

Much of this research has depended on the use of nozzle type simulators, most of which use model 80150 or 80100 Veejet<sup>®</sup> nozzles produced by Spraying Systems Co. <sup>®</sup> (Spraying Systems Co. 2013). By placing Veejet nozzles at a height of 2 meters, with nozzle pressure set to 41 kilopascals (kPa), researchers are able to produce a reasonable

drop size and distribution pattern for mimicking rainfall intensities of greater than 25mm/h (Moore *et al.* 1983). Nozzles operated at these conditions produce 580mm/h of water over a plot, and need be run only briefly to simulate very heavy rainstorms (Moore *et al.* 1983).

In rainfall simulators, droplet size depends on both the water pressure at which the simulator is operated and the distance the droplets are able to fall. As pressure in the simulator drops, droplet size increases; likewise, as water pressure increases, droplet size decreases, but water application rate increases (Shelton *et al.* 1985). Simulators running more than one nozzle at a time may also produce more variable droplet sizes, as drops from crossing nozzle streams may join together (Cerdà *et al.* 1997). In addition, rainfall simulator model designs employing multiple nozzles may not fire at exactly the same pressure if they are fed from the same water system (T. Danielson pers comm. 2011).

## **Section II**

### **The Effects of Rainstorm Events on Soybean Aphid Populations**

## **Introduction:**

The introduction of the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphidida), to North America has increased soybean pesticide applications and production costs throughout North America. First detected in Wisconsin in July of 2000 (Ragsdale *et al.* 2004), soybean aphid (SBA) can now be found in 30 states and 3 Canadian provinces (Ragsdale *et al.* 2011), and is expected to spread to all major soybean production areas in North America (Heimpel *et al.* 2004).

Researchers in North America and China have worked to better understand SBA biology the impact of environmental factors on in a field setting. Biotic factors such as predation and parasitism (Costamagna and Landis 2006, Desneux *et al.* 2006, Kaiser *et al.* 2007) and disease (Koch *et al.* 2010) and abiotic factors such as temperature (Shusen *et al.* 1994, McCornack *et al.* 2004, Zhang *et al.* 2008) are well studied. One environmental phenomena less understood is rainfall, which has been reported to be responsible for population drops in the field by researchers and growers in Minnesota (Ragsdale 2001).

Soybean aphid is most successfully managed using integrated pest management (IPM) (Ragsdale *et al.* 2006). IPM programs typically incorporate insecticide applications only when aphid populations reach or exceed a set economic threshold; these applications can increase profitability of soybean cultivation when SBA are present (Johnson *et al.* 2009). SBA management plans now use foliar organophosphate and pyrethroid pesticide applications, and more recently neonicotinoids (Johnson *et al.* 2009, Song and Swinton 2009, Hodgson *et al.* 2012).

Cost effective insecticide application requires that treatments are not applied until pest population levels are sufficient to threaten crop yield. Ragsdale *et al.* 2007 showed that the economic injury level (EIL) for soybean aphid populations is 674 aphids per plant. The action threshold (AT) to trigger treatment for SBA is 273 aphids per plant, though often treated as 250. This threshold allows for about 7 days' time to treat for SBA before the EIL is reached (Ragsdale *et al.* 2007). Soybean IPM plans using an AT reduce the frequency of pesticide applications on crops and risks to both the environment and human health (Song and Swinton 2009).

When designing economic thresholds for pest management decisions, it is important to assume that environmental factors will have some effect on pest population behavior and growth (Ragsdale *et al.* 2007). Rainstorm events curb the activity of natural enemies, suppressing pest control (Fink and Völkl 1995), or can increase the chances of an epizootic outbreak in pest species (Dean and Wilding 1971, Koch 2011).

The timing of pesticide applications is critical to successful implementation of pest management systems. Environmental factors, such as rainstorm events, may influence the timing of pesticide applications. Applying chemistry shortly before a rainstorm can result in substantial runoff before it can cause mortality, and require the re-application of the pesticide (Willis *et al.* 1994, Fishel 1997).

While there is little definitive research-based evidence as to why pest populations decline after rainfall events, there are three primary hypotheses found in the literature. The first hypothesis is that rain is responsible for insect mortality. Rain has been proposed to kill insects by washing them off of host plants (Hughes 1963, Maelzer 1977,

Moran and Hoffmann 1987, Moran *et al.* 1987), causing direct death by drowning (Ba-Angood and Stewart 1980b), or secondarily by exposing insects to ground predation (Losey and Denno 1998b).

A second hypothesis proposes that soil particle shifting during rainstorms causes mortality in surface or ground dwelling insects (Fye 1973, Montoya *et al.* 2008). Soil may contribute to insect mortality; a field study of aphids observed that aphids knocked from a host plant during storms may become trapped in mud and die (Walker *et al.* 1984). Insects that dwell on a host plant canopy rather than in the soil may be vulnerable to a soil splash effect, in which the soil is launched away from the soil surface by the impact of a raindrop (Salles and Poesen 2000, Legue´dois *et al.* 2005). Soil splash during rainstorm events may kill insects by knocking them from a plant, or, as observed with insect eggs, death results from desiccation as muddy soil dries over the insect (Shade *et al.* 1969).

The third proposed mortality factor is wind, which may kill insects during storms by increasing the force of the impacting rain (Extension 2012), by shaking insects off of plants during wind events (Dean and Wilding 1971, Jones 1979, Ba-Angood and Stewart 1980b), by damaging the host plants or detaching the plant parts that the insect is feeding on (Williams and Whitham 1986), or by blowing dust and dirt particles during high winds (Naranjo and Ellsworth 2005, Howard and Davis 2012).

**Study Focus:**

This study investigated whether the three primary hypotheses (rain, wind, and soil) were responsible for reported declines in soybean aphid populations after rainstorm



events. Our initial hypothesis proposed that rain would be the most critical factor responsible for aphid mortality. We also hypothesized that high winds in combination with rain were to be responsible for mortality as it was thought that wind driven rain may increase the impact of rain on insects.

Three studies were conducted on the effects of rainfall on soybean aphid populations from the summer of 2010 through the winter of 2011. Two studies used rainfall simulators to investigate the effects of rainfall on soybean aphid. In the first study rainfall was simulated on commercial soybean plots in Northern Minnesota in August of 2010 using a tractor and spray boom system. In the second study a rainfall simulator was used to investigate rainfalls effects on soybean aphid colonies reared in a Northwest Research and Outreach center greenhouse. A third study observed the effects of rainfall, wind, plant stage, and soil on soybean aphid populations on commercial soybean fields throughout northwestern Minnesota.

## **Materials and Methods:**

### **Experiment 1: Field Rainfall Simulation:**

This study was conducted on August 8<sup>th</sup> 2010 in a commercial soybean field immediately Northeast of the NWROC station (47°48'15.61" N 96°36'15.32"W) (figure 1). The field plot design used six treatments with four replications of each treatment. The treatments included application of a no-rain control, 1.27cm, 2.54cm, 3.81cm, 5.08cm, and 10.6cm of simulated rain (figure 2). Every application was applied to plots at 5 PSI regardless of treatment rate, larger applications were applied over a longer period of time. High PSI practice applications produced only mist which would not have simulated rain

droplets. Each plot was a rectangle measuring .91meters x 1.52meters. While the plots were originally intended for a random block design, treatments were eventually laid out in ascending volume for each row (figure 2). A random block design risked trapping the tractor and water tank used to supply the simulator in the higher volume application areas. Aphid population counts were taken from each plot before each treatment and immediately after, two hours after, and 24 hours after the treatments.

Water was applied using Teejet® Turbo FloodJet® nozzles (TeeJet® Technologies 2012). The nozzles were advertised to create large droplets, to more closely simulate rain than a misting spray nozzle, and had a wide angle tip which could be used for more uniform coverage over a soybean plant. Water was supplied through a clean spray tank and delivered by a standard spray boom. The rate of water flow was tracked by monitoring water pressure, (the tractor remained stationary during application), and confirmed by a Chaney Instrument 5” capacity easy-read rain gauge (Chaney Instruments 2012) mounted on a wooden stake within the plot. During each count, five plants were randomly selected from each plot for destructive sampling.

## **Experiment 2: Greenhouse Rain Simulation**

A second rain simulation study was conducted in a greenhouse at the Northwest Research and Outreach center. The simulator was based on blueprints for a Norton Ladder Rainfall Simulator (Blanquies *et al.* 2003, Norton and McAfee 2011). These blueprints were provided by Purdue University researcher Darrell Norton and USDA-ARS researcher Scott McAfee (Norton and McAfee 2011) and modified mechanically for integration into a NWROC greenhouse.

The simulator used two VeeJet® 80100 nozzles (chosen for their ability to produce large rain-like droplets and for their use in previous research on insect rain mortality (Moore *et al.* 1983). The design was modified at the NWROC to accommodate greenhouse dimensions and to connect with the greenhouse water supply. The simulator was suspended 5' above the greenhouse tables and was mounted on a baby rocker motor to provide even coverage was provided by a baby rocker motor. Two Veejet 80100 nozzles were installed in the simulator as suggested by Moore 1983 for their ability to create a rain-like droplet and impact force (Moore *et al.* 1983).

In spring of 2011 an aphid colony was founded in a NWROC greenhouse bay; the founding members were provided by a colony maintained at the University of Minnesota. Secondary colonies were maintained in growth chambers on the NWROC property using aphids from the main colony.

Soybean seedlings (V3-V4) were grown in 15 slot trays using Berger BM2 Germination Mix soil (Berger Supply 2011). Each tray was filled with 3-5 plants and was artificially infested with SBA starting at the V2 stage. Aphids were allowed to settle for a minimum of 24 hours prior to the experiment.

For this study four replications of a single 2.54 cm (1") treatment were run on four different aphid trays. Before each treatment each plant within individual tray slots were divided into two levels (bottom and top) as plant size was small and did not allow division of a canopy into three parts. Non-destructive aphid population counts were taken from each plant and level. The tray was then placed under the simulator after it had been allowed to run for a minimum of 60 seconds to build to full pressure. Water output was

monitored by a Chaney glass rain gauge (5") (Chaney Instruments 2011) and the duration of the application was timed by stopwatch.

The tray was then removed and allowed to sit for 5 minutes to allow excess water to drain. After the trays had drained another non-destructive aphid population count was conducted. The tray was then placed in an isolated corner of the greenhouse away from the rest of the aphid colony; after 24 hours of isolation the tray was given another non-destructive count.

### **Experiment 3: Field Observation Study:**

Data for the aphid field observation study was collected from 10 unique sites throughout Northwestern Minnesota. During the 2010 and 2011 summer season data was collected from 31 unique rain events, 27 of which were retained for analysis (figure 3) (table 1). Data from the 2010 field season was collected from 5 soybean crop fields in 3 locations volunteered by approved cooperators. Three fields were owned and run by the University of Minnesota Northwest Research and Outreach center (NWROC) in Crookston. The remaining four fields were privately owned in Fergus Falls and Underwood and loaned for our research on a volunteer basis.

Data from the 2011 season was collected from 8 fields in 4 locations volunteered by approved cooperators. The University of Minnesota provided several NWROC soybean fields not used in the previous year and soybean plots at the University of Minnesota Magnusson Research Farm in Roseau MN. Four privately owned fields were volunteered by Minnesota growers, three in Underwood, and one in Climax MN.

In 2011, weather data was again collected from the National Oceanic and Atmospheric Administration (NOAA) (Commerce) and NWROC sources, and with the addition of two RainWise MKIII-LR portable weather stations (RainWise 2012). The weather stations were solar powered and transmitted data on wind and rainfall to an external data logger. These stations were placed in trial fields during the aphid pre-count, and weather data was collected from them during the post-count.

Weather data was collected for rainfall in mm, average wind speed m/s, and maximum wind speed m/s. Average wind speed was calculated from averaging all available wind readings during the time that rain was recorded to have fallen, with the addition of all wind readings 30 minutes prior to, and 30 minutes after rainfall. If no rain fell during the collection period, all wind speeds were averaged for the period between the pre-count and post-count. Maximum sustained wind speeds were taken as the maximum sustained wind recording during the rain event or during the collection event for no-rain control events. Rainfall data was taken from in-field weather stations when possible and official NOAA sources when in-field data was unavailable.

Data collection events were conducted when weather forecasts by NOAA (NOAA Administration 2010-2011), Weather Underground (Wunderground 2010-2011), and/or USAirnet (US Airnet 2010-2011) predicted a 30% or better chance of rainfall at any active field site(s). The day prior to, or the day of predicted rainfall, (depending on how rapidly rain systems developed), in-field data on aphid populations and plant stage would be collected.

To collect aphid population data, four or more wooden stakes were placed randomly in a field; line-of-sight was maintained between stakes to ensure they could be located for subsequent aphid counts. Stakes were removed after post-rain counts were completed and randomly replaced during pre-counts for the next rainfall event. However, if another rainfall was predicted within 1-2 days of the post rain count the stakes were not removed and the post-rain count for the previous rainstorm event would serve as a pre-rain count for the next event. Plant stage was also recorded for each count, with plant stage being recorded as the average field stage observed and using the University of Wisconsin soybean plant staging guide (Wisconsin Extension 2004).

Three to five plants were randomly selected for destructive aphid counts within a ten foot radius of a stake. Aphids populations were estimated by counting individual aphids for populations smaller than 100; larger populations were counted by 10's until populations exceeded 1000 per plant, after which aphids were counted in groups of 100. Each plant counted was divided into thirds based on node placement on the stem, each third represented a portion of the canopy (lower, middle, upper) and aphid numbers were recorded for each canopy level for each plant counted. This study did not differentiate between various aphid stages and alate versus apterous morphs.

### **Study Locations:**

For the rainfall simulation study one soybean field was selected for the presence of aphids on NWROC grounds (figure 1). For the field observation study, data from five fields and seven individual data collection events were gathered in the summer of 2010

and data from eight fields and twenty five data collections events were gathered in 2011 (see table 1 and figure 3).

Four events not mentioned above were excluded from analysis, three from 2010 and one from 2011 for sites in Crookston, Fergus Falls, and Underwood MN. These events were rejected as anomalies because they produced significant population increases. When calculating proportion survival (post-rain aphid population averages divided by pre-rainfall aphid population averages) these sites showed population increases far greater than 100% for most stakes). These increases were greater than could be adequately explained by normal aphid population growth. Under ideal conditions soybean aphid populations may double every 1.5 days (McCornack *et al.* 2004), but for the events in question population proportion survival increased 2-5 fold between population counts 1-3 days apart and were not explainable by natural population growth in field conditions. Migration of alate soybean aphids via low level wind currents was thought to be the most probable cause for these sudden large population increases.

**Analyses:**

**HYSPLIT Analysis:**

Before removing rainstorm events where aphid population increases were far higher than explainable by normal reproductive increases, Hybrid Single Particle Lagrangian Integrated Trajectory models (HYSPLIT) were used to explore the possibility that an aphid migration event had occurred. The HYSPLIT modeling system may be used to calculate air particle trajectories as well as simulations of dispersion and deposition (Draxler and Hess 1997, 1998, Draxler 1999, Draxler and Rolph 2013, Rolph 2013).

In this study, HYSPLIT was used to evaluate if wind currents passed low over areas of soybean cultivation known to harbor soybean aphid before traveling to a research site between sampling dates (figures 4-7). We speculated that these currents had the highest potential for transporting aphids to our field site. To explore this possibility backwards trajectory models were used to track air currents shortly before and during the period between counts. When designing the HYSPLIT analysis this study speculated that aphids deposited onto field trials locations during storm events would have been picked up from source locations and transported by LLJ within 1-2 days of deposition.

Wind events capable of picking up aphids would need to be low and aphids may have been sourced from multiple wind events during the same period. With this in mind a backwards HYSPLIT analysis was run for the 48 hours leading up to the post-count event. Each analysis tracked three particle wind trajectories (running a new trajectory every 24 hours) during that time period and specifically looked for winds 500-1500m above the ground level. Wind events of greatest interest were those that dipped to low altitudes over known soybean cultivation areas prior to passing over target field sites. All four models indicated low level air currents crossed low over known aphid refuges and may have introduced populations of alate aphids into the target field. This could account for large increases in population, especially if the alates began rapidly producing apterous offspring on the soybean fields.

#### **Soil Analysis:**

After the collection of field trial data it was decided that obtaining data on soil type in each location would be a useful addition to the research. Soil data was collected



from Roseau, Underwood, and Crookston Minnesota. From each site 15 soil core samples were collected and returned to the NWROC for drying and analysis. Each sample was analyzed for general composition with a hydrometer method test to determine the percent sand clay and silt of each sample (Sheldrick and Wang 1993).

Each soil sample was oven dried and approximately 10 grams from each was used to confirm the sample dry weight. For the hydrometer analysis, samples were mixed with 100 ml of a sodium hexametaphosphate solution (50g sodium hexametaphosphate per 1 L deionized water) and 300mL of deionized water and then allowed to sit overnight. Soil samples were re-suspended by mixing with a milkshake mixer and transferred to 1L graduated cylinders and deionized water was added until the cylinder was filled to 1L. Hydrometer readings were taken with a Fisherbrand® soil hydrometer (5/60GL 1.0) (Fisherbrand® 2012) at 40 seconds and 7 hours after pouring the suspension into the graduated cylinder. Percent sand/silt/clay was determined using the following calculations (Sheldrick and Wang 1993).

$$\text{Sand \%} = 100 - (R_{40s} - R_L) * (100 / \text{oven-dried soil Wt. in g})$$

$$\text{Clay \%} = (R_{7h} - R_L) * (100 / \text{oven-dried soil Wt. in g})$$

$$\text{Silt \%} = 100 - (\text{sand \%} + \text{silt \%})$$

It was advised that United States Department of Agriculture (USDA) soil maps also be consulted; accurate soil composition analysis requires precision laboratory equipment and trained soil research technicians (Dr. Albert Sims, UMN Dept. Soil, Water and Climate, pers. comm.). Soil reports were gathered using the USDA Natural

Resources Conservation Service Web Soil Survey site (NRCS 2012). The field maps from this site were used to determine the dominant soil type for each field location.

### **Aphid Population Data:**

#### **Experiment 1: In-Field Rain Simulation Data:**

Data from the 2010 rain simulation trial was compiled into Microsoft Excel (Microsoft 2010). Each set of aphid 3-5 destructive aphid counts was averaged by both individual sampling location (plot) within the field trial and for each population sampling period (visit). A graphical summary of aphid population averages over each visit can be found in figure 8: a-f. Aphid population averages were then used to calculate proportion of aphid survival across each visit...

$$\text{Proportion Survival} = (\text{pre-treatment aphid population average}) / (\text{post-treatment aphid population average})$$

All subsequent post-treatment counts (immediate, 2-hour, and 24 hour) were calculated using the following method:

$$\text{Proportion Survival} = (\text{post-treatment aphid population average}) / (\text{subsequent post-treatment aphid population average})$$

This data was then compiled in excel and formatted for submission into SAS S® statistical software version 9.2 (32) (SAS 2012) for analysis.

A quantile-quantile evaluation of the data (count/previous count) was performed using SAS® software version 9.2 (SAS 2012) and found that the data needed to be normalized. A log<sub>10</sub> transformation of the data was applied to normalize the data before further analysis. The transformed data on aphid population was examined using a random

intercept multiple regression analysis (PROC MIXED) using SAS® software version 9.2 (32) (SAS 2012).

The linear regression model used the following equation:

$$Y_{ij} = \beta_0 + \beta_1 \text{rain treatment} + \beta_2 \text{location} + \beta_3 \text{sample period} + \beta_4 \text{location} * \text{sample period} + \beta_5 \text{location} * \text{rain treatment} + \beta_6 \text{sample period} * \text{rain treatment} + u_i + \Sigma_{ij}$$

- $Y_{ij}$  = proportion survival at field  $i$  at time  $j$ .
- $\beta_1$  rain treatment = The rainfall treatment applied to a plot
- $\beta_2$  location = The location of a plot within the row
- $\beta_3$  sample period = The point at which the population counts were conducted (pre-treatment, post-treatment, 2 hours post-treatment, and 24 hours post-treatment)
- $\beta_4$  location \* sample period = The interaction between location of the plot within the row and the period in which aphid population was sampled.
- $\beta_5$  location \* rain treatment = The interaction between the location of a plot in the row and the rain treatment applied.
- $\beta_6$  sample period \* rain treatment = The interaction between the period when aphid populations were sampled and the rain treatment applied.

