EFFICACY AND NON-TARGET EFFECTS OF AERIAL ULTRA LOW VOLUME MOSQUITO ADULTICIDING WITH WATER BASED UNSYNERGIZED PYRETHROIDS

By

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To my father

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By

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We assessed efficacy and non-target effects of ultra low volume (ULV) aerial adulticiding with two new water-based, unsynergized pyrethroid formulations, Aqua-K-Othrine (FFAST® anti-evaporant technology, 2% deltamethrin) and Pesguard S102 (10% d-phenothrin), against riceland mosquitoes of Greece. A helicopter with GPS navigation technology, real-time weather recording, and spray dispersal modeling (AGDISP) was utilized. Two application rates were tested per formulation that corresponded to 0.75 and 1.00 g AI/ha of deltamethrin and 7.50 and 10.00 g AI/ha of d-phenothrin. The mosquitoes used for the trials were the main nuisance species found in the rice field areas of Thessaloniki, which were primarily *Aedes caspius* Pallas, *Culex modestus* Ficalbi and *Anopheles sacharovi* Favre. The mean mortality observed in caged mosquitoes was ~69.2% and ~64.8% for deltamethrin and d-phenothrin, respectively. Wild mosquito populations decreased up to ~90 % for both products tested. The control mean caged-mosquito mortalities remained low (<20%) during all trials.

Two non-target aquatic organisms were used: 2nd instar mosquito larvae of the genus *Aedes* and adult aquatic beetles of the family Dytiscidae (*Hydroglyphus geminus*). Three terrestrial non-target organisms were used: bees (domesticated hives), family Apidea (*Apis mellifera)*, and two other beneficial insects, families Chrysopidae and Coccinelidae (*Chrysoperla carnae* &

Cryptolaemus montrouzieri, respectively). Very low, not significant non-target mortalities were observed. Also, no bees were observed showing signs of sublethal exposure to insecticides. The beehives exposed to the insecticidal applications performed as well as the control beehives and increased in weight (25-30%), in adult bee population (14-18%), and in brood population (15-19%). During our experiments the bees collected mainly rice pollen and were able to maintain healthy and productive hives.

Our study provided direct evidence that Teflon®-coated slides are not the proper sampling technique for FFAST® anti-evaporant droplets and MgO slides should be preferred over Teflon®. High droplet densities (400-4,000 drops/cm^2) as assessed by the MgO spinning slides, were observed throughout the treatment areas. The application technologies along with the spray dispersal modeling enabled the majority of the treatment areas to receive homogeneous coverage and high droplet densities that resulted in uniform caged-mosquito mortalities.

CHAPTER 1
INTRODUCTION

> No animal on earth has touched so directly and profoundly the lives of so many human beings. For all history and all over the globe she has been a nuisance, a pain, and an angel of death. The mosquito has killed great leaders, decimated armies, and decided the fate of nations. All this and she is roughly the size and weight of a grape seed. (Spielman and D'Antonio 2001, p.15 from The Preface to Mosquito)

It would be impossible to refer to all those times that insects affected the course of human history. Just to name a few, the death of great warriors like Alexander the Great was attributed to malaria, a disease transmitted by mosquitoes. Great empires like the Roman Empire were brought to decline because of the Bubonic plague, a disease transmitted by fleas. The Bubonic plague, also referred to as the Black Death, was responsible for causing 25 to 75 million deaths in Europe alone. A vast number of deaths during wars are attributed to insect borne diseases; in the American civil war an estimate of 40,000 to 100,000 deaths were attributed to dysentery, a disease transmitted by filth-flies (Capinera 2004).

Mosquitoes are on top of the list of the most dangerous insects to humans. Mosquitoes are vectors of serious and deadly diseases such as malaria, yellow fever, dengue and the different types of encephalitis. Approximately 500-700 million human cases of mosquito borne diseases (Drew 2002) with ~3 million deaths occur every year (AMCA 2010). Despite their minute size, and their delicate, vulnerable figure, mosquitoes have managed successfully to survive on planet earth for more than 170 million years. With their unique adaptation mechanisms, they have managed to thrive in almost all kinds of water habitats, from crab-holes and leaf-axils, to subzero tundra wetlands in Arctic. That is one of the main reasons why mosquitoes are so difficult to control. There are approximately 3,200 recognized mosquito species worldwide and the largest number of them still remains to be described (Rutledge 2004).

Because of the major impact mosquitoes can have on people's lives, humans have been developing methods to most effectively control these blood sucking insects. Some of the most commonly used methods include chemical compounds with effective mosquitocidal activities. These compounds are specialized to target the different stages of a mosquito lifecycle. Those targeting the immature stages are generally known as larvicides and those targeting the adult stages are known as adulticides. Both larviciding and adulticiding applications can occur either by ground or by air. The research presented in my study will focus on aerial adulticiding applications for mosquito control.

Aerial adulticiding is a valuable mosquito control technique because it effectively provides rapid reduction of adult mosquito populations over large areas, especially in emergency situations when the threat of disease is at a high level (Clayton and Sander 2002). For example an aircraft daily can cover up to 2,000 ha where as a vehicle mounted fogger can only cover up to 225 ha (Chow et al. 1977). Recent studies in California showed that despite intensive larviciding and ground adulticiding treatments, West Nile Virus (WNV) transmission cycle was successfully disrupted only after aerial adulticiding (Carney et al. 2008).

The success of an aerial adulticide application is dependent on a variety of factors. First, it relies on the toxic properties of the insecticide, which are mediated by the internal detoxification systems that a particular insect species has, but also on the application equipment/technique utilized to deliver the insecticide to the right place, at the right time, and in the right form. The delivery technology of adulticides (Ultra Low Volume technology) (Mount 1979, Mount et al. 1996) involves the atomization of small volumes of product into fine droplets that are meant to remain at suitable densities in the air column for an appreciable length of time to impinge on the flying adult mosquito (Connelly and Carlson 2009). The technology relies on the prevailing

weather conditions to carry the droplets to and through the target zone (Mount et al. 1996). Another important factor that governs the success of an adulticiding mission is the formulation of the product. The formulation regulates the atomization of the product to the right droplet size, and the stability of the droplets subjected to the weather conditions. Last, but not least, the degree of insect exposure to the product plays a significant role and depends entirely on the unique behavioral traits of each mosquito species. The behavioral interaction between the insect and the environment affects greatly the final outcome of an insecticide application (Ruppel 1984).

Along with the use of any insecticidal products that are applied in the environment and, therefore, may come in contact with people or other non-target organisms, comes a responsibility to use insecticides judiciously while protecting both the environment and the people/life within it. The environmental effects of insecticide applications have been continuously studied through direct toxicity studies on beneficial and non-target organisms (Jensen et al. 1999, Dukes et al 2004, Zhong et al. 2004, Breidenbauch & Szalay 2010, Macedo et al. 2010), as well as through the calculation of ecological and human health risk assessments (Peterson et al. 2006, Davis et al. 2007). For an insecticide application to be considered successful, appropriate, and acceptable it needs not only to be effective but also safe (AMCA 2009). The same applies for adulticiding applications.

Currently in the U.S. there is a rather broad variety of adulticiding products, which fall in two categories: organophosphates (malathion and naled), and synergized pyrethroids (pyrethrin, resmethrin, d-phenothrin and permethrin). However, that is not the case for EU countries where there is only one category of adulticides (pyrethroids) with few available active ingredients. In some countries, there are absolutely no products registered for adulticiding, and Greece, one of

the most popular tourist destination worldwide, fell into this category until recently (Hellenic Center for Disease Prevention and Control 2010). To make matters worse, the North region of Greece known as Macedonia faces a serious mosquito problem (Becker et al. 2003), which is a direct result of the extensive rice cultivation in the area in combination with abundant natural bodies of water (salt-marshes, river-deltas, etc) and a warm temperate climate. Unfortunately, due to the unavailability of adulticiding products in combination to the immense populations of mosquitoes in the area, controlling mosquitoes has become a serious challenge (Chaskopoulou et al. 2010).

There are products available in Greece with possibly good ULV adulticiding properties that could be potentially good candidates for ULV adulticiding registrations. These products are water-based, unsynergized pyrethroids. Their appropriateness for use as adulticiding agents by aerial application has not been investigated from the perspective of applicability, efficacy and safety. The overall objective of this research was to assess the applicability of aerial adulticiding with water-based, unsynergized pyrethroids in the agricultural area of West Thessaloniki, Greece in relation to: a) product atomization to the appropriate droplet size and coverage of the target area with sufficient droplet densities to produce efficacious treatments, b) performance of aerial navigation technologies and spray dispersal modeling in relation to consistent coverage of the treatment areas, c) product toxicity to the local mosquito fauna and ability to control the wild mosquito populations, and d) product/application safety to non-target and beneficial organisms naturally occurring within the local agricultural environment.

CHAPTER 2
MOSQUITO CONTROL STATUS–USA VERSUS GREECE

Integrated Mosquito Management

A well-thought, intelligent mosquito control program should employ not just one but all available pest control methods. This combination of methods into one program is called an Integrated Mosquito Management approach (IMM) (AMCA 2009). An IMM program employs a broad variety of tools and methods, both chemical and non-chemical, to control the target pest in the most efficacious and environmentally safe way (AMCA 2009). The most common elements of a typical IMM program are the following: first, continuous surveillance of the mosquito species and their abundance; second, source reduction or environmental manipulation whenever applicable to eliminate the mosquito breeding sites; third, the use of biological or chemical products to target the aquatic immature mosquito stages (commonly known as larviciding), and last but not least, the use of chemical products to target the flying, adult mosquitoes (commonly known as adulticiding) (AMCA 2009). All of these elements are necessary to ensure the best levels of control. The majority of the most sophisticated mosquito control programs employ an IMM approach; however, this has not always been the case.

Mosquito Control in the USA

Before 1900, people lived at the mercy of mosquitoes and wondered whether mosquitoes would ever be controlled. During that time people adapted their life-style to avoid and protect themselves from the mosquito menace. For instance U.S. natives back in the 1560s would cover themselves with red dye to avoid getting bitten by mosquitoes or burry themselves in the ground to be protected from mosquito bites (Patterson 2004). In the 1800s, mosquito-borne diseases, even though they were not known to be associated with mosquitoes back then, would shape migration and economic development in some parts of the South U.S. Investment, immigration,

and trade were limited by disease or rumors of disease (Spielman and D'Antonio 2001). Florida, currently one of the most popular tourist destinations in the world, was once considered uninhabitable and it was one of the last states to become member of the U.S. nation, partially due to the high mosquito nuisance (Patterson 2004). One of the opponents of Florida becoming a member of the U.S., John Radolph of Virginia, declared that, "Florida is a land of swamps, quagmires, of frogs, and alligators, and mosquitoes" (Patterson 2004). Back then people viewed mosquitoes as something to be avoided or simply endured.

The discovery that mosquitoes are vectors of serious life-threatening diseases a little over 100 years ago (Chaves-Carballo 2005) resulted in the initiation of serious efforts to control mosquitoes in the U.S. (Patterson 2004). In 1898 Ronald Ross proved that a particular genus of mosquitoes (*Anopheles*) was responsible for transmitting malaria (Chernin 1988) and a little later, in 1901, the work of Walter Reed and Carlos Finlay proved that the *Aedes aegypti* was the vector of yellow fever (Chaves-Carballo 2005). With these discoveries people were aware of two things: first, mosquitoes transmit diseases, and second, mosquitoes breed in water.

Between 1900-1940, the main approach for mosquito control was manipulation of the mosquitoes' natural breeding environment known as source reduction (Connelly and Carlson 2009). Ditching of high marshes by hand using workers or explosives was conducted to dewater the marsh after several days of rainfall (Connelly and Carlson 2009). Filling (placing earth or hydraulic material to fill mosquito-producing areas) and impounding (construction of earthen dikes to isolate high salt marshes and swamps from adjacent estuary) were two additional source reduction techniques mainly employed in those early development years of mosquito control (FCCMC 2009). Another method utilized was larviciding through the use of waste and diesel oils, and Paris green dust (an arsenical insecticide) (Connelly and Carslon 2009). However, the

first compound with high insecticidal activity used for mosquito control after 1945 was the chlorinated hydrocarbon dichloro-diphenyl-trichloroethane known as DDT (Casida and Quistad 1998).

DDT was brought into the insecticide market in 1939, and it was effective against a wide range of insects but most notably against mosquitoes (Casida and Quistad 1989). Paul Muller received a Nobel Prize in 1949 for his discovery of the insecticidal activities of DDT (Capinera 2004). It is estimated that DDT saved about 15 million human lives from malaria only (Becker et al 2003). DDT was used both as a larvicide and adulticide with high levels of efficacy. However, this did not last long.

In 1947, the first case of resistance to DDT was documented in *Aedes tritaeniorhynchus* and *Aedes solicitans* (Hemingway and Ransom 2000). Along with the development of resistance came environmental concerns associated with insecticide misuse. An example of insecticide misuse took place in St. Lucie County of Florida (Carlson 1962). It involved the spraying of 2,000 acres of saltmarsh with dieldrin to control sand fly larvae. Upon completion of the spraying mission it was concluded that "the minimum over-all kill throughout the marshes was 20-30 tones of fish, or about 1,175,000 fishes, of at least 30 species" (Patterson, 2004). After that incident the use of dieldrin was discontinued in the St. Lucie County, however, this incident presented a strong reminder of the potential harm that pesticides would do to non-target organisms if not used properly.

The growing problem with insecticide resistance and the environmental concerns associated with insecticide misuse encouraged the judicious use of insecticides and forced mosquito control operators/scientists to look for new insecticides as well as develop non-chemical alternatives to mosquito control. After the development of the chlorinated

hydrocarbons, other successful insecticidal groups followed, such as the organophosphates [malathion (1952), chlorpyrifos (1965)], the methyl carbamates [carbaryl (1957), alanycarb (1984)], and the pyrethroids [allethrin (1949), resmethrin (1967), permethrin (1973), deltamethrin (1974)] (Casida & Quistad 1998). Some more recent insecticidal groups are the insect growth regulators such as juvenoids and chitin synthesis inhibitors [methoprene (1975)] (Henrick 2007), and the microbial agents [*Bacillus thurigiensis israelensis – Bti* (1976), *Bacillus sphaericus – Bsph* (1964, 1984)] (Becker et al. 2003). The use of mosquito fish and other biological agents (i.e. fungi) became available for mosquito control, as well (Becker et al. 2003).

Besides the development of new products the understanding of the mosquito behavior and ecology through observation and research transformed mosquito control into a science. The decisions on where, when, and how to control mosquitoes are now based on in-depth understanding and consideration of ecological, biological, and social factors so as to achieve optimum control levels while protecting both people and the environment (Connelly and Carlson 2009). Currently, some of the best mosquito control programs in the U.S. are based on continuous monitoring/surveillance of mosquito populations/species, behavior, mosquito disease, mosquito breeding habitats, environmental changes, as well as mosquito resistance to various products. Implementing of a control plan involves a complex management strategy that takes into account all these elements in choosing the right combination of treatments, the place and time for their proper use to achieve the maximum level of control while minimizing the use of insecticides and protecting the environment and people (AMCA 2009). In the U.S. , after long years of research and development, there is a broad spectrum of tools and techniques available to the mosquito control operators (AMCA 2010). The large spectrum of monitoring/surveillance

tools, insecticidal products, and technologies allows for more complete and flexible mosquito control strategies.

Another important aspect of mosquito control in the U.S. is the development of a sound regulatory and governmental framework supporting mosquito control operations (USEPA 2010). Mosquito control in the U.S. is mostly the government's responsibility; the majority of the mosquito control programs in the U.S. are in the hands of public institutions funded by state or county taxes. Mosquito control is regulated as an essential public service because it "… protects human health, promotes the quality of life of the people, promotes the economic development of the state, and facilitates the enjoyment of Florida's natural attractions by reducing the number of pestiferous and disease carrying arthropods" (The Florida Statutes 2006). The first organized mosquito control institutions in the U.S. were founded in 1913 in New Jersey (New Jersey Mosquito Control Extermination Association) followed by the foundation of the Florida Anti-Mosquito Control Association in 1922 and the California Mosquito Control Association in 1930 (AMCA 2010).

Mosquito control professionals in all areas of research and industry come together under the umbrella of the American Mosquito Control Association, while at the same time individual member states maintain their local mosquito control agencies. The mosquito control academia and industry in the U.S. continue to provide mosquito control services and research while educating people through extension and outreach programs (Connelly and Carlson 2009) on the dangers associated with mosquitoes and the benefits associated with good mosquito control practices and effective protective measures.

Mosquito Control in Greece

Greece was the last European country to eradicate malaria in the 1970's (Bruce-Chwatt and Zulueta 1980). Back in 1942, half of the country's population was infected with the malaria

parasite (Spielman and D'Antonio 2001). To fight against the disease the United Nations provided Greece with DDT and with the help of military veterans a spraying campaign was initiated (Spielman and D'Antonio 2001). The campaign was successful in reducing the disease transmission (whereas before the initiation of the campaign 16% of children tested positive for malaria parasites, none could be found after the campaign, Spielman and D'Antonio 2001). Another important epidemic occurred in 1927 in Athens, where approximately 1 million people were infected with Dengue (Theiler et al. 1960). Also, a West Nile Virus (WNV) epidemic occurred for the first time in Northern Greece in 2010 in the region of central Macedonia. Approximately 261 human clinical cases were reported with 34 deaths (HCDC 2010).

Even though Greece has a long history of mosquito related disease epidemics it is only since 1997 that a serious effort to control the mosquito problems afflicting the residents of Greece was initiated (Becker et al. 2003). A larviciding program was established to target the mosquitoes breeding in the rice field region on the west side of Thessaloniki (Iatrou and Mourelatos 2007, Piakis 2007) the second largest city of Greece with a population of ~2 million people. Form 1997 to 2008 there were mainly 2 products available for mosquito control: the organophosphate, temephos, and the microbial agent, *Bti*. In 2009, temephos was no longer available for the Greek market (ANSES 2009) and a chitin inhibitor was introduced, diflubenzuron. In 2010 an additional product was introduced, a bio-insecticide known as spinosad.

The status of mosquito control in Greece is being seriously challenged due to the scarce availability of mosquitocidal products and applications methods/techniques. As a direct result of a new European regulation (Biocidal Product Directive-BPD) one of the most effective, reasonably priced larviciding products (tememphos) used in Greece for the past 10 year has been

excluded/eliminated from the market. Currently, the 2-3 larviciding products available are either highly priced or limited to 2-3 applications per year (Chaskopoulou 2009).

The EU currently consists of many different countries, including Greece, with different languages and different regulations that control the registration and proper use of insecticides. The BPD is a recent piece of European legislation that has been incorporated into the national law of each member country (European Commission Environment 2010). It will cover all biocidal products including the public health pesticides. The objective of the BPD is to promote free trade, within Europe, of effective biocidal products that do not pose significant risk to human health, non-target organisms, or the environment (Commission of the European Union Community 2006). However, it imposes a heavy cost burden on the manufacturers of a wide range of products, due to the high standards required in the updated registration dossiers Gartiser et al. 2007). The implementation of the BPD could result in many active substances, and their associated products, being withdrawn from the market (Gartiser et al. 2007). In many cases this will lead to the loss of some efficacious and products on purely economic grounds. It is suggested that by 2010 the Biocide market may contain approximately 20-25% of the compounds present in 2005 (Gartiser et al. 2007). Eminent researchers, scientists, and professors globally worry about the effects of BPD on the availability of insecticides and how it would affect the development of insecticide resistance and what would the implications be for vector-borne disease control (Abaasa et al. 2008).

To add to the challenge of scarce availability of mosquitocidal products there is scarce availability of control techniques. There is currently no outdoors ULV treatments allowed for mosquito adulticiding in Greece (Chaskopoulou 2009) and to make matters worse there is the threat for a total ban of aerial applications in Europe (Commission of the European Union

Community 2006), which would imply the elimination of a very useful vector control tool. The banning of aerial spraying in the case of a mosquito-born disease epidemic, when immediate, fast and effective measures must be enforced, could have detrimental consequences to the health and wellbeing of the public. For instance, despite intensive larviciding and ground adulticiding treatments, in California, West Nile Virus (WNV) transmission cycle was successfully disrupted only after aerial adulticiding (Carney et al. 2005), and both reduction of the WNV mosquito vectors and subsequent decrease of WNV activity were achieved (Lothrop et al. 2008). These studies clearly demonstrate the importance aerial mosquito adulticiding in reducing human illness and potential death from mosquito borne diseases.

Retrieved from the EU Parliament draft report on the proposal for a directive to achieve a sustainable use of pesticides "Aerial spraying does not offer environmental benefits over other spraying methods. Derogations should not be possible in areas where residents, and bystanders might be affected, for example highly populated rural areas or near areas used by the public and vulnerable groups such as schools". Recent science has concluded that actually the risks to the environment from aerial mosquito adutliciding operations are much lower than ground operations (Schleier III et al. 2008, Mickle 2005). In addition the weight of scientific evidence from independent research and regulatory agency assessments strongly suggests that exposures to people, mammals, birds, fish are extremely low (Peterson 2010). On the other hand, the weight of scientific evidence shows that the mosquitoes and the pathogens they carry can cause appreciable risks to public and environmental health (Peterson 2010).

Greek mosquito control operators and governmental agencies have been trying to bring in Greece new vector control tools and preserve the use of already existing tools. Since 2008 research is being conducted in Greece to add new methods in the already existing control

strategies through collaborations with American universities and other institutions. Our study is an example of these efforts to improve the mosquito control services in Greece.

CHAPTER 3
EFFICACY OF AERIAL ULTRA LOW VOLUME APPLICATIONS WITH TWO NOVEL WATER-BASED FORMULATIONS OF UNSYNERGIZED PYRETHROIDS AGAINST RICELAND MOSQUITOES OF GREECE

Introduction

Control of adult mosquitoes is an important step in effectively reducing populations of both nuisance and disease-vectoring mosquitoes. Aerial adulticiding effectively provides rapid reduction of adult mosquito populations over large areas, especially in emergency situations when the threat of disease is at a high level. For instance, despite intensive larviciding and ground adulticiding treatments in California, West Nile Virus (WNV) transmission cycle was successfully disrupted only after aerial adulticiding (Carney et al. 2008). Both reduction of the WNV mosquito vectors and subsequent decrease of WNV activity were achieved (Lothrop et al. 2008). These studies clearly demonstrate that aerial mosquito adulticiding can effectively reduce human illness and potential death from mosquito borne diseases.

Aerial mosquito adulticiding is widely used in the United States of America (U.S.) and constitutes an essential tool for many integrated mosquito management (IMM) programs. However, despite the clear advantages of aerial adulticiding in controlling vector populations, currently only one country (Hungary) in the European Union (EU) allows this type of application. The increasing population of disease-vectoring and nuisance mosquitoes in certain European countries and the introduction of new vector species, i.e. *Aedes albopictus* (Enserinc 2007, 2008), has raised concerns about re-occurrence of disease epidemics. The Chikungunya virus outbreak in France during 2005 (Renault et al. 2007) and in Italy during 2007 (European Centre for Disease Prevention and Control 2009), as well as the recent epidemic of West Nile virus in Greece (Hellenic

Centre for Disease Prevention & Control 2010) with ~261 human cases and 34 fatalities reported indicate the need for better vector control. Aerial adulticiding could provide needed mosquito control and help prevent potential disease outbreaks such as these.

Approval of aerial mosquito adulticiding in EU countries, where environmental sensitivities are very high, requires extensive data on both efficacy and safety of proposed products and methods. Water-based ultra low volume (ULV) formulations containing unsynergized pyrethroids are currently the only available products labeled for mosquito adulticiding in some EU countries. These formulations represent a new generation of products with potentially lower environmental effects. Unlike the oil-based, synergized pyrethroids commonly used in the U.S., unsynergized, water-based products seem to be more acceptable in environmentally sensitive regions. This is because water is seen as a more environmentally acceptable diluent for these products than oil, and the synergist used in the U.S. formulations, piperonyl butoxide (PBO), is considered an environmental toxicant in its own right (Weston et al. 2006).

Aqua-K-Othrine and Pesguard S102 are two water-based formulations of deltamethrin and d-phenothrin, respectively, available for mosquito adulticiding in EU countries. These products have not been tested in aerial applications for mosquito adulticiding. Our first objective was to investigate the efficacy of these products applied aerially against several major nuisance and potential disease-vectoring mosquito species of Europe. The rice field region of northern Greece in the prefecture of Central Macedonia (population ~2 million) was chosen as the test site due to the serious mosquito problem that characterizes this region and its close proximity to Thessaloniki, one of the largest urban centres of Central Macedonia with over ~1 million inhabitants.

To improve the precision of the application, the aircraft was equipped with a guidance system utilizing Global Positioning System (GPS), real-time meteorological equipment and a computer spray dispersal model (AGDISP). These technologies allow prediction of the spray movement based on application parameters and meteorological conditions to effectively guide precise treatment of the target area while minimizing off-target drift and non-target effects (Teske et al. 1993, Bilanin et al. 1989). Also, these technologies allow recording of application parameters, such as spray altitude, flight speed and duration, flight line position, and meteorological conditions. However, little has been reported on the performance of these technologies in large scale aerial mosquito adulticiding trials, especially with water-based ULV formulations. Our second objective, therefore, was to examine the performance of these technologies with the water-based ULV formulations in relation to a) consistent coverage of the target area and b) uniform mosquito kill throughout the target area.

Materials and Methods

This work was a two-year study (2008-9) conducted in the agricultural area of West Thessaloniki, Greece. Research permits were obtained from the Greek government and the work was observed by Greek regulatory authorities (Ministries of Rural Development and Food, and Health and Social Solidarity) as well as by AGROLAB S.A, a European laboratory consultancy. The research was certified for Good Experimental Practice (GEP) by the Greek Ministry of Rural Development and Food, so results could be used in registration of products and application technology in Greece and other EU countries. GEP studies are similar to Good Laboratory Practice (GLP) except that they apply to field experimentation.

Insecticides

Two commercially available ULV insecticides registered in Europe for ground mosquito adulticiding were used for the aerial treatments: Aqua-K-Othrine (2% w/w deltamethrin, Bayer Environmental Science, Lyon, France), a water-based, anti-evaporant Film-Forming Aqueous Surface Technology (FFAST®) formulation, and Pesguard S102 (10% w/w d-phenothrin, Sumitomo Chemical Co., Ltd., Osaka, Japan), a water-based microemulsion formulation.

In 2008, two rates were tested with each product based on recommendations of the World Health Organization (WHO) for ULV applications of insecticides for mosquito adulticiding (WHO, 1997) and on the label rates of the specific products for use in other countries where they are registered. For Aqua-K-Othrine, 0.75 and 1.00 g/ha of active ingredient (AI) were tested, corresponding to 37.5 and 50.0 ml/ha of 2% AI formulation, respectively. For Pesguard S102, 7.5 and 10.0 g/ha AI were tested, which corresponds to 75.0 and 100.0 ml/ha of 10% AI formulation, respectively. In 2009, Aqua-K-Othrine was tested at the higher of the two previously tested application rates, and Pesguard S102 was tested at the lower of the two rates. Over the 2 years, for Aqua-K-Othrine the lowest application rate was replicated twice and the highest one five times. For Pesguard S102 the highest application rate was replicated twice and the lowest one five times. A total of 7 spraying trials were conducted with each product. Both formulations were sprayed undiluted.

Experimental Sites

During 2008 there were 2 experimental sites chosen, one treatment site (Area A) where all insecticide treatments were conducted, and one control site where no treatments took place. During 2009 after a request from the Greek government an additional

treatment site was included (Area B) that would allow for each insecticide to be applied at a separate site (Figure 3-1). The treatment sites were open agricultural areas, approximately 1,000 ha in size (~ 4 x 2.5 and ~3.5 x 3.21 km areas A and B, respectively). They consisted of rice, cotton and corn fields (~60% and ~90% rice fields for 2008 and 2009, respectively) and were at least 3 km away from any urbanized areas as required by permits from the Greek government. The GPS coordinates corresponding to the 4 corners of the treatment sites A and B were: a) 40° 34.194’ N, 22° 44.531’ E, b) 40° 35.446’ N, 22° 47.122’ E, c) 40° 36.511’ N, 22° 46.626’ E, d) 40° 35.211’ N, 22° 43.717’ E and a) 40° 35’4.07 N, 22° 47’58.03 E, b) 40° 35’58.20 N, 22° 49’55.79 E, c) 40° 37’29.20 N, 22° 48’56.74 E, and d) 40° 36’36.87 N, 22° 46’55.81 E, respectively. The control sites assigned for both years were similar to the treatments sites consisting mostly of rice fields and located ~3-5 km from the treatment areas.

Application Technologies

Insecticide application was conducted by an aerial contractor company, Air Applications-ASNF (State Airport of Macedonia, Thessaloniki, Greece) using a turbine helicopter (500C model, McDonnell Douglas Helicopters, Mesa, AZ). The helicopter was equipped with a pair of electric rotary atomizers (Micronair AU 6539 model, Micron Sprayers Ltd, Bromyard, Herefordshire, UK). The nozzles were positioned on the lateral booms facing toward the rear of the helicopter (1.4 m out from the aircraft centreline but in line with the center of the main rotor). The nozzles generated droplets within a 38-45 microns volume median diameter (VMD) range. The applications were conducted approximately 30 min after sunset (during high mosquito activity) at an altitude of ~61 m and an air speed of ~145 km/h. The swath width for the first two trials of Aqua-K-Othrine was 152 m but it was increased to 305 m for the remaining five trials, so that

sharp turns that caused concern for pilot safety could be avoided. All seven Pesguard S102 trials were conducted at an intermediate swath of 228 m. Based on the swath, the flow rates were adjusted each time so that the appropriate amount of active ingredient was applied per hectare (Table 3-1).

The helicopter was equipped with a flight guidance GPS system (Wingman™ GX, Adapco, Sanford, FL) which included an onboard moving map screen that displayed aircraft position, spray block boundaries, and flight lines. Additionally, the helicopter was equipped with a real-time meteorology system (AIMMS-20™, Aventech Research Inc., Ontario, Canada) that recorded information such as wind speed and direction, temperature and humidity at the spraying altitude. The real-time meteorological data recorded by the AIMMS-20™ system were automatically processed by the Wingman GPS guidance system. The USDA spray dispersal computer model AGDIPS1 (Bilanin et al. 1989) had previously been run to predict the spray movement based on application parameters and meteorological conditions. Results of numerous spray model runs covering a range of meteorological conditions and application parameters for this specific helicopter and droplet spectrum were incorporated into a “lookup table” into the Wingman system. Spray offsets were calculated and flight lines were positioned automatically through the Wingman/AgDISP lookup table system. As wind speed and direction changed, the system adjusted guidance/offsets for the correct flight line positions for optimum coverage. The pilot followed the flight line guidance provided by the system for each of the spray lines necessary to cover the treatment area.

A ground weather station (Kestrel 4500 NV Weather Tracker, Nielsen-Kellerman Co., Boothwyn, PA) was located in the centre of the treatment site to collect data

throughout the duration of the spray trials. The weather data were recorded and retrieved from both the aircraft and the ground weather station.

Insects

Wild strains of mosquitoes were field-collected as larvae from rice fields and other natural aquatic habitats outside the treatment area and reared to adulthood in the laboratory (25°C, >60% RH). The mosquito larvae were kept in pans (53.3 x 40.6 cm) filled with deionized water and aerated with aquarium pumps. Larval mosquito diet consisted of a 2:3 liver:yeast powder medium. Adult mosquitoes were supplied a diet of 10% sucrose solution in water. For the spray trials, we used adult females of the major nuisance mosquito species found in the agricultural area of western Thessaloniki. These species were primarily *Aedes caspius* Pallas, *Culex modestus* Ficalbi, and *Anopheles sacharovi* Favre.

Assessment Bioassay

Mosquito trapping devices: CDC miniature light traps baited with dry ice and octenol were utilized for mosquito trapping. Treatment efficacy was determined by comparing numbers of adult mosquitoes trapped in CDC traps pre-and post-treatment. For pre-treatment collections, the traps were deployed the night before each adulticidal application and were collected the following morning. For post-treatment collections the traps were deployed the night after each adulticidal application and were collected the following morning. For each pre-and post-application sampling, a total of 3-6 CDC light traps were utilized: 2-4 traps in the treatment area, and 1-2 traps in the control area. The treatment traps were placed at central locations within the treatment area to avoid mosquito infiltration from the surrounding untreated area.

Sampling stations: Eight to fourteen sampling stations were deployed homogeneously at pre-assigned locations within each treatment site. Five sampling stations were placed in the control area. All sampling stations were deployed and handled in a similar manner. Each sampling station contained an adult mosquito cage, and a droplet sampling device as described below (and non-target sampling as described in Chapter 4).

Mosquito cages: One modified WHO cylindrical cage (13 cm in length, 4 cm in diameter) with 0.2 cm mesh screen wire (WHO 2006) was suspended on a 1.2 m high stake in each sampling station. Approximately 2 hours before each spray application, ~30-50 female adult mosquitoes were aspirated from the rearing cages into the WHO field cages. The cages were placed in the field ~15 min before the spray application commenced and remained there until 30 min after the spraying was completed to ensure enough time for the entire insecticidal cloud to pass through the intended area. Approximately 60 min post spraying, all cages were returned to the laboratory and the mosquitoes were transferred into clean containers to avoid secondary insecticide pickup. Mosquito mortality was determined 12 hours after the treatment.

Droplet sampling device: Magnesium oxide (MgO) coated acrylic slides (3 mm wide x 75 mm long) (McMaster-Carr, Atlanta, GA) were freshly prepared for each trial by burning ~10-cm pieces of magnesium strips (Fisher Scientific Company, Pittsburg, PA) approximately 5 cm beneath a row of parallel slides supported on a metallic stand. The smoke released from the burning strips formed a thin layer of a white, powdery, uniform coating on the slide surface. The slides were checked against the light for coating

irregularities before being used in the tests. Slides with scratches, dirt and uneven coatings were discarded.

Two slides were placed 18 cm apart on a cross arm mounted to a custom-made spinner, which was placed on top of the 1.2-m high stake at each sampling station. The spinner motor (Premotec, MJK Inc., Waterbury, CT) operated at ~590 rpm, resulting in a slide speed of 5.6 m/s. The slides remained in the field for the same duration as described for the mosquito cages. After completion of the trial, the slides were returned to the laboratory where the size and density of the droplets impacting the slides (as indicated by their craters in the MgO powder layer) were measured under a compound microscope (FM200, American Scope, Chino, CA) at 100x magnification. At least 100 droplets were measured per slide. Also, to ensure proper nozzle performance, low altitude spraying (~2 m above the spinner) was conducted prior to each trial to measure size of the droplets produced by the spraying nozzle.

The Volume Median Diameter (VMD) was chosen to describe droplet size range. When calculating the VMD for each slide sampler, the diameter (D) rather than the diameter cubed (D^3) for each droplet was used to correct for the collection efficiency variation between droplets of different sizes, as suggested by Yeomans (1949). Because the collection efficiency of droplets on spinning slides is directly related to the diameter squared (D^2), removal of D^2 from the volume (D^3) calculation compensates for collection efficiency variability (Yeomans 1949). The spread factor for droplets impacting upon MgO slides were 0.86, 0.80, and 0.75 for droplet sizes >20, 15-20, and 10-15 microns, respectively (May 1950).

Statistical Analysis

All statistical analyses having the insecticides as treatments were performed with the SAS software package (SAS Institute Inc., Cary, NC). Because results were similar for the 2 doses per insecticide tested in the first year, the low dose of Aqua-K-Othrine and the high dose for Pesguard S102 were dropped for the second year test and were not included in the statistical analysis. Five replicates were analyzed per insecticide dose. Data were adjusted for control mortality using Abbott's formula (Abbott 1925). One-way ANOVA was performed to determine the effect of the insecticide treatment on percent caged-mosquito mortality, and percent wild mosquito population decrease. Data were arcsine-square-root-transformed before ANOVA. Means were separated using Student-Newman-Keuls (P = 0.05; [SAS Institute 2003]).

Also, a General Linear Model (GLM) procedure was used to analyze the caged-mosquito mortality with application event as the block factor and cage location (1-14 for the year of 2008, 1-8 for 2009, Area A & B, respectively) as the main effect. Significance was assigned at $P < 0.05$, and means were compared using Tukey test.

Results

Efficacy

Caged-Mosquito Mortalities: The low dose of Aqua-K-Othrine and the high dose of Pesguard caused mean caged-mosquito mortalities of 67.7 and 67.4%, respectively (Table 3-2). The high application rate of Aqua-K-Othrine killed an average of 69% of the mosquitoes with mortalities as high as 97% in some cages. The low application rate of Pesguard killed an average of 65% of the mosquitoes with mortalities as high as 90.5%. No statistically significant differences were observed between the two insecticide

treatments; however, the insecticide treatments caused higher caged-mosquito mortality than that observed in the control areas (≤15%).

Effect of Location on Caged-Mosquito Mortalities: For both insecticide treatments, there was some effect of location in caged mosquito mortalities for the 2008 trials (Figure 3-3). Specifically, there was a statistically significant difference on mortalities between 3 groups of cage-locations. The first and largest group with similar mortalities consisted of 9 cage-locations (mean % mortalities 66-75), the second group consisted of 4 cage-locations (% mean mortalities 55-65) and the last group consisted of a single cage-location, an outlier (% mean mortality 79). For those trials conducted during the year of 2009 there was no statistically significant difference in caged-mosquito mortalities between all 8 cage-locations for both insecticide treatments, indicating a more homogeneous treatment of the targeted/treatment areas.

Wild Mosquito Mortalities (natural population control): The low dose of Aqua-K-Othrine and the high dose of Pesguard resulted in mean mosquito population decrease of 62 and 78%, respectively between pre-and port-treatment trap nights (Table 3-3). The high rate of Aqua-K-Othrine caused a mean population decrease of 76.5 %, and the low application rate of Pesguard caused a mean population decrease of 78 %. Both rates caused mosquito population decrease which was significantly higher than that observed in the untreated control area. Specifically, the % mean population decrease in the untreated control area was a negative number (- 3.4 %) indicating that there was an increase in numbers of mosquitoes trapped between pre- and post-treatment nights.

Application Technologies

Weather Data and Predicted Offsets: The prevailing winds at all times were generally from the south with some variation between SW or SE (Table 3-1). The wind

speed at application height ranged from ~3.6 up to ~7.0 m/s, which corresponded to offsets (as calculated by the AGDISP model software) ranging from 1,000 to 1750 m, respectively. Ground-level wind speeds were lower, as expected, ranging from ~0.7 up to ~3.35 m/s. The average temperature ranged from 24-26°C and 23-25°C at application height and ground level, respectively. The relative humidity at ground was generally >80%, and at flight height, it ranged from 61 to 86%.

Droplet Sizes and Densities: When the droplets were collected immediately upon release from the nozzle, their VMDs ranged between 35 and 40 microns for both formulations tested. However, the VMDs of the droplets collected at ground level during each trial were in the range of 10-14 microns. For all 14 trials, droplets were collected in high numbers throughout the treatment area. The droplet densities inside the treatment areas ranged between 700 and 4,000 drops/cm^2 on the rotating slides.

Discussion

This is the first report of efficacy of 2 unsynergized, water-based pyrethroids, Aqua-K-Othrine (2% deltamethrin) and Pesguard S102 (10% d-phenothrin), applied aerially for control of adult mosquitoes. Both products provided similar level of control, however, the amount of AI/ha of d-phenothrin was 10-fold higher than the one for deltamethrin. Previous studies with Aqua-K-Othrine applied as a ground ULV space spray at similar AI rates to that in our study, 0.5-1 g AI/ha, demonstrated high efficacy (caged-mosquito mortalities exceeding 77%) against a variety of mosquito species such as *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* (WHO 2006). However, reports on use of deltamethrin involve residual surface sprays (Cilek & Hallmon 2006) and not space sprays because not many deltamethrin formulations are globally available for outdoors space spray and no formulations are available in the U.S. Our study provides

evidence that water-based deltamethrin products have high potential as space sprays. Similar formulations to the ones used in our experiments should be considered for further testing and registration in the U.S., and other countries where mosquito adulticiding is needed.

Although results on the efficacy of ULV space spraying with Pesguard S102 are not currently available in the scientific literature, a study using a similar water-based microemulsion formulation showed that Pesguard 102 (5% d-phenothrin, 5% d-allethrin), when applied at the rate of 10 g AI/ha, caused high mortality of caged *Aedes* and *Culex* adult mosquitoes (Yap et al. 1997). Additionally, there are several studies of both ground and aerial applications with synergized, oil-based formulations of d-phenothrin (Groves et al. 1994, Lesser 2002, Meisch et al. 2005, 2007), using EPA rate guidelines for space sprays and ranging from 1.36 to 4.00 g AI/ha (U.S. EPA 2008). The AI rates tested in our study followed the WHO recommended range of 5-10 g AI/ha (WHO 1997), which is about 2.5-4 times higher than the application range for products used in the U.S. Compared with other d-phenothrin studies, our study with unsynergized product showed similar efficacy data despite the large difference in the AI rates applied. The presence of piperonyl butoxide (PBO) synergist in the U.S. formulations may explain why similar efficacy to Pesguard S102 was obtained with lower AI rates, as PBO is known to significantly increase the toxicity of pyrethroids (Roberts 1981, Floore et al. 1990, 1992) and improve their efficacy in field trials (Yap et al. 1978).

Space spray efficacy studies typically use cage bioassays to determine insecticide efficacy and optimum application rate. However, caged-mosquito mortalities often do not reflect the insecticidal impact on wild mosquito populations, with caged mosquitoes

experiencing higher mortalities than wild populations (Miller et al. 1982, Khoo and Sutherland 1985, Townzen et al. 1987). In our study, caged-mosquito mortalities were consistently lower than wild-mosquito mortalities estimated by trap counts, for both products tested (Figure 3-2). The cage design and screen size can prevent up to 70% of the insecticide from reaching mosquitoes inside the cage (Hoffman et al. 2008). This lower exposure to the insecticide could explain the lower mortalities in caged mosquitoes that we and other investigators observed in efficacy studies. Alternatively, significant mosquito mortality in cages can be caused by secondary exposure to insecticide deposited on the screen (Barber et al. 2006), and could lead to overestimating actual mortalities. Thus, caged-mosquito mortalities often do not serve as an accurate representation of wild population mortalities, and other additional mosquito monitoring methods should be used.

High densities of droplets reached the treatment area during all trials, validating the AGDISP model's prediction. The VMDs of the droplets collected at ground level (10-14 microns) during each trial were consistently smaller when compared with the droplets collected at the nozzle (35-40 microns). This decrease in the droplet size range at ground level could be attributed to the fact that bigger droplets may have deposited out faster, thus being unavailable for collection by sampling devices, or due to partial shrinkage of the water-based droplets caused by evaporation due to prolonged exposure to atmospheric conditions.

In general, cage location seemed to have some effect on caged-mosquito mortalities indicating that the insecticide covered some parts of the area more thoroughly. Specifically, for those trials in 2008, the mortalities seemed to be higher (66-75%) for

those cages located downwind from the spray lines and tended to be lower for those cages located upwind (55-65%). The one cage (cage 6) with by far the highest average mortality was the most downwind location during the majority of the trials, suggesting that a more dense insecticide cloud covered the downwind portion of the treatment area. During the 2008 trials, there were high winds in the range of 3.5-7 m/s and the model possibly underestimated the offsets for winds of that magnitude. Nevertheless, during the 2009 trials location seemed to have no effect on caged-mosquito mortalities, indicating a uniform coverage of the treatment areas. Uniform coverage prevents the possibilities of under-spraying leading to the survival of mosquito populations and over-spraying leading to potential environmental problems. Uniformity of mosquito mortality over the treatment area also indicates that the spray dispersal model predictions provided important guidance to the pilot.

Overall, the aerial adulticiding treatments conducted over the rice field area of west Thessaloniki provided high mosquito mortalities. The application technologies along with the spray dispersal modeling enabled the majority of the treatment areas to receive homogeneous coverage and high droplet densities that resulted in uniform caged-mosquito mortalities. The results of this study provide additional evidence that aerial application of water-based pyrethroids, when done properly, can result in targeted treatments and high mosquito control levels, even in a heavily infested mosquito environment such as the rice fields. When mosquito borne disease epidemics are increasing, vector-control tools that can provide immediate, effective, and environmentally acceptable control practices assume greater importance. These vector

management practices should be available both as a preventive measure and an emergency tool for mosquito control.

Table 3-1. Application parameters and weather conditions during aerial adulticiding trials with 2 water-based products in Thessaloniki, Greece.

Spray Date	Prevailing wind direction	Average wind speed (m/s)		Average temperature (°C)		Relative humidity (%)		Product	Application Rate (ml/ha)	Flow rate (L/min)	Coverage (ha/min)	Swath width (m)
		Ground	Aerial	Ground	Aerial	Ground	Aerial					
07/28/08	SSW	0.7	3.6	25	26	>90	69	AKO	50.0	1.75	35.0	152.4
08/02/08	SSE	1.8	5.2	25	26	>80	61	AKO	37.5	1.31	35.0	152.4
08/19/08	S	2.0	5.4	25	28	>80	68	AKO	50.0	3.50	70.0	304.8
08/26/08	S	1.6	4.8	25	28	>80	72	AKO	37.5	2.63	70.0	304.8
08/29/08	SSW	0.9	3.8	25	26	>90	80	PESG	100.0	5.25	52.5	228.6
09/02/08	SSE	2.7	6.2	25	24	>80	70	PESG	100.0	5.25	52.5	228.6
09/05/08	S	2.5	5.0	25	24	>80	86	PESG	75.0	3.94	52.5	228.6
09/10/08	S	3.4	7.0	25	24	>90	75	PESG	75.0	3.94	52.5	228.6
07/15/09	SSE	2.4	7.0	25	28	>80	55	PESG	75.0	3.94	52.5	228.6
07/22/09	SSE	1.5	7.0	23	26	>80	-*	AKO	50.0	3.50	70.0	304.8
07/29/09	SSE	3.6	7.0	24	26	>80	60	PESG	75.0	3.94	52.5	228.6
08/09/09	SSW	1.2	4.0	25	26	>90	69	AKO	50.0	3.50	70.0	304.8
08/13/09	SSW	1.4	6.0	24	26	>90	-*	PESG	75.0	3.94	52.5	228.6
08/20/09	S	0.8	3.7	24	26	>90	-*	AKO	50.0	3.50	70.0	228.6

AKO; Aqua-K-Othrine.
PESG; Pesguard S102.
* Aircraft humidity sensor due to a malfunction did not record data during the flight.

Table 3-2. Caged mortality of mosquitoes exposed to aerial adulticiding treatments with 2 water-based products in Thessaloniki, Greece.
AKO; Aqua-K-Othrine.

Treatment	Application Rate (ml/ha)	% Caged-Mosquito Mortality		
		n	Mean [a] ± Std Error	% Mortality Range (min-max)
AKO	37.5	2	67.7*	48.7-83.3
	50.0	5	69.29 ± 1.10 A	53.7-97.1
PESG	75.0	5	64.80 ± 3.43 A	47.4-90.5
	100.0	2	67.4*	44.0-91.0
CONTROL	0.0	10	11.3 ± 5.2 B	0.0-18.0

PESG; Pesguard S102.
[a] Means within a column followed by the same letter are not significantly different (P = 0.05, Student-Newman-Keuls [SAS Institute 2003]).
*Not included in statistical analysis.

Table 3-3. Population change of wild mosquitoes exposed to aerial adulticiding treatments with two water-based products in Thessaloniki, Greece.

Treatment	Application Rate (ml/ha)	n	% Mean [a] Decrease [b] ± Std Error
AKO	37.5	2	61.5*
	50.0	5	76.5 ± 11 A
PESG	75.0	5	78 ± 9 A
	100.0	2	89*
CONTROL	0.0	10	- 3.4 ± 16 B

AKO; Aqua-K-Othrine.
PESG; Pesguard S102.
[a] Means within a column followed by the same letter are not significantly different (P = 0.05, Student-Newman-Keuls [SAS Institute 2003]).
[b] Population decrease was determined with comparison between numbers of pre-and post-treatment adult mosquitoes trapped in CDC traps placed within experimental sites and deployed 24 hrs before and after each spraying trial.
*Not included in statistical analysis

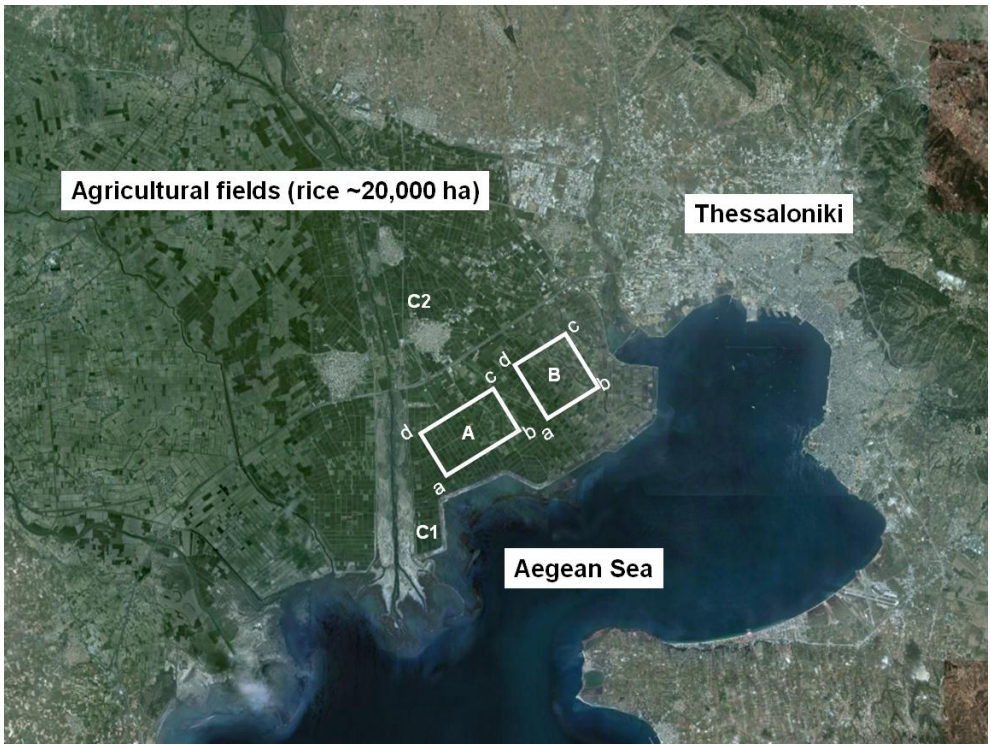
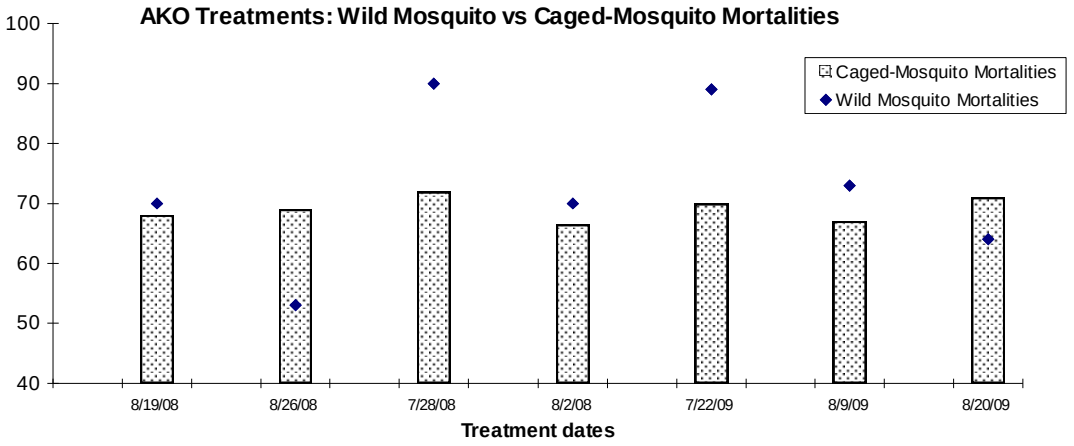


Figure 3-1. Two satellite images showing Thessaloniki and the agricultural region of Northern Greece with the 2 treatment (A and B) and control (C1 and C2) areas.



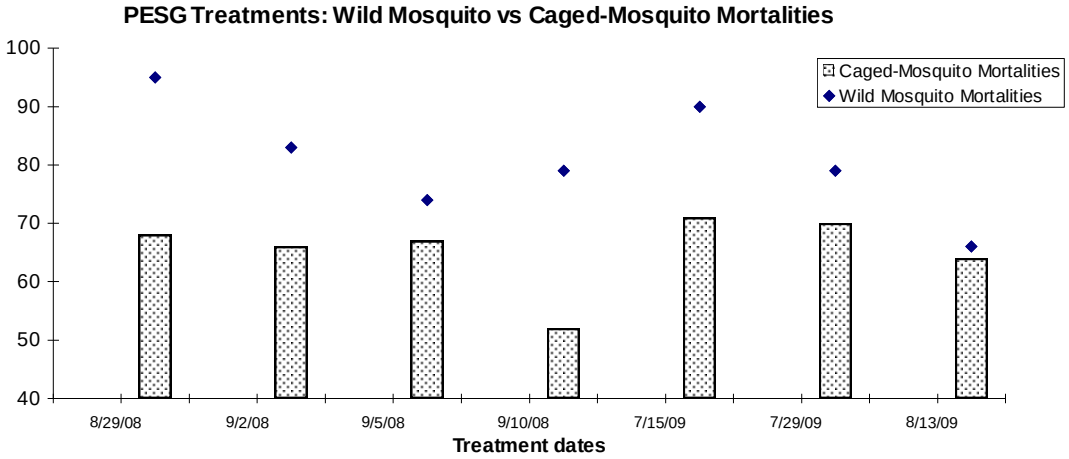


Figure 3-2. Percent mean caged-mosquito mortalities and percent wild mosquito population mortalities for each one of the 7 aerial treatments with Aqua-K-Othrine (AKO) and the 7 aerial treatments with Pesguard (PESG).

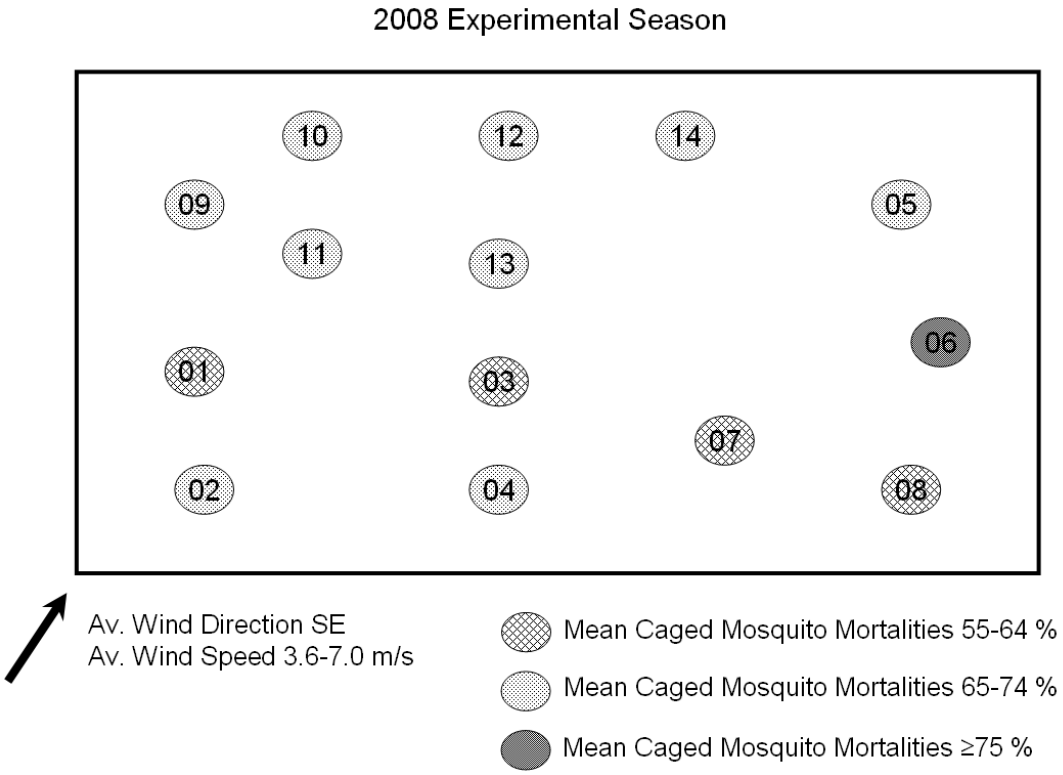


Figure 3-3. Mean caged mosquito mortalities per location (1-14) for all 8 trials conducted during 2008. Locations with different background designs are significantly different (P<0.05, Tukey test, [SAS Institute 2003]). For all trials during the summer of 2009, there were no statistically different mean caged-mosquito mortalities based on location.

CHAPTER 4
NON-TARGET EFFECTS OF AERIAL MOSQUITO ADULTICIDING WITH WATER-BASED UNSYNERGIZED PYRETHROIDS TO HONEY BEES AND OTHER BENEFICIAL INSECTS IN THE AGRICULTURAL ECOSYSTEM OF NORTHERN GREECE

> The 1960's was the beginning of a change, a time of unrest in this country. *Silent Spring* by Rachel Carson was published. Earth Day 1970 marked the beginning of environmental awareness in the form of laws and regulations. Some of these laws and regulations were long overdue and some were unreasonable. Suddenly it was fashionable to be an environmentalist. I resent this in many ways because I feel we in mosquito control were the first environmentalists when it was not the thing to be. We cleaned up after careless builders moved out of a newly developed area and eased the drainage problems that caused mosquito breeding. We established ponds and ditches in marshes and uplands that were beneficial to wildlife and increased productivity on marshes. We were the first biologists who knew the value of marsh and that it was not just a vast wasteland to be filled… This is a success story. Many of our distinguished colleagues of the past managed to get their message across. It is now our responsibility to carry the message to the future – Judy Hansen. (Patterson 2009, p. 222 from The Mosquito Crusades)

Introduction

Mosquitoes undoubtedly constitute one of the world's most serious nuisance and disease related pests that are responsible for the suffering of more than 500-700 million people globally (Drew 2002), with ~ 3 million deceased annually (AMCA 2010). Since the association was made between mosquitoes and disease (Chaves-Carballo 2005), approximately 100 years ago, science has evolved to produce advanced tools to be utilized in the most efficient ways to fight against the mosquito menace (FCCMC 2009). The most recent trend is the development of products and techniques that are specifically targeted against mosquitoes while minimally affecting non-target beneficial organisms. These products may be biorational agents (Ware 1989) specifically toxic to mosquitoes, or broader action, "traditional" insecticides, which, when used judiciously at the proper time, dose, and with the proper application technologies, can produce efficacious results with negligible impact on non-target organisms.

Aerial Ultra Low Volume (ULV) applications for mosquito adulticiding involve applying low rates of insecticides from an aircraft (10-100 ml/ha depending on the formulation, Latham

and Barber 2006) in the form of an aerosol space spray, utilizing an efficient droplet size range ($Dv_{0.5}$ <60 µm, $Dv_{0.9}$ <100 µm as per label recommendations), to target the flying adult mosquitoes. In order to maximize the spray efficacy the applications occur during crepuscular or nocturnal hours when mosquitoes are mostly active, while non-target beneficial organisms, such as bees and butterflies, are not (FCCMC 2009). The effects of organophosphates and synergized pyrethroids ULV formulations applied by air have been continuously studied through direct toxicity studies on beneficial and non-target organisms (Jensen et al. 1999, Dukes et al 2004, Zhong et al. 2004, Breidenbauch & Szalay 2010, Macedo et al. 2010).

Ultra Low Volume formulations containing unsynergized pyrethroids are currently the only available products with a label for outdoors ULV mosquito adulticiding in some Europena Union (EU) countries. These formulations represent a new generation of products with potentially lower environmental effects (Groome et al. 1989). Unlike the oil-based, synergised pyrethroids commonly used in the U.S., unsynergized, water-based products seem to be more acceptable in environmentally sensitive regions. Aqua-K-Othrine and Pesguard S102 are two water-based, unsyngerized formulations of deltamethrin and d-phenothrin, respectively, available for mosquito adulticiding in Greece, a member of EU. In chapter 3, I described the effectiveness of these formulations as adulticides and determined effective dose. However, the formulations must be considered not only for their efficacy against the target vectors but also for their safety to beneficial non-target organisms.

Both deltamethin (type II pyrethroid) and d-phenothrin (type I pyrethroid) are known to be highly toxic to beneficial terrestrial insects such as bees (LC50s 0.05 and 0.0005 ug/bee for deltamethrin and d-phenothrin, respectively, NPIC 2010) and aquatic insects such as *Daphnia magna* (LC50s 0.113 and 4.5 ug/L of water for deltamethrin and d-phenothrin, respectively, Xiu

et al. 1989, WHO 2002). There are no studies currently available reporting acute effects of aerial ULV applications of unsynergized deltamethrin and d-phenotrhin to terrestrial and aquatic non-target insects.

Despite the large body of literature on the effect on bees of insecticides used in agricultural applications (Johansen et al. 1983, Shires et al. 1983, Shires et al. 1984, Morandin & Winston 2003), little is available on the effects of mosquitocidal applications (Zhong et al. 2003b, Zhong et al. 2004, Boyce et al. 2007). Most of the literature on the effects of ULV pyrethroid mosquitocidal treatments involves the immediate exposure of caged bees to the insecticidal cloud during crepuscular hours (Colburn & Langford 1970, Womeldorf et al. 1974, Caron 1979, Pankiw & Jay 1992, Boyce et al. 2007). This type of field bioassay does not take into account the natural biology of the bees, which return in their hives during the night (Seeley 1996). These experiments do not provide information on the realistic effects of the treatment to the bee populations. Zhong et al. (2004) investigated the effects of aerial ULV applications of the organophosphate naled on beehives; however, the effects of aerial pyrethroid ULV application on beehives have not been investigated.