The error terms are defined as  $\Sigma_{ij} \sim N(0, \sigma^2)$  and  $U_i \sim N(0, \sigma^2)$  with both error terms treated as independent. The analysis was tested the significance of water treatment, location within the rows, sample, and interactions between these factors to aphid proportion survival.

## **Experiment 2: Greenhouse Rain Simulation**

The results of the greenhouse rain simulation were compiled in a table (table 2) and a graph (figure 9) with Excel version 2010 (Microsoft Co. Redmond, Washington) (Microsoft 2010) to detect differences between pre and post-aphid population averages. Aphid populations were averaged by tray and sample period for each of the four trials in which the one inch rain treatment was applied.

## **Experiment 3: Field Observation Data:**

Data from both the 2010 and 2011 field seasons were compiled in Excel version 2010 (Microsoft Co. Redmond, Washington) for initial analysis (Microsoft 2010). Graphical representations of the data were compiled in excel and may be found in figure 10 (A-L). Each set of 3-5 aphid counts were averaged by individual stake plot. Each stake average was given a unique ID and was treated as the basic unit of measurement for the experiment. From these stake plots, proportion of aphid survival was calculated for each stake plot as...

Proportion Survival= (average post-rain aphid count)/(average pre-rain aphid count)

These data points were normalized with a  $\log_{10}$  transformation in Excel (Microsoft 2010) and confirmed normal with a quantile-quantile test using SAS® software Version 9.2 (32) (SAS 2012).

All data was analyzed with SAS® software Version 9.2 (32) (SAS 2012). The data was analyzed with a mixed model regression, with a random intercept model based on an existing model structure found in SAS® for mixed models (Littell *et al.* 2006). The multiple linear regression model was constructed using the following equation.

$Y_{ij} = \beta_0 + \beta_1 \text{rain} + \beta_2 \text{average wind} + \beta_3 \text{location} + \beta_4 \text{plant stage} + \beta_5 \text{average starting population} + \beta_6 \text{rain} * \text{average wind} + \beta_7 \text{rain} * \text{maximum wind} + u_i + \Sigma_{ij}$

- $Y_{ij}$  = % mortality at field i at time j.
- $\beta_1 \text{rain}$  = The amount of rainfall in mm that fell on the site between the pre-rain and post-rain count.
- $\beta_2 \text{average wind}$  = The average recorded wind speed recorded during the interval that rainfall was recorded. If no rainfall was observed this was recorded as the average wind speed between the pre and post count interval.
- $\beta_3 \text{location}$  = The field site where data was collected.
- $\beta_4 \text{plant stage}$  = The recorded plant stage during the pre to post-count interval.
- $\beta_5 \text{average starting population}$  = The average aphid population at the pre-count.
- $\beta_6 \text{rain} * \text{average wind}$  = The interaction between rainfall and average recorded wind speed (m/s)
- $\beta_7 \text{rain} * \text{maximum wind}$  = The interaction between rainfall and the maximum recorded wind speed during rainfall. If no rainfall was observed the maximum recorded wind speed between the pre and post-count interval was used.

The error terms are defined as  $\Sigma_{ij} \sim N(0, \sigma^2)$  and  $U_i \sim N(0, \sigma^2)$ , error terms are both treated as independent.

The model is designed to measure possible contributing factors for significance in relation to proportion of aphid survival. Specifically the model is proportion of survival = soil type by location, sample, plant stage, rainfall (in mm), aphid pre-count average, average wind speed (in m/s), maximum sustained wind speed (in m/s), average wind

speed\*rainfall, maximum sustained wind speed\*rainfall. Factors included in the model are location (used to determine the significance of soil type, which varied by location), plant stage (treated as a character rather than numeric effect), pre-count average (used to investigate if pre-rain populations had any effect on survival during rainstorm events).

Weather factors included in the study were rain (in mm). Average sustained wind (m/s) recorded as the average of all wind readings taken during the duration of rain, including readings directly before and after rainfalls and all time between multiple showers if they occurred. For several collection events no rain occurred and all wind readings between pre and post-counts were used to obtain an average wind speed.

Because stake location was randomly chosen in the sampled fields, the factor “stake” was treated as a random effect. Both the model and the random effect were selected based on suggestions from Michael Soma of the UofM Statistical Consulting Service and the input from NWROC and UofM Entomology department research staff members. Model design was taken from “SAS for Mixed Models” (Littell *et al.* 2006) significance was determined using a P=0.05 threshold.

## **RESULTS:**

### **HYSPLIT Analysis:**

Four events (figures 4-7) were ultimately removed from the data set due to large population increases between pre and post-count sampling. Three of these rainstorm events were collected in 2010 were removed, one from Fergus Falls MN, one from Underwood MN, and one from Crookston MN from the data set. A single rainstorm event from 2011 in Crookston was also removed. HYSPLIT analysis was run for each from

500-1500 meters above ground level based on the analysis of potential LLJ events performed by Zhu (Zhu *et al.* 2006) in which trajectories from 600m to 1800m were considered as possible events for transporting aphids (Zhu *et al.* 2006). That study explored aphid migrations in relation to LLJ events and found a significant correlation between the events and aphid capture rates as well as outbreak of aphid vectored disease. In our study, we attempted to discern if extreme increases of aphid populations between counts as seen in 4 events might be explainable by aphid migrations on LLJ's.

The Fergus Falls event was for a 2010 storm event with sampling on the 21<sup>st</sup> and the 23<sup>rd</sup> of July (figure 4). The model was run backwards for 48 hours from the 23<sup>rd</sup> and tracked trajectories from 500-2000m above ground level. Aphid pickup would most probably have occurred along the red trajectory where aphids would have been picked up over Iowa and South Dakota on the 22<sup>nd</sup> before being deposited early on the 23<sup>rd</sup>.

A HYSPLIT model for an Underwood site was run backward from the 2<sup>nd</sup> of August 2010 to the 29<sup>th</sup> of July (figure 5). The long period between pre-count and post-count was the result of several near-miss rainstorm events which were predicted but never arrived. A post-count was conducted on the 2<sup>nd</sup> of August after a light rain to explore what had happened to the population. Large population growth was observed, but it was unclear if this was due in total to natural population growth or to a migratory event and so a HYSPLIT analysis was conducted.

Two trajectories may have introduced aphids into the area between counts. The first is a trajectory that started on the 29<sup>th</sup> in southeastern Minnesota, and traveled through northern and central Iowa before straddling the North Dakota and Minnesota border and

passing over the target field on the 31<sup>st</sup> of July. The second was a trajectory that started in eastern South Dakota on the 31<sup>st</sup> of July and traveled through Iowa and Nebraska before traveling up western Minnesota to the target location. Each of these states has extensive soybean cultivation and sustained seasonal soybean aphid populations.

Another HYSPLIT trajectory model was run for a NWROC field sampled in Crookston MN from the 15<sup>th</sup> through the 18<sup>th</sup> of August 2010 (figure 6). A single trajectory may have introduced aphids into the target field. This trajectory started in Canada on the 16<sup>th</sup> and passed over known soybean cultivation areas in the Dakotas where it circled around and traveled into Minnesota before passing over the target field early on the morning of the 18<sup>th</sup>.

A final HYSPLIT analysis was run for a 2011 field data collection event for a NWROC field in Crookston MN from the 13<sup>th</sup> of July to the 16<sup>th</sup> of July (figure 7). Two trajectories may have introduced aphids into the target field. The first trajectory began on the 13<sup>th</sup> in Michigan and passed through Iowa and Minnesota to reach the target field on the 15<sup>th</sup>. The second trajectory began in Missouri and passed along the border of Nebraska and Iowa, through the eastern Dakotas, and into Northern Minnesota early on the morning of the 16<sup>th</sup>. This pathway traveled over a large number of soybean production areas and may have acquired aphids later deposited on the sampled field.

**Soil Analysis:**

Sample results for all trials were considered inconclusive as composition results conflicted widely, with samples from the same site differing widely in their sand/silt/clay ratios. Subsequent sampling events at the same locations yielded the same extremely varied results. Variability in analysis results resulted from the methods used. Further



input from NWROC soil researchers revealed that soil analysis performed outside highly controlled laboratory settings often results in conflicting and inaccurate readings (Dr. Albert Sims pers. Comm). Instead, soil data was compiled from existing USDA Web Soil Survey map results.

Soil analysis as evaluated from field maps by the USDA Web Soil Survey site can be found in table 3. Of the 5 classified sites, no sites shared the same mix of predominant soils. Sites varied between being predominantly clay and predominantly loam, and secondary soil type varied from silty to sandy. The NWROC sites in Crookston MN were found to be composed primarily of silty clay. The field site in Climax MN was a silty clay loam mix. The 2011 Underwood MN fields were composed of a sandy loam. The Fergus Falls site was predominantly a loam soil, with no major secondary soil type present. The Roseau MN research farm site had a very fine sandy loam soil type.

### **Experiment 1: Field Rainfall Simulation:**

Of the various factors explored in the field rainfall simulation only two factors were found to be significant to changes in soybean aphid populations. The first of the two significant factors was the sample period and second was the sample period\*treatment interaction. Both these factors were significant to the  $p=.001$  level (table 4). The sample factor was used to detect the differences in aphid proportion survival during the four sampling periods (pre-count, immediate post-count, 2 hour post-count, and 24 hour post-count). The sample\*treatment interaction was included in the analysis to detect if there were significant differences in soybean aphid proportion survival as a result of the application of various water treatments.

The plot location within a row (which was not randomized) was not significant in the analysis. Other non-significant factors to the  $p=0.05$  level include the location\*sample period interaction, the water treatment levels applied to the plots were also found to be non-significant, and the location\* water treatment interaction.

A graphical analysis of the data See figure 8 (a-f) reveals aphid averages following a consistent pattern over the 4 counts. Averages dropped for all treatments except the 12.7mm treatment on the immediate post-treatment count. Aphid population averages rebounded by the 2-hour post-treatment count, some substantially higher than the pre-treatment count. Populations then consistently drop by the 24 hour post-treatment count. Control treatment population averages behaved in the same manner as the various treatment options and suggests that aphid populations were not significantly influenced by water treatment.

### **Experiment 2: Greenhouse Rain Simulation Study:**

No formal statistical analysis was run on the data set produced for the greenhouse rain simulation study. The data set produced for this experiment was small and had no data from control trials. Sustained experimentation was prohibited by recurrent aphid colony collapse due to attack by parasitoids and predators. Attacks occurred during the experimental trials used to construct the dataset presented in this paper, therefore ascribing any change in aphid population to an abiotic factor such as rainfall is erroneous. Both the table and graphical results of the experiment show a visible reduction in aphid populations after the application of a 1 inch rainfall in 3 out of the 4 trials conducted (table 2, figure 9). These population reductions were visible in the immediate post-count

data. For 2 of the 3 trials aphid populations were reduced by more than half of their original population.

Despite observing a visible decline in the immediate post-treatment counts of the soybean aphid populations, these populations returned to near original levels by the 24 hour post-treatment counts. In the 3<sup>rd</sup> and 4<sup>th</sup> trials, populations had partially recovered to their original levels, while in the 2<sup>nd</sup> trial the average aphid population had exceeded its original level. Only the 1<sup>st</sup> trial saw no reduction in population between the pre and post-treatment counts and the average aphid population increased between the pre and post counts and the post and 24 hour post counts.

### **Experiment 3: Field Observation Study:**

A number of factors were found to be non-significant in the field observation study (table 5). The soybean plant stage factor, which included both early and mid-stage reproductive stages was not significant ( $p=.05$ ) in this study. However, the analysis indicated plant stage was nearly significant with a p-value of 0.09. Further research on plant stage needs to be conducted to determine if it can be a conditionally significant factor. This study only evaluated plants infested at reproductive stages with closed or mostly-closed canopies. Research on vegetative stage soybean may find that open canopies are significant to soybean aphid proportion survival.

Rainfall, measured in mm, and the interaction between the rainfall and average wind speed (measured in m/s) factors were also found to be non-significant at the  $p=.05$  level. While the interaction between rainfall and average wind speed was not significant, average wind speed as an individual factor was significant to the  $p=.05$  level. Further, the

interaction between rainfall and the maximum recorded sustained wind speed (mm) was also significant. The location factor, used to detect significance in the differences between fields, including soil type, was found to be significant in the analysis. Aphid pre-count populations, which detected significance in aphid populations and crowding effects on proportion survival were also significant.

A graphical analysis of the data (figure 10 a-1) reveals the difficulty in predicting aphid population changes even when rain and wind data is available. Populations often increased even after a rainstorm event, regardless of severity. However, given the significance of wind and maximum wind and rain interactions in the data analysis to proportion survival, we speculate that these populations did not increase as quickly as they would have in the absence of a storm event.

Of note, aphid populations at the Roseau research site declined even in the absence of rainfalls, but did decline during periods of significant maximum wind recordings. Also of note is the extreme similarity in changes to aphid population over time for the 3 2011 Underwood fields. These fields were each about ½ mile apart but ranged in layout from an open field planting, to a shared corn/soy planting, and a third field bordered by a shelter belt.

### **Discussion:**

#### **HYSPLIT Modeling:**

The HYSPLIT models of four outlying observational data sets suggest the possibility of aphid migration events using low level jetstreams (figures 4-7). These events showed large increases in aphid proportion survival, larger than would be

expected from aphid reproduction for a 24-48 hour interval between aphid counts. In each model, there was at least one wind trajectory that passed at low altitudes over known soybean production areas in the hours before traveling over targeted field sites. These trajectories arrived prior to the post-count data sample for each eliminated trial.

The blue trajectory in figure 4 passes over the field site in question just as a rain system that deposited 8.89mm of precipitation passed through. Afternoon rainfalls occurred on the 15<sup>th</sup> and 16<sup>th</sup> of July 2011 just as a series of two trajectories passed low over the target field (figure 6). The presence of rainfall, which is reported to deposit aphids riding wind events, makes a stronger case for the possibility of migration events. The frequency of migrations, both regional and long-distance, by soybean aphid in Minnesota is not well known. Migrations occurring between seasonal host-switching migrations are generally prompted by plant stress and high population densities. All four suspected events occurred between mid-July and Mid-August of 2010 and 2011 when aphid populations are typically going through a seasonal population boom. This natural seasonal population shift may have increased the likelihood of aphid migrations during sampling events, and explain why suspect events were so temporally clustered.

**Soil Analysis:**

Soil type varied widely by the region in which the sample sites were chosen. Most of the soil types were a variety of loam, a relatively equal mix of sand, silt, and clay for basic loams. Most sites were a silty or clay loam which are finer particle mixes and have a higher water holding capacity than straight clay or sand. These soils are slower to drain than larger particle soils like sand and will stay muddier longer (Extension 1999a).

Field observations agree with these findings, these soils were often very muddy for several days after a storm. These soils may provide a muddy trap for any aphids washed from a host plant onto the soil surface and ultimately drown or starve trapped aphids. Location/soil type was significant in the field observation study, which we believe to be the result of differences in soil composition between field sites. Further studies of rainstorm events on soybean grown in a more sand dominant soil could observe a lower rate of mortality than seen in the field observation experiments if aphids are able to walk back to the host plant after the passing of a rainstorm.

### **Experiment 1: Field Rainfall Simulation**

Field simulation observations indicate aphid populations only marginally reduced immediately after the application of simulated rain, regardless of treatment level (figure 8 a-f). Populations returned to or were greater than their original levels by the 24 hour post treatment count. Treatment was non-significant as an individual factor, but may still have had an effect on aphid populations as an interacting factor with sample period, shown to be significant in the analysis (table2). The significance of the sample-treatment interaction could be explained by interference from the non-random block design of the experiment and natural aphid immigration and emigration within the observed field. When aphid counts were conducted, the numbers of apterous versus alate morphs were not differentiated, nor were the various aphid instars.

Alate aphid migration may have occurred in and out of the trial plots, or leaf to leaf migration by walking apterous aphids prompted by disturbance from sampling, tractor disturbance, and treatment application may account for the statistical significance

in sample. It may be that treatments with rain, or heavy rain, prompt more movement and lead to a significant difference in the number of aphids between sample events. Alleys between plots were also populated by soybean infested plants, some of which were damaged by the tractor as it moved through the field. Damage to the alley plants may have prompted aphid movement off of damaged plants and onto plants in plots where water treatments were applied.

The results provided by the field simulation trials present an interesting glimpse into the impact of rainfall on aphids. The field simulation trial was conducted on a windless and very heated day and used a single trial with four replications. It does provide a case for rain having some effect on aphid populations, but this effect was observed to be limited. Additionally, we can't disprove that the experimental layout did not interfere with the results. We also note that the results from the field observational study conducted from 2010-2011 stress that the inclusion of other factors like wind or the presence of a different soil type or plant stage, may significantly affect the number of aphids returning to their host plants.

The destructive nature of the sampling, coupled with the damage created by tractor driven rain application, limited the use of repeated trials in the in-field simulation. The damage to the field used in this trial was extensive enough to prompt some financial compensation to the NWROC for lost revenue. It should also be noted that the nozzles applied in the simulator were only able to simulate a single type of rain, generating a smaller droplet than produced in some rain showers. The application of other types of

nozzles mimicking different droplet size, and dropped from different heights may also result in a significant change in the observed outcome of simulation.

### **Experiment 2: Greenhouse Rain Simulation Study**

Simulated rainfall appeared to cause a visible reduction in aphid populations in the greenhouse simulation study. However, this population reduction was only temporary, and populations were observed to recover by the 24 hour post-treatment count. It is unclear if this was a result of aphids returning to host plants if they were washed off or if this was caused by the production of more offspring on the host plants by survivors of the rainfall application.

The greenhouse simulator was more closely modeled after the accepted standards of rainfall research as determined by soil science, though the height at which the droplets were launched was still lower than the accepted standard and the water pressure in the greenhouse system was extremely variable. Further trials with this simulator were attempted, but aphid populations within the greenhouse were frequently decimated by infestations of natural enemies and parasitoids. Secondary colonies at different locations within the station were likewise eradicated before sustained aphid populations could be found on soybean plants. Further trials both in-field and within greenhouse systems, with accurate rain simulators are needed to further explore the significance of rainfall.

As with the field simulation trial, the greenhouse rain simulation did not differentiate between alate and apterous aphids. During a year's worth of attempts the number of alates present in the greenhouse was observed to be very low and many trials



were aborted due to aphid populations being actively preyed upon by the time of the post-treatment count.

However there are advantages to the greenhouse simulation trials, mainly the ability to limit the variability of factors such as wind, soil type, temperature, and the time and amount of rainfall. Aphid counts could also be conducted with low risk of aphid migration, and individual plants or plant groups could be revisited without the need for destructive sampling.

### **Experiment 3: Field Observation Study:**

In the field observation study, wind is a significant factor, both as an individual factor (average sustained wind) and as an interaction with rain (rainfall\*maximum sustained wind). However, rainfall as an individual factor was not significant, and the interaction between average sustained wind and rainfall was also non-significant. Wind and rain were conditionally significant factors, only as interactions, which was reflected by a lot of research presented in the literature review. Many studies indicated aphid population declines in the wake of windstorms or after rainfalls with high winds.

Maximum wind was most often recorded during the rainstorm rather than in the time recorded before or after the storm event. At our Roseau site, aphid population averages declined during periods with no rain, but high maximum wind readings. It makes sense given the observations of other studies and our own hypothesis that high winds can have a negative impact on aphid populations. Average wind being significant as an individual factor instead of an interacting series of factors reflects the findings of previous publications (Jones 1979, Trumble 1982, Williams and Whitham 1986). These

studies have observed that windstorms, even low speed sustained winds, were enough to dislodge aphids on host plants. Rainfall and wind driven rains were also described as sources of aphid mortality in other published studies.

Exactly at what point wind and rain are conditionally significant to soybean aphid survival in soybean systems remains unclear. Given that average sustained wind is significant our primary assumption is that wind with or without rainstorms has a significant effect on aphid mortality. However, the result that average recorded wind is not significant as an interaction with rain while the maximum sustained wind/rain interaction is prompts us to restructure our hypothesis. We now speculate that that wind as a factor has an overall significance to aphid survival in the field, but only when wind speeds are sufficient to dislodge aphids from their host plants. We believe this to be especially true of gusting winds during rainstorm events, however further research on this phenomena is required to determine how much wind is needed to dislodge soybean aphids from a host plant.

Location of researched fields was significant, though whether it was due to soil effects such as soil splash or soil texture and saturation, or other unknown factors is unclear. Soil was seen frequently to splash on the underside of soybean leaves within the field, occasionally on the undersides of leaves on the upper canopy, where soybean aphids tend to congregate. With younger stage soybean plants (vegetative stages) this effect may be devastating to soybean aphid colonies.