Measurement of the toxicity of pesticides to bees has relied largely on the acute mortality due to direct exposure of the insects to the insecticide (Colburn & Langford 1970, Boyce et al. 2007). However, mortality may only be a partial measure of the deleterious effects of the insecticides. In addition to direct mortality, the sublethal effects of insecticides on bee physiology and behavior must, also, be considered. Sublethal effects are defined as effects (behavioral or physiological) on individuals that survive exposure to a pesticide (Desneux et al. 2006). The sublethal effects could result in problematic beehive performance even in those cases where there is no apparent post treatment bee mortality.

In any given day, bees from a colony will perform several activities such as collect water, gather pollen and nectar from a variety of plant species. Possible insecticide residues on any nectar and pollen source for bees could contaminate the hive and stress or kill bees (Desneux et al. 2006). While bees collect pollen from all types of plants, they show preference for certain types of pollen. The most attractive pollen to bees is one that provides the best nutrients for bee development (Daluz et al. 2010). The bees preference to certain types of pollen may increase or decrease the likelihood of them coming in contact with those plant species present within the insecticide treatment areas. Knowledge of the pollen species that the experimental bees collect during an experimental season may provide important information on whether the bees have foraged and collected pollen from the plant species dominant within the treatment areas, and whether bees may have come in contact with possible insecticide residues.

To address all the above issues, the first objective of this study was to evaluate the acute/immediate toxic effects of aerial applications of 2 water-based unsynergized mosquito adulticiding products to aquatic and terrestrial non-target insects, including bees, occurring in abundance in the rice-field ecosystem of North Greece. The second objective of this study was to investigate sublethal effects of these 2 water-based unsynergized mosquito adulticiding products on the performance of beehives exposed to the aerial treatments. The final objective was to identify the pollen species collected from the bees form exposed hives in order to determine the major foraging activity/plant preference of the bees.

Materials and Methods

This work was a two-year study (2008-9) conducted in the agricultural area of West Thessaloniki, Greece. Research permits were obtained from the Greek government and the work was observed by Greek regulatory authorities (Ministries of Rural Development and Food, and Health and Social Solidarity) as well as by AGROLAB S.A, a European laboratory consultancy.

The research was certified for Good Experimental Practice (GEP) by the Greek Ministry of Rural Development and Food, so results could be used in registration of products and application technology in Greece and other EU countries. GEP studies are similar to Good Laboratory Practice (GLP) except that they apply to field experimentation.

Insecticides

Two commercially available ULV insecticides registered in Europe for ground mosquito adulticiding were used for the aerial treatments: Aqua-K-Othrine (2% w/w deltamethrin, Bayer Environmental Science, Lyon, France), a water-based, anti-evaporant Film-Forming Aqueous Surface Technology (FFAST®) formulation, and Pesguard S102 (10% w/w d-phenothrin, Sumitomo Chemical Co., Ltd., Osaka, Japan), a water-based microemulsion formulation.

In 2008, two rates were tested with each product based on recommendations of the World Health Organization (WHO) for ULV applications of insecticides for mosquito adulticiding (WHO, 1997) and on the label rates of the specific products for use in other countries where they are registered. For Aqua-K-Othrine, 0.75 and 1.00 g/ha of active ingredient (AI) were tested, corresponding to 37.5 and 50.0 ml/ha of 2% AI formulation, respectively. For Pesguard S102, 7.5 and 10.0 g/ha AI were tested, which corresponds to 75.0 and 100.0 ml/ha of 10% AI formulation, respectively. In 2009, Aqua-K-Othrine was tested at the higher of the two previously tested application rates, and Pesguard S102 was tested at the lower of the two rates. Over the 2 years, for Aqua-K-Othrine the lowest application rate was replicated twice and the highest one five times. For Pesguard S102 the highest application rate was replicated twice and the lowest one five times. A total of 7 spraying trials were conducted with each product. Both formulations were sprayed undiluted.

Experimental Sites

During 2008, there were 2 experimental sites chosen, one treatment site (Area A) where all insecticide treatments were conducted, and one control site where no treatments took place. During 2009, because beehives were added in the experiments and the bees had to be exposed to each insecticide separately, an additional treatment site was included (Area B) that would allow for each insecticide to be applied at a separate site (Figure 4-1). The treatment sites were open agricultural areas, approximately 1,000 ha in size (~ 4 x 2.5 and ~3.5 x 3.21 km areas A and B, respectively). They consisted of rice, cotton and cornfields (~60% and ~90% rice fields for 2008 and 2009, respectively) and were at least 3 km away from any urbanized areas as required by permits from the Greek government. The control sites assigned for both years were similar to the treatment sites consisting mostly of rice fields and located ~3-5 km from the treatment areas.

Application Technologies

Insecticide application was conducted by an aerial contractor company, Air Applications-ASNF (State Airport of Macedonia, Thessaloniki, Greece) using a turbine helicopter (500C model, McDonnell Douglas Helicopters, Mesa, AZ). The helicopter was equipped with a pair of electric rotary atomizers (Micronair AU 6539 model, Micron Sprayers Ltd, Bromyard, Herefordshire, UK). The nozzles were positioned on the lateral booms facing toward the rear of the helicopter (1.4 m out from the aircraft centreline but in line with the center of the main rotor). The nozzles generated droplets within a 38-45 microns volume median diameter (VMD) range. The applications were conducted approximately 30 min after sunset (during high mosquito activity) at an altitude of ~61 m and an air speed of ~145 km/h. The swath width for the first two trials of Aqua-K-Othrine was 152 m but it was increased to 305 m for the remaining five trials, so that sharp turns that caused concern for pilot safety could be avoided. All seven Pesguard S102 trials were conducted at an intermediate swath of 228 m. Based on the swath, the flow rates

were adjusted each time so that the appropriate amount of active ingredient was applied per hectare (Table 3-1).

The helicopter was equipped with a flight guidance GPS system (Wingman™ GX, Adapco, Sanford, FL) which included an onboard moving map screen that displayed aircraft position, spray block boundaries, and flight lines. Additionally, the helicopter was equipped with a real-time meteorology system (AIMMS-20™, Aventech Research Inc., Ontario, Canada) that recorded information such as wind speed and direction, temperature and humidity at the spraying altitude. The real-time meteorological data recorded by the AIMMS-20™ system were automatically processed by the Wingman GPS guidance system. The USDA spray dispersal computer model AGDISP1 (Bilanin et al. 1989) had previously been run to predict the spray movement based on application parameters and meteorological conditions. Results of numerous spray model runs covering a range of meteorological conditions and application parameters for this specific helicopter and droplet spectrum were incorporated into a "lookup table" into the Wingman system. Spray offsets were calculated and flight lines were positioned automatically through the Wingman/AGDISP lookup table system. As wind speed and direction changed, the system adjusted guidance/offsets for the correct flight line positions for optimum coverage. The pilot followed the flight line guidance provided by the system for each of the spray lines necessary to cover the treatment area.

Non-target Insects

Aquatic

Two aquatic non-target insects were used: mosquito larvae and aquatic beetles. Wild insects were field-collected from rice fields and other natural aquatic habitats outside the treatment area and were reared in the laboratory (25°C, >60% RH). Mostly second instar larvae of the genus *Aedes* were used for all experimental trials. Although adult mosquitoes were the

target insect in these applications, their aquatic larval stage is not an intended target and is an abundant representative of the most sensitive non-target aquatic organisms. The genus *Aedes* was chosen because the larvae are characterized by synchronous developmental stages so, when collected from the wild, the majority of the larvae were of the same instar. The second instar was chosen because of its high sensitivity to insecticidal compounds, and, specifically, pyrethroid compounds.

The adult *Hydroglyphus geminus* Fabricious (Coleoptera: Dytiscidae) beetles are semi-aquatic insects of approximately 1-1.5 mm in length. They are predacious insects naturally occurring in large numbers in rice fields where mosquito larvae are one of their main target preys; therefore, they are considered beneficial insects. In the laboratory, the beetles' diet consisted of live mosquito larvae.

Terrestrial

Three terrestrial non-target insects were used: lace-wings, beetles, and bees. Green lace-wing larvae, *Chrysoperla carnae* Stephens (Neuroptera: Chrysopidae) are very active, gray or brownish and alligator-like with well-developed legs and large pincers which they use to trap their prey. They range in size from <1-8 mm. They are highly predacious and feed on several species of agricultural pests such as aphids, spider mites (especially red mites), thrips, whiteflies, eggs of leafhoppers, moths, and leafminers, small caterpillars, beetle larvae, and the tobacco budworm.

For the experiment, *Chrysoperla* larvae were imported from a European biological control company (Koppert, Berkel en Rodenrijs, Netherlands). The larvae were received as 1st ands 2nd instars in Koppert bottles and were maintained in plastic rearing containers in an incubator at 15°C (Figure 4-2). For the experiments, 3rd and 4th instar larvae were used. Approximately 24 hrs

prior to the spraying trial, the larvae were moved from the incubator to the laboratory at 23°C in order to acclimate them for field exposure. Lacewing larvae were fed with *Ephestia kuhniella* Zeller (Lepidoptera: Pyralidae) eggs and aphids. The use of aphids as a food source significantly reduced the degree of cannibalism in the rearing containers.

Adult beetles, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) are small (about 3-4 mm long), dark brown lady beetles with a tan to orange head and posterior. *Cryptolaemus. montrouzieri* are particularly voracious predators and are known to be efficient biological control agent for mealybugs. They have been observed to feed on other herbivorous insects such as aphids and scales. For the experiments adults *C. montrouzieri* were purchased from a Greek biological control company (BioInsecta, Thessaloniki, Greece). The adults were received the day of the experiment and were used immediately.

The common European honeybees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), were exposed to the treatments within their hives. Fifteen hives (Langstroth, ~20 x 28 x 18 cm) were obtained from the Aristotle University of Thessaloniki and were delivered to the experimental sites (5 hives in Area A, 5 hives in Area B, and 5 hives in the control area) 2 weeks prior to the experimentation to allow for bee acclimatization. The hives were located in the centre of the treatment sites to ensure exposure to the majority of the insecticidal cloud (Figure 4-1). Previous studies have shown that hives located well within an insecticide treated area sustain more losses than hives situated beyond the treated area, at the edge or outside (Anderson and Atkins 1968). The hives were left in the experimental sites for the entire summer season during which the trials were conducted (~2.5 months). Upon delivery each hive contained a minimum of 4 brood frames and ~12,000 adult bees and was provided with artificial nectar substitute that bees could freely access in the case of insufficient food sources within their new environment.

Assessment Bioassay

Sampling stations: Eight to fourteen sampling stations were deployed at pre-assigned locations within each treatment site (Figure 4-1). Five sampling stations were placed in the control area. All sampling stations were deployed and handled in a similar manner. Each sampling station contained holding devices for each one of the non-target insects except for the bees, which were in their hives.

Aquatic insects: Every sampling station had one open 237-ml container (American Plastics, Chattanooga, TN) filled with 200 ml deionized water for each of the 2 aquatic non-target organisms. The containers with water were placed in the field ~15 min before the spray application commenced and remained there until ~30 min after the spraying was completed to ensure enough time for the entire insecticidal cloud to pass through the intended area. Approximately 60 min post spraying, all containers were returned to the laboratory and approximately 40 *H. geminus* adult beetles and 40 *Aedes* sp. mosquito larvae were placed in separate containers from each sampling station. Mortality was determined 12 hours after the insects were added to the containers. The non-target insects were added to the containers in the laboratory to avoid unnecessary stress during travel from the field to the lab (U.S. EPA 1996), as significant non-target mortality can be caused by vibration of containers as the vehicles move on rough, uneven roads.

Terrestrial insects: Every sampling station had one open 237-ml container (American Plastics, Chattanooga, TN) containing 10 *Cr. montrouzieri*, and two containers containing 5 *Chr. carnae* each. The reason why *Chr. carnae* individuals were divided into 2 containers was to prevent cannibalism resulting from overcrowding. The preparation of the containers with the insects was initiated approximately 2 hours prior to each trial. The containers were handled as described above for aquatic non-target insects.

The beehives were monitored for presence of dead bees the morning before and 12 hrs post-spraying and at weekly intervals (for a total of 10 weeks). All of the beehives were fitted with entrance traps to collect the dead bees. In addition to bee mortality, the performance/development of the beehives was monitored throughout the summer season. The adult bee and brood population along with the weight of each hive was recorded once a week. The presence of black shiny bees (indicative of bees exposed to sublethal quantities of insecticide) was also recorded by removal of each frame from the hives and careful observation for the presence of unusual looking adult bees. Also, pollen was collected weekly. One pollen entrance trap was deployed in one hive per treatment area (Figure 4-3). Pollen species composition was conducted to identify the plants with the highest appeal to the foraging bees.

Statistical Analysis

All statistical analyses having the insecticides as treatments were performed with the SAS software package (SAS Institute Inc., Cary, NC). Because results were similar for the 2 doses per insecticide tested in the first year, the low dose of Aqua-K-Othrine and the high dose for Pesguard S102 were dropped for the second year test and were not included in the statistical analysis. Three to five replicates (depending on the insect species) were analyzed per insecticide dose. One-way ANOVA was performed to determine the effect of the insecticide treatment on percent non-target insect mortality. Data were arcsine-square-root-transformed before ANOVA. Means were separated using Student-Newman-Keuls ($P = 0.05$; [SAS Institute Inc. 2003]).

Also, a paired t-test was performed to determine if the insecticide treatments had any detrimental effect on the development of the beehives ($P = 0.05$; [SAS Institute Inc. 2003]). The adult bee population, brood population, and the weight of the beehives before the treatments (beginning of the summer season) and after the treatments (end of the summer season) were compared for each treatment area and the control area.

Lastly, one-way ANOVA was performed to determine whether there was any significant difference in beehive performance between the 3 areas tested (treatment Area A, treatment Area B and the control Area C). The percent change in adult bee population, brood population, and hive weight between the initial data (beginning of the summer season - pre-spraying) and final data (end of the summer season - post-treatment) was compared across the three areas. Data were arcsine-square-root-transformed before ANOVA. Means were separated using Student-Newman-Keuls (P = 0.05; [SAS Institute Inc 2003]).

Results

Aquatic Insect Acute Mortality

For the two non-target aquatic species, very low mortalities were observed which were not significantly different from the mortalities of the non-target controls. No mortalities were observed for the *H. geminus* beetles for both products at both doses tested. The control mortalities were slightly higher but not significantly different than the treatment mortalities. Similarly, for *Aedes* larvae, very low or no mortalities were observed, which were not significantly different than the control mortalities (Table 4-1).

Terrestrial Insect Acute Mortality

Both terrestrial non-target insect species had very low mortalities when exposed to either one of the products tested. Specifically, the mean *Ch. carnae* mortality was 2.50 % and 1.67 % when exposed to Aqua-K-Othrine and Pesguard, respectively. Similarly, the mean *Cr. montrouzieri* mortality was 1.88 % and 3.33 % for Aqua-K-Othrine and Pesguard, respectively. Control mortalities remained low and were not significantly different than the treatment mortalities for both products tested. There were no unusual acute mortalities observed on adult bees collected on the traps fitted at the entrance of each hive. Average daily mortality never

exceeded 10 dead bees per hive (Figure 4-4). Also, no shiny bees (bees with fallen setae - indicative of sublethal exposure to insecticide) were observed in any of the hives.

Beehive Performance

The beehives in the 2 treatment areas performed as well as the beehives in the untreated, control area, and increased in weight (25-30%), adult bee population (14-18%), and in brood population (15-19%). There was no statistically significant difference in the percent increase between the two treatment (Area A, and Area B) and the control (Area C) areas (Figure 4-5). When comparing the development of the beehives before and after the aerial treatments for each one of the treatment areas, there was no significant increase in adult bee population for all 3 areas (Area A, Area B, and Area C, Table 4-2). However, there was statistically significant increase in brood for the treatment area B and the control area. Similarly, there was a significant increase in the total weight of the beehives in all 3 areas.

Pollen Identification

A total of 950 gr, 890 gr, and 750 gr of pollen from a single hive were collected from Area A, Area B, and the control area, respectively (Figure 4-6). Along with the pollen we were able to identify what initially looked like a type of creamy grains, as rice flower anthers (Figure 4-7). Pollen was identified utilizing pictorial keys based on color and structure (Table 4-3). At least 7 different pollen species were collected by the bees during the experimental season. By far the plant species with the highest preference was rice, *Oryza sativa*, followed by *Tribolium*.

Discussion

In our study of non-target organisms, very low mortalities post-treatment were observed for all non-target insects, *Aedes* sp. larvae, *Ch. carnae* larvae, *H. geminus* and *Cr. montrouzieri* adult beetles exposed to both products tested. These results suggest that very low ground deposition of insecticides occurred during our trials. Mosquito larvae in particular are known to

be very susceptible to pyrethroid insecticides, with LC_{50}s for *Aedes* species as low as 0.07 and 0.56 µg AI/L of water for deltamethrin and d-phenothrin, respectively (U.S. EPA 2009). Our results agree with previous research which concluded that when mosquito adulticides are delivered in appropriate droplet size, similar to the one used in the research presented here, they result in low ground deposition levels with no measurable negative effect to aquatic non-target species (Dukes et al. 2004). Similar results were obtained with aerial ULV night spraying that resulted in low insecticide deposition and low *Ochlerotatus taeniorhynchus* larvae mortality (Barber et al. 2008). Another study showed that aerial ULV treatments of pyrethrin, malathion, and permethrin for mosquito control had no significant effects on aquatic insects or fish naturally occurring in seasonal wetlands (Jensen et al. 1999).

Normal daily bee mortality reported in the literature varies from 20-25 bees/hive (Delabie et al. 1985), to 30-80 bees/hive (Gary & Mussen 1984), and ≤100 bee/hive (Johansen 1977). Very low, not significant bee mortalities, which did not exceed the lowest reported normal daily mortality (<20 bees/hive), were observed in our experimental beehives. Honeybees are highly susceptible to both products tested, with LD_{50s} 0.05 (24 h mortality) and 0.005 µg/bee (48 h mortality) for deltamethrin and d-phenothrin, respectively (NPIC 2010). Studies have shown that the timing of an insecticide application (diurnal versus nocturnal) has significant effects on bee mortality and subsequent hive performance (Anderson and Atkins 1968, Byrne and Waller 1990). It was shown that pesticides caused higher losses to bees when applied during day (Byrne and Waller 1990). Because our spraying trials occurred during late evening hours, when the vast majority of the bees are not actively foraging and are sheltered inside their beehives (Seeley 1995), the bees were minimally exposed to the insecticide. This is probably one of the main

reasons why no significant adult bee mortalities were observed the morning following each treatment.

Insecticide residues on water, or any nectar and pollen source for bees could contaminate the hive and stress or kill the bees. For example, sublethal doses of parathion were shown to alter bee foraging activity by slowing flight speed and affecting the time sense of foraging bees (Desneux et al. 2007). Communication of direction and distance information by dancing bees was also adversely affected (Desneux et al. 2007). These effects would significantly reduce beehive productivity. In laboratory studies it has been shown that several chemicals either reduce bee egg hatch or brood production (Erickson & Erickson 1983). In another more recent study, the disorienting effects of deltamethrin on the homing ability of foragers were demonstrated (Desneux et al. 2007). Possible insecticide residues on water bodies and plants exposed to our aerial treatments could have affected bee behavior and subsequently reduced beehive productivity. In our study, the beehives in the treated areas performed as well as those in the untreated control area and increased in adult bee population, brood, and weight. This indicates that insecticide deposits from our treatments were very low and did not have any biologically significant effect on the experimental bees foraging within the treatment areas. It is important to state that by the end of the experimental season the beehives in both treatment and control areas had reached maximum capacity and the colonies, in their attempt to expand, constructed new combs on the cover of the hives (Figure 4-8).

Honeybees typically show preference for pollen rich in protein content. Pollen with a protein level ≥25% has been recognized as excellent quality pollen. This type of pollen normally is found in plants that are zoophilous (pollinated by animals, such as bees) (Somerville 2005). Pollen with <20% protein content has been described as of poor quality and is normally found in

anemophilous (wind-pollinated) plant species (Somerville 2005), such as species of the family Graminae (Schmidt and Bothma 2005). Some of the world's most important crops, such as rice, are members of this family (Schmidt and Bothma 2005). Typically bees do not prefer to collect pollen from anemophilous plants, probably because of its unpalatability and unsuitability for normal hive development (Pernal and Currie 2002). Approximately, 90% of our treatment areas consisted of rice cultivation (pollen protein content 13.5). Interestingly, the experimental bees collected mainly rice pollen and were able to maintain healthy and productive hives. This is the first report in literature with evidence that cultivated bees will collect pollen from rice, when it is their most readily available pollen source, and will manage to maintain a healthy hive. This surprising/non-anticipated finding provided us with evidence that the experimental bees were foraging on the rice, the main plant species within our treatment areas and, therefore, became exposed to possible insecticide residues deposited on the rice as a result of our aerial treatments.

With the increasing prevalence of mosquito borne pathogenic diseases in the European community, such as the most recent West Nile Virus epidemic in North Greece with 261 human cases and 34 deaths (Hellenic Centre for Disease Prevention and Control 2010), pyrethroids, such as d-phenothrin and deltamethrin, will be more widely used to control adult mosquitoes. There are public concerns about environmental effects of such increased uses of pyrethroids. It is therefore crucial to utilize proper insecticide application techniques along with knowledge mosquito biology to result in properly targeted, efficacious insecticide treatments while protect the environment and the life within it.

Table 4-1. Mortalities of non-target organisms exposed to the aerial adulticiding treatments with 2 water-based products, Pesguard and Aqua-k-Othrine, in Thessaloniki, Greece.

Treatment	Application Rate (ml/ha)	% MortalityMean [a] ± Std Error			
		Hydroglyphus adults	*Aedes* sp. larvae	*Cryptolaemus* adults	*Chrysoperla* larvae
AKO	37.5	0	0.08*	-	-
	50.0	0	2.20 ± 0.96 A	1.88 ± 1.01 A	2.50 ± 1.09 A
PESG	100.0	0	0.00*	-	-
	75.0	0	3.09 ± 0.93 A	3.33 ± 1.15 A	1.67 ± 0.78 A
CONTROL	0.0	0	2.80 ± 1.47 A	1.00 ± 1.00 A	3.33 ± 1.59 A

AKO; Aqua-K-Othrine.
PESG; Pesguard S102.
[a] Means within a column followed by the same letter are not significantly different (P = 0.05, Student-Newman-Keuls [SAS Institute Inc. 2003]).
*Not included in statistical analysis (replicates=2). For those tests included in the statistical analysis 3≤replicates≤5.

Table 4-2. Development of beehives before and after exposure to aerial adulticiding treatments with 2 water-based products, Pesguard and Aqua-K-Othrine, in Thessaloniki, Greece.

Treatment	Mean [a] adult population per hive ± Std Error (frames)		Mean [a] Brood population per hive ± Std Error (frames)		Mean [a] weight per hive ± Std Error (kg)	
	Before	After	Before	After	Before	After
AKO	8.8 ± 2.7 A	10.4 ± 0.5 A	6.0 ± 1.2 A	6.9 ± 1.1 A	23.4 ± 2.5 A	30.4 ± 4.7 B
PESG	8.4 ± 1.5 A	9.60 ± 1.1 A	6.2 ± 0.8 A	7.4 ± 0.9 B	24.0 ± 3.0 A	30.0 ± 2.9 B
CONTROL	10.0 ± 0.0 A	10.40 ± 0.5 A	6.2 ± 0.8 A	7.6 ± 1.1 B	22.2 ± 3.4 A	26.6 ± 3.2 B

AKO; Aqua-K-Othrine.
PESG; Pesguard S102.
[a] Means before and after the treatments within a row followed by the same letter are not significantly different (P = 0.05, Student-t-test [SAS Institute Inc. 2003]).

Table 4-3. Type of pollen collected by the experimental bees located in the center of the treatment and control areas during the summer of 2009.

Pollen	Area A	Area B	Area Control
Oryza sativa	78.7 %	77.6%	78.0%
Tribolium sp.	5.0%	7.8%	7.8
Carduus sp	3.1%	3.2%	0.8%
Portulaca sp	4.1%	0.9%	8.6%
Vicia sp	5.9%	6.6%	3.1 %
Pistacia sp	0.7%	0.7%	0.4%
Tribulur sp	1.3%	2.1%	1.8
Other	1.02%	1.1%	1.2%

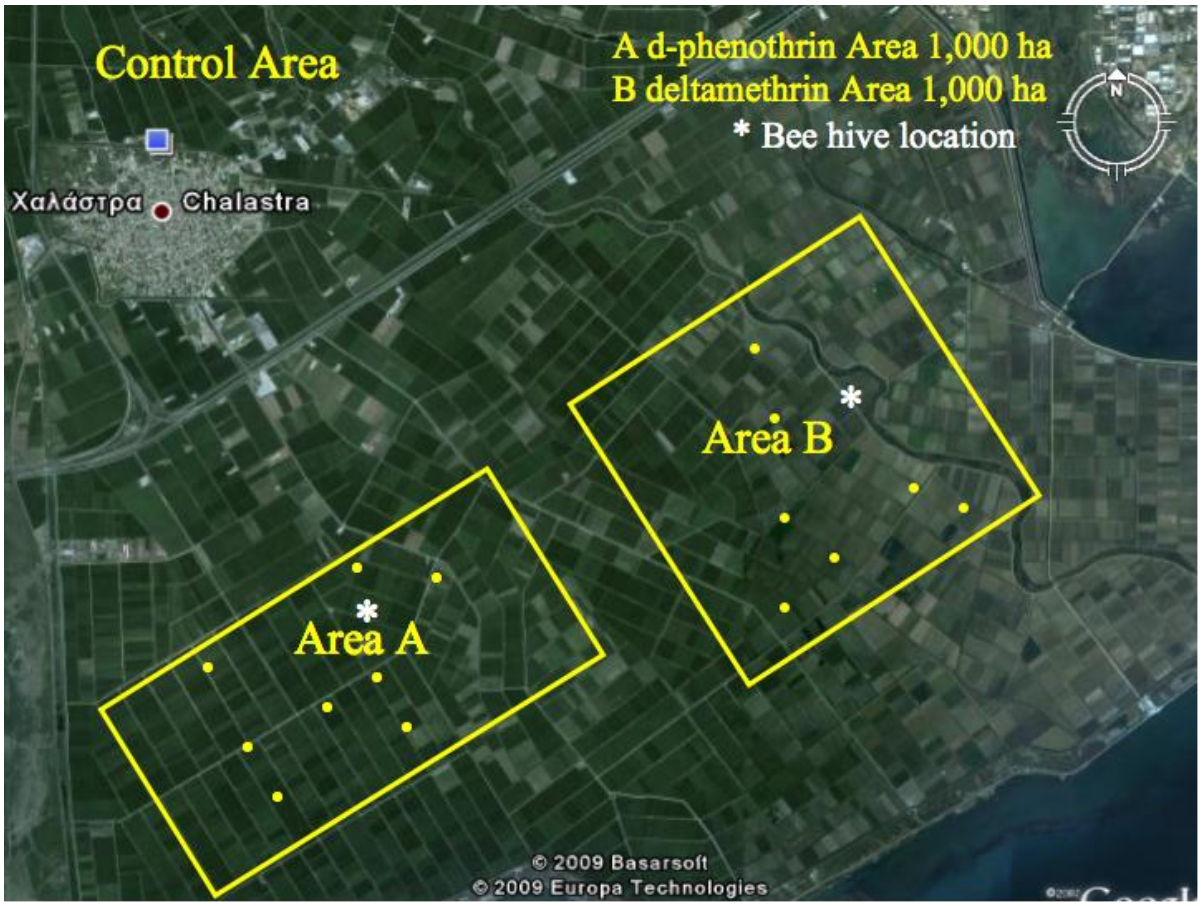


Figure 4-1. A satellite image of the 2 treatment areas (2009 experimental season) located in the agricultural area of West Thessaloniki, Greece. The locations of the sampling stations along with the location of the beehives are portrayed in the image.

A

B

Figure 4-2. Rearing and maintenance procedure for *Chrysoperla carnae*. A) Transferring insects from their shipping bottles into their modified rearing containers. B) Placing containers in the rearing chamber with controlled temperature and humidity.

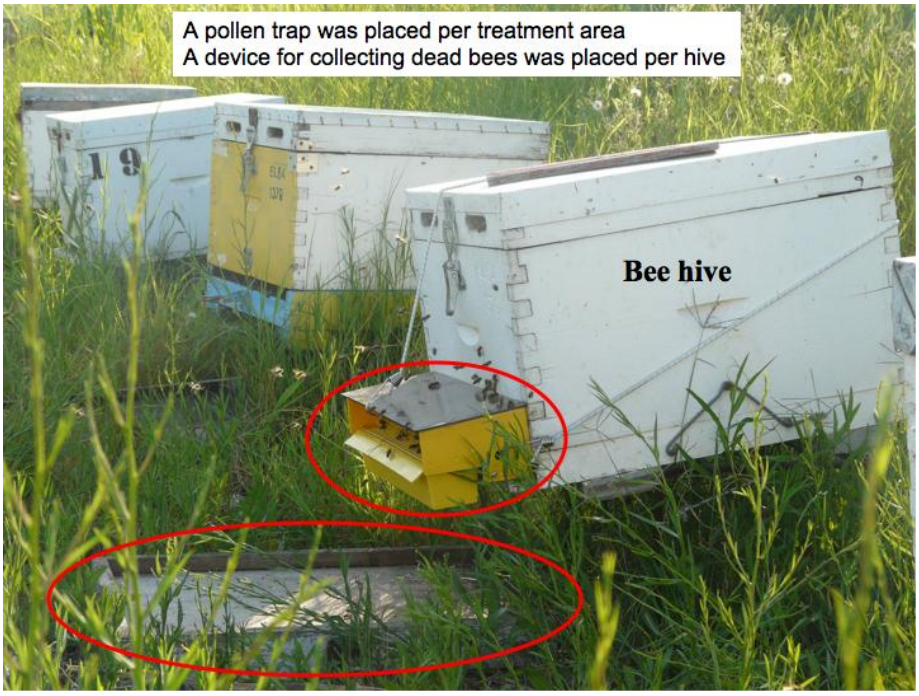


Figure 4-3. Experimental beehives assembled with a pollen trap and a device for collecting dead bees.

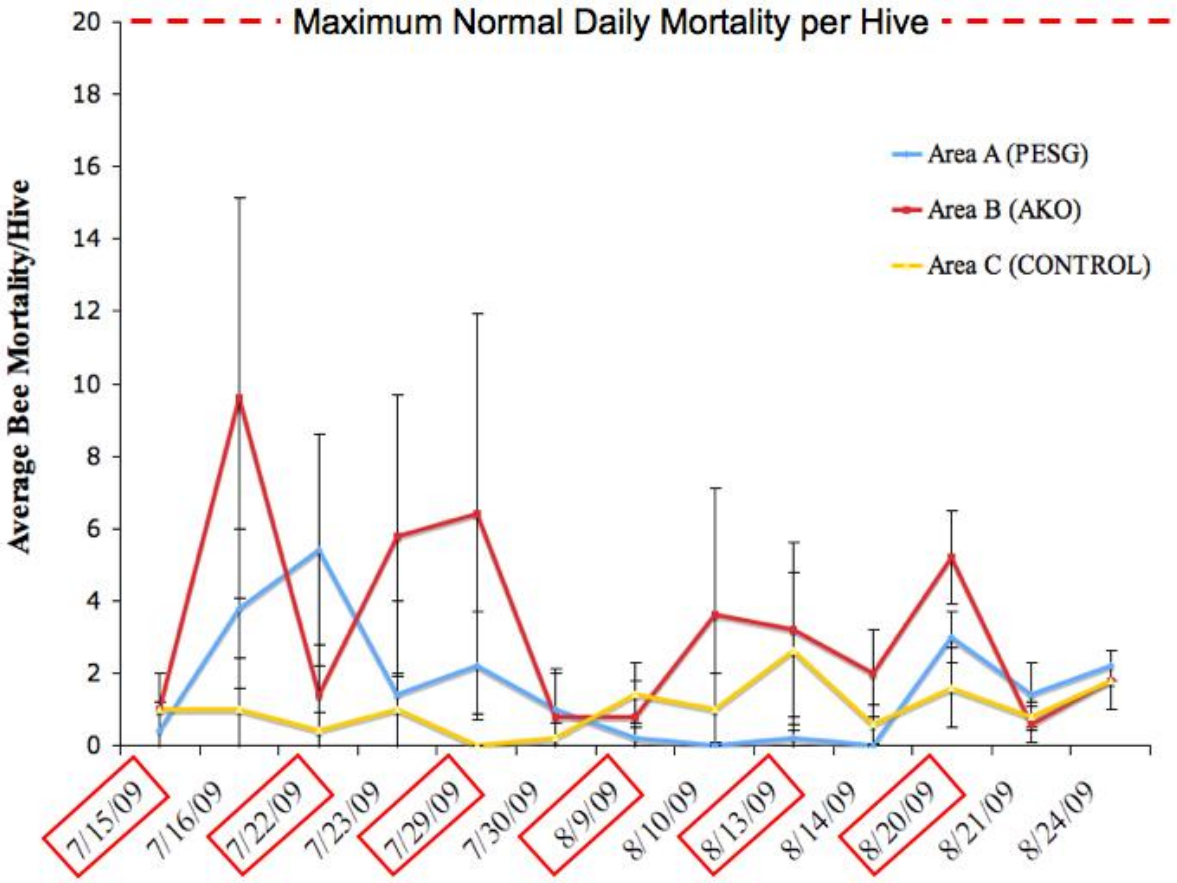


Figure 4-4. Average daily bee mortality sampled the morning before (in red boxes) and the morning following each spray application.

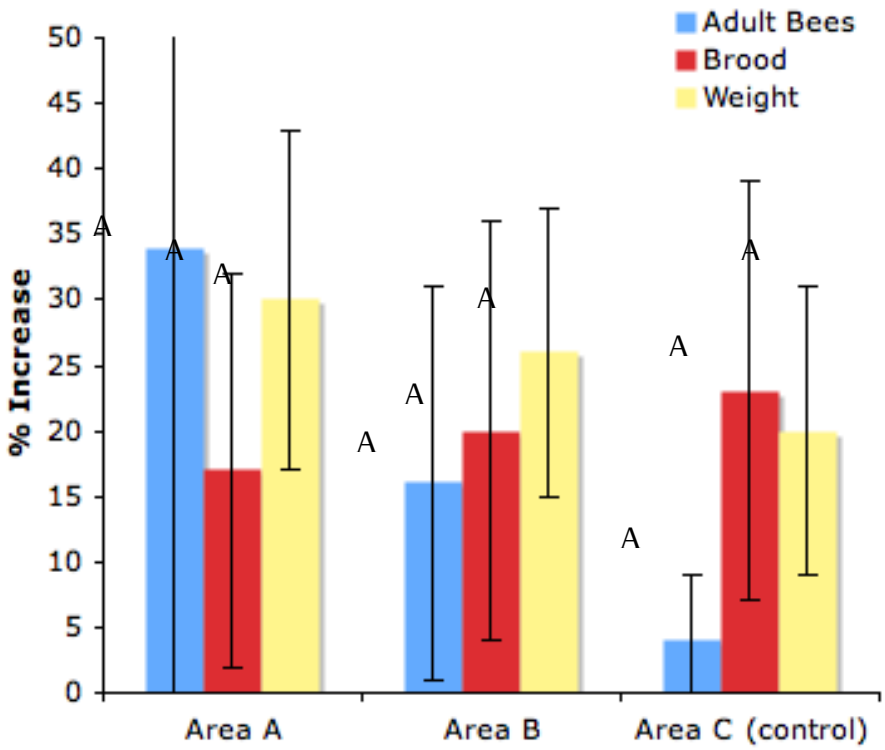


Figure 4-5. Mean percent increase, between the initial data (beginning of the summer season - pre-spraying) and final data (end of the summer season - post-treatment), in adult bee population, brood population, and weight for the experimental hives (N = 5 per area) located in the two treatment areas (A: treated with PESG & B: treated with AKO) and the untreated control area (C: no treatment). Columns with the same letter are not significantly different (P = 0.05, Student-Newman-Keuls [SAS Institute Inc. 2003]).

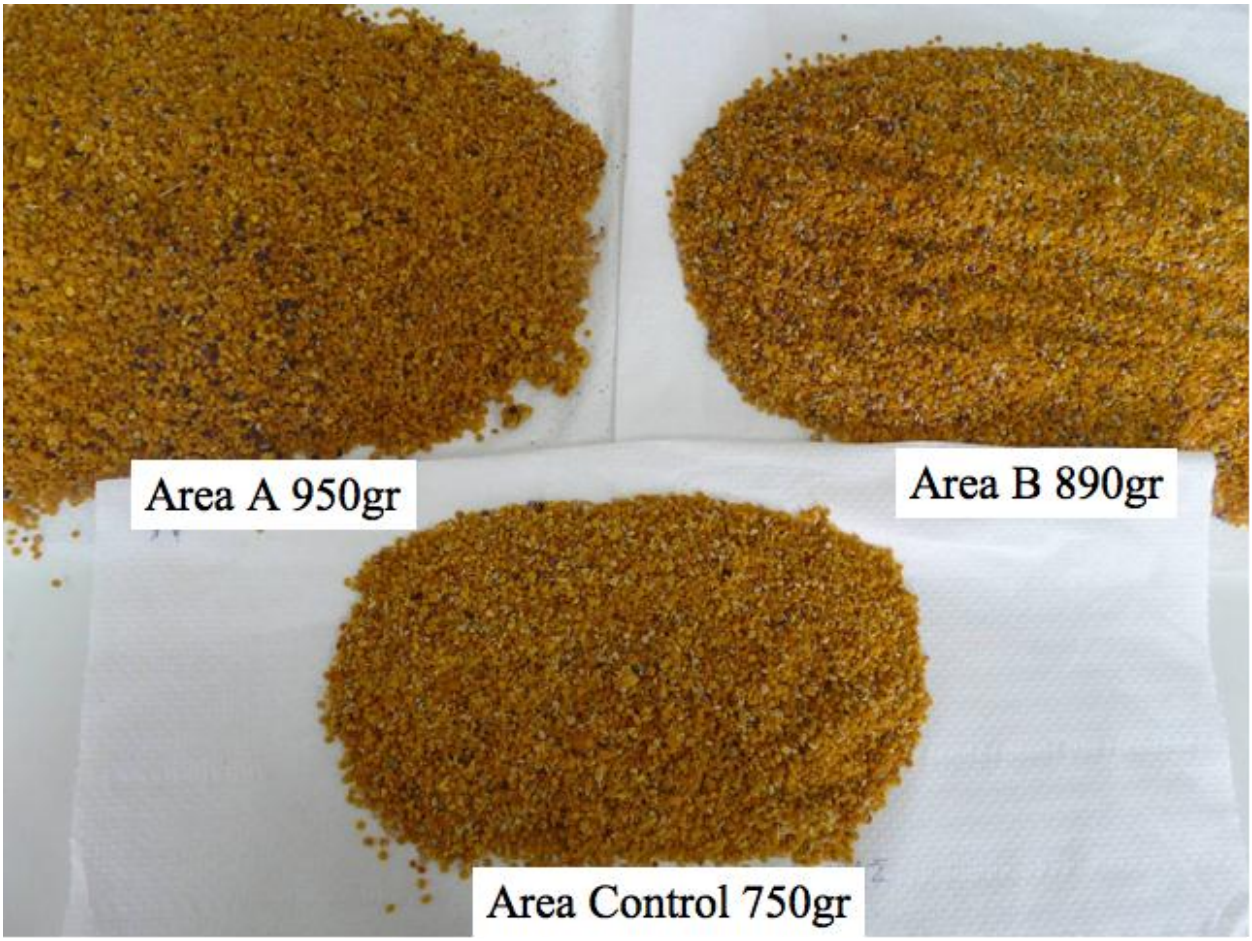


Figure 4-6. Total amount of pollen collected from the two treatment areas and the untreated control area.

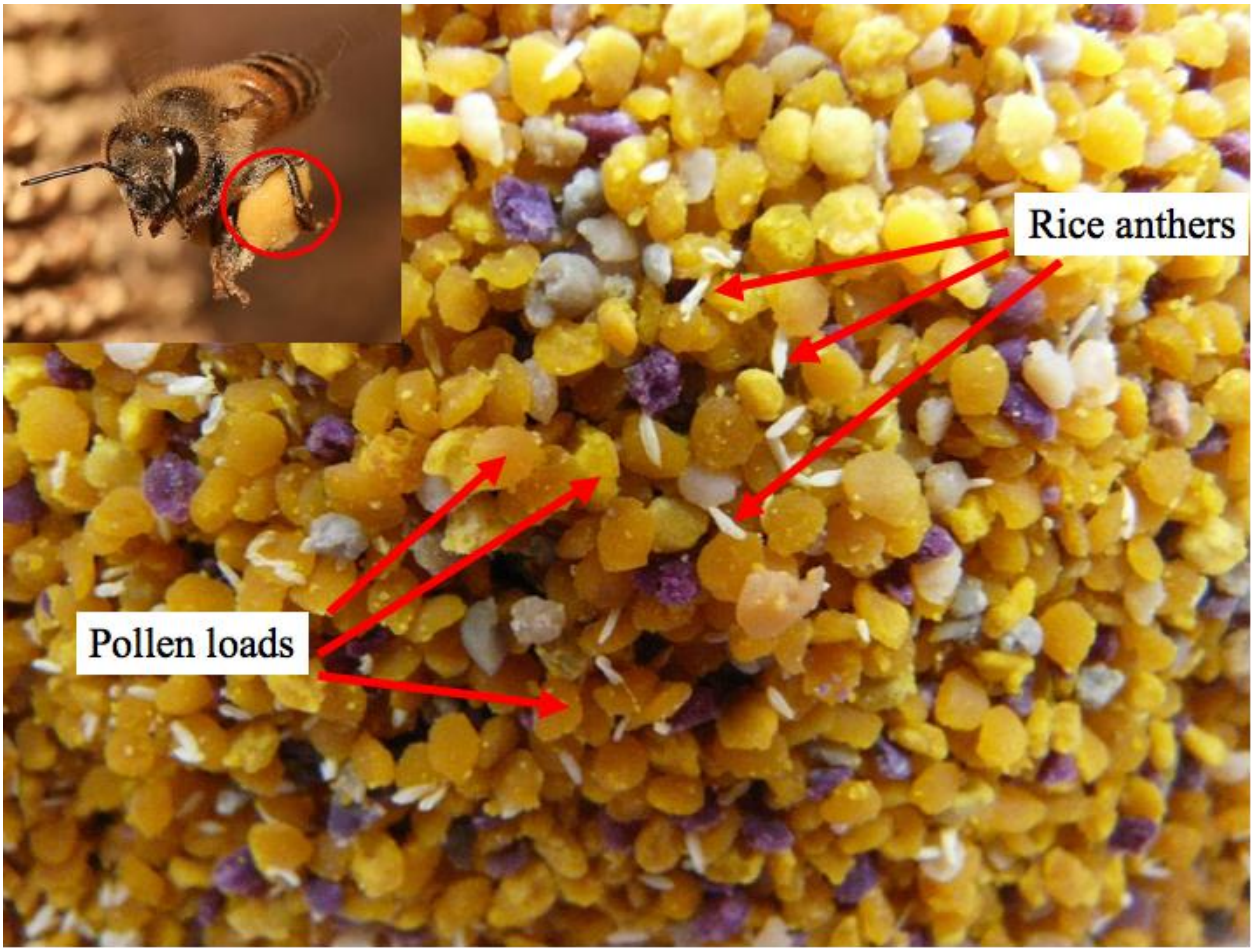


Figure 4-7. Close-up image of the pollen loads along with the rice anthers collected from the agricultural area of West Thessaloniki, summer of 2009.

Figure 4-8. An example of an overcrowded experimental beehive. The bees were constructing new combs (as indicated within the 3 circular areas) on the cover and on the sides of the hive.

CHAPTER 5
DROPLET SAMPLING OF AN OIL-BASED AND TWO WATER-BASED ANTI-EVAPORANT ULV INSECTICIDE FORMULATIONS USING TEFLON® AND MAGENSIUM OXIDE COATED SLIDES

Introduction

The efficacy of an aerosol, ultra low volume (ULV) insecticidal spray (space spray) in targeting flying insects, such as mosquitoes, depends primarily on the size of the droplets contained within the aerosol cloud (Mount 1970). For mosquito adulticiding, the optimum droplet size was shown to be 5-10 microns for ground spraying and 10-25 microns for aerial spraying (Mount 1970); however, most insecticidal aerosols contain a wide range of droplet sizes. In order to acquire a good understanding of how these droplets affect the targeted insect under field conditions, droplet size distribution needs to be assessed in the most accurate possible way. There are various methods available for sampling aerosol droplets in the field and for estimating their size distribution (Rathburn 1970, Matthews 1975, Brown et al. 1990). One of the most commonly used methods is by impaction on rotating glass microscope slides due to its simplicity, affordability, and ease of use.

The coating of the slides used for droplet impaction varies based on the formulation characteristics of the insecticide. For oil-based insecticides, Teflon® coating was found to be a suitable material for droplet impaction (Anderson and Schulte 1971). Teflon® is oleophobic and allows the formation of oil-based droplets that remain in a round shape and do not coalesce, thus facilitating accurate measurements (Anderson and Schulte 1971). However, droplets on the Teflon® surface are susceptible to the effects of temperature and humidity, so aging of the slides may cause droplet shrinkage through evaporation. Fortunately, conventional oil-based products are relatively non-volatile and have been shown to retain their original diameters for extended periods of time on Teflon® slides (Anderson and Schulte 1971). On the other hand, water-based

insecticides are more prone to volatilization/evaporation and for that reason a magnesium oxide (MgO) slide coating has been the coating of choice for water-based insecticidal droplets (May 1945, May 1950, Rathburn 1970, Matthews 1975). The slides are prepared by burning MgO strips beneath the slides to create a white, powdery surface (Matthews 1975). When using the MgO slides, the impression that the droplets create upon impaction on the slide is measured, not the actual droplets. This technique has the advantage in that the impressions left by the water-based droplets colliding with the slide surface are permanent and allow for a more precise size determination, even as the droplets shrink due to evaporation (Rathburn 1970).

In recent years a new group of insecticidal formulations was developed with water as a solvent but with none of the disadvantages due to evaporation of conventional water-based sprays. These formulations are pre-formed emulsions in water that utilize an anti-evaporant technology (known as Film Forming Aqueous Spray Technology-FFAST®) to protect the water-based droplets from evaporating (Groome et al. 1989). This technology works by forming a stable, protective film of long-chain alcohols around the droplets (1-2 sec after atomization), which decreases the rate of evaporation. Insecticides utilizing this technology had evaporation rates higher than a conventional oil-based product but lower than a conventional water-based one (Groome et al. 1989).

Technology has not been developed to perform droplet sampling with these new hybrid insecticides, which behave neither like conventional water-based insecticides nor like oil-based ones. In the past, both Teflon® and MgO coatings have been used to sample droplets of the FFAST® products (Brown et al. 1998, Mickle and Brown 1999, Mickle and Brown 2000, Mickle and Brown 2001, Brown et al. 2003, Lothrop et al. 2007). However, experiments for direct comparison of these two coatings in determining the volume median diameters (Dv_{50s}) of

FFAST® droplets have not been conducted. Also, the appropriateness of Teflon® as a FFAST® droplet impaction surface has not been investigated in relation to droplet shrinkage, and the behavior of the droplets immediate after impaction on the Teflon® surface under different environmental conditions has not been considered.

Our first objective was to estimate the Dv_{50} and the 90% diameter (Dv_{90}) of one oil-based and 2 water-based, anti-evaporant aerosols using Teflon®- and MgO-coated slides and determine whether the aging of the droplets on the slides (up to 60 min) has any significant effect on Dv_{50} and Dv_{90} calculations. Our second objective was to observe and record the behavior of droplets (changes in size, shape, and texture) from the same products immediately after impaction, and through time, on Teflon®-coated slides under different temperature and relative humidity conditions. In addition to Teflon® spider web was used to suspend the droplets and their behavior was observed and recorded through time.

Materials and Methods

Insecticides

Three commercially available Ultra Low Volume (ULV) formulations registered for mosquito adulticiding were used in all experiments; one oil-based formulation, Permanone 30-30 (30% permethrin, Bayer Environmental Science, Research Triangle PK, NC), and two water-based, FFAST® formulations, AquaReslin (20% permethrin, Bayer Environmental Science, Research Triangle PK, NC), and Aqua-K-Othrine (2% deltamethrin, Bayer Environmental Science, Lyon, France). The latter product is registered in the European Union but not currently available for use in the U.S. All compounds were used undiluted.

Spraying Equipment and Droplet Sampling

A prototype, portable ULV-generator (London Fogger Inc., Long Lake, MN) was used to atomize all compounds. The generator operated with a flow rate of ~60 ml/min.

Battery-powered slide rotators (Compact Aerosol Droplet Sampler Model 312, John W. Hock Company, Gainesville, FL) were used for droplet sampling. Rotators were operated at a speed of 550 rpm. Three to four rotators were placed side by side to create a sampling line on a stand ~1 m from the ground. In order to create a relatively uniform spraying cloud, the generator was hand-held facing the rotators and moved at the same height and along-side the sampling line at a constant distance (~2m). In the rare case where not enough droplets reached the slides, the spraying pass was repeated. For the entire experiment spraying was conducted outdoors during morning hours in an open field, low-wind area next to the laboratory facilities to allow for quick transfers of the equipment into and out of the laboratory.

Microscope glass slides (25 x 75 x 1 mm) were either coated with Teflon® plastic film (BioQuip Products Inc., Rancho Dominguez, CA) or purchased plain (Fisher Scientific Company, Pittsburg, PA) and coated with MgO. The MgO glass slides were freshly coated by burning ~ 10 cm pieces of magnesium strips (Fisher Scientific Company, Pittsburg, PA) approximately 5 cm beneath a row of parallel placed, plane glass slides supported on a metallic stand. The smoke released from the burning strips formed a thin layer of a white, powdery, uniform coating on the slide surface. The slides were checked against the light to search for irregularities on the coating before being used in the tests. Slides with scratches, dirt and uneven coatings were discarded.

Natural spider web was collected from the field the day of the experimentation and was carefully positioned and adequately stretched between two sticks to create a surface area of ~5x5 cm. The sticks functioned as a supportive frame and were used to handle the web during the experimentation. The web was stabilized on a stand ~1 m from the ground and spraying was conducted as described above. Spider web was used to verify that changes on the droplet

behavior (if any) are not a result of the interaction between the slide surface and the insecticide formulation.

Dv_{50}/Dv_{90} Calculation Using Teflon®- and MgO-Coated Slides

A total of 3 slide rotators were used in this experiment. Each rotator contained one Teflon®- and one MgO-coated slide simultaneously to ensure that both coating types received an equivalent droplet sample during spraying. After spraying was completed the rotators with the slides still attached were moved in the laboratory. Once in the laboratory the slides were removed from the rotators and placed on the microscope stage one by one so the droplets could be measured. The droplets on both coating types were measured immediately after spraying, and at 60 min post-spraying for both coating types. During the 60 min period, the slides were kept on their holding racks in the laboratory where the temperature and humidity remained relatively constant (~20°C & 55% RH, Fisher Scientific Traceable Thermometer, China). The droplet sizes were determined under a VanGuard compound microscope (model 14867FLi, Vee Gee Scientific, Kirkland, WA) at 200x magnification with an ocular micrometer calibrated so that each division measured 4.5 microns. The diameters of at least 200 droplets were measured per slide for a total of 3 Teflon®- and 3 MgO-coated slides per product tested. For those droplets that did not have a spherical shape, 2 measurements were made corresponding to the narrowest and widest dimension of the droplet. The average of these 2 measurements was used as the droplet diameter.

When estimating the Dv_{50} and Dv_{90} values, the correction for volume diameter was done using the diameter (D) value rather than the volume diameter (D^3) associated with each droplet, as suggested by Yeomans (1949). This was done to compensate for the decrease in the rate of deposition on the slide surface as the droplet size decreases. The spread factor for the 3

compounds on Teflon® was 0.61. The spread factors on MgO coating were 0.86, 0.8, and 0.75 for droplet sizes >20, 15-20, and 10-15 microns, respectively (May 1945).

Droplet Behavior on Teflon®-Coated Slides and on the Spider Web

A total of 4 slide rotators were used in this experiment, each rotator containing a pair of Tefon®-coated slides. The droplet behavior on Teflon®-coated slides was observed immediately after spraying and at 10 min intervals for 60 min. The effects of three different scenarios on droplet behavior were investigated, including any change in size, shape and texture. For the first scenario the slides were kept spinning at conditions of high temperature (>25°C) and humidity (>80% RH) resembling spinning slides in the field during a night spray. The second scenario was identical to the first one except that the slides were not spinning. For the third scenario the slides were stationary in their slide racks at conditions of low temperature (<20^{0}C) and humidity (<55%RH) resembling a laboratory where slides would normally be stored for droplet measurement after a night spray trial. To create and maintain conditions of high temperature and humidity, the rotators along with the slides were transferred into the insectary where all assessments were made for the two first scenarios. All the assessments for the third scenario were made in the laboratory where the temperature and humidity remained low at all times.

In order to observe droplet behavior, the slides were moved immediately after spraying to the microscope (200x magnification) and the droplet behavior was recorded through time by photographing a set field of ~ 25 droplets every 10 minutes for 60 minutes. The same field of droplets on each slide was captured every time. This procedure was repeated over the 8 different slides and a total of 100-200 droplets were recorded per product for each one of the 3 scenarios. The slides were on the microscope stage only long enough to capture the images and were immediately returned to the conditions described above. A digital microscope camera (DCM500, Hangzhou Huaxin IC Technology Inc., Zhejiang, China) with the corresponding

computer software (ScopePhoto 3.0 Image Processing, Hangzhou Huaxin IC Technology Inc., Zhejiang, China) was used to capture, manage and save the image files.

The images of the droplet fields and of a calibrated stage micrometer (1 division = 4.5 microns) were printed at the same scale on standard copy paper or transparent acetate sheets (216 x 279 mm), respectively. The stage micrometer on the acetate sheet was used to measure the droplets on the printed images of the droplet fields.

In order to observe droplet behavior on the spider web, the web was moved immediately after spraying into the laboratory and stabilized on a petri dish under the microscope. Then the droplet behavior was recorded through time by filming a set field of ~ 10 droplets immediately after spraying and for the following 10 min. The only product sprayed and observed on the spider web was Aqua-K-Othrine because it was the only product that showed significant changes in droplet size, shape, and texture (see below).

Statistical Analysis

All statistical analyses were performed with the SAS software package (SAS Institute; Cary, NC). A paired t-test was performed to determine the effect of droplet aging on the calculation of droplet Dv_{50} and Dv_{90} values for droplets sprayed on MgO and Teflon®-coated slides (P = 0.05; [SAS Institute Inc 2003]). The Dv_{50} and Dv_{90} values of the droplets immediately upon impaction on the slides were calculated and compared with the Dv_{50} and the Dv_{90} values of the droplets 60 min post impact.

Results

Dv_{50} /Dv_{90} Calculation Using Teflon®- and MgO-Coated Slides

The mean Dv_{50} and Dv_{90} values of insecticide droplets at 0 and 60 min after impaction on Teflon® (Figure 5-1) and MgO (Figure 5-2) -coated slides were calculated for all 3 products tested. There were no significant differences in both Dv_{50} & Dv_{90} estimates on MgO slides at 0

min and 60 min for all three products tested (Table 5-1). On Teflon® coated slides, the only product that showed significant difference between 0 min and 60 min in both Dv_{50} and Dv_{90} estimates was Aqua-K-Othrine. Specifically, both values corresponding to Dv_{50} and Dv_{90} at 60 min decreased by approximately 50% when compared to the values at 0 min. For the other 2 products, AquaReslin and Permanone, time did not have any significant effect on of Dv_{50} and Dv_{90} values.

Droplet Behavior on Teflon®-Coated Slides and on Spider Web

The diameter of Aqua-K-Othrine droplets decreased significantly during all 3 experimental conditions (dry environment/non-spinning slides, moist environment/non-spinning slides, moist environment/spinning slides) (Figure 5-3). The shape of the droplets got distorted as the droplets dried (Figure 5-8).

Under high humidity conditions, immediately upon impaction, there were some unusually large Aqua-K-Othrine droplets, that were non-spherical and contained of smaller inner globules. These droplets were dominant on the slide surface (Figure 5-6). Within the first ~2 minutes, these non-spherical formations gradually shrunk into spherical droplets (Figure 5-6). The spherical droplets decreased further in size within the next 60 minutes, with the majority of the shrinkage occurring during the first 10 minutes after impaction on the slides. The initial non-spherical formations were not present on the slides at dry conditions. However, a similar event, involving a change in the texture of the initial droplets, was observed when the droplets were suspended on the spider web (Figure 5-7). The initial droplets appeared grey under the microscope. As the time passed, a movement was observed on the surface of the droplets and their color gradually changed. Within ~2 min after impingement on the web, the droplets lost their grey color and became white.

The diameter of AquaReslin droplets remained almost unaffected under all 3 experimental conditions (Figure 5-4). However, the droplets lost their spherical shape (Figure 5-8) as the droplets dried. No change in droplet texture was observed during the 3 different experimental conditions studied.

Permanone droplets did not change in size, shape and texture under all 3 experimental conditions (Figure 5-5).

Discussion

In previous studies with FFAST® anti-evaporant water-based products some inconsistencies between Dv_{50s} estimated using Teflon®- and MgO-coated slides have been demonstrated. The Teflon® slides produced a measured Dv_{50} that was approximately ½ the Dv_{50} measured by using MgO slides (Mickle and Brown 2000, Brown et al. 2003). Mickle & Brown (2001) showed that characterization of their nozzle using a laser wind tunnel in the laboratory, yielded a Dv_{50} nearly 5-fold greater than what was measured in the field using Teflon® slides. Findings by these authors suggest that possible evaporation of the aqueous formulation may have resulted into smaller droplets being measured on the Teflon® slides. However, our study provides direct evidence that FFAST® droplets, despite containing anti-evaporant ingredients, will shrink significantly on Teflon®-coated slides.

Specifically, Aqua-K-Othrine droplets lost their spherical shape and exhibited significant shrinkage on the Teflon®-coated slides 60 minutes post spraying. On the other hand, despite losing their spherical shape, AquaReslin droplets on Teflon® coated slides did not shrink significantly within 60 min post spraying. Although, both Aqua-K-Othrine and AquaReslin formulations contain the same FFAST® anti-evaporant technology (Groome et al. 1989), these formulation had different evaporation rates. This difference in evaporation rate could be attributed to the different physical properties (i.e. vapor pressure) of the chemicals contained in

the formulations. The higher the vapor pressure of a substance, the higher is its evaporation rate (Krieger 2001). The two formulations contained different concentrations of 2 different active ingredients (2 % deltamethrin and 20 % permethrin for Aqua-K-Othrine and AquaReslin, respectively), and different compositions of inert ingredients and synergists (i.e. AquaReslin contains 20% piperonyl butoxide (PBO) and there is no reported concentration of naphtha on the MSDS, whereas Aqua-k-othirne contains no PBO and up to 25% naphtha) (AquaReslin & Aqua-K-Othrine MSDS). Deltamethrin and permethrin have low vapor pressures (1.5×10^{-8} and 2.15×10^{-8} mmHg respectively, NPIC 2010). Piperonyl butoxide has an even lower vapor pressure (0.26×10^{-8} mmHg, NPIC 2010). On the other hand naphtha has a high vapor pressure (5 mmHg, CDC 1978). The higher concentration of active ingredient (permethrin), the presence of PBO (20%), and possibly the lower concentration of naptha in the formulation of AquaReslin may have contributed to the lower evaporation rate of this product compared to Aqua-K-Othrine. Permanone droplets maintained their shape and size on Teflon®-coated slides throughout all experimental conditions. This observation is in agreement with previous studies on the suitability of Teflon® as a coating surface for oil-based droplet impaction (Anderson and Schulte 1971).

The principle behind the FFAST® anti-evaporant technology relies on the formation of a protective long chain alcohol layer on the insecticide droplet. As each droplet forms, long chain alcohol molecules rapidly migrate to the surface of the droplet where they align to form a protective layer which retards evaporation (Groome et al. 1989). The hydrocarbon tail (hydrophobic group) of each alcohol molecule is on the outer surface of the layer and their hydrophilic head is on the inner surface. It takes <1 sec for the formation of the layer (Groome at al. 1989). The change in texture of the Aqua-K-Othrine droplets observed under high humidity conditions and within 2 minutes after impaction both on the Teflon®-coated slides and on the

spider web corresponds to the time for movement of the long-chain alcohol molecules to the surface of the droplets. However, this movement should take place in <1 sec, and the droplet texture change in our experiment took up to 2 min after slide impaction. This delay in the alcohol chain layer formation may be due to the high humidity conditions, which may have delayed the molecule movement process.