Soil texture and drainage may be the more important features to the significance of location. Each site was predominantly a loamy soil with a high silt content, though the

type of loam varied (see table 4). Loam and silty clay loam textured soils (Fergus Falls Climax and Crookston have a high water holding capacity than do sandy loam soils (found in Underwood and Roseau). These clay and silty clay soils remained muddy for days and often had small pools of water which would have trapped and drowned aphids washed from host plants. Soils with a higher sand content may allow aphids washed from host plants to return to hosts by walking/climbing.

Aphid starting population was also found to be significant, which may mean that population levels per plant may influence aphid location on the host, as in high populations. Displacement from ideal feeding locations on host plants may lead to aphids being in less protected regions of the plant canopy and aphids may more easily be washed from the host.

A differentiation between life stages and morphs in future studies may reveal different results under similar conditions. Rainstorm events could have greater impact if they arrive during periods where there are greater proportions of aphid alate morphs, such as during seasonal migrations or when stressors trigger the generation of more alates. Migrating aphids may be deposited by rainstorms onto fields and leading to rapid population increases in the field.

Rainstorm events arriving during periods after aphid migrations, or during seasonal population increases where there is a greater number of juvenile aphids may mean rapid population declines if juveniles are more vulnerable to rainstorm conditions. Further studies looking at rainstorm event impacts in relation to seasonal changes in

aphid morphs, population, and movement, could aid in the explanation of why results in this study are conditional.

**Conclusions:**

Traditionally, studies have only explored the effects of 1-2 factors during rainstorm events, generally rainfall and wind (Ba-Angood and Stewart 1980b, Walker et al. 1984, Williams and Whitham 1986, Moran and Hoffmann 1987, Moran et al. 1987, Mann et al. 1995, Narayandas and Alyokhin 2006), with occasional publications on the role of soil on insect mortality (Fye 1973, Montoya et al. 2008). The importance of many of the individual factors explored in this study appear less significant than interactions between two or more factors during any given storm, though the significance of individual factors under the right conditions can't be discounted by this study. The results of this study do imply that a more complex system of individual and interacting factors is responsible for insect mortality during rainstorm events than originally explored in scientific publications.

This study can conclude that there are detectible changes in aphid populations in the wake of rainstorms, but only in the presence of a correct combination of rainfall and wind, or high winds. Moreover, these changes may rapidly disappear as aphid populations recover either by a return of dislodged aphids to the host plant, repopulation of survivors, or repopulation by migration. As with population decline, this population regeneration may be closely linked with the abiotic factors active during storm event. That is, aphids dislodged from plants may not die if rainfall and wind do not strike with enough force, or if soils drain quickly, allowing for aphids to return by walking.

Only further study will resolve the question, which is the most significant factor or combination of factors in the reduction of soybean aphid populations during rainstorm events. This research may have further applications in the development of predictive aphid population models in field conditions. Ultimately, a better understanding of the abiotic forces active during rainstorm events, and their impact on aphids and other small arthropods may lead to more accurate modeling and IPM management decision making. That such a universal force on arthropods remains poorly understood is grounds enough for further research.

**Section 3: The Effects of Rainstorm Events on Soybean Aphid Within-Plant  
Distribution**

## **Introduction:**

Aphid distribution and movement have long been a subject of study in the field of Entomology. Research has found a number of biotic and abiotic factors may prompt changes in apterous aphid distribution and movement or the generation of alate aphids. However, aphid movement as the result of abiotic factors associated with rainstorm events is not well understood. Among the aphids whose within-plant distribution is thought to be impacted by rainfall is the soybean aphid *Aphis glycines* Matsumura (SBA).

Many species of aphids, including SBA, have complex life cycles, alternating between generations of sedentary apterous aphids with high fecundity and lower fecundity migratory alate generations (Wang *et al.* 1962, Ragsdale *et al.* 2004). Alate generations produced between seasonal migration cycles are generated in response to ecological stressors, including crowding and reduction in plant nutritional quality (Lees 1967, Hodgson *et al.* 2005).

With the maturation of soybean in the fall, the apterous viviparous female SBA produce a generation of alate males and alate females (gynopara) which then migrate to primary hosts. The gynopara produce a wingless egg laying generation of females (ovipara) which mate with migrant males and lay eggs which will overwinter on the primary host (Ragsdale *et al.* 2004).

Apterous soybean aphids hatch during the spring bud break on Rhamnus buckthorn hosts (Michel *et al.* 2011) and produce several apterous generations before producing a migratory alate viviparous generation (Ragsdale *et al.* 2004, Ostlie 2011).

These alate viviparous aphids migrate to secondary hosts during mid to late spring and produce an apterous viviparous generation, triggered, in part by increasing photoperiod (Hodgson *et al.* 2005). All further within-season generations are primarily sedentary apterous viviparous females until the fall migration back to the primary host (Ragsdale *et al.* 2004, Venette and Ragsdale 2004, Ragsdale *et al.* 2011).

**Study Purpose and Overview:**

This study seeks to assess if higher volumes of rainfall prompt a downward shift in aphid population distribution in the host-plant canopy. We speculated that SBA are washed from higher regions during rainstorms and that survivors on the lower canopy, along with any offspring they produce, lead to a post-rainstorm distribution concentration favoring the lower portions of the host-plant canopy. This study was initiated based on observations communicated to us by Dr. Dave Ragsdale. We speculated that, as with mortality, a number of factors, influenced aphid distribution after storm events. These include rain, wind, and rain and wind interactions.

To explore which factors are responsible for changes to soybean aphid within-plant distribution three studies were conducted at the University of Minnesota Northwest Research and Outreach Center in Crookston MN. In the first study, a preliminary rain simulation trial was completed in the field to determine if distribution changes were visible after applying different levels of simulated rainfall. In the second study, aphid population surveys were taken in fields in the summer of 2011 at various canopy heights within soybean plants in commercial soybean fields to determine if natural rainfall or other abiotic factors were responsible changes in within-plant distribution on different



host-plant canopy levels. A third controlled greenhouse study was conducted to assess the effect rainfall and wind had statistically on soybean aphid distribution in a controlled greenhouse setting.

## **Materials and Methods:**

### **Experiment 1 Rain Simulation Field Study:**

This study was conducted to determine if rainfall initiated changes to soybean aphid distribution in a field setting. The data generated by this study was the same data generated for an analysis of rainfall in aphid mortality in section 2 of this thesis. A Northwest Research and Outreach Center commercial soybean field (47°48'15.61" N 96°36'15.32"W) (figure 1) was selected for a simulation trial due to its close proximity to site farm equipment and easy access to water. Plants were R4-R5 and canopies were closed. A non-randomized block design (figure 2) was flagged within the field on August 9<sup>th</sup> 2010. Each plot was .91meters x 1.52meters and separated by other plots by 10 foot alleys. Three to five destructive whole-plant aphid population counts were taken from each plot before and immediately after each treatment; further counts were taken two hours after treatment, and 24 hours after the treatments. Counts were divided by canopy level, with each plant visually separated into equal thirds by node placement.

Plots received one of six treatments, a no-rain control, 1.27cm, 2.54cm, 3.81cm, 5.08cm, and 10.6cm of simulated rain (comparable to 0", .5", 1", 1.5", 2", and 4" rain event) (figure 2). Rain was applied via a 3-point spray boom using a triple rinsed spray tank filled with Crookston city water. Spray nozzles used to apply treatments were Teejet® Turbo FloodJet® nozzles (Teejet® Technologies 2012). The nozzles were

advertised to create large droplets, which were chosen to more closely simulate rain than a misting spray nozzle, and had a wind angle tip which could be used for more uniform coverage over a plot. Application times were short, ranging from less than a minute to several minutes to apply the prescribed amount of water to a plot. The rate of water flow was tracked by monitoring water pressure, (the tractor remained stationary during application, with overlapping nozzle output on the boom used to more evenly cover a plot), and confirmed by a Chaney Instrument 5” capacity easy-read rain gauge (Chaney Instruments 2012) mounted on a wooden stake within the plot.

## **Experiment 2 Field Observation of Aphid Movement**

An observational field study of aphid movement in relation to rainstorm events was conducted in commercial soybean fields in Crookston MN, Climax, MN, Roseau MN, and Underwood MN. This experiment uses the same pool of data from 2011 field data used in the field observation experiment on aphid mortality in section 2 of this thesis. Fields in Crookston were donated by the NWROC, all other fields were volunteered by local growers. The day before or the day of a predicted rain event (if weather changed rapidly) a minimum of 5 stakes were randomly placed in a target field. At each stake, 3-5 destructive aphid counts were collected; each plant sampled was divided into thirds based on node placement and aphid populations for each third of a soybean plant were recorded. This data was also used for further analysis in chapter two of this paper.

When weather reports by the National Oceanic and Atmospheric Administration (NOAA) (NOAA Administration 2010-2011), Weather Underground (Weather

Underground 2010-2011), or USAirnet (US Airnet) predicted a 30% or better chance of rainfall a data collection event was initiated. Further weather data was collected during or after the rainstorm event. In 2010 all weather data was collected from NOAA and NWROC weather records, while in 2011 weather was collected from NOAA sources and NWROC sources. In 2011, in-field data was also collected using two RainWise MKIII-LR portable weather stations (RainWise 2012). These were solar powered stations that recorded weather data to NOAA standards and transmitted the data to external data loggers. Stations were placed in the field prior to the pre-count, and weather data was downloaded from the data logger during the post-count.

Weather data was collected for rainfall in mm, average wind speed m/s, and maximum wind speed m/s. Average wind speed was calculated from averaging all available wind readings 30 minutes prior to the recorded storm through 30 minutes after the last record of rainfall. If no rain fell during the collection period wind readings were averaged for the pre-count through the post-count collection. Maximum sustained wind was the fastest wind reading m/s taken during the duration of the storm, or during the collection period if no rain had fallen. Rainfall data was taken from in-field weather stations when possible and official NOAA sources when in-field data was unavailable.

### **Experiment 3: Greenhouse Rainstorm Simulation:**

A rain simulator study on aphid movement in relation to rainstorm events was conducted in a NWROC greenhouse in the winter of 2011. This data was also used to analyze the role of rainfall in aphid mortality during simulated rainstorms that was presented in section 2 of this thesis. The rainfall simulator was constructed by adapting

plans for a Norton Ladder Rainfall Simulator (an accepted standard in soil science for accurately simulating rainfall (Blanquies *et al.* 2003, Norton and McAfee 2011) in a small greenhouse setting. Blueprints of the original rainfall simulator design were provided by Darrell Norton of Perdue University and Scott McAfee of USDA-ARS (Norton and McAfee 2011).

The design was modified at the NWROC to accommodate greenhouse dimensions and to connect with the greenhouse water supply. The simulator was suspended 5' above the greenhouse tables and was mounted on a baby rocker motor to provide even coverage was provided by a baby rocker motor. Two Veejet 80100 nozzles were installed in the simulator as suggested by Moore 1983 for their ability to create a rain-like droplet and impact force (Moore *et al.* 1983).

Aphid susceptible soybean seedlings were sprouted in 15 slot trays filled with Berger BM2 Germination Mix soil (Berger Supply 2011) and matured to V3 or V4. Each tray was planted with 5 seeds but produced between 3-5 plants per slot. These seedlings were artificially infested with 10 adult SBA per plant starting at the V2 stage. An aphid colony was maintained in the greenhouse with aphids donated from colony maintained Dr. George E. Heimpel of the University of Minnesota. Seedlings were infested with the aphid covered leaves of older plants, though no deliberate infestation was performed for at least 24 hours before any trial to allow aphids time to settle.

Four replications of a single 2.54 cm (1") treatment were run on four unique aphid trays. Prior to applying a water treatment each plant within individual tray slots were divided into a bottom and top level as plants were small and did not allow for the division

of a canopy into three parts as with the in-field simulation study. Non-destructive aphid population counts were taken from each plant and level for each sample event. The simulator was prepared by running water through the system for a minimum of 60 seconds to build water flow to full pressure. After preparation the trays were then placed under the simulator and left there until the desired amount of water had been applied.

Water pressure remained variable during application, so water application was tracked by a Chaney glass rain gauge (5") (Chaney Instruments 2011). When the application was complete, the tray was removed and allowed to sit for approximately 5 minutes to allow excess water to drain, after which a post-count was conducted. The tray was then placed in an isolated corner of the greenhouse and allowed to sit for 24 hours, after which a second post-count was conducted.

### **Analysis:**

#### **Experiment 1: Rainfall Simulation Trial:**

Aphid numbers were averaged by canopy level (bottom, middle, and top, with each host plant divided into thirds by number of nodes present) and by plot and sample period. Proportional survival was then calculated for each plot and sample period by taking each successive average aphid post-treatment count and dividing it by the previous sample. These data were then  $\log_{10}$  transformed to normalize the data which was indicated by a quantile-quantile plot of the data.

A multiple linear regression analysis was performed using PROC MIXED SAS® software version 9.2 (32) (SAS 2012). The analysis tested for significance in location on the plant, the plant canopy was divided into bottom, middle, and top, and classified as

level in the analysis. The linear regression also looked for significance in plot location within the rows, visit, treatment, or interactions between these factors had significant effects on the results. The linear regression model used the following equation:

$$Y_{ij} = \beta_0 + \beta_1 \text{ locrow} + \beta_2 \text{ sample} + \beta_3 \text{ locrow} * \text{ sample} + \beta_4 \text{ level} + \beta_5 \text{ locrow} * \text{ level} + \beta_6 \text{ sample} * \text{ level} + \beta_7 \text{ rain treatment} + \beta_8 \text{ locrow} * \text{ rain treatment} + \beta_9 \text{ sample} * \text{ rainfall treatment} + \beta_{10} \text{ level} * \text{ rainfall treatment} + u_i + \Sigma_{ij},$$

- $\beta_1 \text{ locrow}$  = Location of the plot within the row.
- $\beta_2 \text{ sample}$  = Designates the aphid population sampling period, pre-rainfall treatment to 24 hour post-treatment.
- $\beta_3 \text{ locrow} * \text{ sample}$  = Analyses interaction between location of the plot within the row and the aphid population sampling period.
- $\beta_4 \text{ level}$  = Analyses the significance of aphid populations at the 3 canopy levels, lower, middle, and upper.
- $\beta_5 \text{ locrow} * \text{ level}$  = Analyses for significance in interactions between the location of the plot within the row and canopy levels.
- $\beta_6 \text{ sample} * \text{ level}$  = Analyses for significance in the interaction between sample period and canopy level.
- $\beta_7 \text{ rain treatment}$  = The amount of water in mm applied to each plot.
- $\beta_8 \text{ locrow} * \text{ rain treatment}$  = Analyses for the significance of interactions between location of the plot and the rainfall treatment.
- $\beta_9 \text{ sample} * \text{ rainfall treatment}$  = Analyses for the significance of interactions between the aphid sample period and the rainfall treatment.

- $\beta_{10}$ level\*rainfall treatment=Analyses for the significance between the canopy level and rainfall treatment.

$Y_{ij}$ =proportion survival at field i at time j.

The error terms are defined as  $\Sigma_{ij} \sim N(0, \sigma^2)$  and  $U_i \sim N(0, \sigma^2)$  with both error terms treated as independent.

Data analysis results are compiled in table 6 and a graphical compilation of the data can be found in figure 11:a-f.

### **Experiment 2: Field Observation of Aphid Movement**

Data for each set of 3-5 aphid counts were averaged for each stake by level. Data on the proportion of aphid survival (used to detect if aphid populations had shifted by level after rainfall) was calculated by dividing the post-count average by pre-count for each collection event by canopy level. These were normalized with a  $\log_{10}$  transformation and confirmed normally distributed with a quantile-quantile test.

A multiple linear regression model (PROC Mixed) was run with SAS® software version 9.2 (32) (2012). The regression sought to determine if rainfall, wind, location, or interaction effects had any significant effect on survivor distribution on the host-plant.

The model used the equation was:

$$Y_{ij} = \beta_0 + \beta_1 \text{location} + \beta_2 \text{sample} + \beta_3 \text{stage} + \beta_4 \text{level} + \beta_5 \text{rainfall} + \beta_6 \text{average wind} + \beta_7 \text{maximum wind} + \beta_8 \text{precount average} + \beta_9 \text{rain} * \text{average wind} + \beta_{10} \text{rain} * \text{maximum wind} + u_i + \Sigma_{ij},$$

- $\beta_1$ location=The location of the field site, also used as a placeholder for varying soil types.
- $\beta_2$ sample=The sampling period, pre and post-rain count.

- $\beta_{3\text{stage}}$ =The plant stage during the sampling period.
- $\beta_{4\text{level}}$ =The canopy level of each subsample, lower, middle, and upper canopy.
- $\beta_{5\text{rainfall}}$ =The amount of rainfall during the interval between sampling periods.
- $\beta_{6\text{average wind}}$ =The average recorded wind speed during the sampling interval.
- $\beta_{7\text{smaximum wind}}$ =The maximum recorded wind speed during the sampling interval.
- $\beta_{8\text{precount average}}$ =The average aphid population during the pre-rain count.
- $\beta_{9\text{rain*average wind}}$ =Analysis of the interaction between rainfall and average wind speed.
- $\beta_{10\text{rain*maximum wind}}$ =Analysis of the interaction between rainfall and maximum wind speed.

$Y_{ij}$ =% mortality at field i at time j

The error terms are defined as  $\Sigma_{ij} \sim N(0, \sigma^2)$  and  $U_i \sim N(0, \sigma^2)$  with both error terms treated as independent.

Because stake location was randomly chosen in the sampled fields, the factor stake was treated as a random effect. Both the model and the random effect were selected based on suggestions from Michael Soma of the UofM Statistical Consulting Service and the input of these authors. Model design was taken from “SAS For Mixed Models” (Littell *et al.* 2006) Significance was determined using a P=0.05 threshold.

Results of the statistical analysis are compiled in table 7 and graphical representations of the data may be found in figure 12 (a-m).



### **Study 3: Greenhouse Rainstorm Simulation**

No formal statistical analysis was run for the greenhouse rain simulations. Formal analysis was forgone due to the small size of the data set and the concern that large proportion predation and parasitism on the studied aphid subjects would skew the results of any statistical analysis. Instead the data for the 4 one inch rain applications was graphed to explore for visual patterns. Data from the rain simulation trials were compiled into a table 8 and figure 13.

#### **Results:**

##### **Experiment 1: Rainfall Simulation Trial**

A number of factors were revealed to be significant in relation to aphid distribution on the various plant canopy levels in the multiple regression analysis to the  $p=.05$  level (table 1). Among these are the sample period (pre-count, post-count, 2 hours post-count, and 24 hours post-count), the interaction between plot location within the row and sample period, the interaction between sample period and aphid proportion survival by canopy level, and the interaction between sample period and treatment applied to the plot. While the analysis looked for significance to  $p=.05$ , three factors were significant to  $p=.001$ . The sample and canopy level was only significant to the  $p=.05$  level with a  $p$ -value of .0263.

Other factors and their interactions explored in this study were not found to be significant. Individual factors found to be non-significant include the location of a plot within the block rows, the canopy level, and the simulated rainfall. A number of interacting factors was also found to be non-significant, including the plot row location

interacting with plant canopy level, the plot row location interacting with water treatment, and the plant canopy level interaction with water treatment (table 1).

A visual analysis (figure 11a-f) of the graphs from the experiment reveals that in many cases, but not all, there was an increase in lower and middle canopy populations. However, there is not an overt decrease in upper canopy aphid populations in relation to individual treatments, indicating that rainfall alone does not have a significant effect on survivor distribution within the plant canopy.

### **Experiment 2 Field Observation of Aphid Movement**

A number of factors were found to be non-significant in this study. Individual non-significant factors included location of the field, plant stage, plant canopy level (divided into a bottom, middle and top level), rainfall (mm) average wind (m/s), and maximum recorded wind (m/s). Two interaction factors, the rainfall average wind interaction and the rain maximum wind interaction were also found to be non-significant.

Two factors were found to be significant to aphid survivor distribution as aphid proportional survival across three canopy levels of host-plants between sample periods. These factors are the sample collection period as an individual factor and the pre-count average aphid population, also as an individual factor. Both these factors were significant to the  $p=.01$  level. The sample period had a  $p$ -value of .007 and pre-count average had a  $p$ -value of .001.

A graphical compilation of the data (figure 12 a-m) reveals that aphid populations tended to increase or decrease uniformly across all levels of the canopy regardless of conditions of exposure.