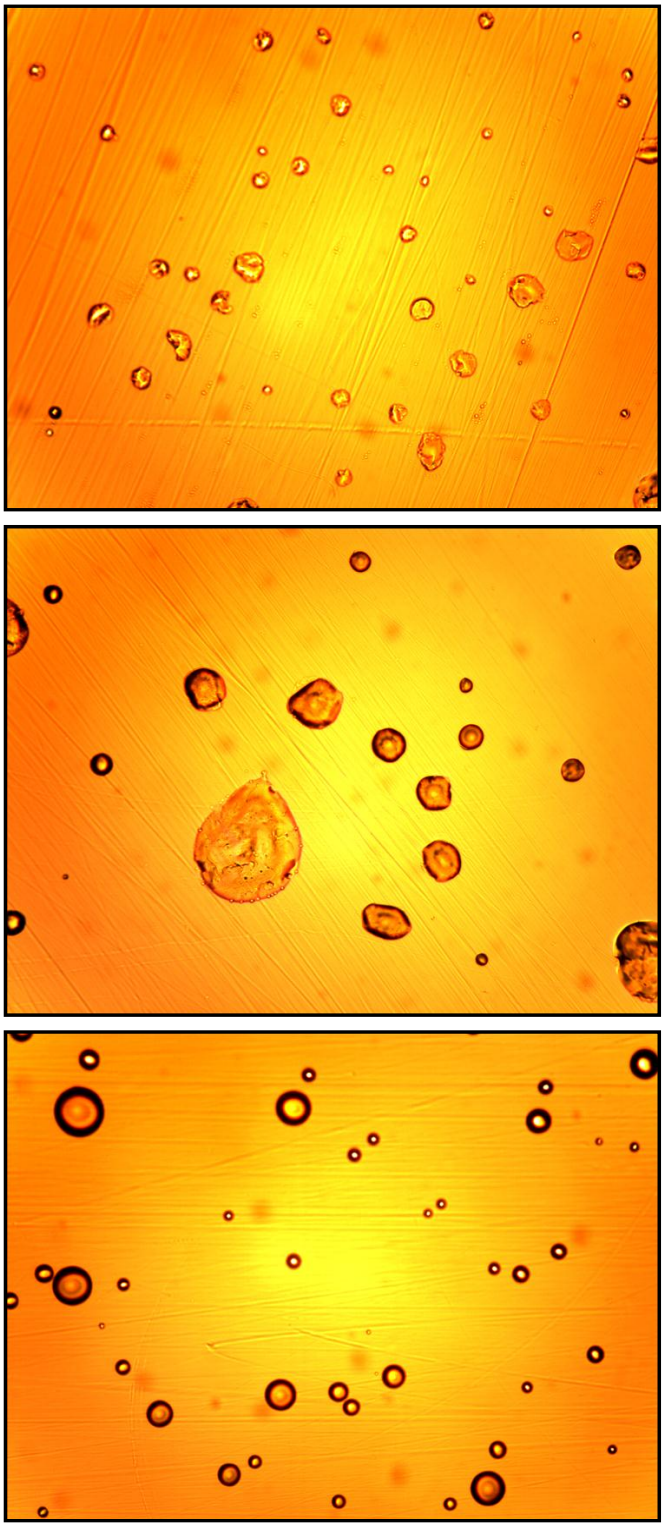
Our study provided direct evidence that Teflon®-coated slides are not the proper sampling technique for Aqua-K-Othrine FFAST® droplets and MgO slides should be preferred over Teflon®. In the case of AquaReslin FFAST® droplets, Telfon-coated slides are adequate in determining Dv_{50}s. However, the droplets need to be measured within 60 min after impaction on the slides. Beyond this time period significant shrinkage may occur and result in reduced Dv_{50} and Dv_{90} values.

Magnesium oxide slides, on the other hand, have several disadvantages. Some of the most important disadvantages are the requirement of a long preparation time (Anderson & Schulte 1971), and their high susceptibility to dust and other airborne contaminants, which may leave imprints on the slide surface. A new technology is needed for a more accurate droplet sampling of FFAST® anti-evaporant insecticides, that behave neither like conventional water-based insecticides nor like oil-based ones. Until such technology is developed, droplet sizes of these insecticides should be measured using MgO-coated slides because MgO slides, even though not perfect, are the most appropriate slides available for sampling FFAST® anti-evaporant insecticide droplets.

Table 5-1. Mean Dv_{50} and Dv_{90} values for Permanone, Aqua-K-Othrine and AquaReslin droplets on MgO and Teflon®-coated slides immediately (0 min) and 60 min after impaction to slides.

Product	Mean[a] Dv_{50} ± Std Error				Mean[a] Dv_{90} ± Std Error			
	Teflon®		MgO		Teflon®		MgO	
	0 min	60 min	0 min	60 min	0 min	60 min	0 min	60 min
Permanone	5.7 ± 0.2	5.6 ± 0.1	6.7 ± 0.1	7.0 ± 0.1	11.1 ± 0.3	11.0 ± 0.2	13.9 ± 0.8	14.0 ± 0.2
Aqua-K-Othrine	8.5 ± 0.1	4.3 ± 0.8*	6.2 ± 0.3	5.9 ± 0.2	25.8 ± 5.0	9.4 ± 1.4*	18.7 ± 1.0	17.3 ± 0.4
AquaReslin	7.2 ± 0.5	6.2 ± 0.4	7.4 ± 0.2	7.8 ± 0.3	18.9 ± 4.2	11.9 ± 0.8	14.9 ± 0.2	15.0 ± 0.2

[a]Mean Dv_{50} and Dv_{90} values at 60 min marked with * are significantly smaller than corresponding values at 0 min (P = 0.05, t-test [SAS Institute Inc 2003]).



A

B

C

Figure 5-1. Images of insecticide droplets on Teflon® slides under the microscope. A) Aqua-K-Othrine. B) AquaReslin. C) Permanone.

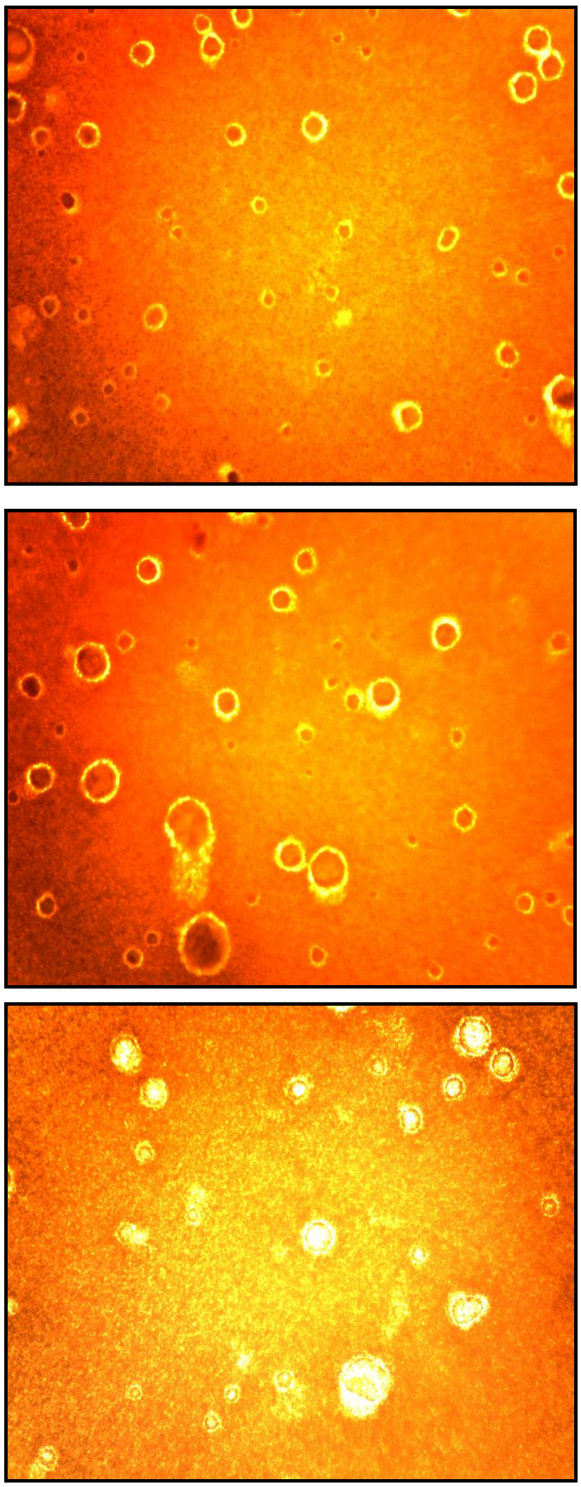


Figure 5-2. Images of insecticide droplets on MgO slides under the microscope. A) Aqua-K-Othrine. B) AquaReslin. C) Permanone.

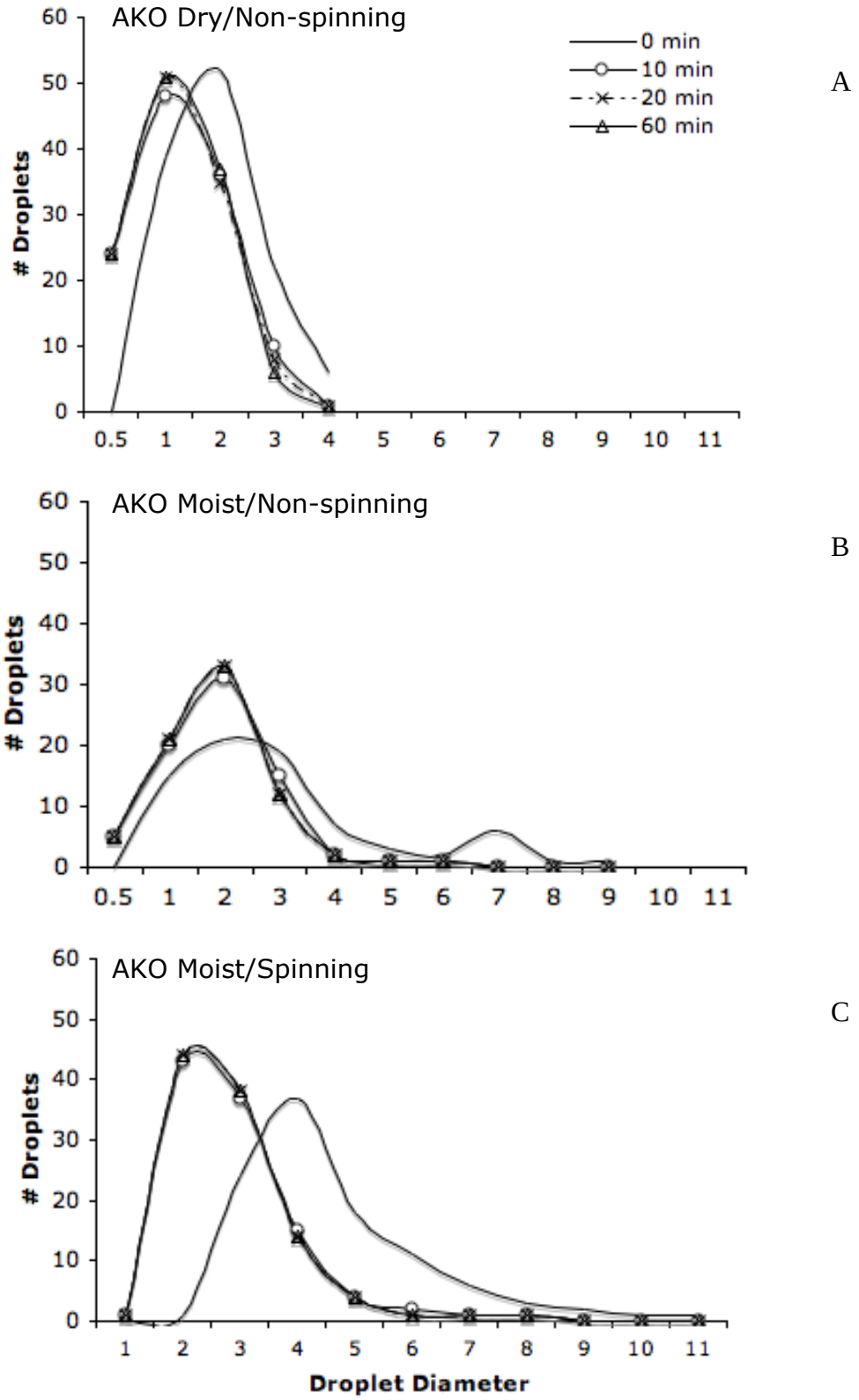


Figure 5-3. Droplet diameter (in microns) distribution for Aqua-K-Othrine at 0, 10, 20, and 60 min. A) Dry, non-spinning scenario. B) Moist, non-spinning scenario. C) Moist, spinning scenario.

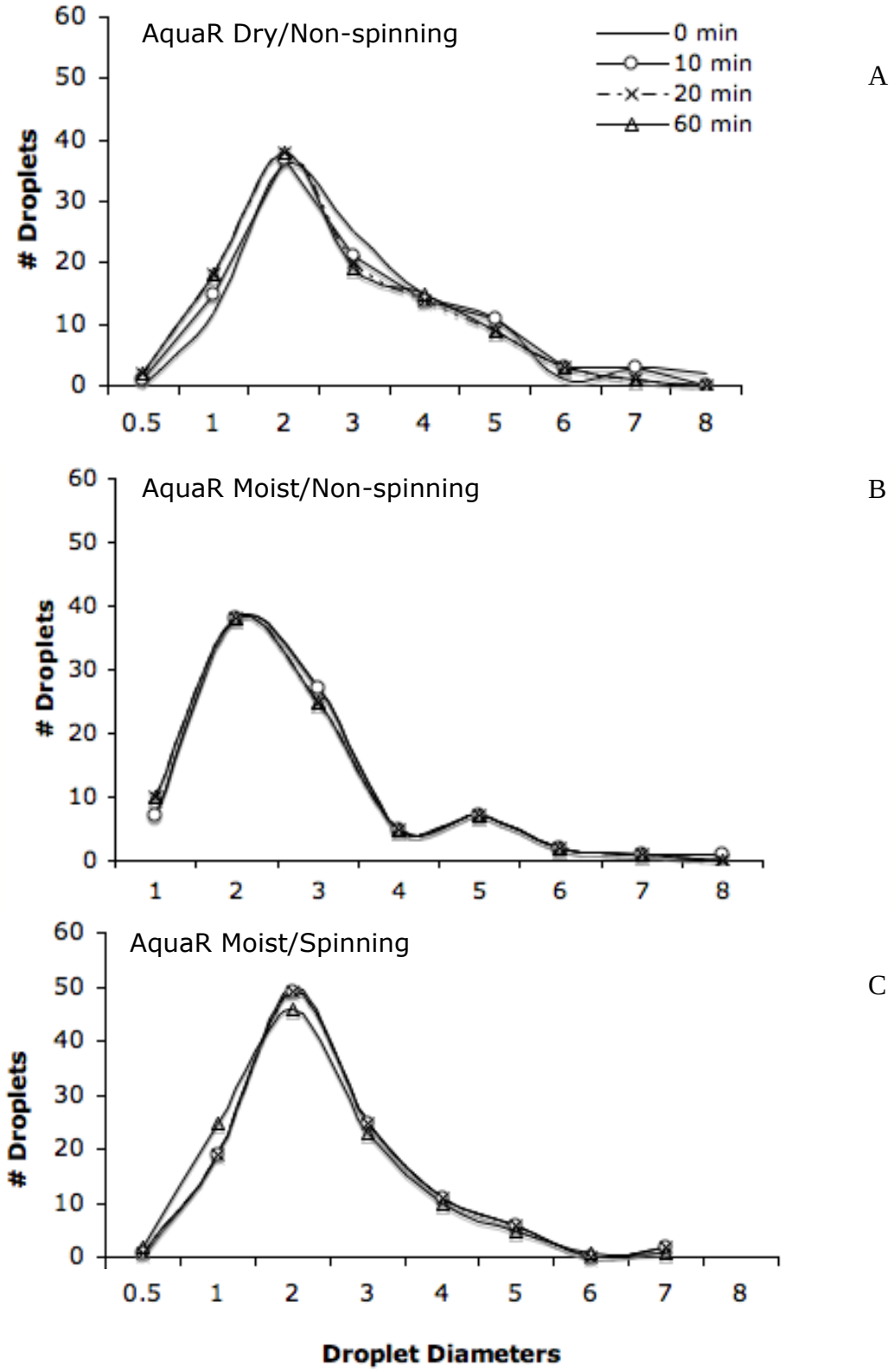


Figure 5-4. Droplet diameter (in microns) distribution for AquaReslin at 0, 10, 20, and 60 min. A) Dry, non-spinning scenario. B) Moist, non-spinning scenario. C) Moist, spinning scenario.

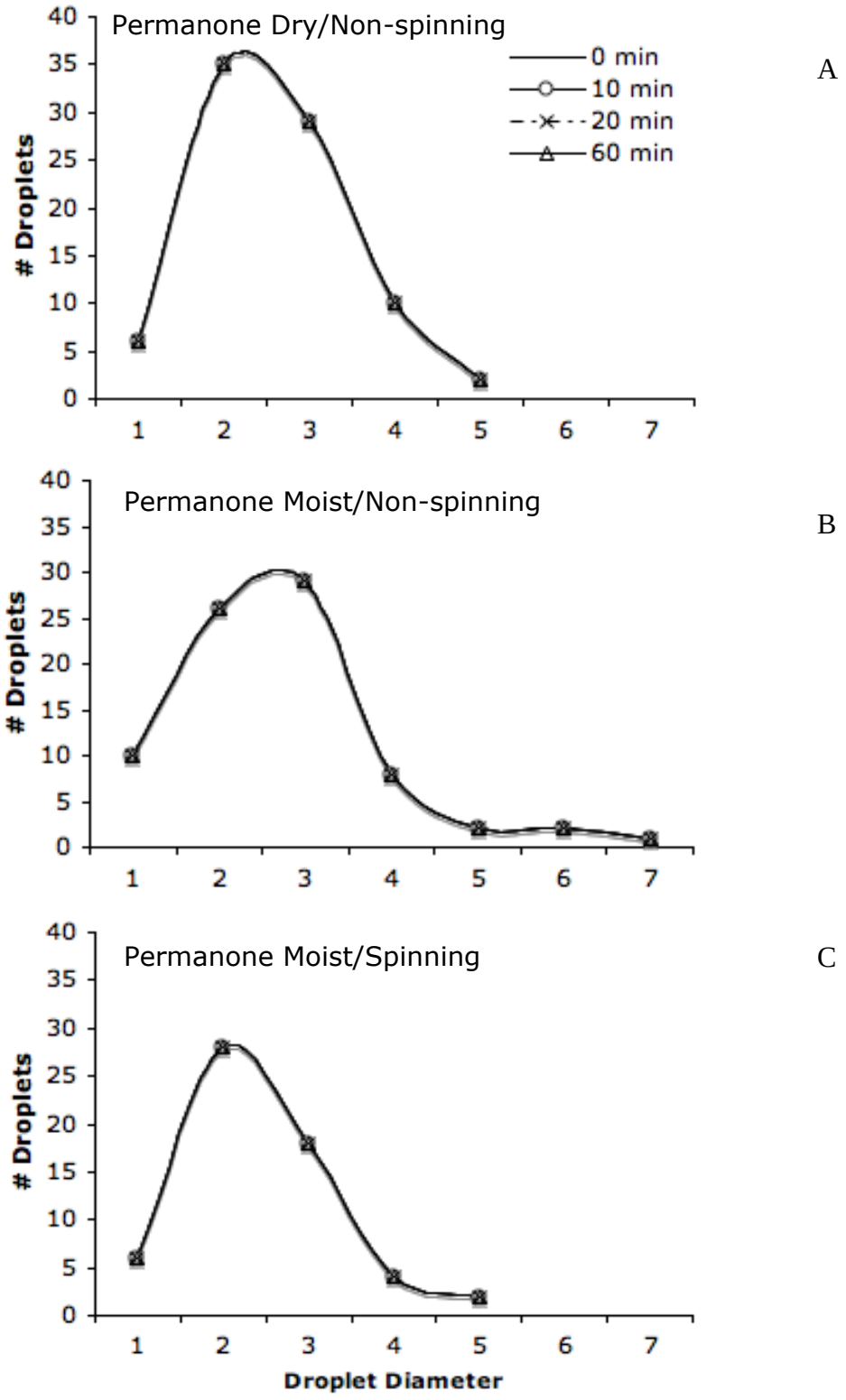


Figure 5-5. Droplet diameter (in microns) distribution for Permanone at 0, 10, 20, and 60 min. A) Dry, non-spinning scenario. B) Moist, non-spinning scenario. C) Moist, spinning scenario.

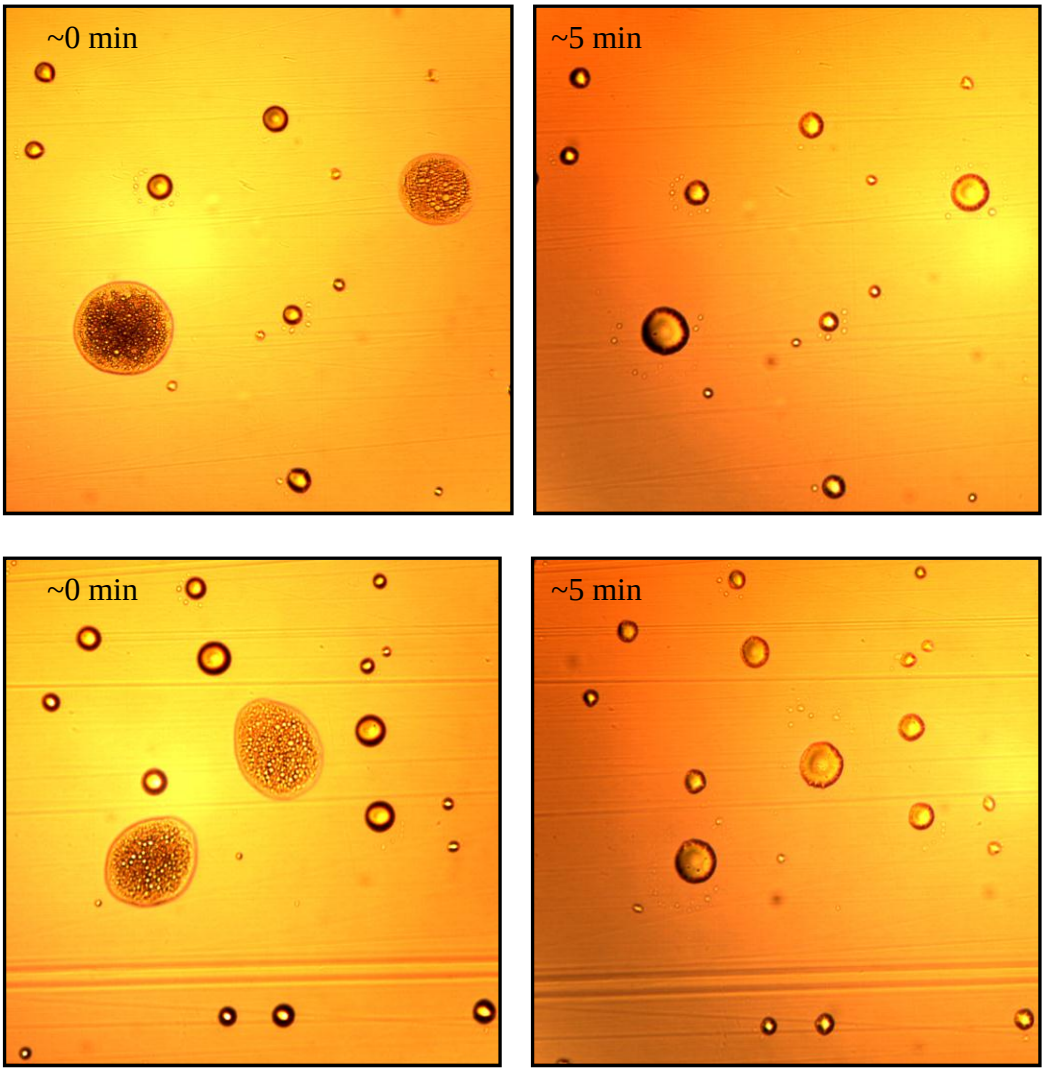


Figure 5-6. Images of Aqua-K-Othrine droplets on Teflon® slides under the microscope. A) At 0 min and 5 min. B) At 0 and 5 min.

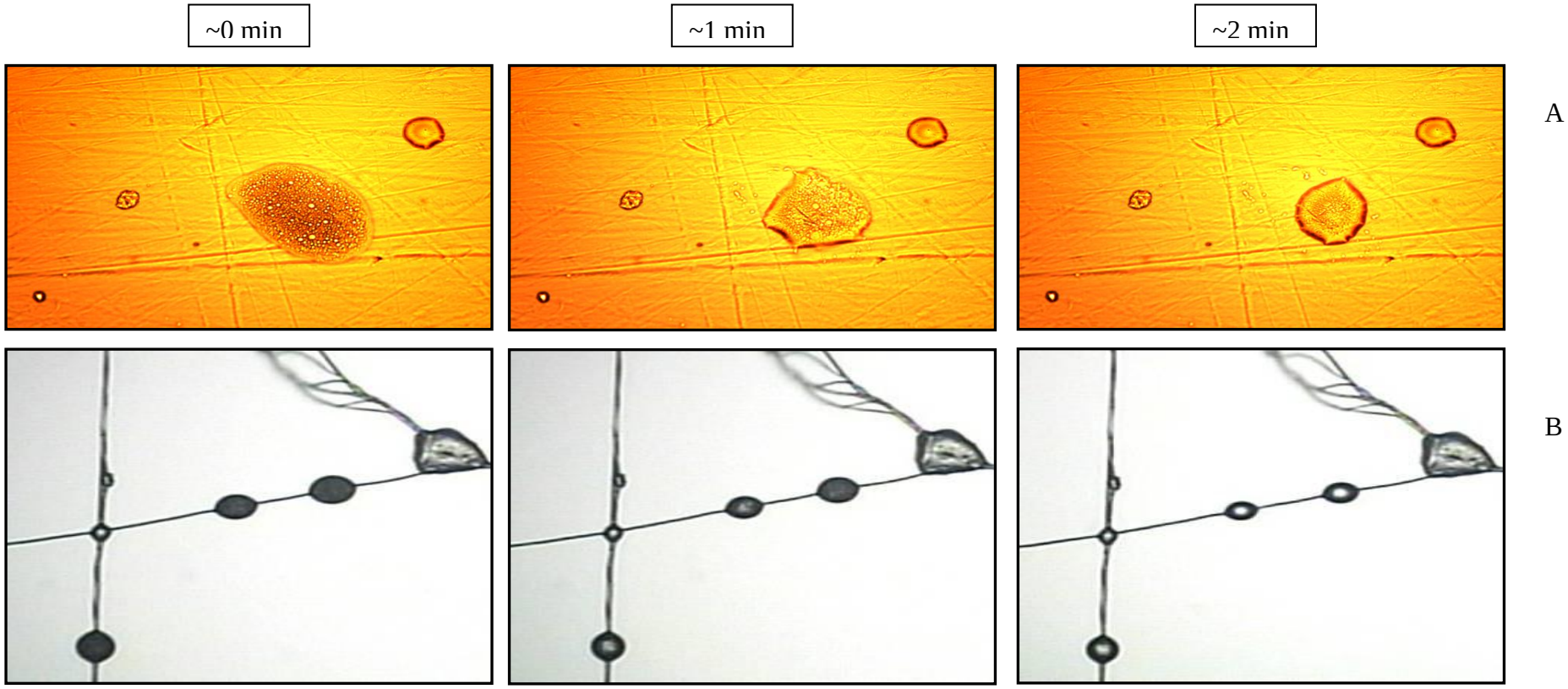


Figure 5-7. Aqua-K-Othrine droplets at 0, 1, and 2 minutes. A) On Teflon® slides. B) Suspended on spider web.

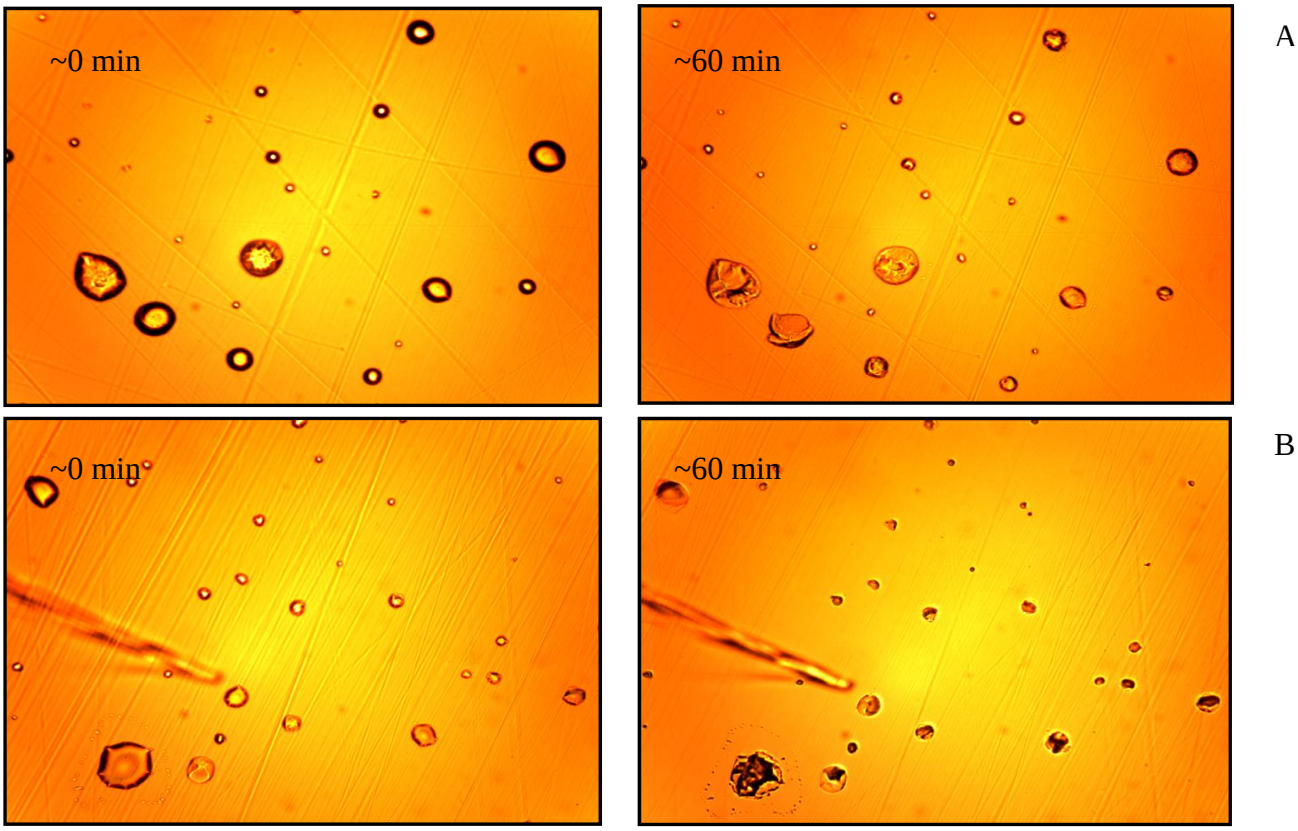


Figure 5-8. Insecticide droplets on Teflon®-coated slides at 0, and 60 minutes. A) AquaReslin droplets. B) Aqua-K-Othrine droplets.

CHAPTER 6
AGRICULTURAL DISPERSION (AGDISP) MODEL

The Model and the History

The use of mathematical modeling to predict the dispersion and deposition of aerially applied spray plumes through the atmosphere and upon the ground aims to improve the efficiency, precision, and safety of insecticide applications (Brown and Mickle 2003). There are several models available for this purpose. Before I summarize what each one of these models does I would like to refer to the 3 phases that describe the movement of a spray plume from the moment it is released from the nozzle (Brown and Mickle 2003, Latham 2004): 1.) The aircraft vortices phase (aircraft wake effects), during which the spray plume is caught up by the energy of the vortices and it is being carried downwards until the energy of the vortices dissipates, 2.) The atmospheric dispersion phase, during which the spray plume is being subjected to the general atmospheric turbulence and weather conditions and 3.) The sedimentation phase, during which the spray droplets are primarily subjected to gravity and gradually sink. Wind speed/direction and atmospheric stability influence the movement of the plume during all 3 phases (Figure 6-1).