### **Study 3: Greenhouse Rainstorm Simulation:**

Data for the third experiment was graphed to determine if there was any apparent visual pattern to aphid movement when exposed to rain (figure 13). The graph shows aphid population counts (averaged by canopy level over the entire tray) by sample. The results appear to indicate that the aphid populations did vary between samples but this variation was not constant across trials. There was a population drop in the top level of the canopy between the pre-count and immediate post-count samples for trials two and three, but populations appeared to recover to near pre-count levels by the third sample period. Trial one displayed an overall population increase between the pre and post-count, while trial four revealed a general population shift from the bottom to the top canopy level between counts.

Aphid population averages at each canopy level and sample were also placed in a table format (table 8). Overall populations appeared to shift down the canopy for the first two trials and up the canopy for the fourth trial. The third trial experienced an overall population decrease over both levels of the canopy. Populations for all trials appeared to return by the third sample to levels at or above the pre-count level.

### **Discussion:**

#### **Experiment 1: Rainfall Simulation Trial**

The results of this study disagree in part with our original hypothesis that both rain and wind events lead to changes in aphid survivor distribution in the plant canopy. In this study where the focus was on rain and conducted on a nearly windless day, we found that rain is not as clearly significant to soybean aphid distribution as we originally

believed. Water treatment on its own was not significant, even with the application of large quantities of rain onto the aphids, nor was it significant in the level-treatment interaction. However, rainfall was significant in the sample-treatment interaction.

The former result implies that rainfall did not have a statistically significant effect on aphid survivorship at different levels of the host-plant canopy. The latter implies that the proportion survival of aphids on plants does change in relation to the treatment. The significance of the sample-treatment interaction suggests that there was a difference in aphid proportion survival during the various sample counts based on the application of various water treatments. This may indicate that an application of simulation rainfall does not cause a noticeable change in apterous aphid survivor distribution, but that the alate aphids are moving between canopy levels or leaving host plants entirely.

In this study, alates and new juveniles were present during the sampling but no differentiation was made between alate and apterous aphids and adult versus juvenile aphids. As juveniles were not differentiated, it may also be the result of alate aphids leaving new offspring at the upper canopy after rainstorm events.

Several additional factors must be considered in the evaluation of this study. The study was carried out in a single trial with four replications all on the same plant stage and with identical exposure to ambient weather conditions. The trial itself was conducted on a windless and exceedingly warm day (in excess of 90°F). While a study on soybean aphid distribution and survival did not find temperature to be significant to aphid distribution (Brosius *et al.* 2010), we cannot discount interaction significance between temperature and other factors explored in this study. The replication of this experiment

while varying factors such as ambient temperature, wind speed, and plant stage may yield very different results.

The simulator itself is another piece to consider. The spray boom used to apply water was suspended only a foot over the top of plants in a soybean plot and produced somewhat small droplets. Rain is an exceedingly variable phenomenon (Strangeways 2011) and while the experiment simulated a steady, small drop sized rain in windless conditions, a heavy rain with wind would might have a stronger negative effect.

### **Experiment 2: Field Observation of Aphid Movement**

Field observation data on the significance of rainstorm events on aphid population movement did not detect statistical significance in either wind or rain to survivor distribution on host-plants. This is reflected in the graphical representations of the data wherein there was no clear pattern of distribution change and that changes were present whether or not a storm event had occurred. Several of the data events included sample periods where no rainstorm event arrived though it was predicted. That both wind and rain remain non-significant with the inclusion of these “controls” is especially surprising given that several soybean researchers have reported larger concentrations of soybean aphids at lower portions of the plant canopy in relation to rainstorm events.

In this case we are unable to reject the null hypothesis, that rain and winds have no effect on aphid survivor distribution. Instead, biotic factors were probably responsible for changes in aphid distribution seen in this study. An explanation may be found in the result that the aphid population at the pre-rain count was significant. Aphid population relocation can result from increasing aphid density (Hodgson 1991), and as the host plant

matures (McCornack *et al.* 2008). Higher populations are more likely to stress a host plant and reduce nutritional quality, often increasing the propensity for alate dispersal and a higher production rate of alate aphids (Johnson 1965, Sutherland and Mittler 1971, Hodgeson *et al.* 2005, Lombaert *et al.* 2006).

Given that much of the data was collected in July and August, a time of aphid population boom, aphid populations may have been sufficient to encourage migration throughout the study. The Roseau research site did not apply pesticide to suppress SBA populations which remained in the thousands per plant for the duration of the study and noticeably reduced plant quality. Other sites had periods of high aphid density, possibly sufficient to encourage alate dispersal and production. This study did not differentiate between apterous and alate morphs and cannot disprove the possibility of permanent migration away from sampled plants.

Another biotic factor, the presence of predators, may be responsible for some of the changes in distribution observed in this study. Predator numbers and diversity were not collected during sampling, and cages were not used to exclude aphid predators. Use of cages for predator exclusion may interfere with the effects of rain and wind (Lee *et al.* 2005). Predators have been observed to affect the distribution of soybean aphid populations on host plants (Brosius *et al.* 2010).

### **Study 3: Greenhouse Rainstorm Simulation**

The third experiment indicated some population distribution shifting as the result of an exposure to a 1” rainfall under controlled greenhouse conditions. While no formal

analysis was run on such a small data set, there do appear to be differences in aphid population after the application of simulated rain.

In part, the differences may be overestimated due to low aphid populations per plant as all trays averaged under 50 aphids per canopy level. However, this experiment was conducted on young soybean plants (v3-v4) with open and unsheltered canopies. Plant stems were also weaker than in more mature reproductive plants and the raindrops produced by the VeeJet® nozzles (Spray Systems Co. 2013) were much larger than those produced in the field simulation study. When the simulator was run on younger plants they were observed to shake and bend, and may have more exposed aphids at all canopy levels to more directly to raindrop impact.

This study provides a brief but important glimpse into population shifting in relation to rainstorm events on young soybean plants. The previous two studies could not significantly link rainstorm events to changes in distribution, but these studies were conducted on mature plants with closed canopies.

Further trials for this study were planned to explore the impact of various rain treatments alone and in tandem with wind. However, parasitoids and natural enemies frequently infested the greenhouse systems collapsed both greenhouse and growth chamber aphid colonies and made further research impractical. It should also be noted that alate aphids were present in the greenhouses during trials.

**Conclusions:**

Results from these three studies indicate that soybean aphid population distributions shift on the canopy over time but not necessarily as a result of rainstorm events. An in-field rain simulation study and in-field observation study indicated that

rainfall had no significance to aphid distribution though there was some difference in aphid populations between visits. These differences could be attributed to migration or dispersal initiated by factors other than rainfall and wind or mortality as a result of the rainstorm events. Biotic factors, such as predators, were present in all three studies and can't be ruled out as the primary factor influencing movement.

The greenhouse rain simulation study indicates that rainfall has a visually detectible, though temporary, impact on aphid distribution on young soybean plants. Whether this impact would hold significant with further trials remains unclear, but the possibility that the vulnerability of younger plant stages promotes greater rates of aphid dispersal and redistribution is intriguing.

Of equal note is the rapid redistribution, wherein aphid populations return to nearly original distribution levels within 24 hours. Aphid dispersal and migration is a much studied subject but the possibility that alate aphids redistribute to their original locations could not be found in a literature search. Most descriptions of dispersal and migration appear to indicate a one-way trip. However, the relatively low cost of an alate dispersing down a node or two on a host does not bar soybean aphids from making a return trip up a plant to reintroduce aphids to the nutrient-rich upper canopy.

Further research on alate soybean aphid in-plant dispersal, both in laboratory and field settings, is needed before a working hypothesis on the subject can be presented. This research has led the authors to conclude that alate soybean aphids are naturally mobile within and between plants. This movement may be independent of abiotic factors such as rainfall and wind on reproductive host plant stages with developed canopies. In



vegetative plant stages aphids might be more exposed to abiotic stressors and therefore may migrate down a canopy, though aphids will rapidly return to their original feeding locations when conditions are again favorable.

New research on this subject should work to determine what intensity of stressor is required at a set host stage to initiate aphid movement. Further research should also investigate how long it might take for aphid populations to redistribute to their original locations. Researchers may eventually conclude that abiotic factors have a predictable influence on aphid movement, but further research is needed before such a conclusion can be made. Continuation of this research may lead to the refinement of aphid sampling methods in field conditions, especially if sampling is carried out after a rainstorm event.

**Tables and Figures:**

**Table 1**  
**Overview of Locations and Frequency of Field Observation Events 2010-2011**

<b>Location</b>	<b>2010</b>		<b>2011</b>	
	<b>Field(s)</b>	<b># Events</b>	<b>Field(s)</b>	<b># Events</b>
<b>Climax</b>	0	0	1	3
<b>Crookston</b>	2	3	1	6
<b>Roseau</b>	0	0	3	6
<b>Underwood</b>	0	0	3	9
<b><i>Total</i></b>	2	3	8	24

**Table 2: In-Greenhouse Rain Simulation Aphid Populations by Sampling Period**

<b>Trial</b>	<b>Pre-Treatment</b>	<b>Post-Treatment</b>	<b>24 Hours Post-Treatment</b>
1	43.5	50.2	70.2
2	91.7	63.7	97.9
3	13.2	4.7	6.4
4	7.6	3.5	6.4

**Table 3: Overview of In-Field Rainfall Observational Field Trial Soil Types by Field Location**

<b>Dominant Soil Type By Location</b>	
<b>Crookston</b>	Bygland Silty Clay
<b>Climax</b>	Silty Clay Loam
<b>Underwood</b>	Arvilla Sandy Loam
<b>Fergus Falls</b>	Loam
<b>Roseau</b>	Very Fine Sandy Loam

**Table4: Compiled Results of the Aphid In-Field Rain  
Simulation Analysis of Factors Influencing Aphid Mortality**

<b>2010 In-Field Rain Simulation Results</b>			
Effect	DF	F	P
Location in Row	3	0.47	0.7062
<b>Sample</b>	2	21.53	<b>0.0001</b>
Location*Sample	6	1.62	0.1769
Treatment	5	0.45	0.8094
Location*Treatment	15	0.88	0.5922
<b>Sample*Treatment</b>	10	4.37	<b>0.0008</b>

**Table 5: Compiled Results of the In-Field Observation Study Multiple Regression Analysis on Factors Influencing Aphid Mortality**

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<b>Effect</b>	<b>DF</b>	<b>F Value</b>	<b>P Value</b>
<b>Location/Soil Composition</b>	7	5.98	<i>&lt;.0001</i>
<b>Stage</b>	5	1.94	0.09
<b>Rain</b>	1	0.41	0.52
<b>Average Wind</b>	1	10.98	<i>0.001</i>
<b>Ave Starting Pop</b>	1	6.81	<i>0.01</i>
<b>Rain*Ave Wind</b>	1	1.99	0.16
<b>Rain*Max Wind</b>	1	7.92	<i>0.006</i>

**Table 6: Compiled Results of the In-Field Rain Simulation Multiple Regression Analysis on Factors Influencing Survival By Canopy Level**

**2010 In-Field Rain Simulation  
Trial Results**

<b>Effect</b>	<b>DF</b>	<b>F Value</b>	<b>P-Value</b>
Locrow	3	0.44	0.7266
<b>Sample</b>	2	79.87	<b>&lt;.0001</b>
<b>Locrow*Sample</b>	6	5.31	<b>&lt;.0001</b>
Level	2	0.35	0.7034
Locrow*Level	6	0.89	0.5052
<b>Sample*Level</b>	4	2.84	<b>0.0263</b>
Trt	5	0.81	0.5455
Locrow*Trt	15	0.38	0.9833
<b>Sample*Trt</b>	10	11.55	<b>&lt;.0001</b>
Level*Trt	10	0.46	0.9112

**Table 7: Compiled Results of a Multiple Linear Regression Model Analysis on Factors Influencing Aphid Distribution from In-Field Observational Data from 2011**

<b>Effect</b>	<b>DF</b>	<b>F-Value</b>	<b>P-Value</b>
Location	5	0.68	0.639
<b>Sample</b>	1	7.15	<b>0.007</b>
Stage	5	0.39	0.856
Level	2	1.1	0.334
Rain	1	0.26	0.61
Ave Wind	1	0.02	0.88
Max Wind	1	0.01	0.926
<b>Pre-count Average</b>	1	11.29	<b>0.001</b>
Rain*AveW	1	0.01	0.926
Rain*MaxW	1	0.06	0.806

**Table 8: Compiled Results of Observed Aphid Population Averages by Canopy Level During In-Greenhouse Rainfall Simulation Trials**

<b>Count</b>	<b>Trial 1</b>		<b>Trial2</b>		<b>Trial 3</b>		<b>Trial 4</b>	
	<b>b</b>	<b>t</b>	<b>B</b>	<b>t</b>	<b>b</b>	<b>t</b>	<b>b</b>	<b>t</b>
<b>1</b>	0.85	18.90	3.90	39.90	4.11	1.32	2.40	0.73
<b>2</b>	2.02	20.61	3.10	27.90	1.59	0.41	1.13	1.30
<b>3</b>	1.80	30.44	3.43	41.80	2.08	0.59	1.40	1.20



Figure 1: The location (highlighted in blue) of the single trial rain simulation conducted at the Northwest Research and Outreach Center in Crookston MN in 2010.



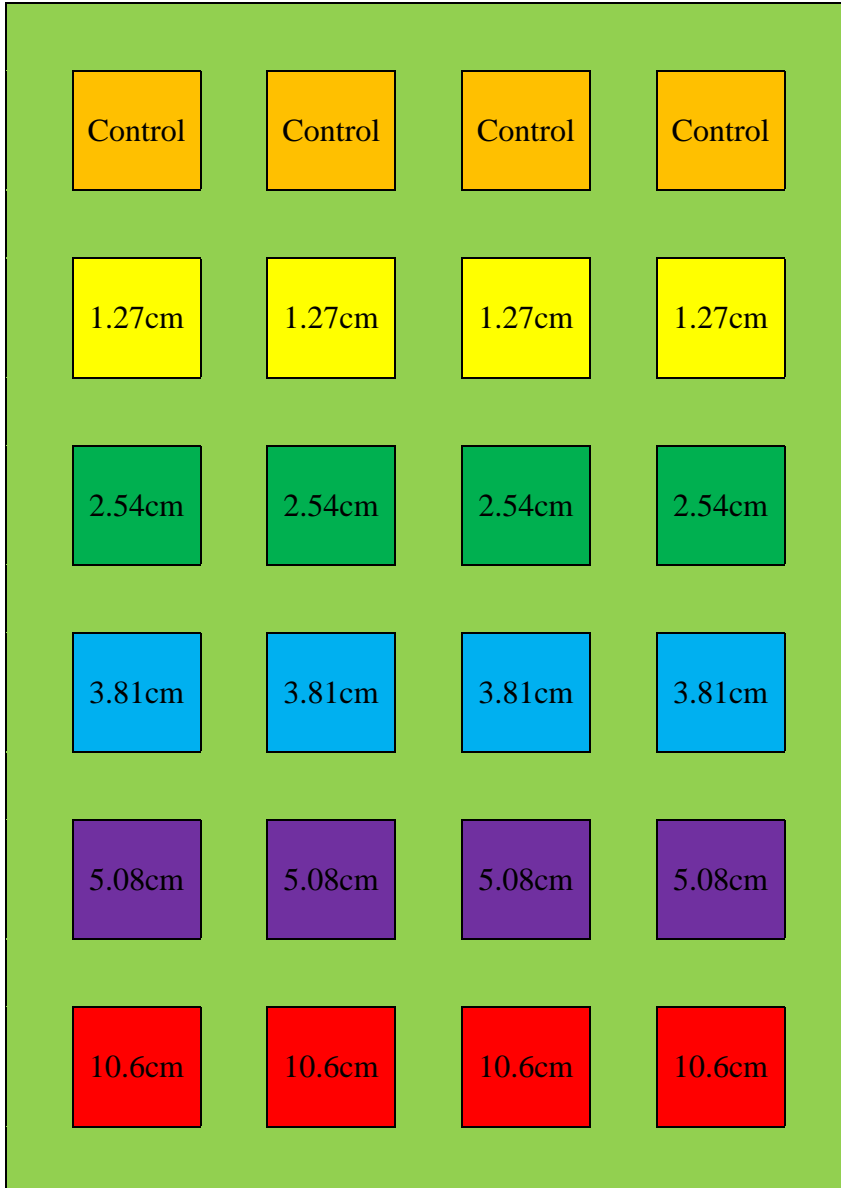


Figure 2: The trial map for the rain simulation study. Plots were not randomly blocked as the large volumes of water for the higher rate applications applied to the trials risked trapping our tractor and water tank in mud.



Figure 3: A comprehensive map of all Minnesota field site locations for both the 2010 (pinned in red) and 2011 (pinned in yellow) seasons. Fields were located near Crookston, Fergus Falls, and Underwood in 2010. In 2011 fields were located near Climax, Crookston, Roseau, and Underwood. This image includes sites that were later removed due to suspected migration events.

NOAA HYSPLIT MODEL  
 Backward trajectories ending at 0000 UTC 23 Jul 10  
 GDAS Meteorological Data

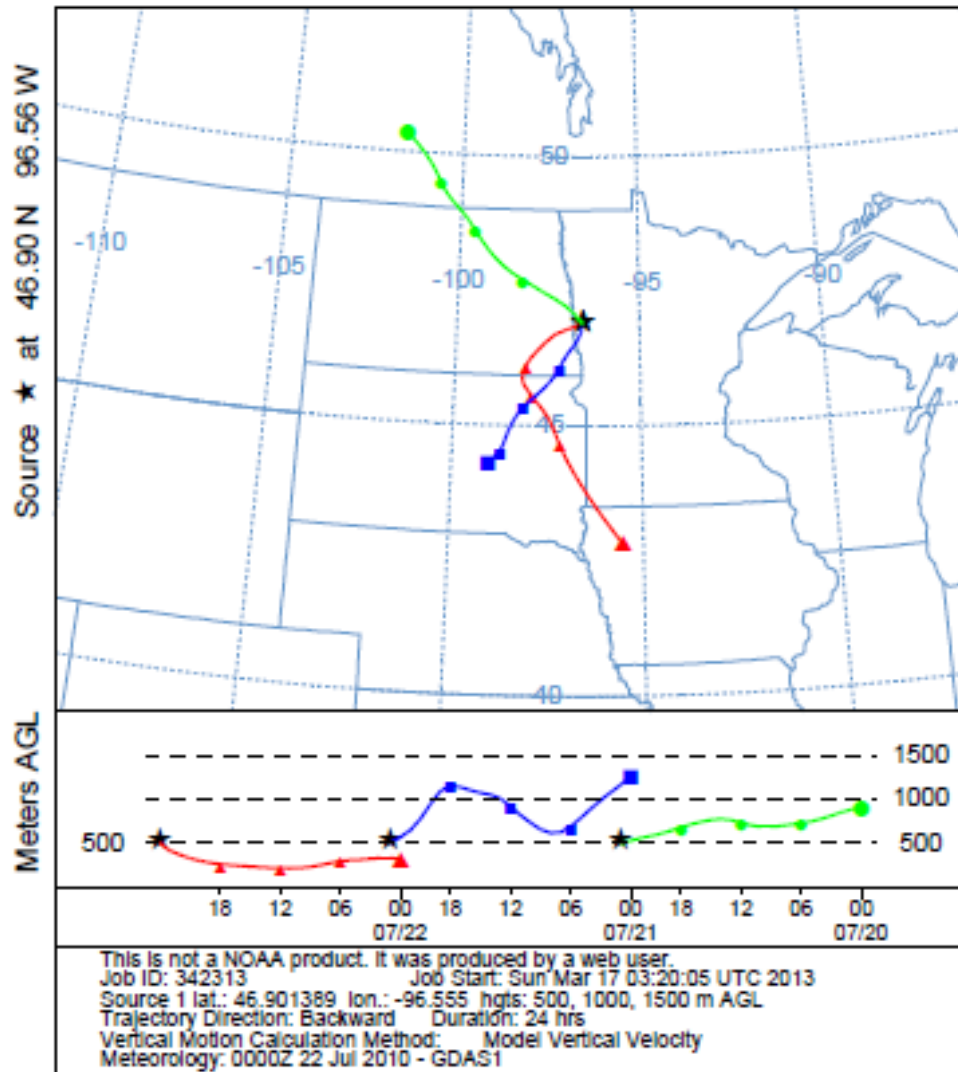


Figure 4: A HYSPLIT model of a Fergus Falls field site for a 2010 storm event with counts from the 21<sup>st</sup> to the 23<sup>rd</sup> of July.