The models calculate the effects that determine the spray plume movement during those phases in order to predict the droplets' trajectories from the moment they leave the nozzle until they deposit on the ground. The first model, known as Forest Service Cramer-Barry-Grim (FSCBG), was developed by the USDA Forest Service in cooperation with the U.S. Army (Teske et al. 1993). The FSCBG was incorporated with the Agricultural Dispersal model (AGDISP, Bilanin et al. 1989). The AGDISP is a "near wake" or a "Lagrangian" model that uses a set of Lagrangian equations to predict the movement of the droplets based on the aircraft wake turbulence (vortices) (Teske and Thistle 1999). The original FSCBG is a "far wake" or

“Gaussian” model that solves a Gaussian diffusion equation to predict further dispersion of the spray plume and ground deposition based on the atmospheric conditions (Teske 1996). Recently, the Spray Drift Task Force (SDTF) (a consortium of 38 agricultural chemical companies established in 1990) and the United States Environmental Protection Agency (EPA) have cooperated to extend AGDISP into a regulatory model framework (AGDRIFT) to be used for product registration (for agricultural aerial applications) and to develop mitigative measures to protect environmentally sensitive areas (Johnson 1997). A lot of studies were conducted (more than 300 applications were made in 10 field studies covering a wide range of application practices) to validate the AGDRIFT model for evaluating agricultural aerial applications out to 2,600 feet (~793 m). These studies were designed and conducted by scientists from universities, research institutions and the EPA. The main objective of these studies was to quantify environmental drift exposure from aerial agricultural spray applications. These studies were conducted at low altitudes, utilizing large droplet spectra (100-500 micron in diameter), with narrow swaths, and they looked at drift exposure/ground deposition out to 2,600 feet (~793m) (Johnson 1997).

AGDISP in not considered a validated model for mosquito control aerial adulticiding applications because, even though is has been field validated under the parameters that characterize aerial applications for agriculture, it has not been field validated under the parameters that characterize aerial applications for mosquito adulticiding. Below I list the main differences between agricultural and mosquito adulticiding aerial applications (Figure 6-2):

1.) Aerial spraying for agriculture is considered a deposit spray. The targeted treatment area is the foliage on the ground, the field plot. The main goal for this application is to successfully get the majority of the spray plume to deposit on the foliage, homogeneously

covering the field while minimizing off target drift. Aerial spraying for mosquito adulticiding is considered a space spray. The target for this application is not a surface on the ground, but it is the space (the volume of air) above ground where mosquitoes will be actively flying (Latham and Barber 2007). In contrast to agricultural sprays, mosquito adulticiding relies on drift and aims to minimize insecticide deposit. For mosquito adulticiding the definition of drift is not the undesired movement of droplets outside the treatment area, as in the case of agricultural sprays, but is the continuous suspension and movement (mixing) of the droplets horizontally and vertically within the target zone (mosquito flight activity zone, within ~50 feet of the ground surface). The longer the droplets remain airborne and "drift" within the target zone the higher the chances are that they will impinge on the flying adult mosquitoes.

2.) Agricultural sprays are large volume sprays (most of the time diluted in a solvent), utilizing large droplets, which are less likely to drift and more likely to deposit fast. On the other hand, mosquito adulticiding sprays are ultra low volume sprays (ULV) (most of the time pure product is applied) utilizing very small droplets (Dv_{50} < 50 microns) that are less likely to deposit and more prone to drift.

3.) Agricultural sprays are low altitude applications (5-20 meters) with narrow swaths (15-30 meters). Mosquito adulticiding sprays are high altitude sprays (30-90 meters) with wide swaths (150-500 meters).

4) Agricultural sprays are mainly daytime operations, where as mosquito adulticiding sprays are nighttime operations.

AGDISP Insecticide Deposition and Transport Aloft Predictions for Aerial Mosquito Adulticiding Trials of Water-based Formulations and Comparison with Field Collected Data

During the field trials, the helicopter was equipped with a flight guidance GPS system (Wingman™ GX, Adapco, Sanford, FL) which included an onboard moving map screen that

displayed aircraft position, spray block boundaries, and flight lines. Additionally, the helicopter was equipped with a real-time meteorology system (AIMMS-20™, Aventech Research Inc., Ontario, Canada) that recorded information such as wind speed and direction, temperature and humidity at the spraying altitude. The real-time meteorological data recorded by the AIMMS-20™ system were automatically processed by the Wingman GPS guidance system. The USDA spray dispersal computer model AGDISP1 had previously been run to predict the spray movement based on application parameters and meteorological conditions. Results of numerous spray model runs covering a range of meteorological conditions and application parameters for this specific helicopter and droplet spectrum were incorporated into a "lookup table" utilized by the Wingman system software. Spray offsets were calculated and flight lines were positioned automatically through the Wingman/AGDISP lookup table system. As wind speed and direction changed, the system adjusted guidance/offsets for the correct flight line positions from the incorporated lookup table based on the average wind speed and direction from the previous flight line for optimum coverage. The pilot followed the flight line guidance provided by the system for each of the spray lines necessary to cover the treatment area. For the field trials there were 2 assessments made: biological effect of the spray (caged mosquito mortality) and droplet densities.

The AGDISP 8.15 model (Figure 6-3) was used to simulate the field trials. Two different outputs of AGDISP for each aerial trial were calculated and are presented below: insecticide deposition (amount of insecticide material landing on the ground surface) and insecticide transport aloft (amount of insecticide material passing though the air column above the ground, Figure 6-4).

The factors which mainly affect the movement of insecticidal droplets include: the spray altitude of the aircraft, the speed of the aircraft, the type of the aircraft, the type of the boom and nozzle system used to discharge the spray material and the sizes of the droplets produced, the swath width, the type and density of the ground canopy, wind speed and direction, relative humidity, atmospheric stability, and spray material characteristics (Potter et al. 1999).

To be able to get as realistic output values as possible from the model, the flight parameters entered in the model were identical to the actual flight parameters (those corresponding to the field trials) with some exceptions: the wind direction/speed value in the model remained constant (it was the average wind speed and direction as measured by the weather station on the helicopter) and it was chosen for one altitude, the spray altitude (in reality the wind direction/speed was constantly changing in space and time), the atmospheric stability value was set at overcast (even though stability varied in the field), the type of the canopy entered was none (in reality the type of the canopy was a combination of rice fields and corn fields), in the material characteristics the products were considered for simplicity reasons as non-volatile (in reality the products will slowly evaporate). Also, AGDISP outputs are approximations of the real world sprays from lower altitudes where aircraft vortices bring the spray plume close to the ground, rather than high altitude sprays where atmospheric turbulence plays an important role in the spray plume dispersion.

For the reasons described above the numerical output values predicted from the model for depositions and transport aloft should not be viewed as absolute values of insecticide deposition and dispersion.

Pesguard Trial 1, 07/15/ 2009

Based on the model's prediction the stations receiving the highest insecticide concentration (insecticide aloft) are the stations outside the treatment area on the downwind edge of the

treatment block. Specifically, the stations 1000 m away from the downwind edge of the block received the highest concentration (~0.0083 mg/cm^2 of product, Figure 6-5). Inside the treatment area, stations 1 and 2, at the most downwind portion of the spray block, received the highest concentration (~0.005 mg/cm^2) followed by station 3 (~0.003 mg/cm^2), stations 4,5, and 6 (~0.001 mg/cm^2), and, lastly, stations 7 and 8 (~0 mg/cm^2). Also, based on the predictions of the model the peak deposition (~17 g/ha of product) occurred inside the treatment block (~400 m from the downwind edge, Figure 6-6).

Based on the data retrieved from the field, the stations with the highest mosquito mortalities were the stations inside the treatment area and in the most downwind portion of the spray block (stations 1,2, & 3, Figure 6-7). Also, these stations had high droplet densities, though not higher than the densities corresponding to stations in the middle of the spray block (stations 4,5, & 6). It is important to mention that droplet densities do not necessarily correspond to volume of product per cm^2 and cannot be directly compared from station to station since they are characterized by a different size distribution. The droplet densities inside the treatment area ranged from 720-1,255 drops per cm^2.

The stations outside the treatment area, both downwind and upwind, had very low mortalities and low or 0 droplet densities (ranged from 0-486 drops per cm^2). This comes in disagreement with the model prediction for the outside/downwind stations, which were those exposed to the highest insecticide concentration.

Pesguard Trial 2, 07/29/2009

The model output for the second PESG trial is identical to the first one because the same application parameters were used and the average wind speed and direction value was the same for both 2 spray nights. Based on the model's prediction the stations receiving the highest insecticide concentration (insecticide aloft) are the stations outside the treatment area on the

downwind edge of the treatment block. Specifically, the stations 1000 m away from the downwind edge of the block received the highest concentration (~0.0083 mg/cm^2 of product, Figure 6-8). Inside the treatment area, stations 1 and 2, at the most downwind portion of the spray block, received the highest concentration (~0.005 mg/cm^2) followed by station 3 (~0.003 mg/cm^2), stations 4, 5, and 6 (~0.001 mg/cm^2), and, lastly, stations 7 and 8 (~0 mg/cm^2). Also, based on the predictions of the model the peak deposition (~17 g/ha of product) occurred inside the treatment block (~400 m from the downwind edge, Figure 6-9).

Based on the data retrieved from the field, the stations with the highest mosquito mortalities were the stations inside the treatment area, in the middle of the spray block (stations 4,5,6 & 3) followed by the stations in the most downwind portion of the spray block (stations 1,2, & 3, Figure 6-10). The stations in the middle of the spray block had the highest droplet densities, ranging from 1,190-2,000 drops per cm^2. The stations with lowest droplet densities (683-820 drops/cm^2) were those located in the most upwind portion of the spray block (stations 7 & 8).

The stations outside the treatment area, both downwind and upwind, had very low mortalities and low or 0 droplet densities (ranged from 0-420 drops per cm^2). This comes in disagreement with the model prediction for the outside/downwind stations, which were those exposed to the highest insecticide concentration.

Pesguard Trial 3, 08/13/2009

Based on the model's prediction the stations receiving the highest insecticide concentration (insecticide aloft) are the stations outside the treatment area on the downwind edge of the treatment block. Specifically, the stations at 1000 m away from the downwind edge of the block received the highest concentration (~0.004 mg/cm^2 of product, Figure 6-11). Inside the treatment area, stations 1 and 2, at the most downwind portion of the spray block, received the highest

concentration (~0.003 mg/cm^2) followed by station 3 (~0.002 mg/cm^2), stations 4,5, and 6 (~0.0008 mg/cm^2), and, last, stations 7 and 8 (~0 mg/cm^2). Also, based on the predictions of the model the peak deposition (~25 g/ha of product) occurred inside the treatment block (~300 m from the downwind edge, Figure 6-12).

Based on the data retrieved from the field, the majority of the stations within the treatment area had high mosquito mortalities ranging from 72-88 %. The majority of the stations, also, had high droplet densities (ranging from 936-2,733 drops per cm^2, Figure 6-13), with the most upwind stations having the lowest droplet densities (936 and 1,010 drops per cm^2). This PESG trial is the one with the highest droplet densities inside the treatment area when compared with the first 2 aerial trials.

The stations outside the treatment area, both downwind and upwind, had very low mortalities and low or 0 droplet densities (ranged from 0-480 drops per cm^2). This again comes in disagreement with the model prediction for the outside/downwind stations, which were those exposed to the highest insecticide concentration.

Aqua-K-Othrine Trial 1, 07/22/2009

Based on the model's prediction the stations receiving the highest insecticide concentration (insecticide aloft) are the stations outside the treatment area on the downwind edge of the treatment block. Specifically, the stations at 1000 m away from the downwind edge of the block received the highest concentration (~0.013 mg/cm^2 of product, Figure 6-14). Inside the treatment area, station 1, at the most downwind portion of the spray block, received the highest concentration (~0.009 mg/cm^2) followed by station 2 (~0.0075 mg/cm^2), stations 3 and 4 (~0.005 mg/cm^2), stations 5 and 6 (~0.0037 mg/cm^2), and last stations 7 and 8 (~ 0.002 mg/cm^2). Also, based on the predictions of the model the peak deposition (~11 g/ha of product) occurred inside the treatment block (~250 m from the downwind edge, Figure 6-15).

Based on the data retrieved from the field, all stations inside the block had high mosquito mortality ranging from 74-100% (Figure 6-16). There is no apparent trend on mortality levels based on station location. The droplet densities inside the treatment area ranged from 764-1,753 drops per cm^2. During this trial the droplets for 3 stations could not be read because of dust from the field roads deposited on the slides.

The stations outside the treatment area, both downwind and upwind, had very low mortalities and low or 0 droplet densities (ranged from 0-340 drops per cm^2). This comes in disagreement with the model prediction for the outside/downwind stations, which were those predicted to have the highest insecticide concentration.

Aqua-K-Othrine Trial 2, 08/09/2009

Based on the model's prediction the stations receiving the highest insecticide concentration (insecticide aloft) were the stations inside the treatment area on the most downwind portion of the treatment block. Specifically, station 1 received the highest concentration (~0.011 mg/cm^2 of product, Figure 6-17), followed by station 2 (~0.01 mg/cm^2), stations 3 and 4 (~0.008 mg/cm^2), stations 5 and 6 (~0.004 mg/cm^2), and last stations 7 and 8 (~ 0.00257 mg/cm^2). Also, based on the predictions of the model the peak deposition (~11 g/ha of product) occurred inside the treatment block (~380 m from the downwind edge, Figure 6-18). The stations outside the treatment area at 500 and 1000 m from the downwind edge also received high insecticide concentrations (in the range of 0.0095-0.0105 mg/cm^2). The stations outside the treatment area at 500 and 1000 m from the upwind edge received the lowest insecticide concentrations, however, unlike the rest of the trials these values were above 0 (~0.0009 mg/cm^2).

Based on the data retrieved from the field, all stations inside the block had high mosquito mortalities ranging from 77-100% (Figure 6-19). There is no apparent trend on mortality levels based on station location. This is the spray trial with the highest overall caged mosquito

mortalities and the highest overall droplet densities within the treatment area. The droplet densities inside the treatment area ranged from 903-2,113 drops per cm^2. This is also the trial with the lowest average wind speed (4 m/s).

The stations outside the treatment area, both downwind and upwind, had very low mortalities and low or 0 droplet densities (ranged from 0-417 drops per cm^2). This comes in disagreement with the model's prediction for the outside/downwind stations, which were predicted to have high insecticide concentrations at similar levels as the stations inside the treatment area. Interestingly, droplets were detected on the slides located outside from the treatment area 500 m from the upwind edge (200 drops/cm^2). This coincides with the model's output, which predicted for the first time insecticide concentrations >0 at those locations.

Aqua-K-Othrine Trial 3, 08/20/ 2009

Based on the model's prediction the stations receiving the highest insecticide concentration (insecticide aloft) were the stations inside the treatment area on the most downwind portion of the treatment block (stations 1 & 2) and outside the treatment area at 500 and 1000 m from the most downwind edge. Specifically, station 1 received the highest concentration (~0.011 mg/cm^2 of product, Figure 6-20), followed by station 2 (~0.0095 mg/ cm^2). Also, based on the predictions of the model the peak deposition (~11 g/ha of product) occurred inside the treatment block (~200 m from the downwind edge, Figure 6-21). The stations outside the treatment area at 500 and 1000 m from the downwind edge received, also, high insecticide concentrations (in the range of 0.0095-0.0105 mg/cm^2). The stations outside the treatment area at 500 (~0.001 mg/cm^2) and 1000 m (0 mg/cm^2) from the upwind edge received the lowest insecticide concentrations, however, unlike the rest of the trials these values were above 0 for those stations 500 m upwind. The only other trial that showed some insecticide available outside and in the upwind portion of

the spray block was conducted on the 08/09/2009. Compared with the rest of the trials, those of 08/09 and 08/20 have the lowest wind speed (~4 m/s) in common.

Based on the data retrieved from the field, all stations inside the block had high mosquito mortalities ranging from 72-94% (Figure 6-22). There is no apparent trend on mortality levels based on station location. The droplet densities inside the treatment area ranged from 830-1,944 drops per cm^2.

The stations outside the treatment area, both downwind and upwind, had very low mortalities and low or 0 droplet densities (ranged from 0-402 drops per cm^2) This comes in disagreement with the model's prediction for the outside/downwind stations, which were predicted to have high insecticide concentrations at similar levels as the stations inside the treatment area. There were no droplets detected on the slides located outside from the treatment area 500 and 1,000 m from the upwind edge.

Conclusions

During all 6 aerial trials droplets were sampled in the stations located both inside and outside the treatment areas. The majority of the droplets, by far, were collected from the stations inside the treatment and ranged from 683-2733 drops/ cm^2. The stations outside the treatment area on the upwind side had no droplets at all, whereas the stations outside and on the downwind side received up to 486 drops/ cm^2. The caged mosquito mortalities inside the treatment area ranged from 67-100%, whereas the mortality outside the treatment area was low (~ 4-20%). It seems that during the low wind trials higher droplet densities were observed within the treatment areas.

Based on the model predictions for all 6 trials the stations receiving the highest insecticide concentrations were the stations inside the treatment area closest to the most downwind edge and the stations outside the treatment area at 500, and 1,000 m from the most downwind edge.

However, when looking at the actual field measurements of droplet densities, the stations with the highest concentrations were the stations inside the treatment area close to the center and the most downwind edge. The stations outside the treatment area at 500 m from the downwind edge had an average of 1/3 decrease in droplet densities when compared with the stations inside the treatment area. There were no droplets detected on the stations outside the treatment area at 1,000 m from the downwind edge for all 6 trials.

This disagreement between the model output and the actual field assessments can be attributed to the fact that the model was set for open environment with no canopy inputs. However, in real field conditions, the spray blocks are composed of forested areas, tree lines, buildings and other types of construction and vegetation that could significantly filter and dilute the spray plume as it travels downwind. In the research described here, our spray blocks and the surrounding areas are composed of agricultural fields, such as rice, cotton, and corn. The majority of the fields inside the treatment block are rice fields (90%), where as the area outside and downwind from our spray block is composed mainly of corn fields. Corn fields are a very dense type of vegetation that can easily reach >3 m in height. In addition to that their leaves are covered with a minute layer of dense hair. All these factors, vegetation density, height, surface type, could significantly filter a good portion of the spray plume resulting in less insecticide available in the air. Previous studies have shown that vegetation can significantly filter deposition (deposition was 7.5 times higher in the open compared to the vegetation area) and concentration of the insecticide in the air (a 67% decrease in droplets/cm^2 was observed in the vegetated area) (Brown et al. 2005). The model we used did not take into account the canopy effects and therefore could not accurately predict the dilution of the insecticide cloud as a result of filtration from canopy or other type of blockage.

In conclusion, the Wingman navigation system with the AGDISP spray dispersion model increased the efficiency of the spray trials by placing high droplet densities inside the treatment areas that resulted in good caged-mosquito mortalities. However, droplets were sampled outside the treatment block and could have been detected in higher numbers or even further downwind if it was not for the presence of dense vegetation. Our finding comes in agreement with previous research which showed that vegetation can significantly filter a high portion of the insecticide plume.

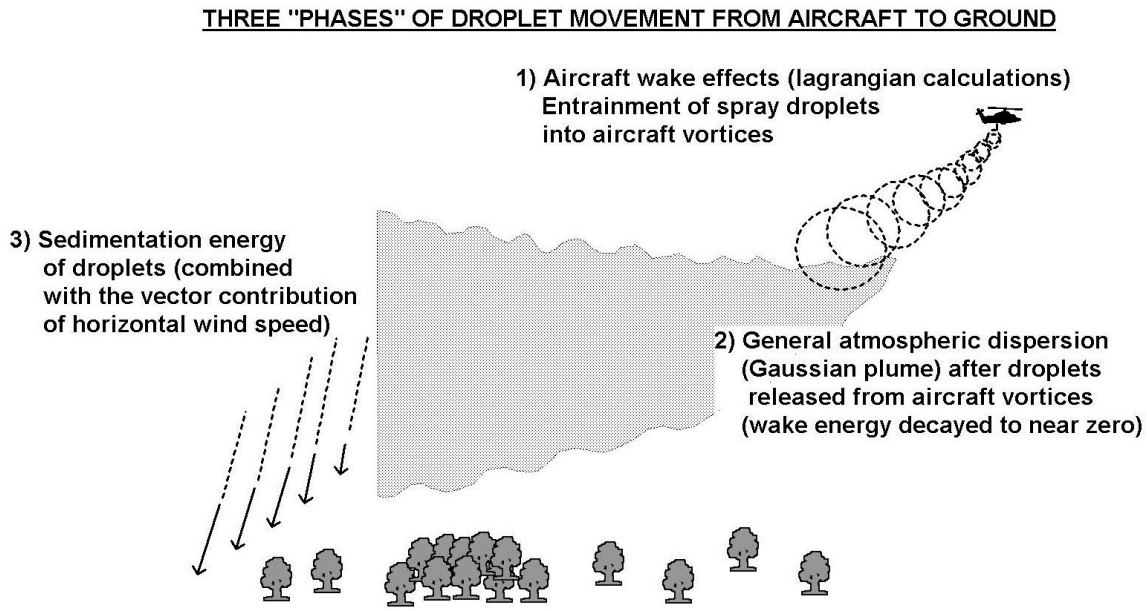


Figure 6-1. The three phases of insecticide droplet movement from an aircraft

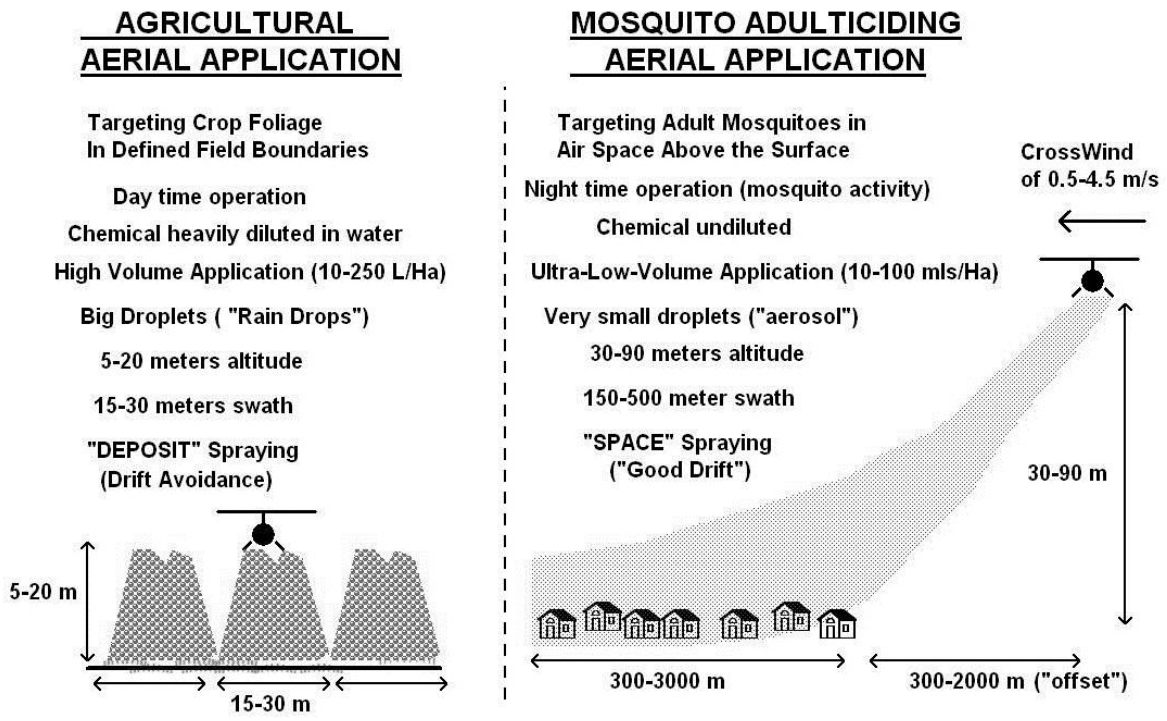


Figure 6-2. Differences in application parameters between agricultural and mosquito adulticiding aerial applications.

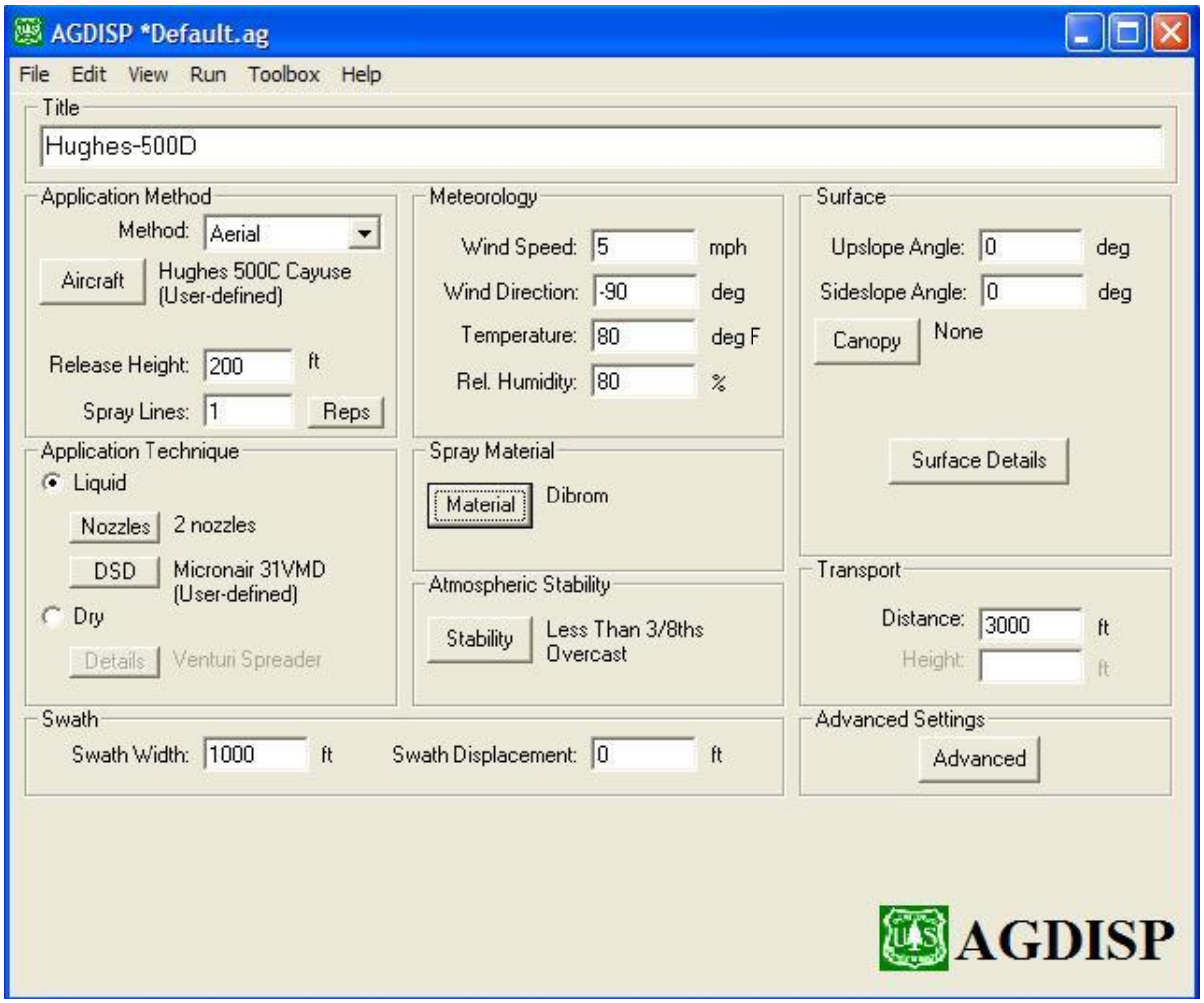


Figure 6-3. AGDISP computer spray fate model version 8.15.

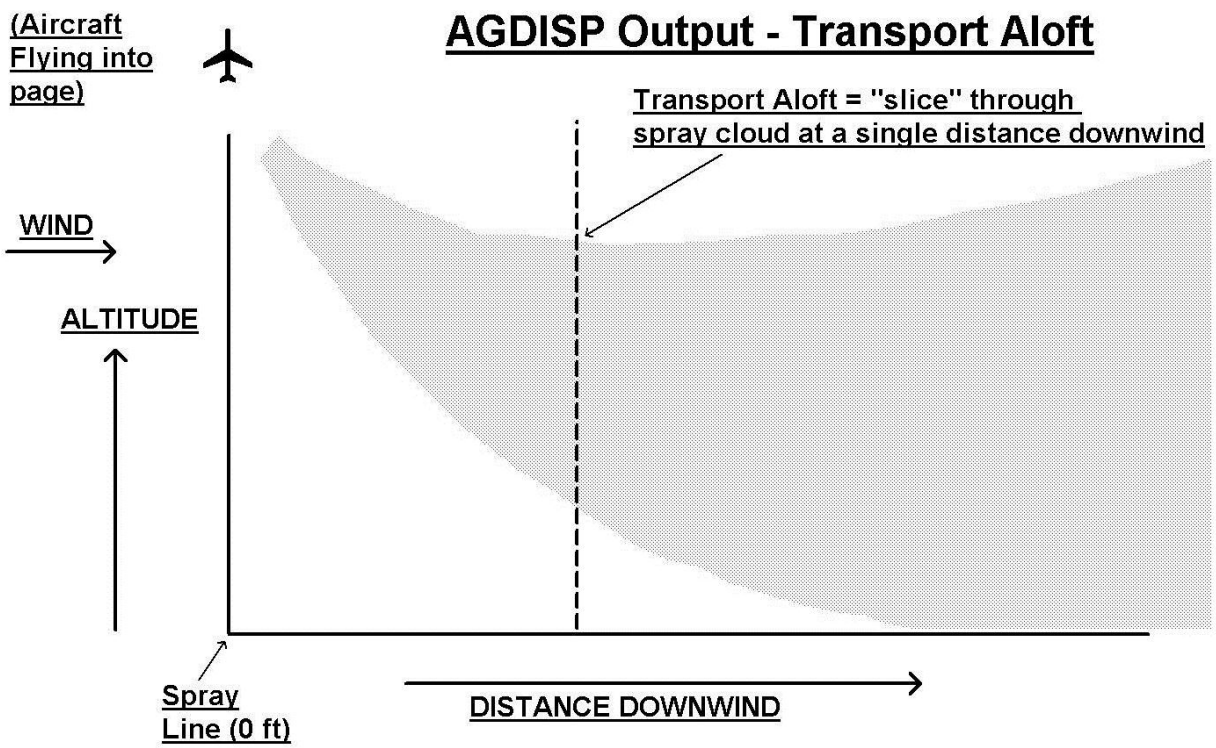


Figure 6-4. AGDISP output value: transport aloft.

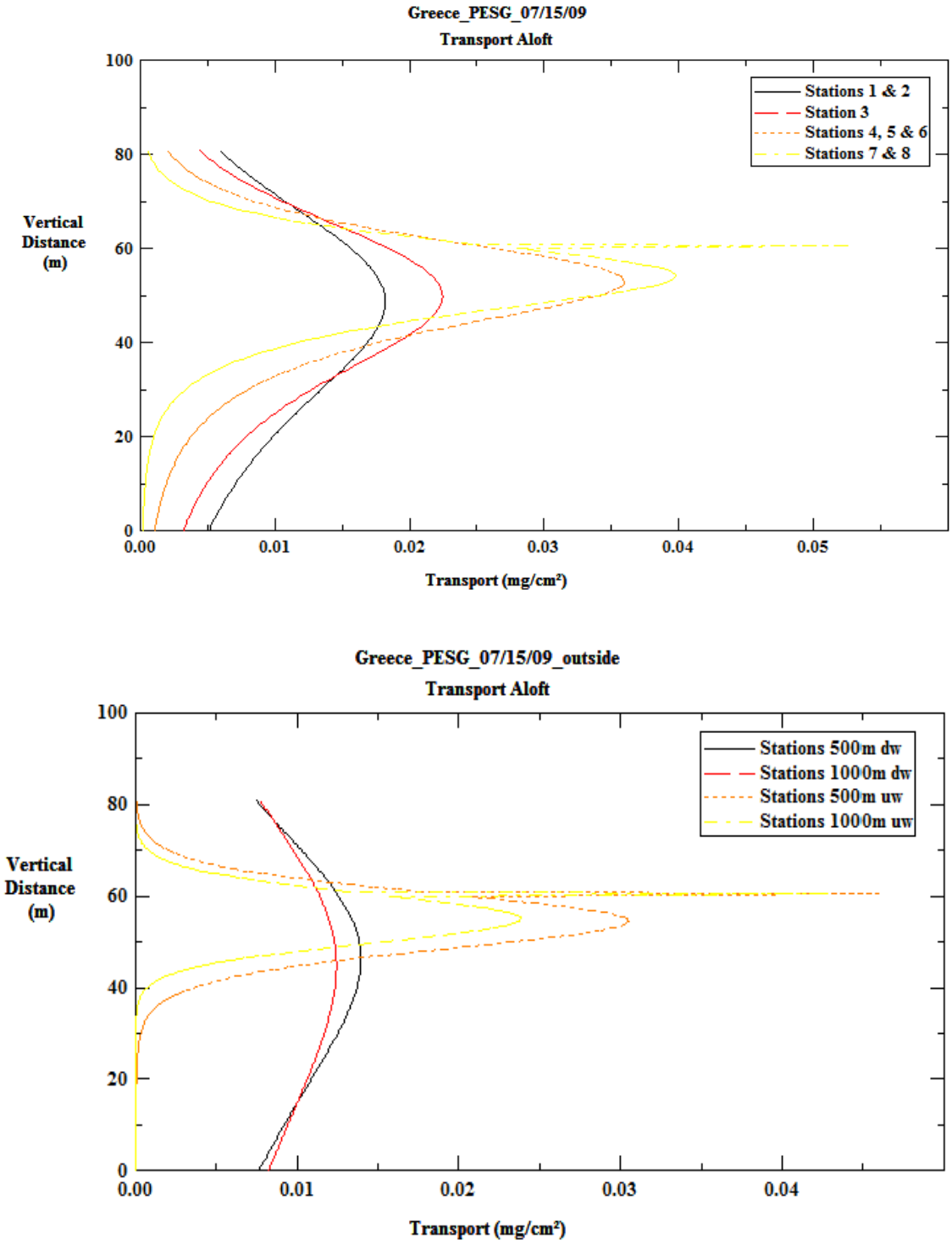


Figure 6-5.Trial with PESG on 07/15/09: Insecticide transport aloft values based on AGDISP predictions for all the station locations inside (graph at the top) and outside (downwind: dw, upwind: uw) the treatment area.

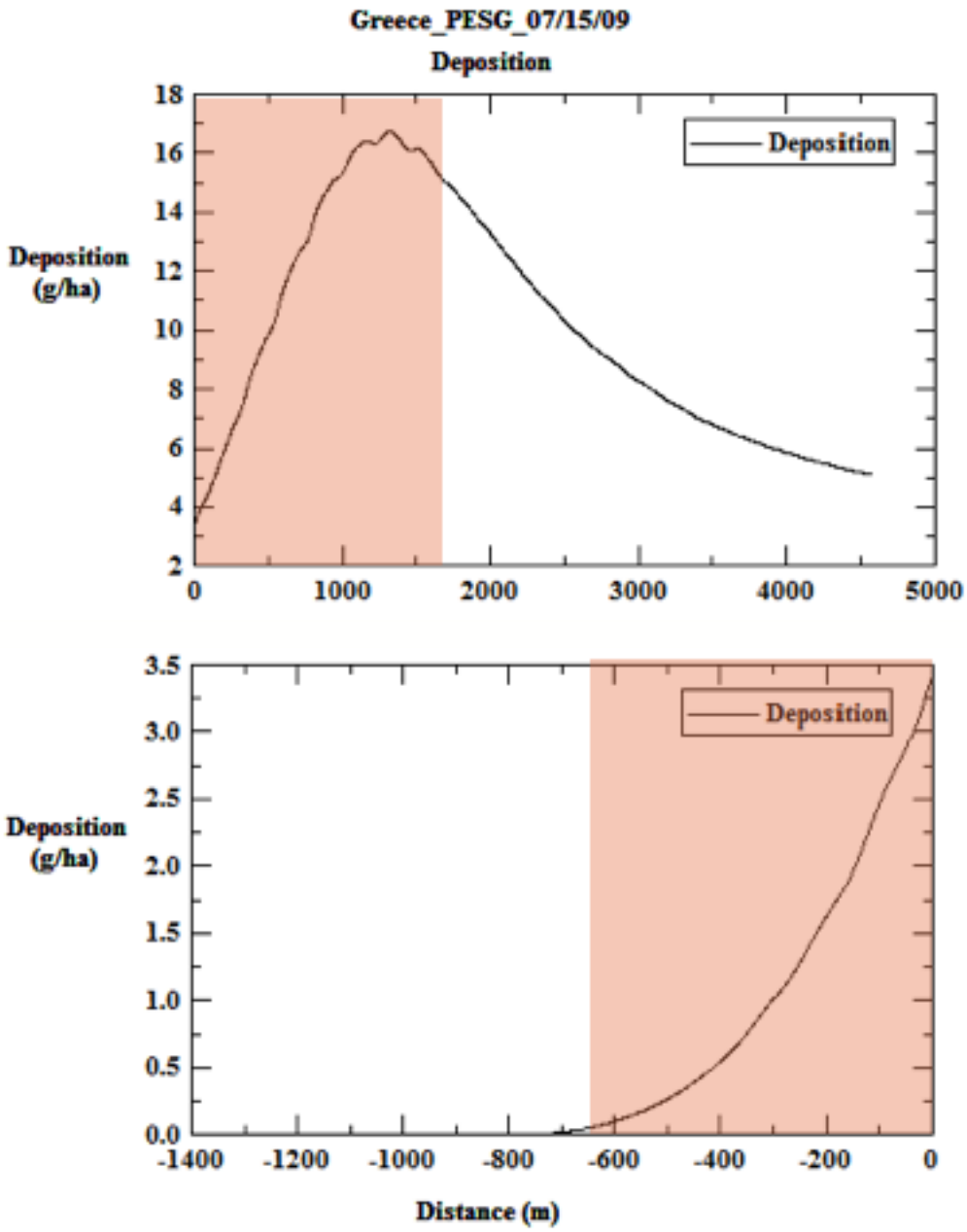


Figure 6-6. Trial with PESG on 07/15/09: Insecticide deposition values based on AGDISP predictions inside and outside the treatment area. The shadowed area corresponds to values inside the treatment area.

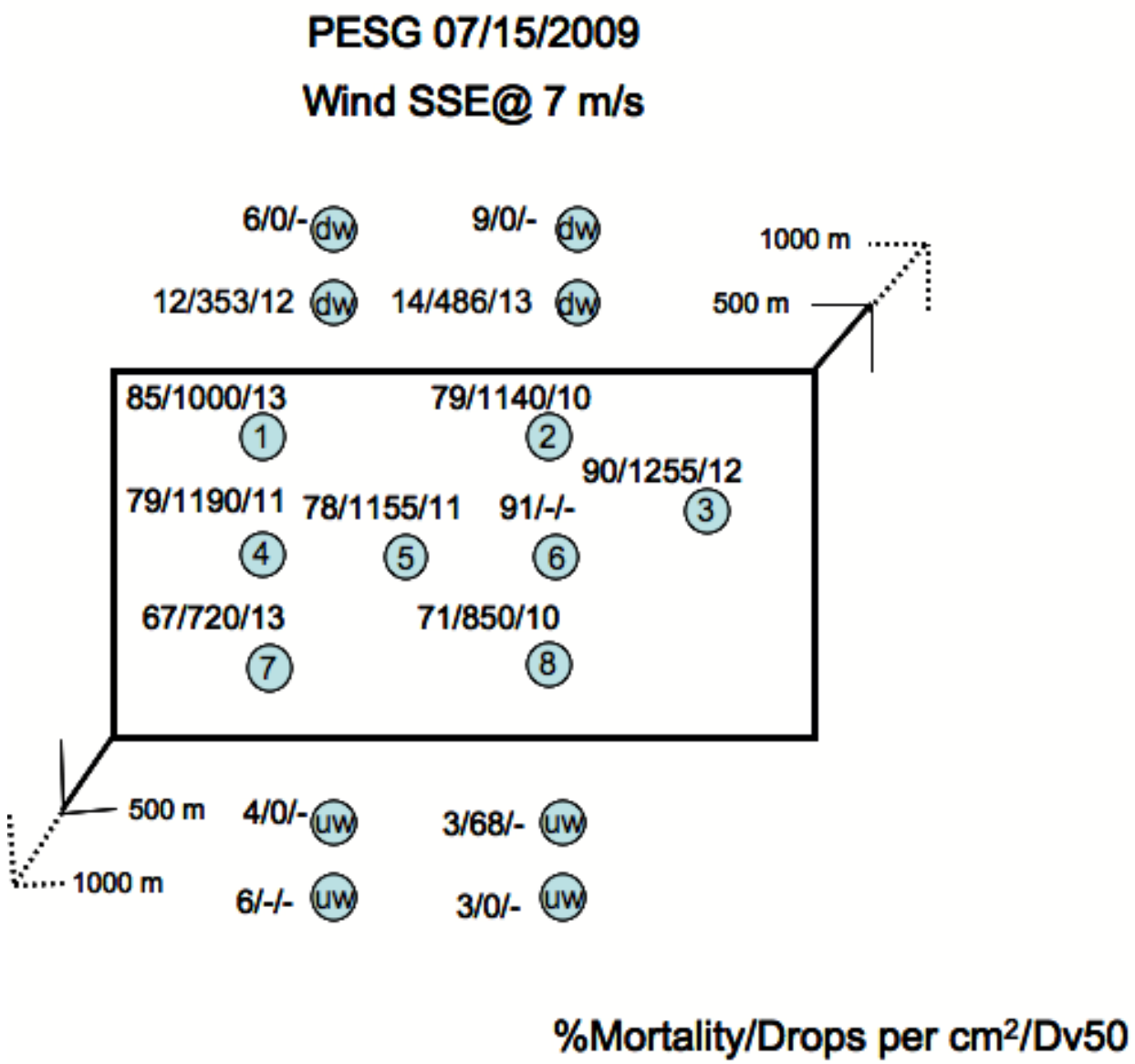


Figure 6-7. Trial with PESG on 07/15/09: Caged-mosquito mortalities, droplet densities along with the corresponding Dv_{50} for all the stations located inside and outside (downwind: dw, upwind: uw) the treatment area. Slides from sampling stations with not enough droplets to determine Dv_{50} (<100 drops/cm^2) or slides that were destroyed because of dust from the field are represented with - .

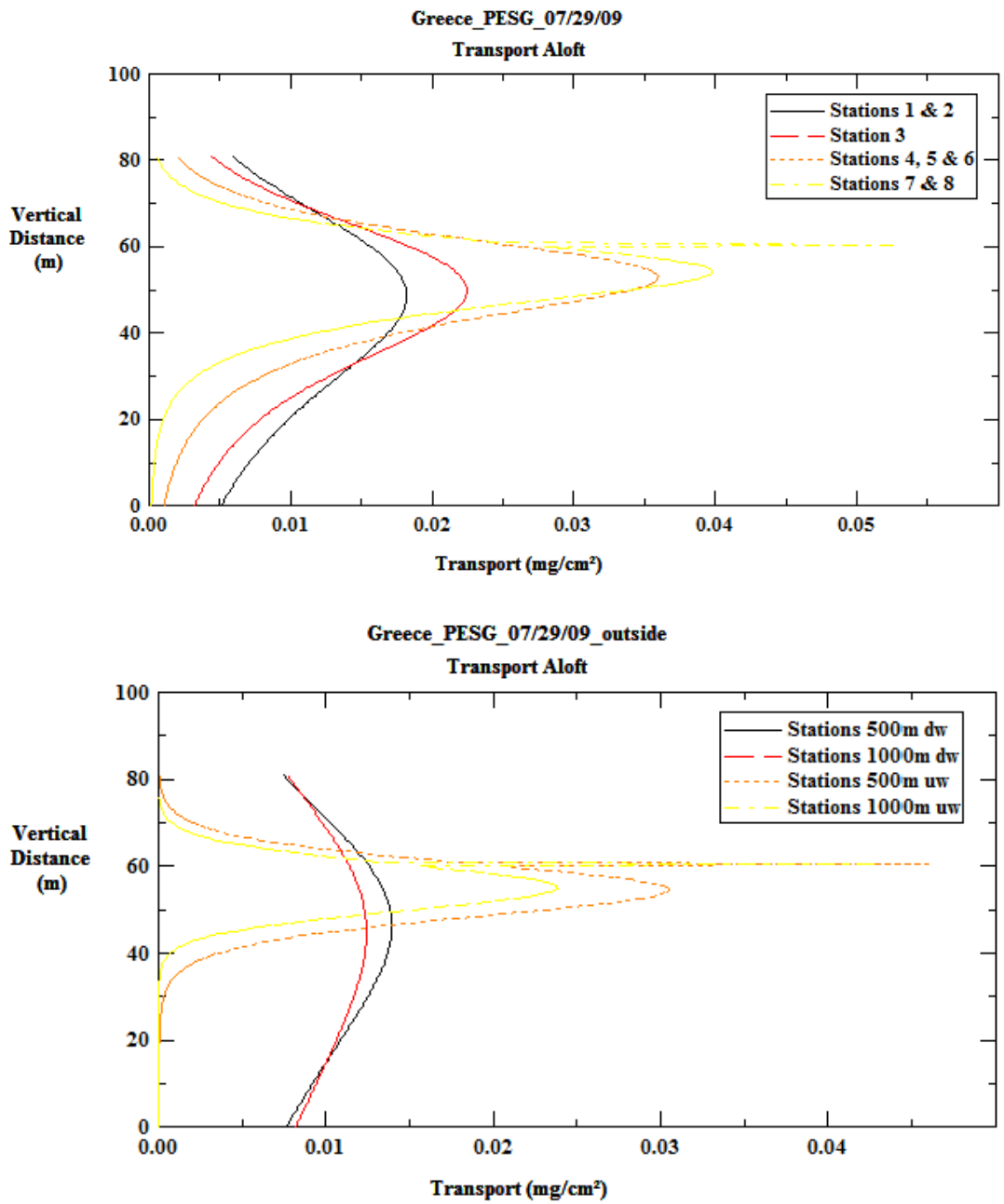


Figure 6-8. Trial with PESG on 07/29/09: Insecticide transport aloft values based on AGDISP predictions for all the station locations inside (graph at the top) and outside (downwind: dw, upwind: uw) the treatment area.

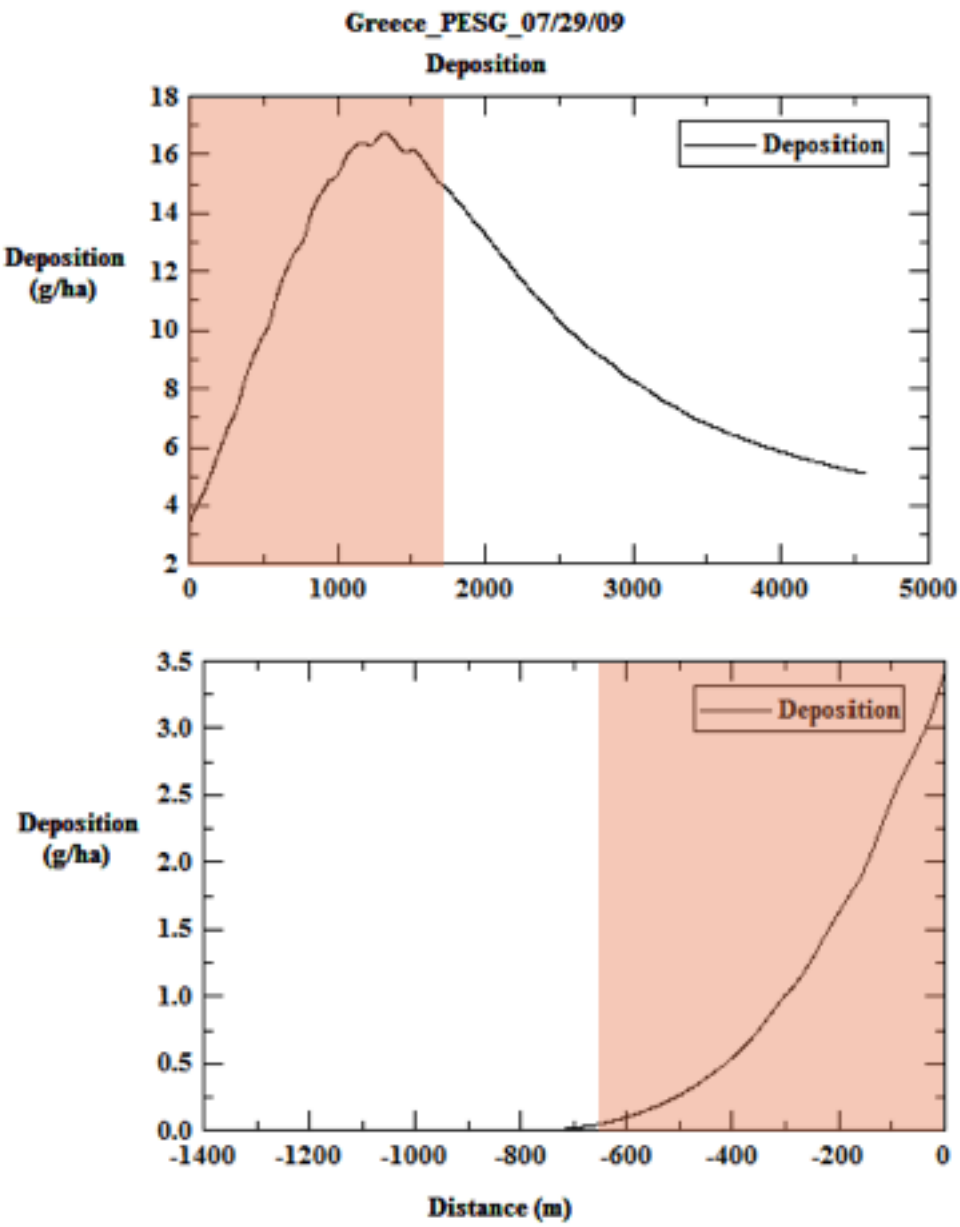


Figure 6-9. Trial with PESG on 07/29/09: Insecticide deposition values based on AGDISP predictions inside and outside the treatment area. The shadowed area corresponds to values inside the treatment area.

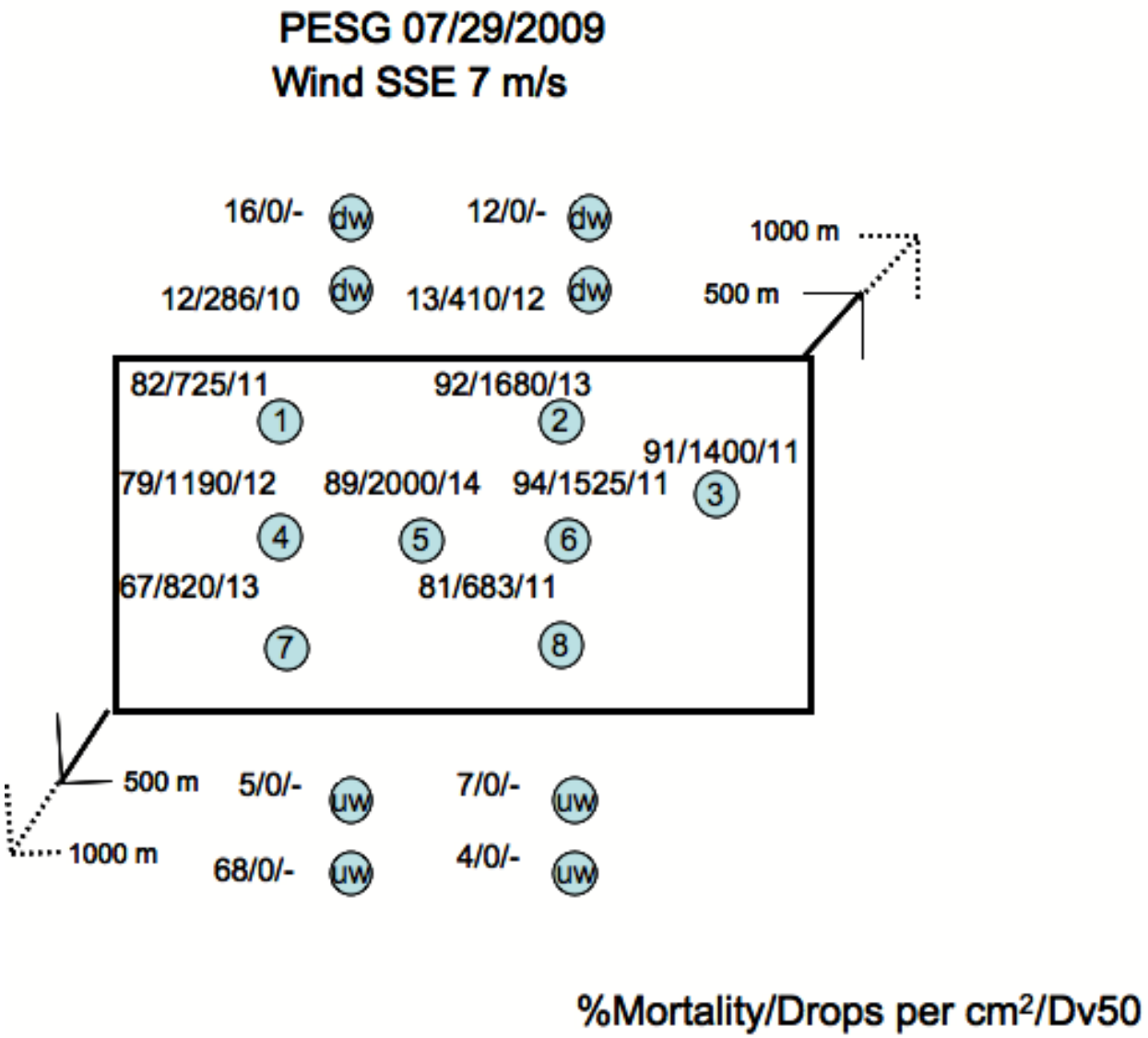
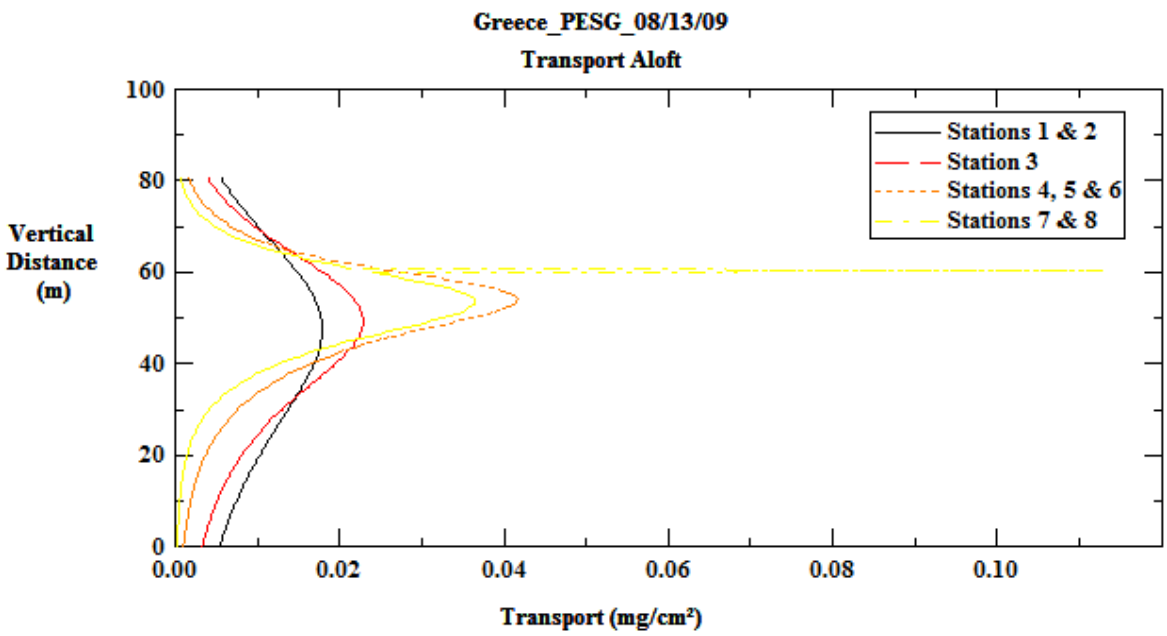


Figure 6-10. Trial with PESG on 07/29/09: Caged-mosquito mortalities, droplet densities along with the corresponding Dv_{50} for all the stations located inside and outside (downwind: dw, upwind: uw) the treatment area. Slides from sampling stations with not enough droplets to determine Dv_{50} (<100 drops/cm^2) or slides that were destroyed because of dust from the field are represented with - .



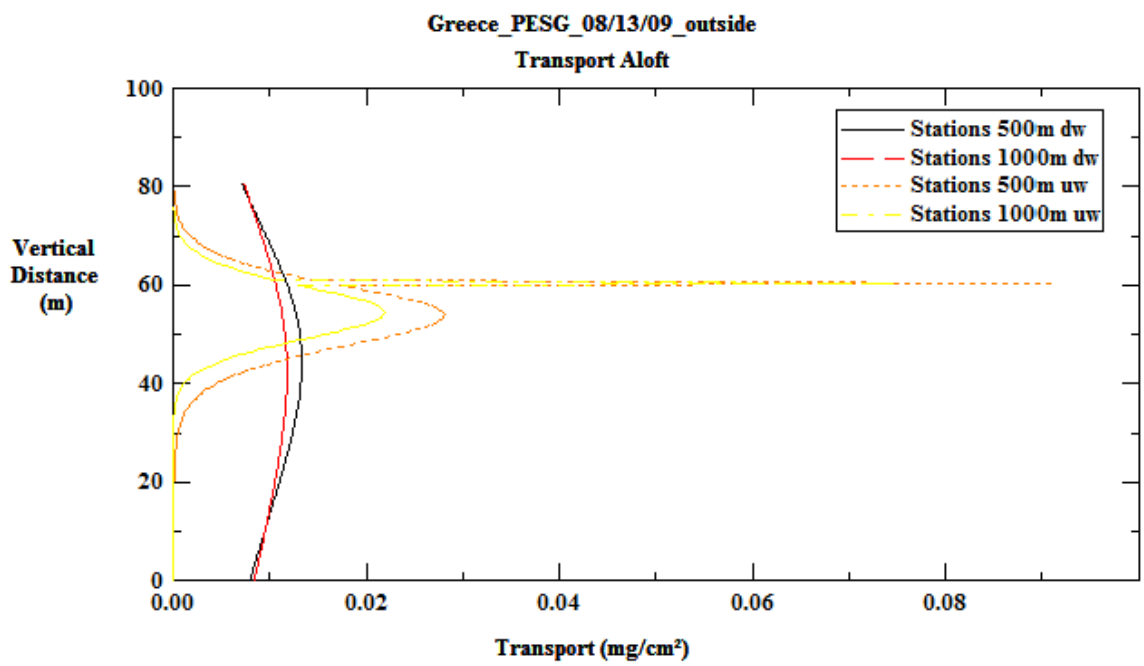


Figure 6-11. Trial with PESG on 08/13/09: Insecticide transport aloft values based on AGDISP predictions for all the station locations inside (graph at the top) and outside (downwind: dw, upwind: uw) the treatment area.

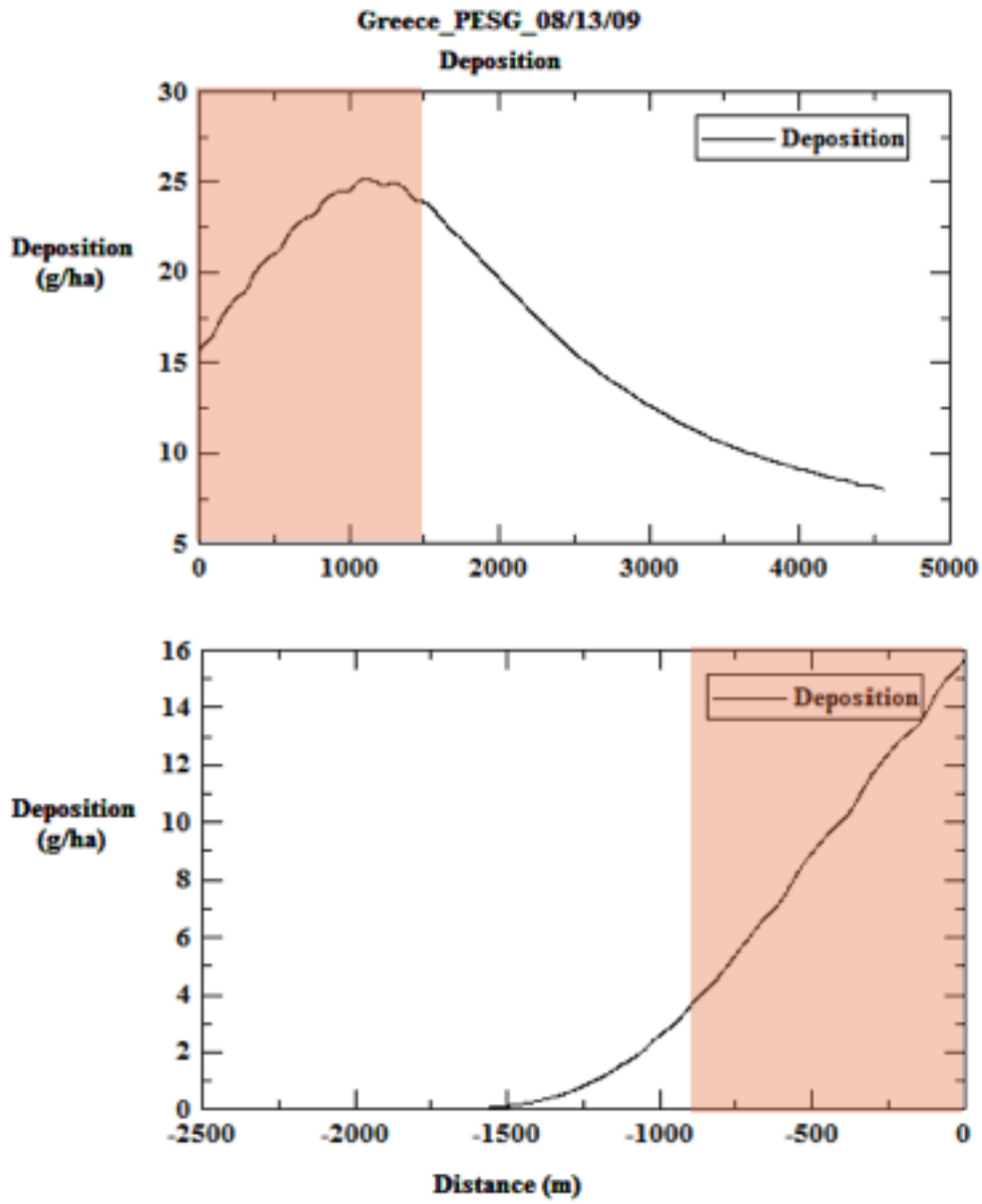


Figure 6-12. Trial with PESG on 08/13/09: Insecticide deposition values based on AGDISP predictions inside and outside the treatment area. The shadowed area corresponds to values inside the treatment area.