NOAA HYSPLIT MODEL  
 Backward trajectories ending at 0000 UTC 02 Aug 10  
 GDAS Meteorological Data

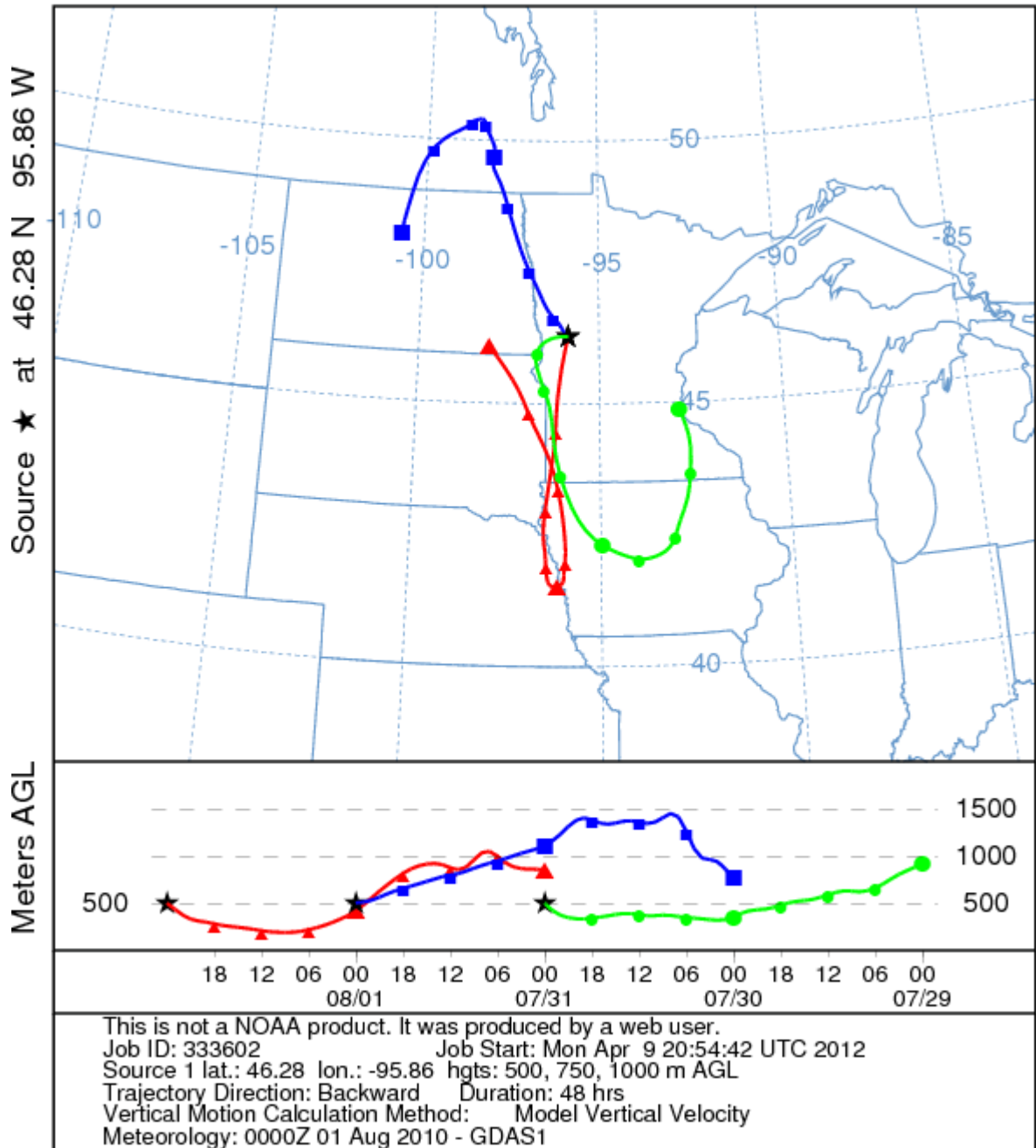


Figure 5: A HYSPLIT model of an Underwood field site with counts from the 29<sup>th</sup> of July to the 2<sup>nd</sup> of August 2010.

NOAA HYSPLIT MODEL  
 Backward trajectories ending at 0000 UTC 19 Aug 10  
 GDAS Meteorological Data

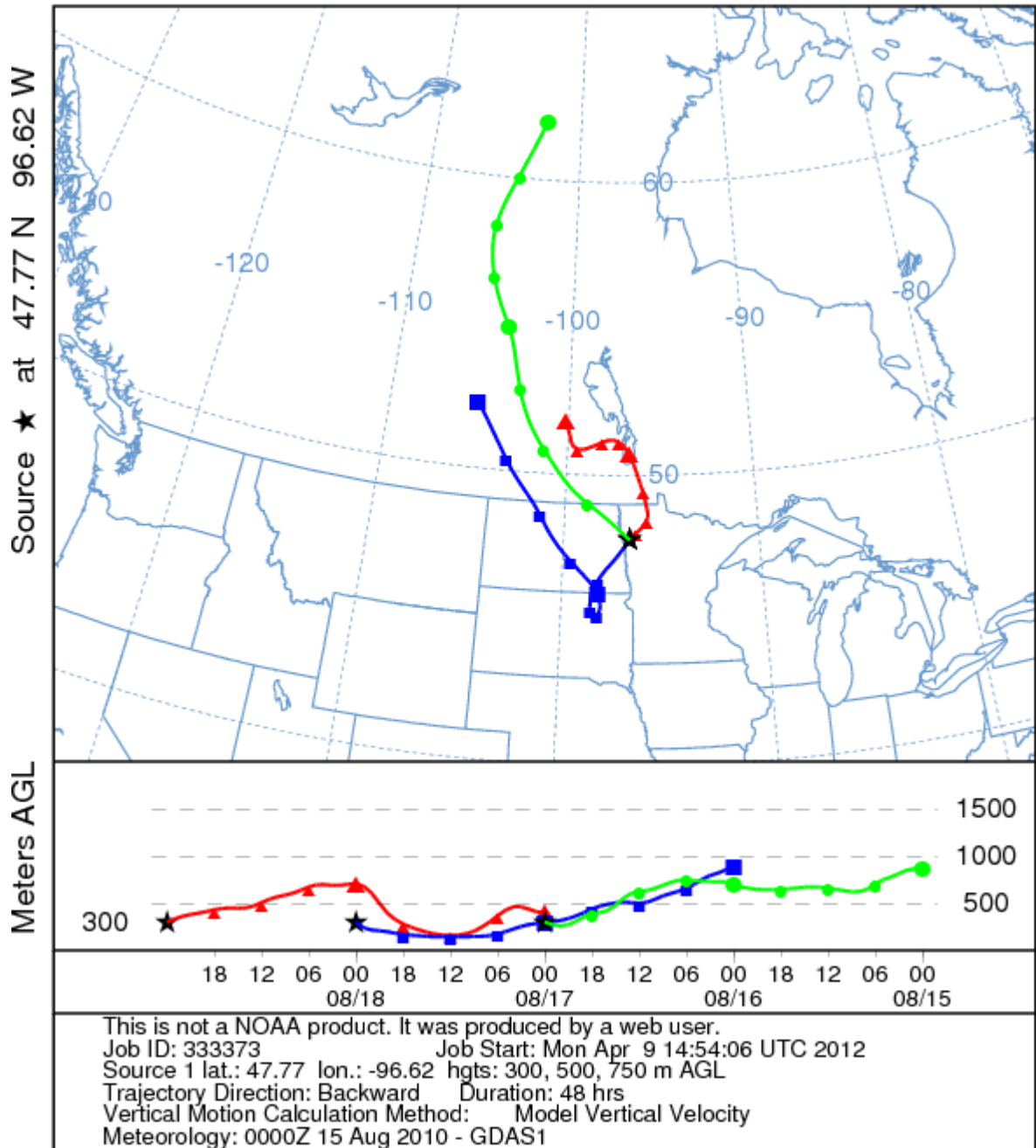


Figure 6: A HYSPLIT trajectory model for a 2010 NWROC field in Crookston MN running from the 15<sup>th</sup> to the 18<sup>th</sup> of August.

NOAA HYSPLIT MODEL  
 Backward trajectories ending at 2300 UTC 16 Jul 11  
 GDAS Meteorological Data

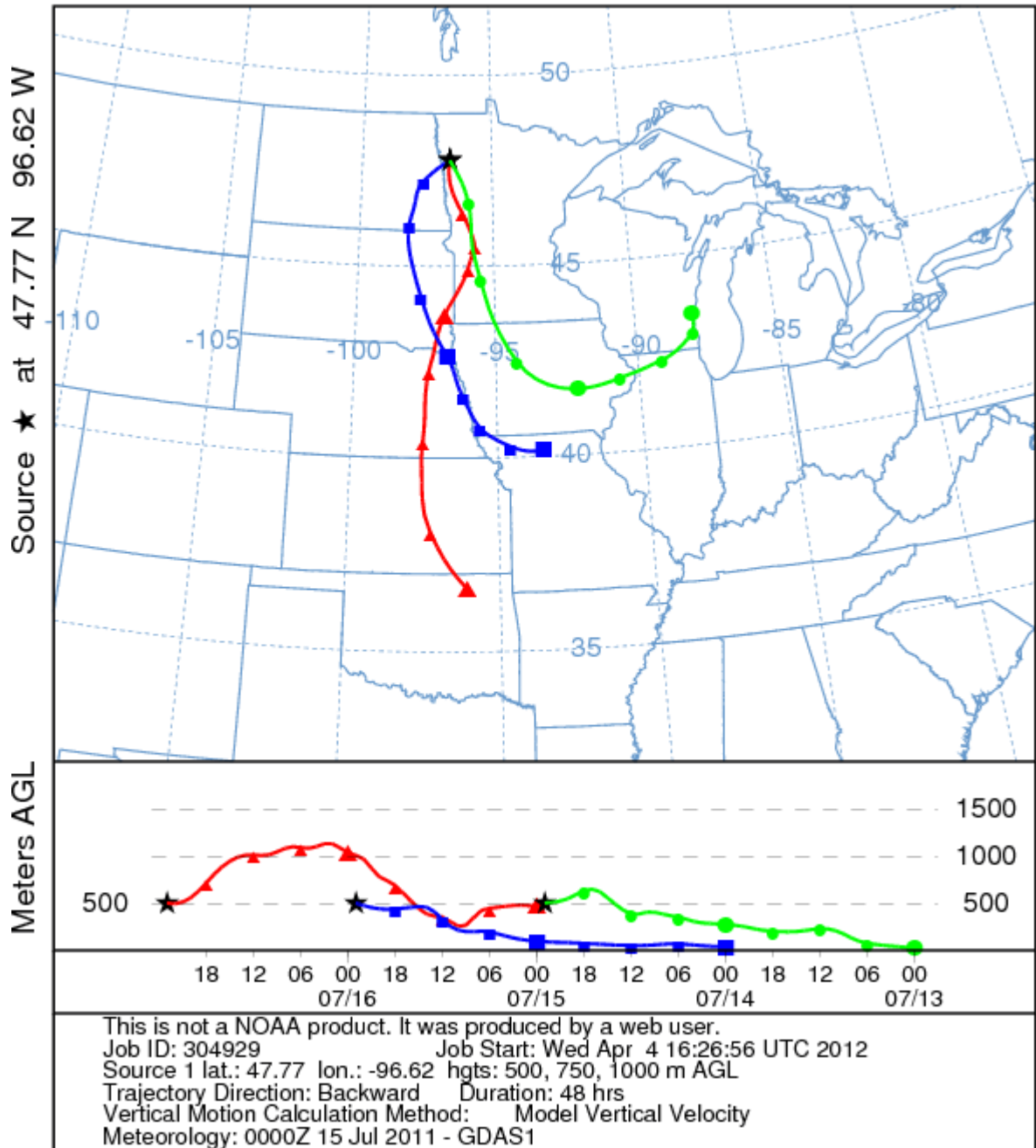
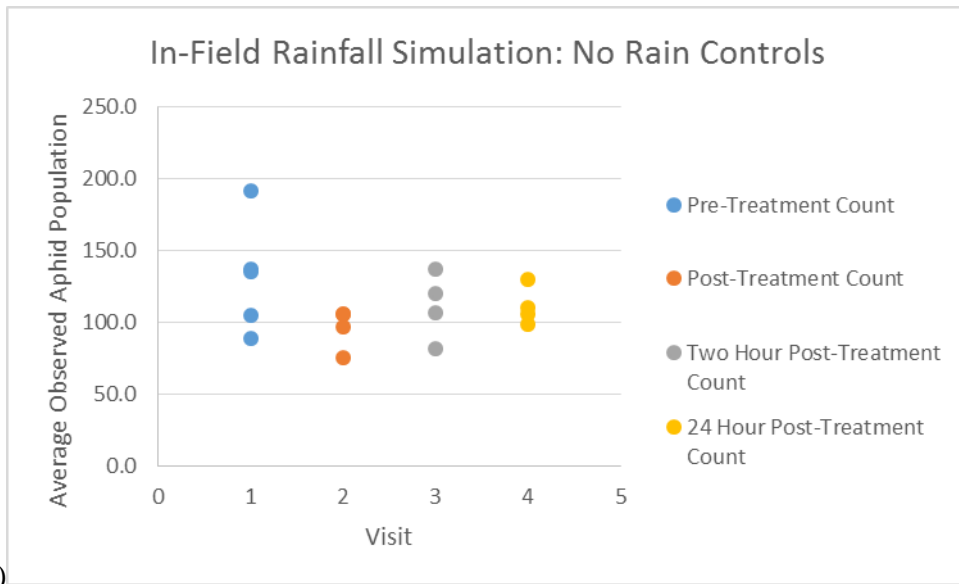
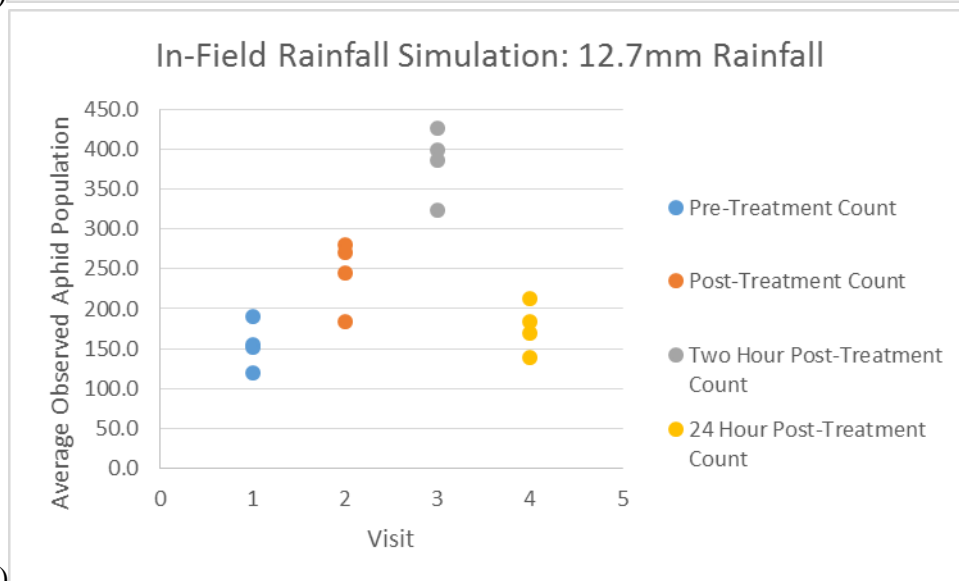


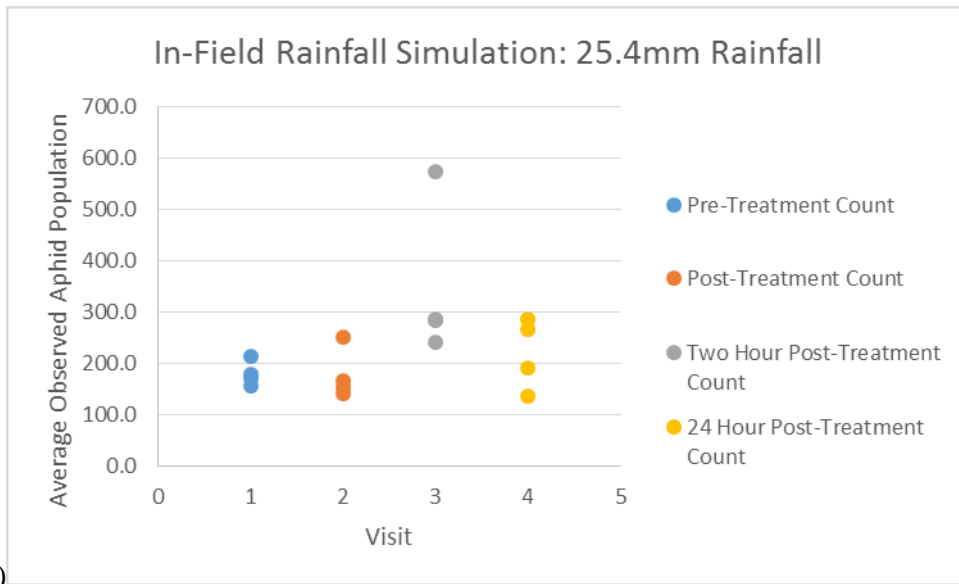
Figure 7: A HYSPLIT model of a 2011 Crookston MN from the 13<sup>th</sup> of July 2011 to the 16<sup>th</sup> of July 2011.



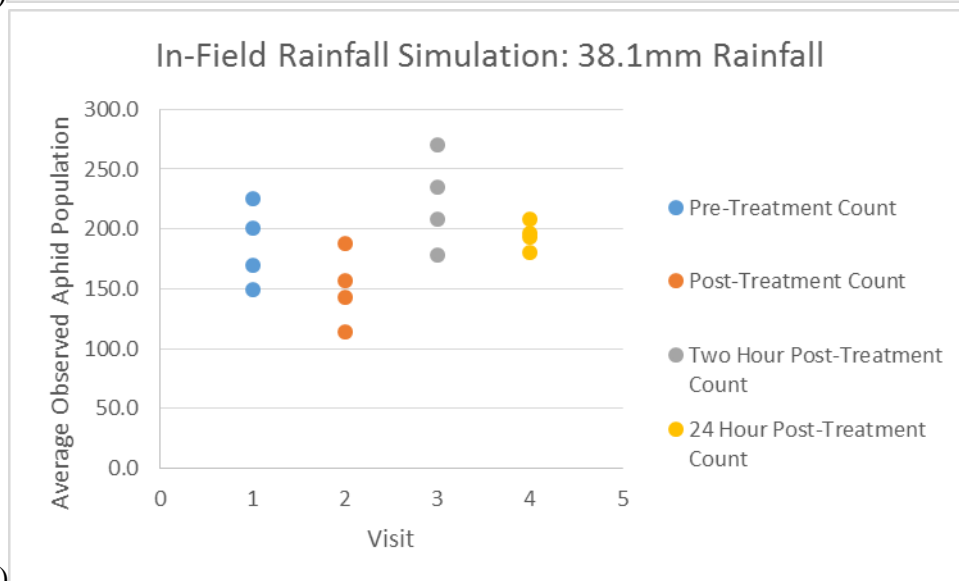
a)



b)

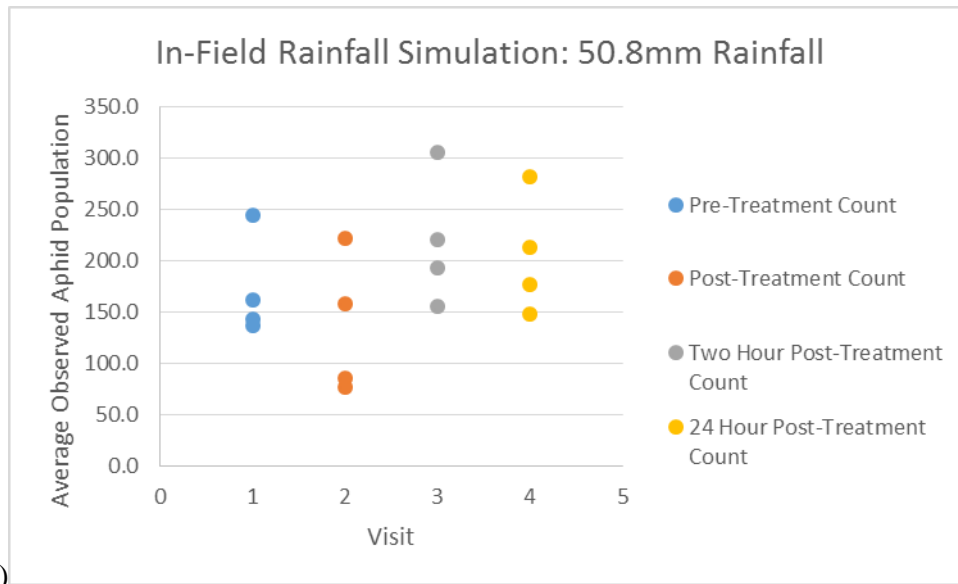


c)

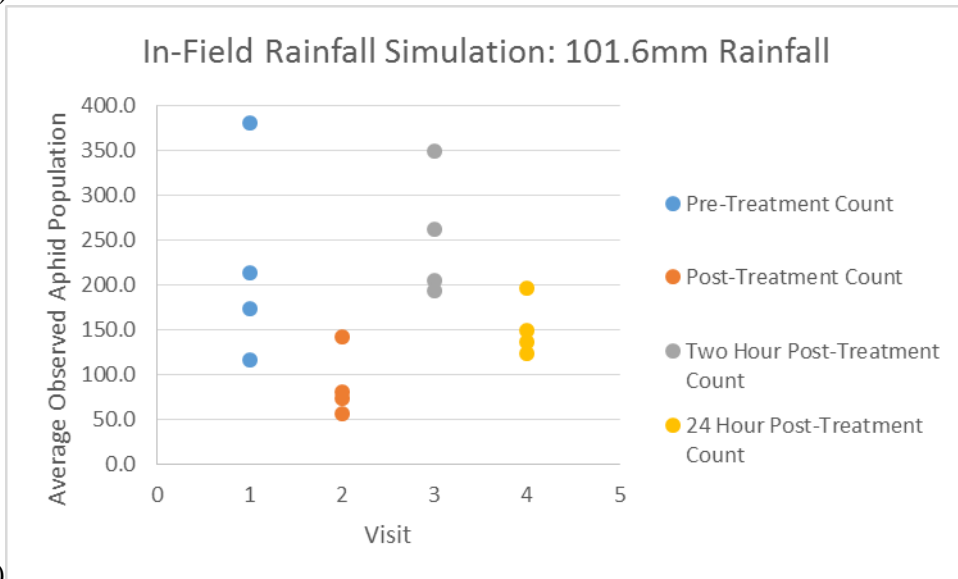


d)





e)



f)

Figure 8 (a-f): Summarizes the results on an in-field rainfall simulation conducted in Crookston MN in the summer of 2010. Each table represents observed aphid population averages for each of the 4 replications of each rain treatment applied to plots in the trial. Aphid counts were taken over the course of four visits, pre-rain treatment, post-treatment, 2 hours post-treatment, and 24 hours post-treatment.

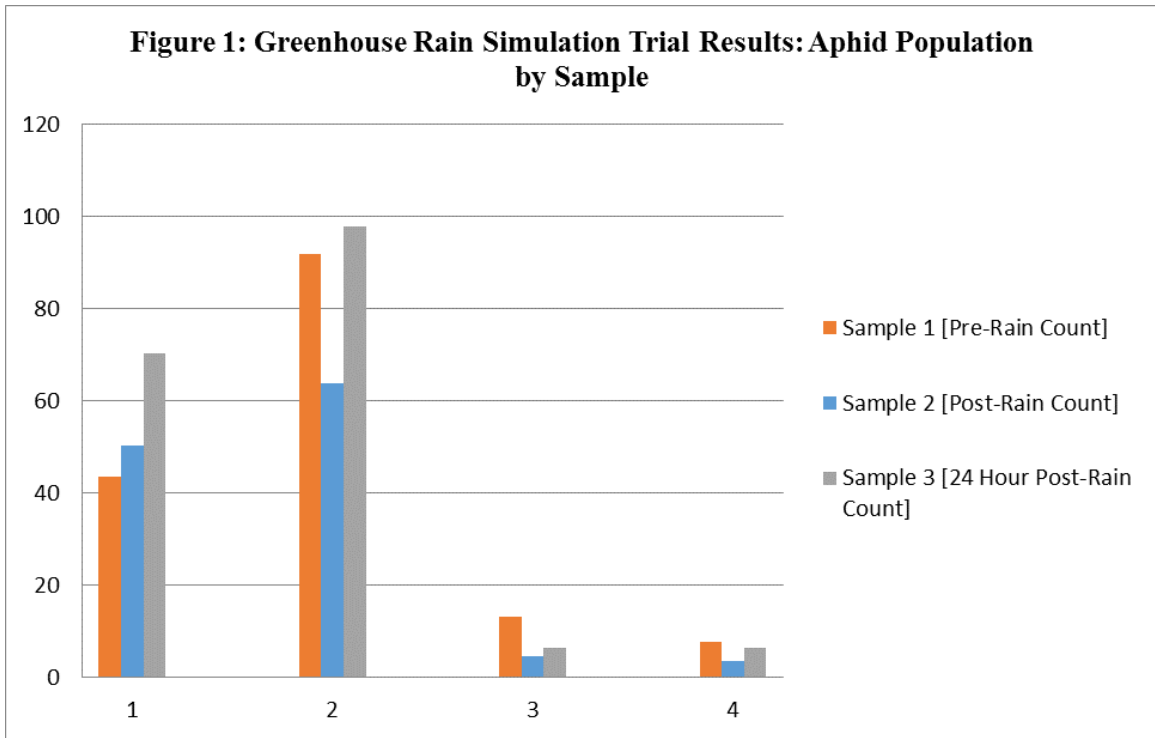
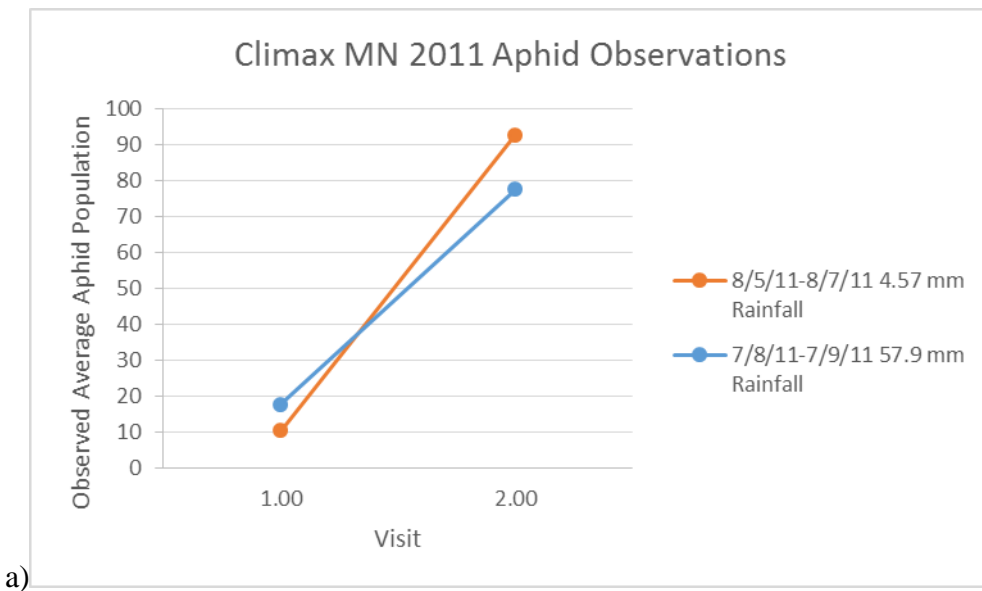
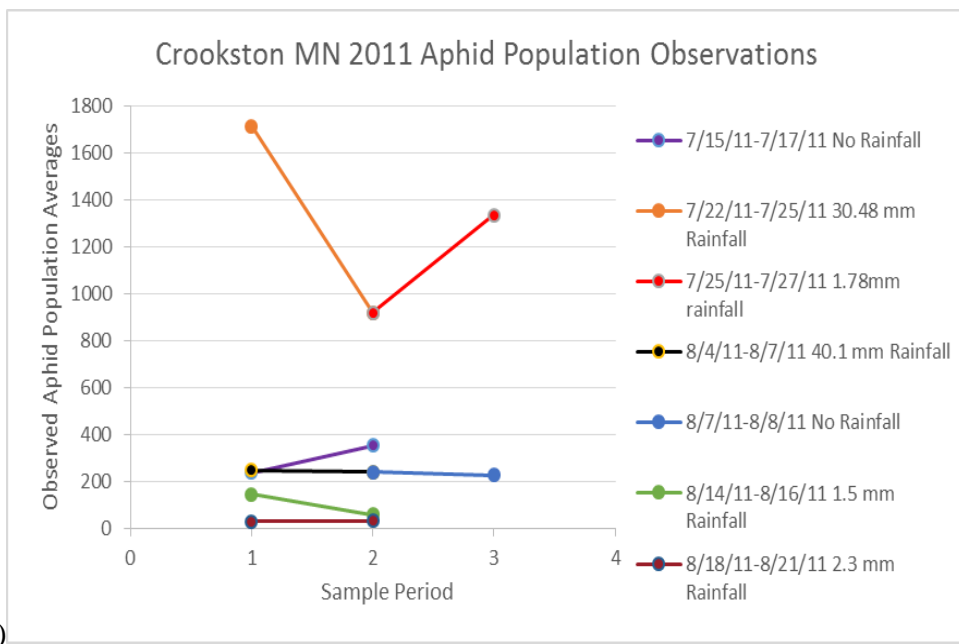
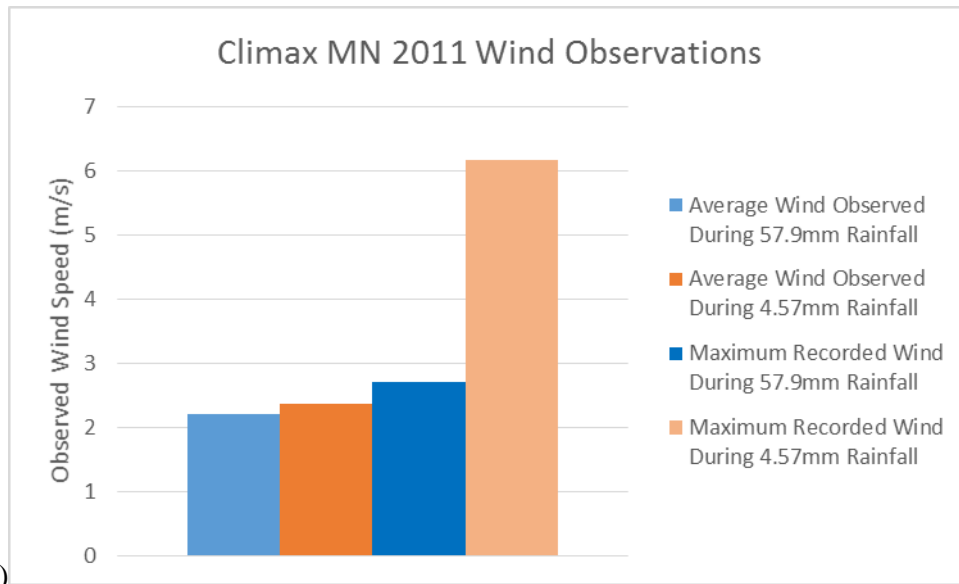


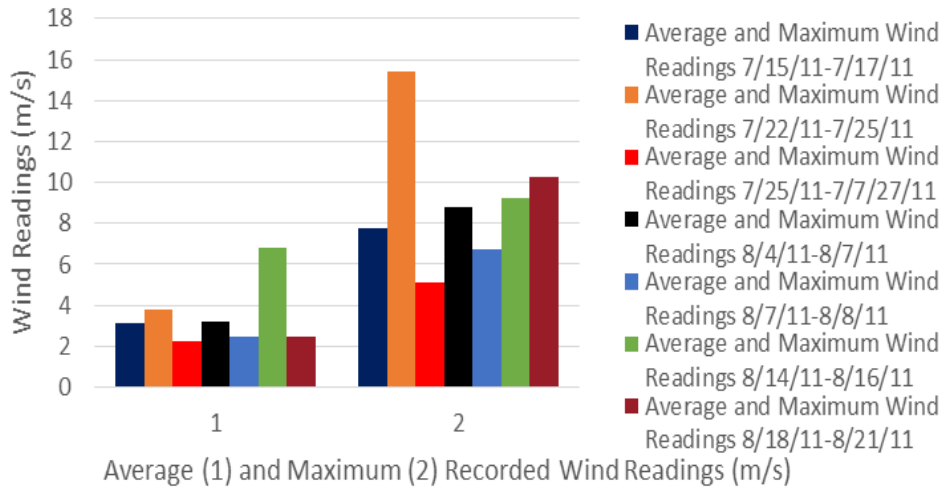
Figure 9: A compiled summary of averaged aphid populations by canopy level for 4 in-greenhouse rainfall simulation trials conducted at a NWROC station in 2011.



a)

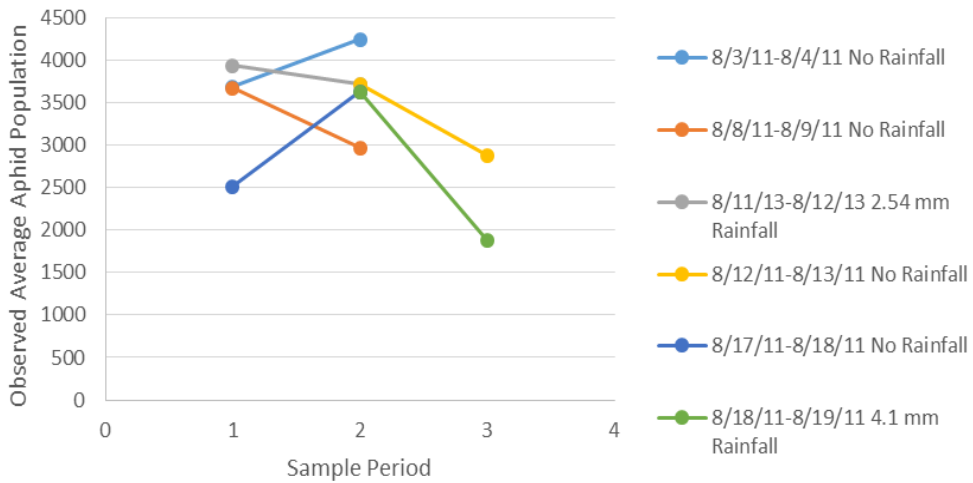


### Crookston MN 2011 Average and Maximum Wind Readings

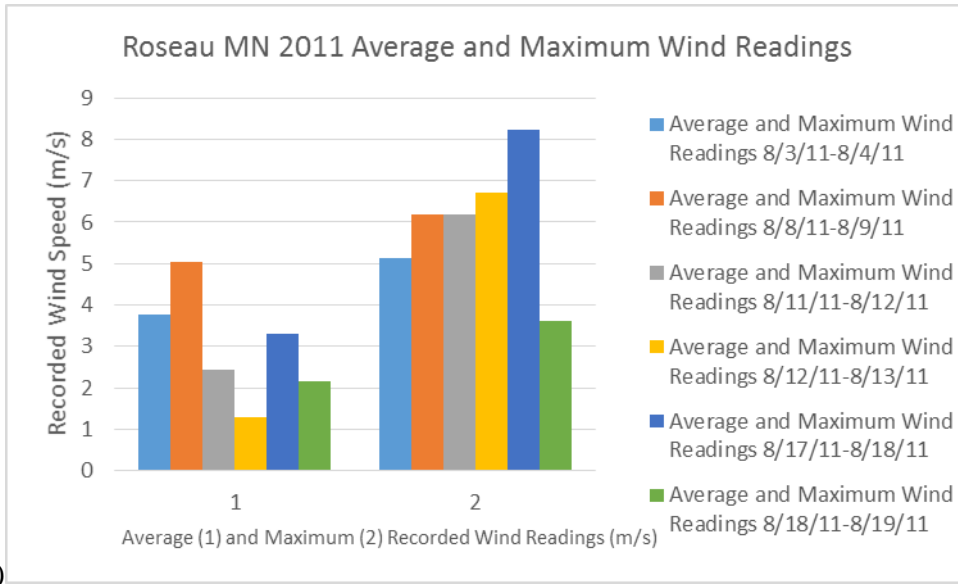


d)

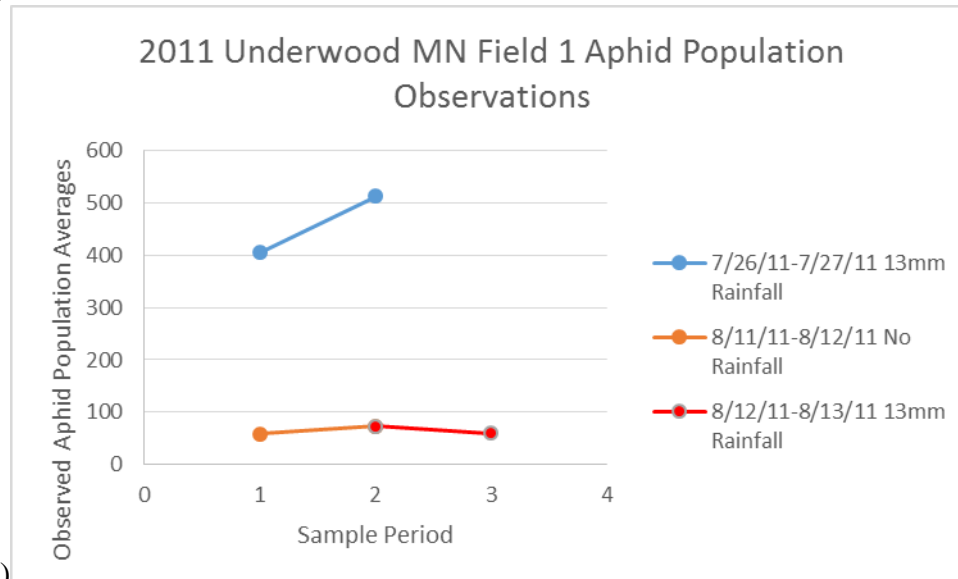
### Roseau MN 2011 Aphid Population Observations



e)

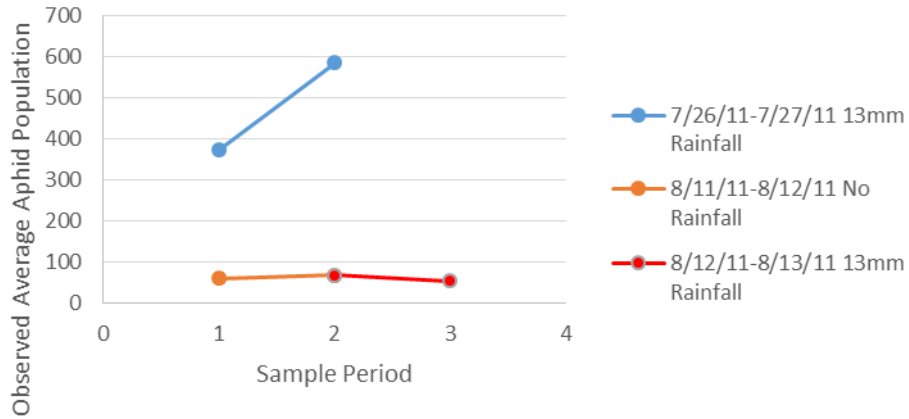


f)



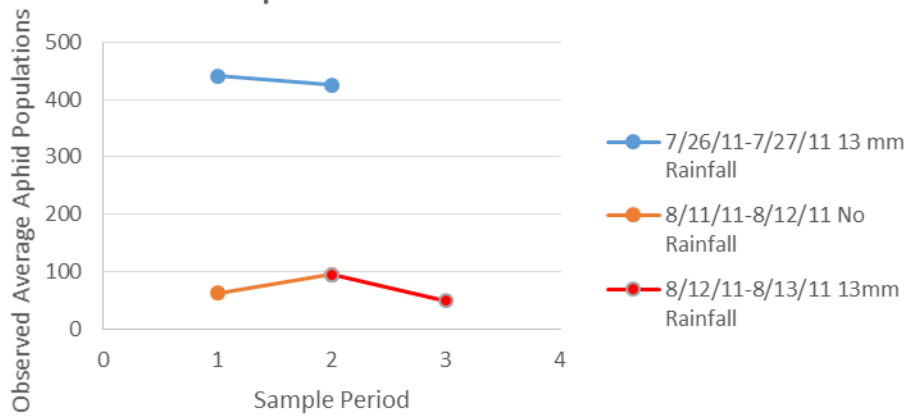
g)

### 2011 Underwood MN Field 2 Aphid Population Observations

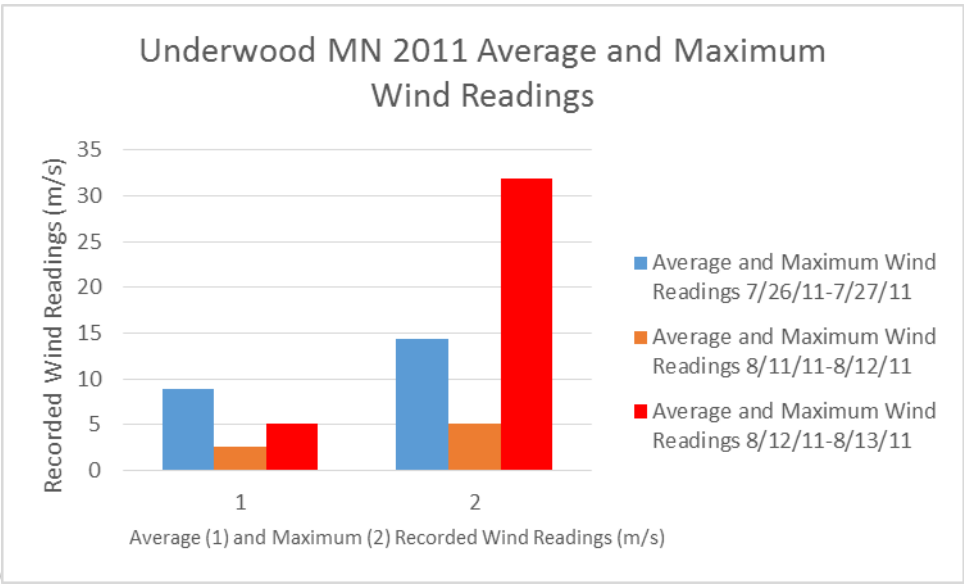


h)

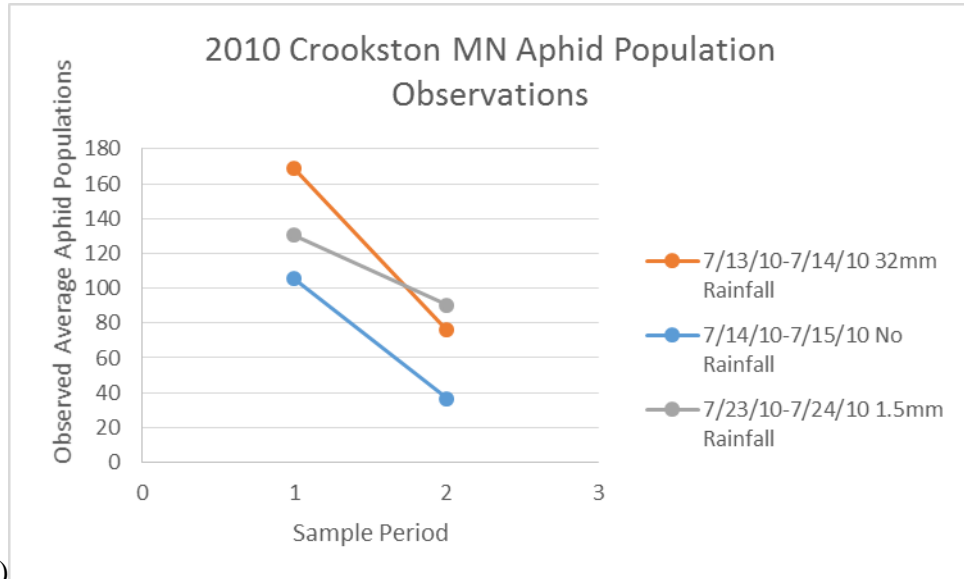
### 2011 Underwood MN Field 3 Aphid Population Observations



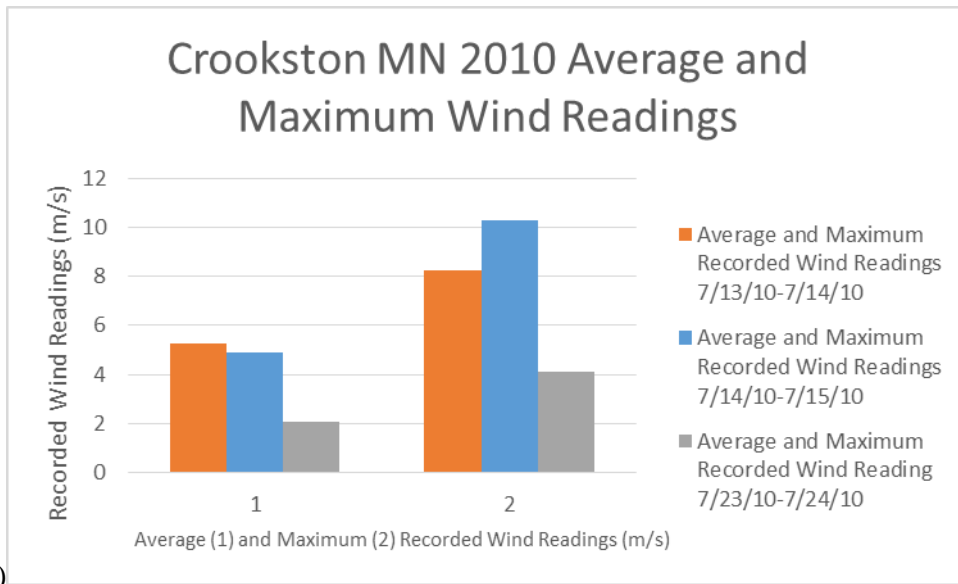
i)



j)

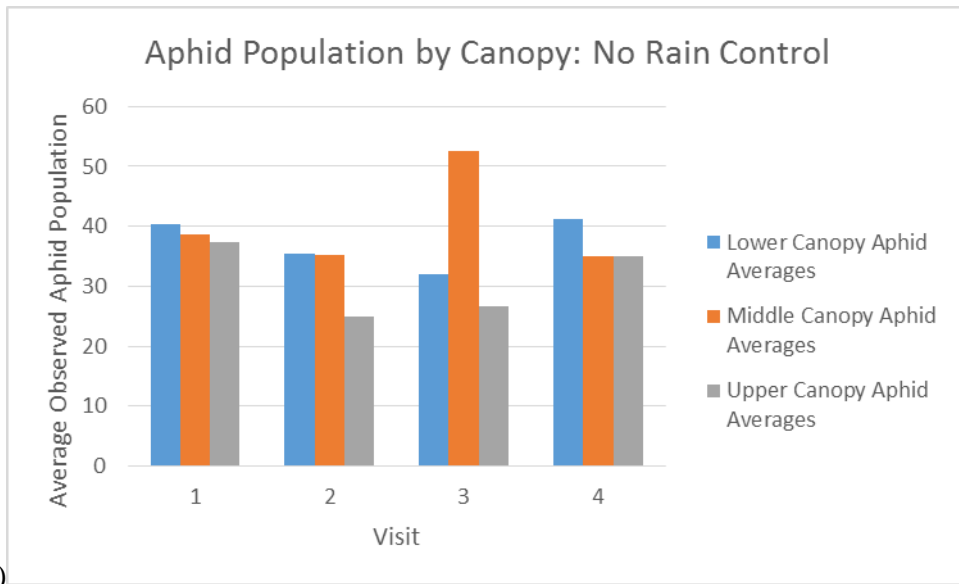


k)

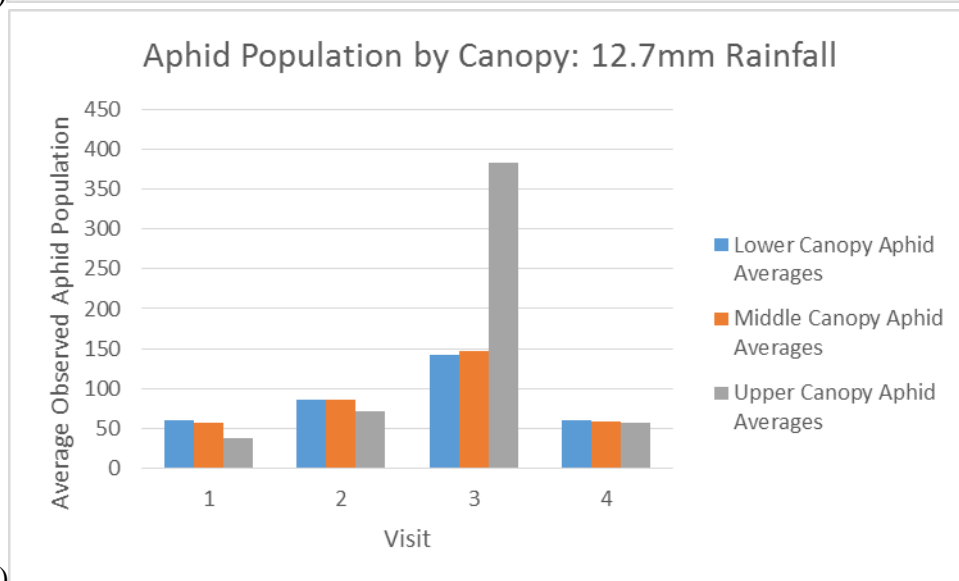


1) Figure 10 (a-1): A graphical summary of observed aphid population averages and corresponding wind readings by site and visit for aphid field observations taken from the summer of 2010 through 2011 in various field sites throughout Minnesota.

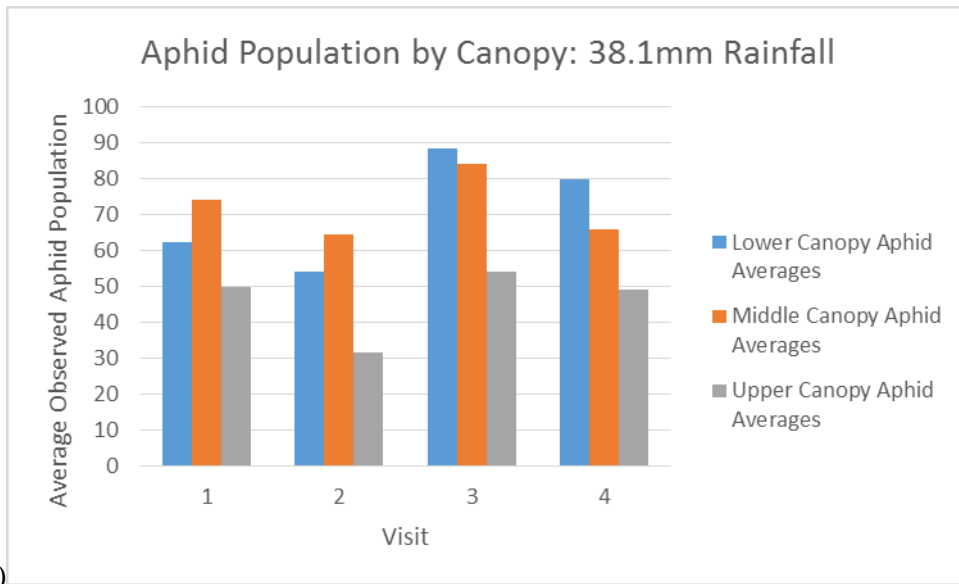




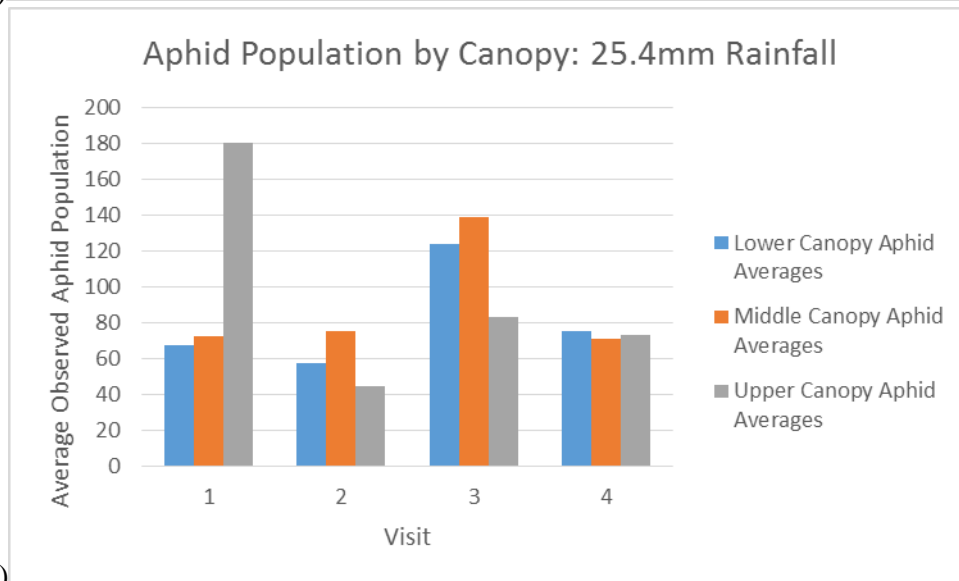
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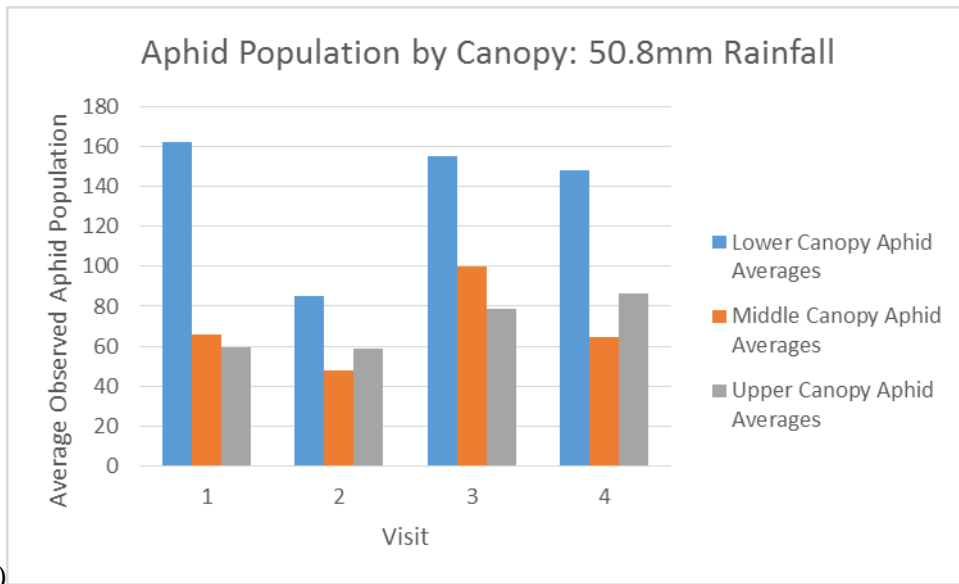
b)



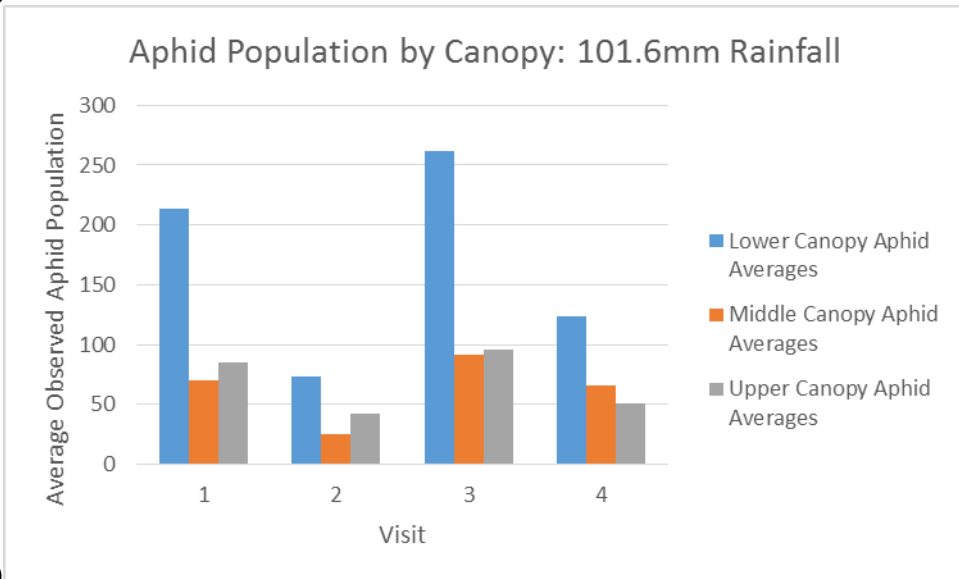
c)



d)

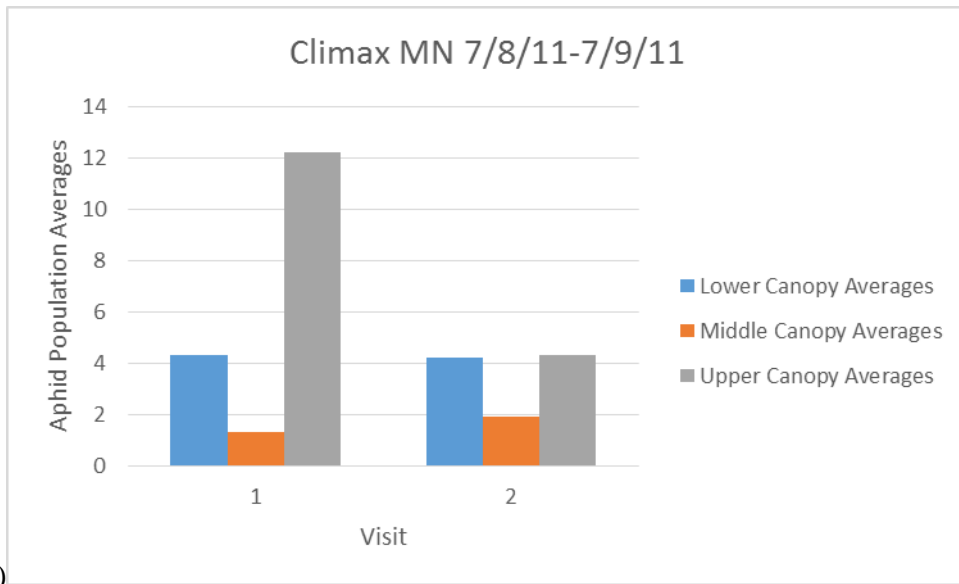


e)

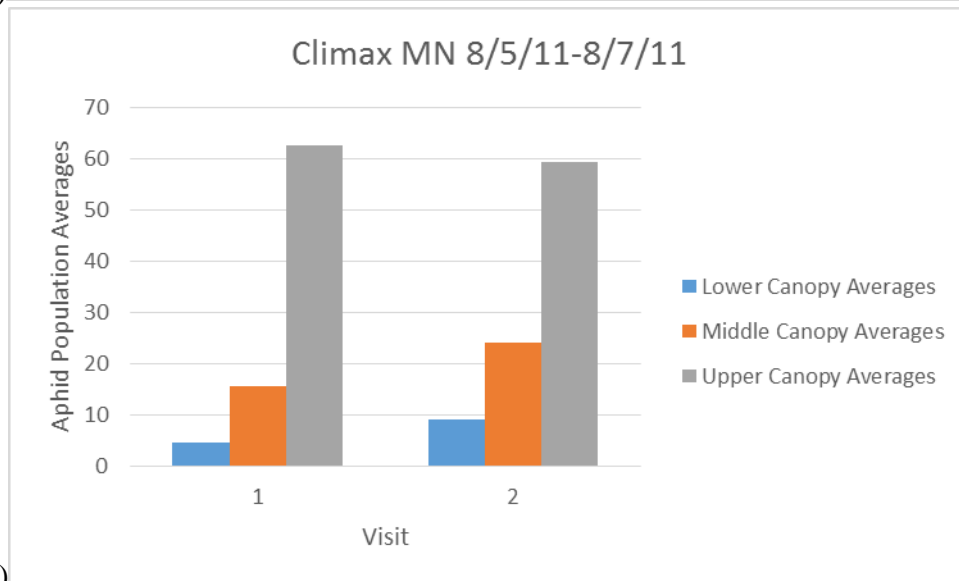


f)

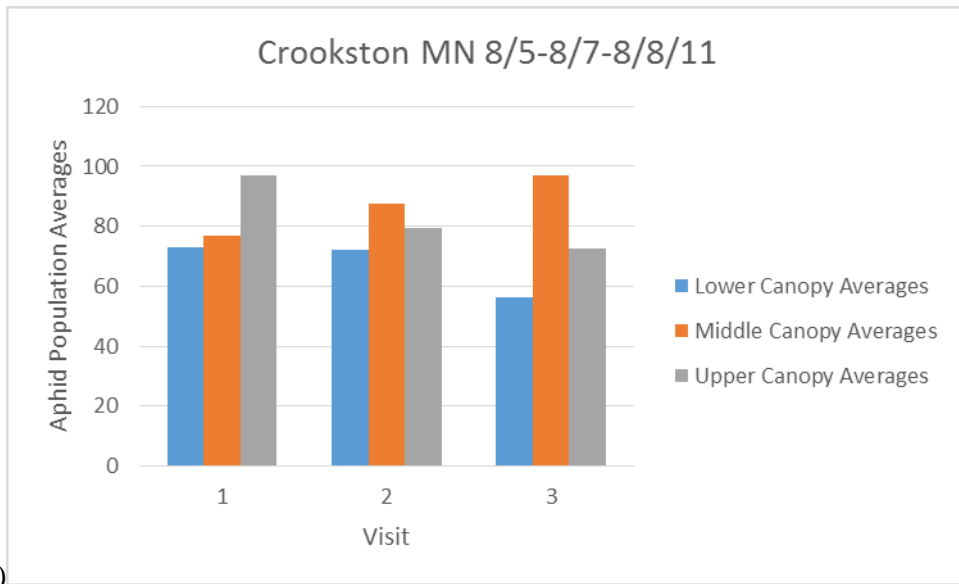
Figure 11 (a-f): A graphical summary of changes to aphid canopy distribution over 4 sampling periods during an in-field rainfall simulation trial in August 2010 in a NWROC field in Crookston MN.



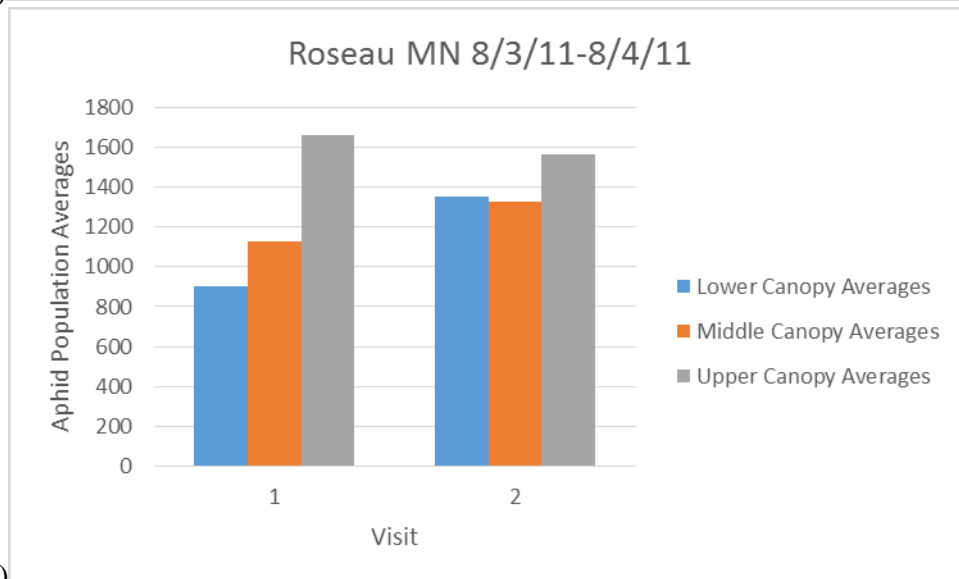
a)



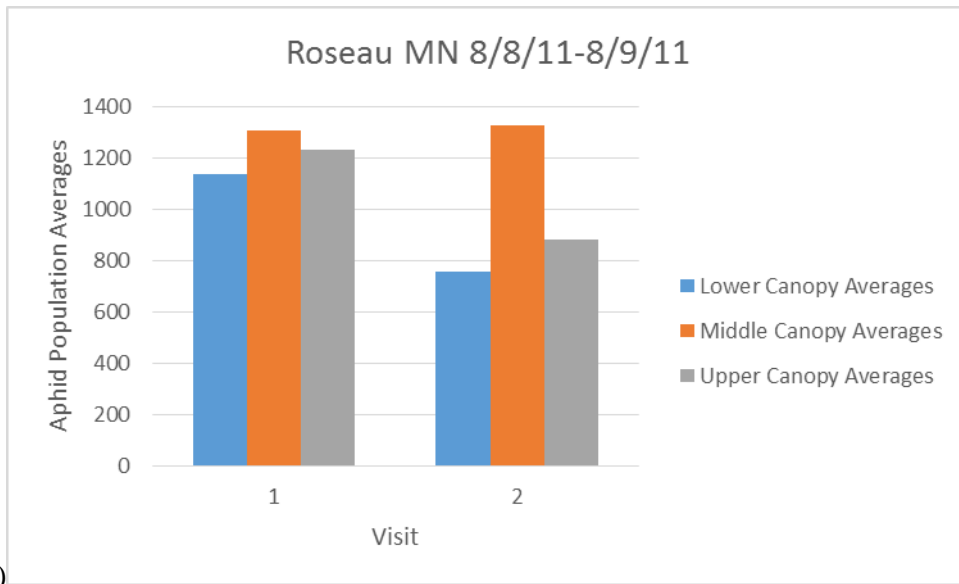
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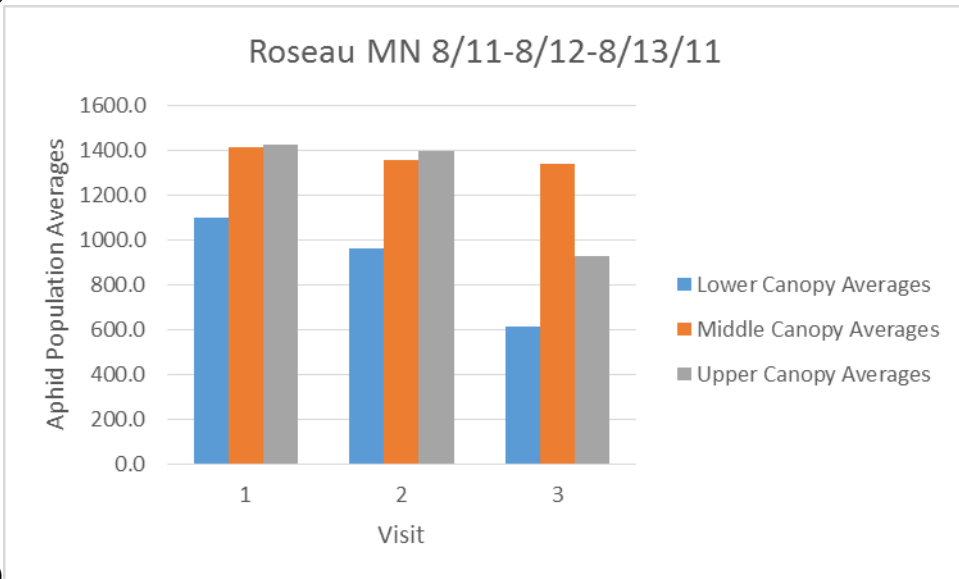
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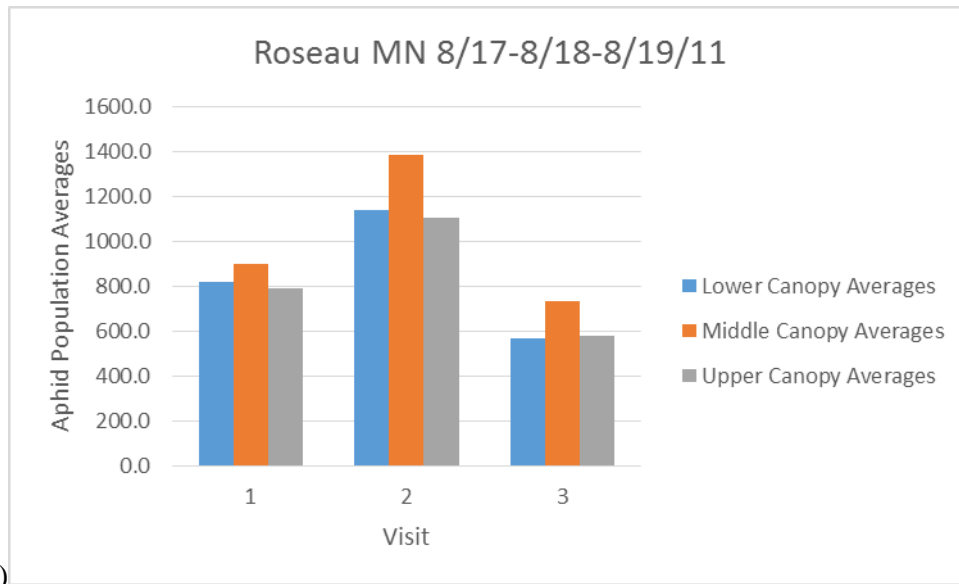
d)



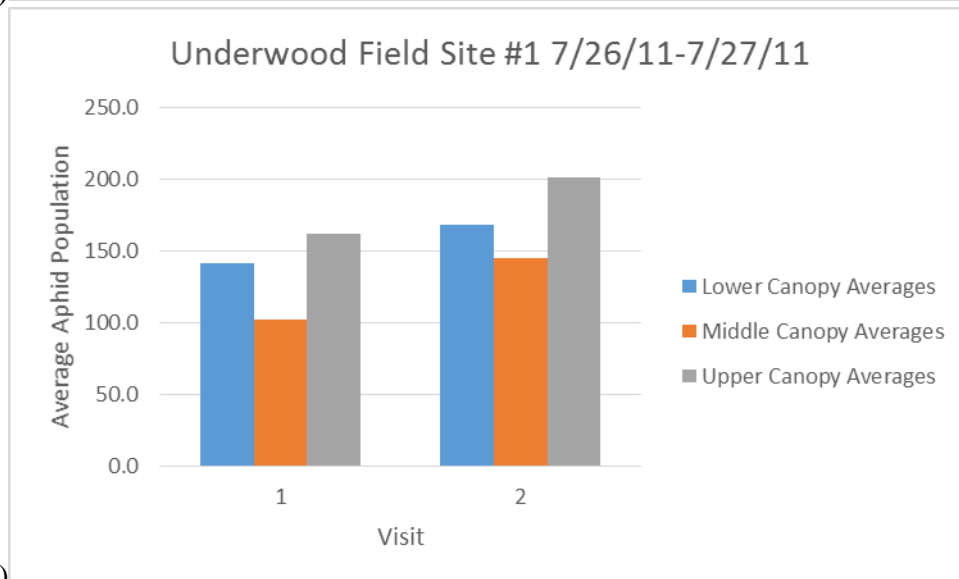
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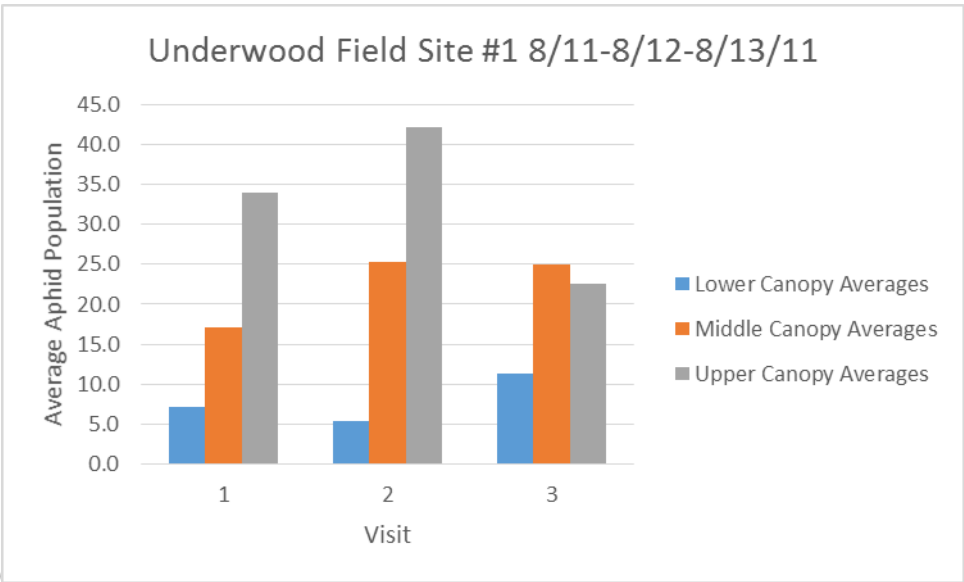
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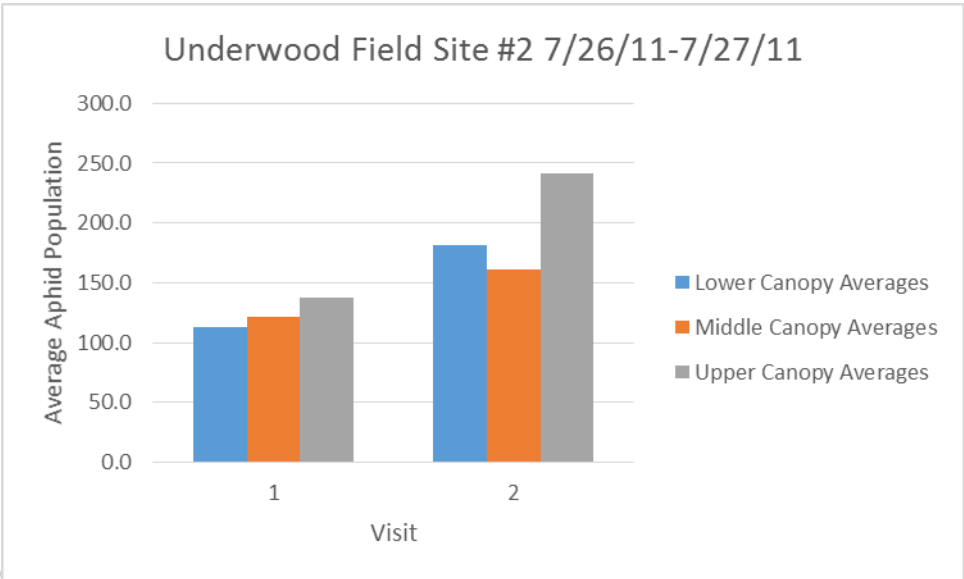
g)



h)

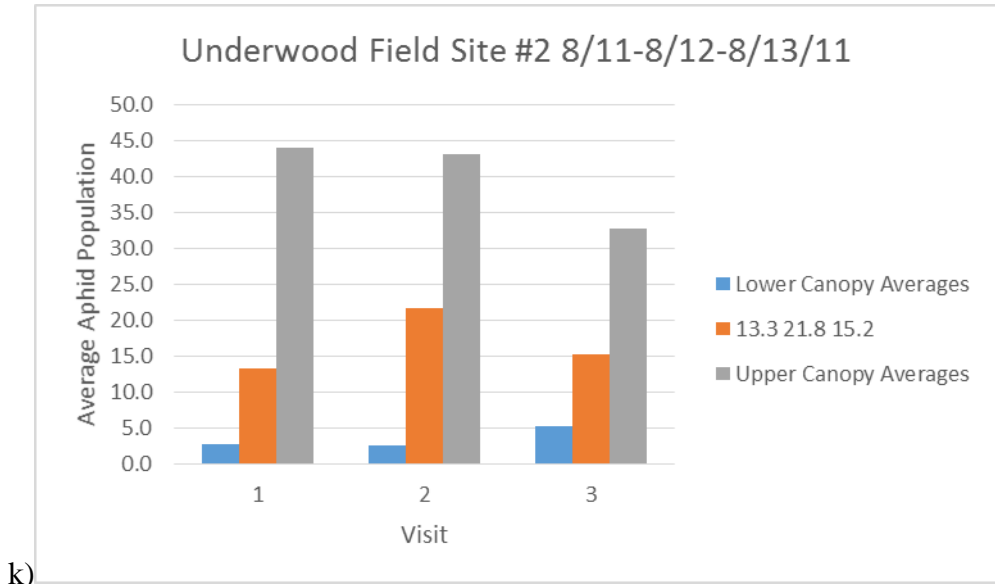


i)

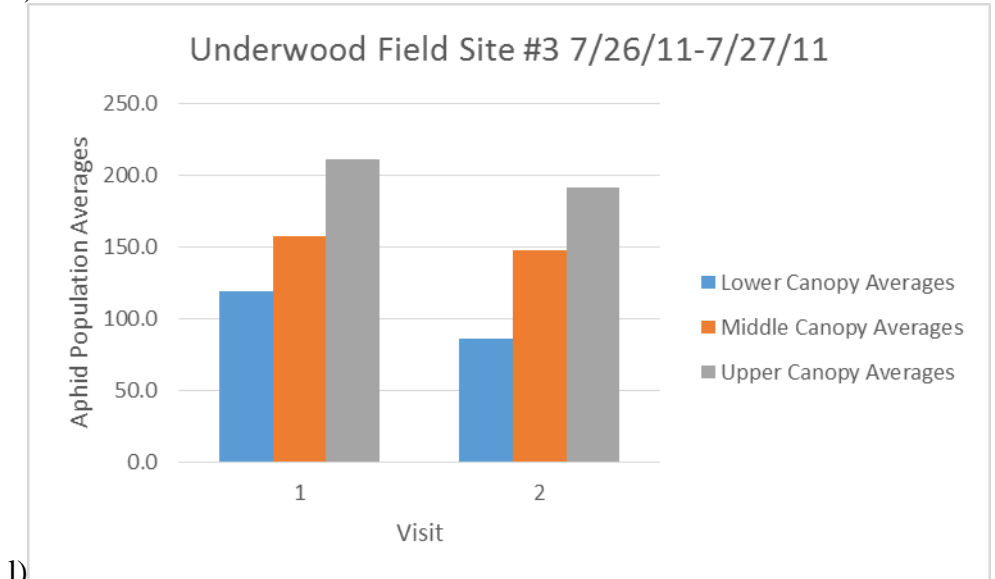


j)





k)



l)



m) Figure 12 (a-m): A graphical summary of changes in aphid population distribution by canopy level during all in-field rainfall observational events.

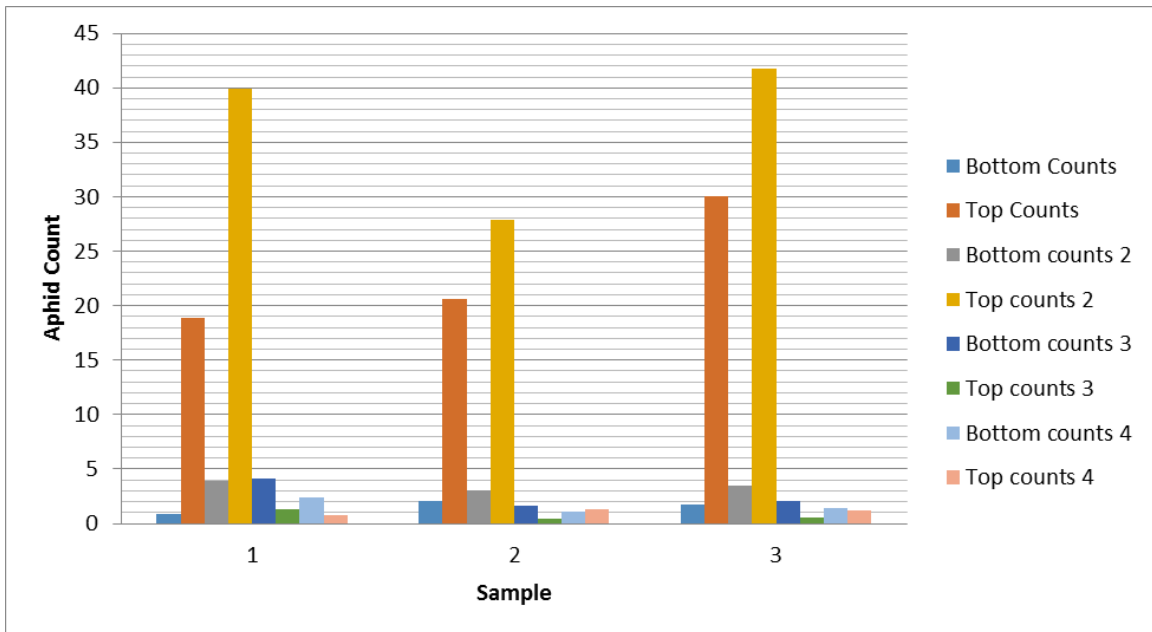


Figure 13: Rain simulation trials results for rainfall simulation on *Aphis glycines* in a NWROC greenhouse. Plants ranged from V2 to V3 plant stage and were small enough that counts were divided between the bottom and top trifoliates. Bottom and top counts were averaged for each of the four trials and represented in the above bar graph.

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