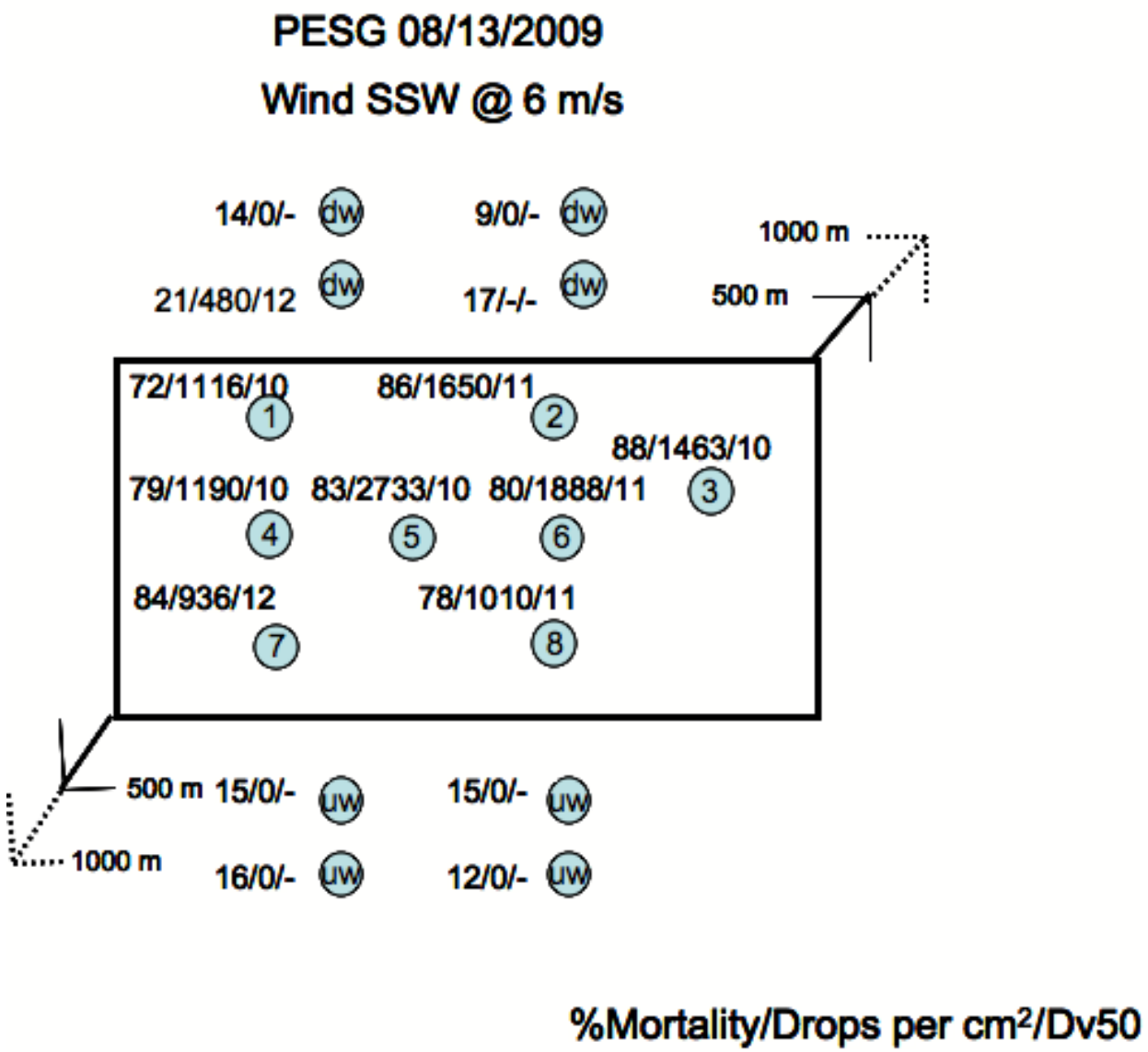


Figure 6-13. Trial with PESG on 08/13/09: Caged-mosquito mortalities, droplet densities along with the corresponding Dv_{50} for all the stations located inside and outside (downwind: dw, upwind: uw) the treatment area. Slides from sampling stations with not enough droplets to determine Dv_{50} (<100 drops/cm^2) or slides that were destroyed because of dust from the field are represented with - .

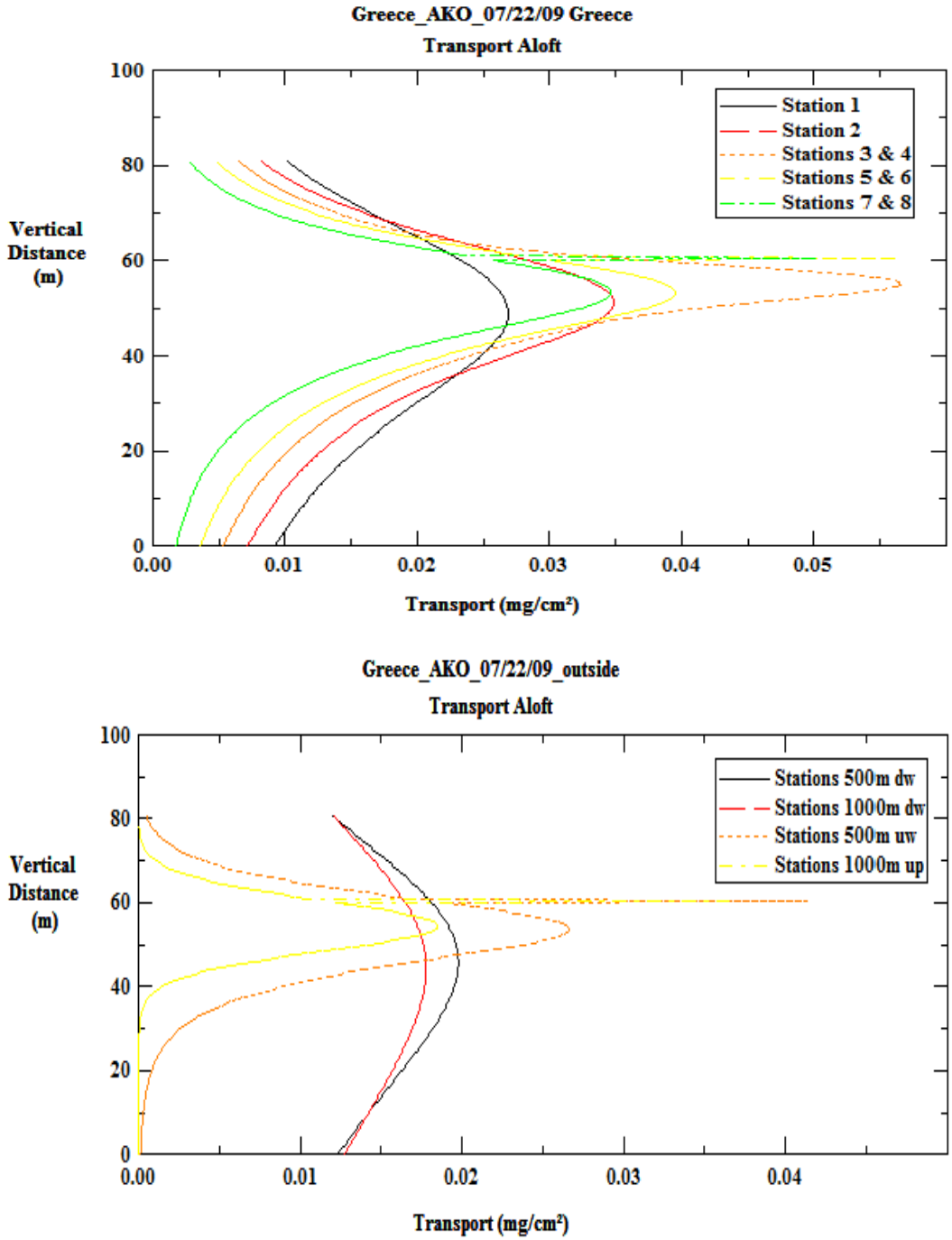


Figure 6-14. Trial with AKO on 07/22/09: Insecticide transport aloft values based on AGDISP predictions for all the station locations inside (graph at the top) and outside (downwind: dw, upwind: uw) the treatment area.

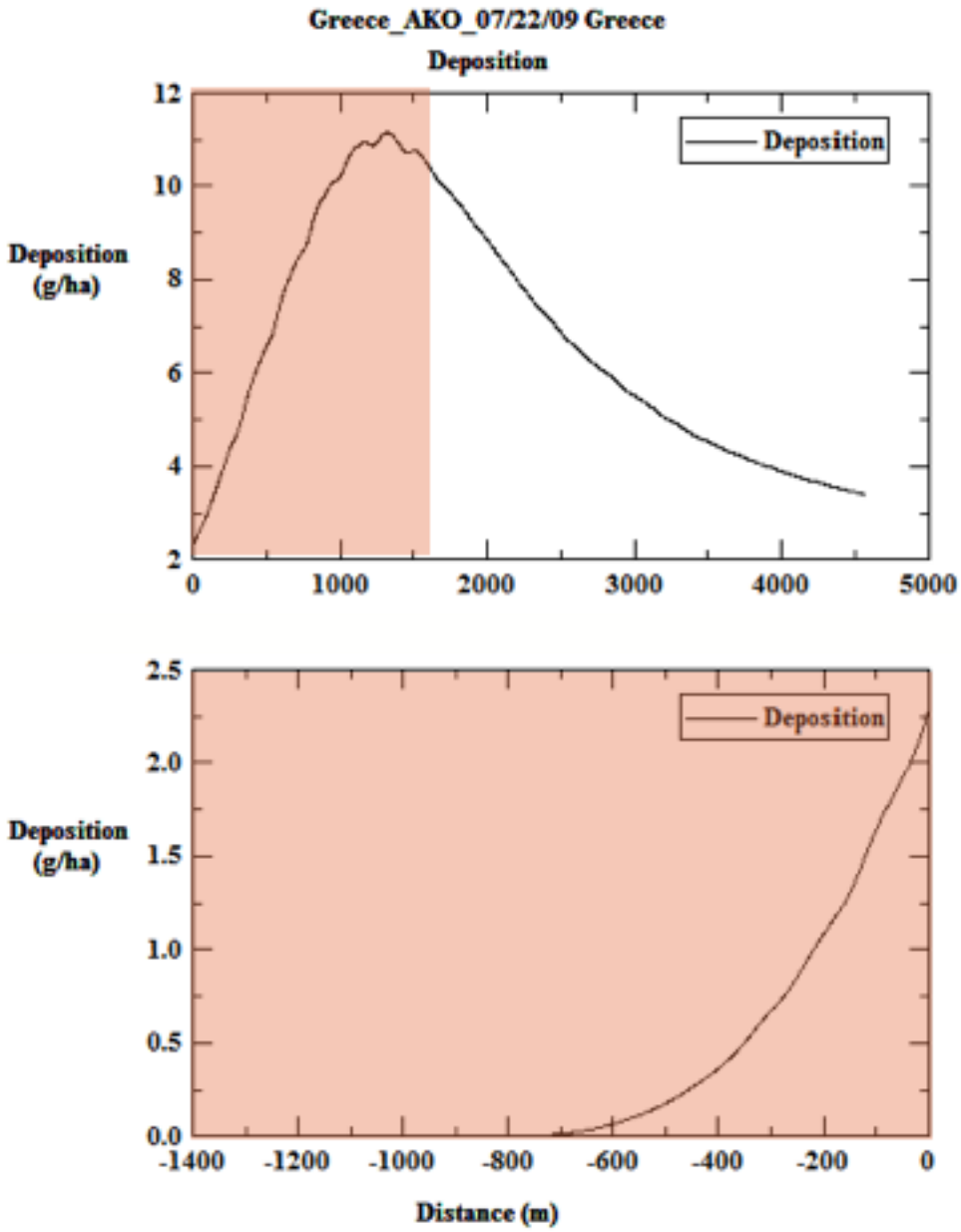


Figure 6-15. Trial with AKO on 07/22/09: Insecticide deposition values based on AGDISP predictions inside and outside the treatment area. The shadowed area corresponds to values inside the treatment area.

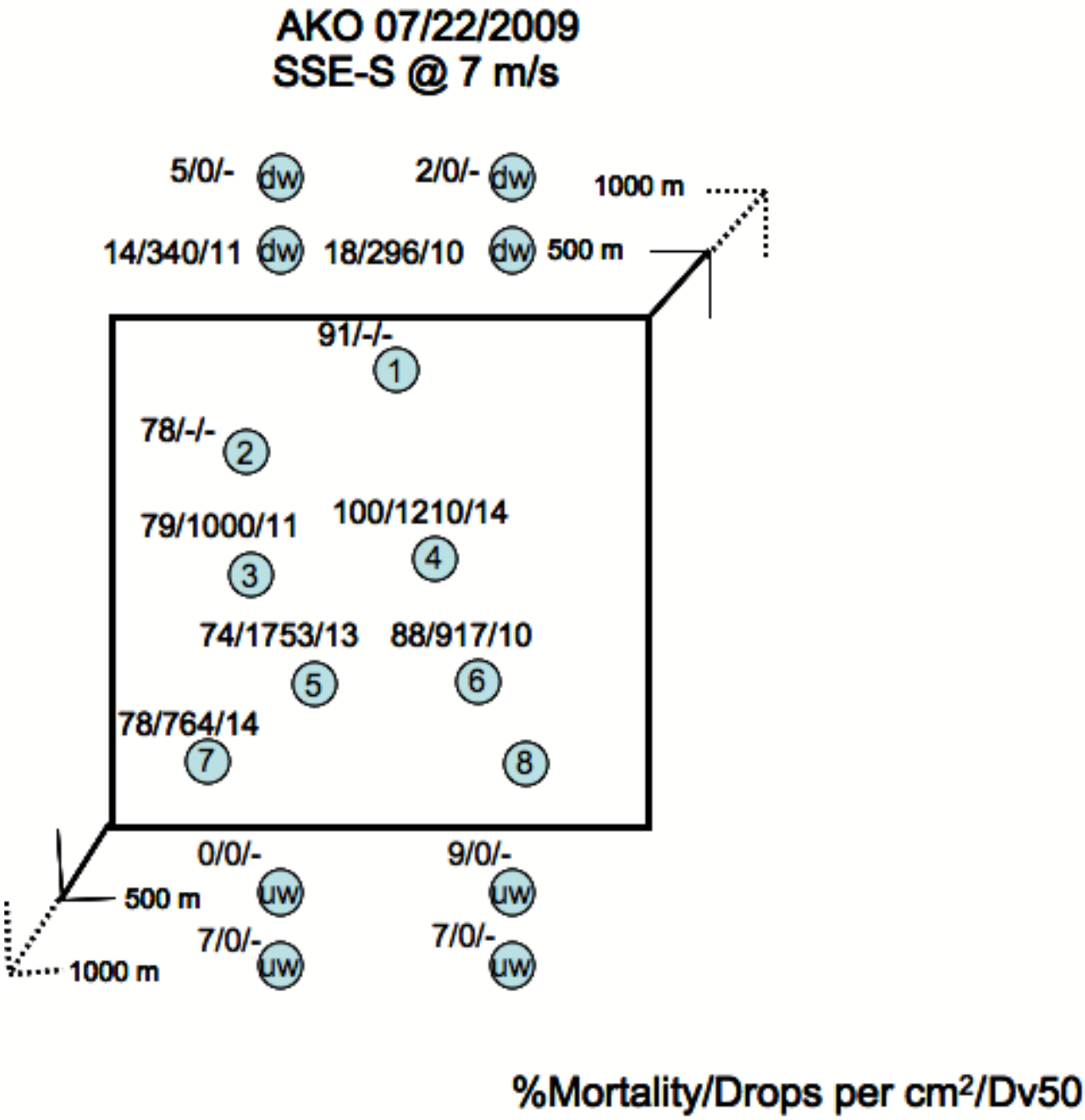
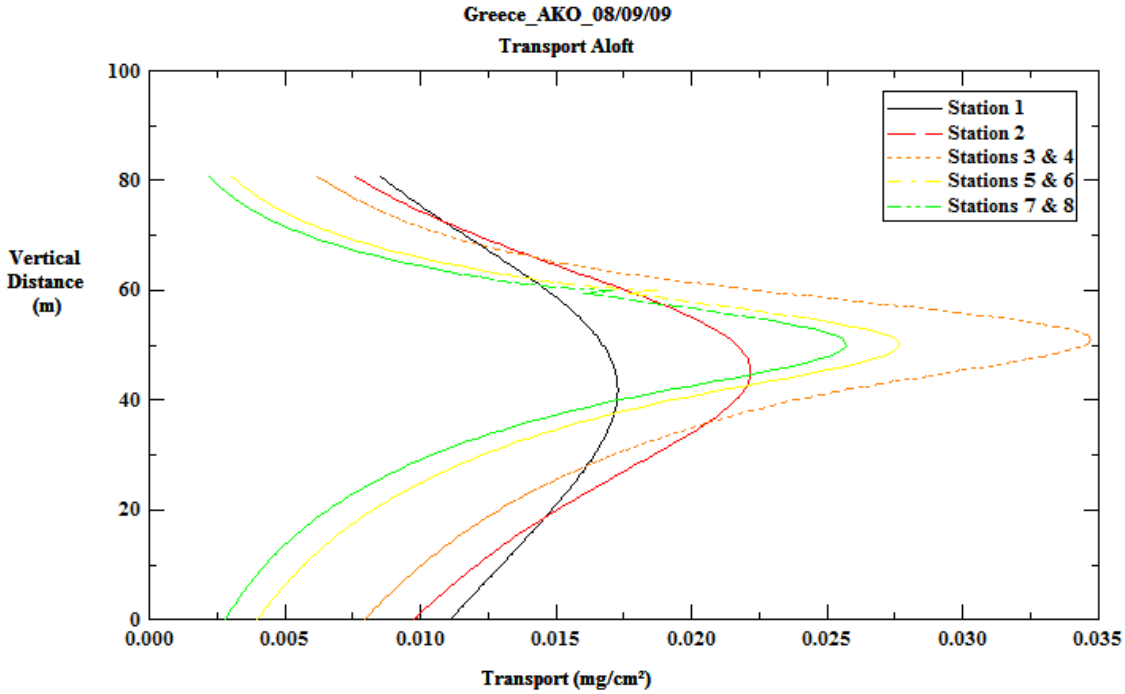


Figure 6-16. Trial with AKO on 07/22/09: Caged-mosquito mortalities, droplet densities along with the corresponding Dv_{50} for all the stations located inside and outside (downwind: dw, upwind: uw) the treatment area. Slides from sampling stations with not enough droplets to determine Dv_{50} (<100 drops/cm^2) or slides that were destroyed because of dust from the field are represented with - .



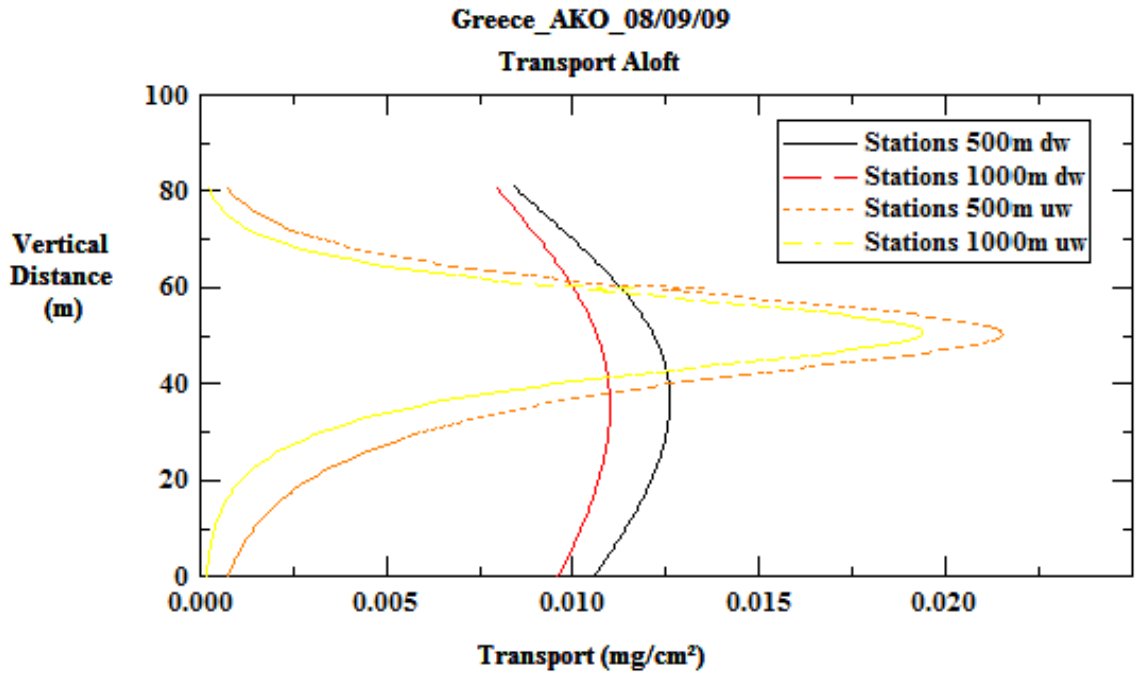


Figure 6-17. Trial with AKO on 08/09/09: Insecticide transport aloft values based on AGDISP predictions for all the station locations inside (graph at the top) and outside (downwind: dw, upwind: uw) the treatment area.

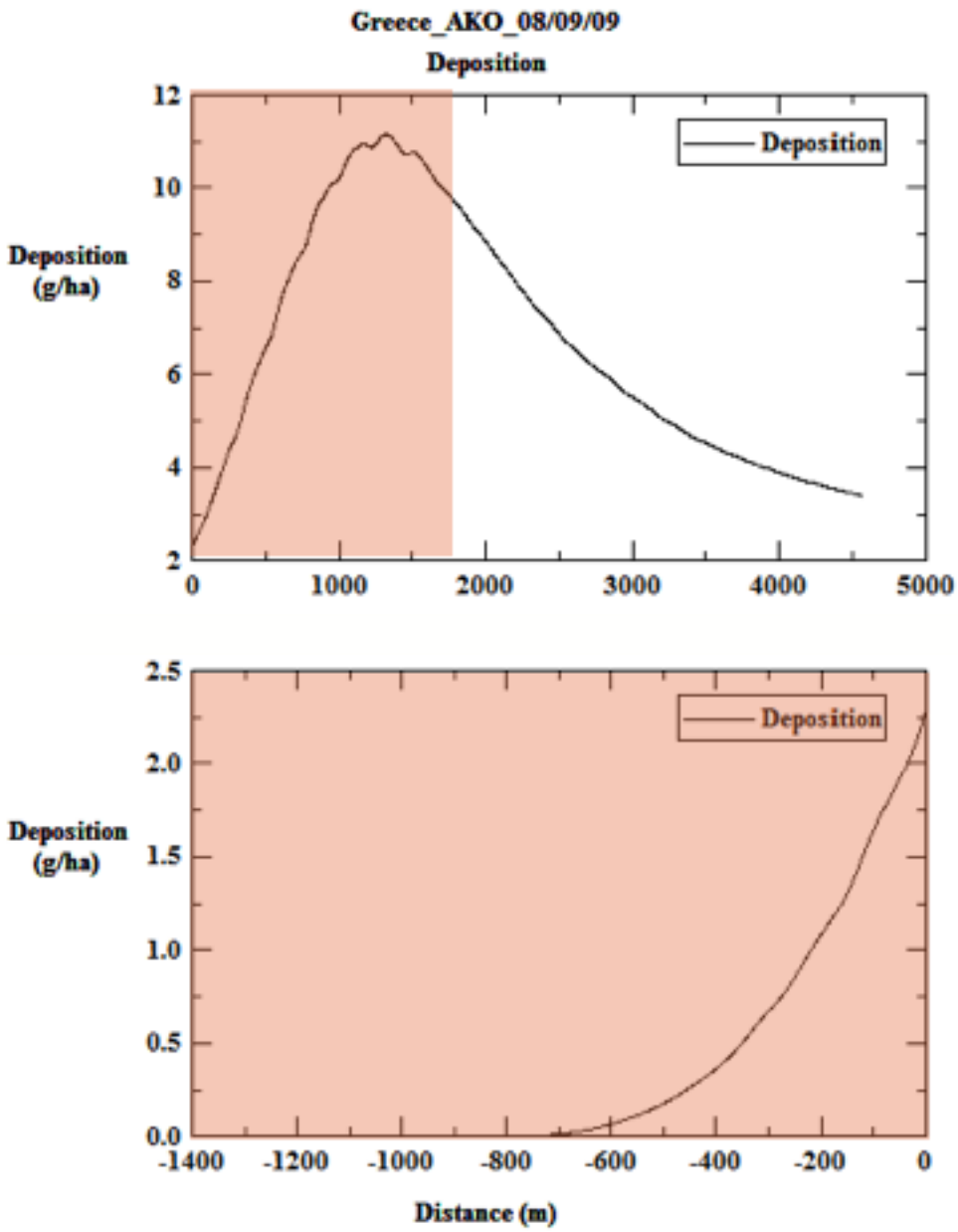


Figure 6-18. Trial with AKO on 08/09/09: Insecticide deposition values based on AGDISP predictions inside and outside (downwind: dw, upwind: uw) the treatment area. The shadowed area corresponds to values inside the treatment area.

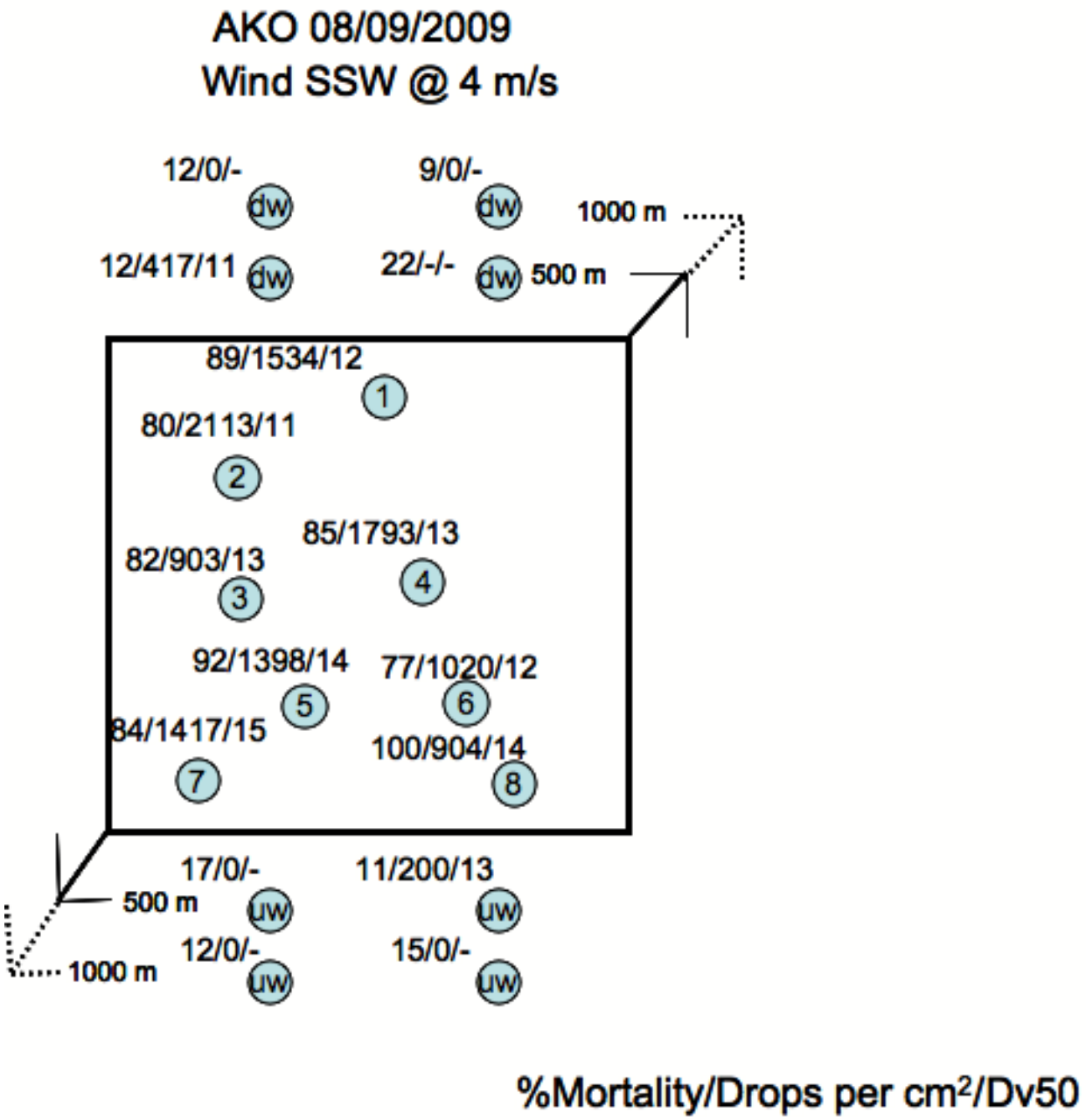


Figure 6-19. Trial with AKO on 08/09/09: Caged-mosquito mortalities, droplet densities along with the corresponding Dv_{50} for all the stations located inside and outside (downwind: dw, upwind: uw) the treatment area. Slides from sampling stations with not enough droplets to determine Dv_{50} (<100 drops/cm^2) or slides that were destroyed because of dust from the field are represented with - .

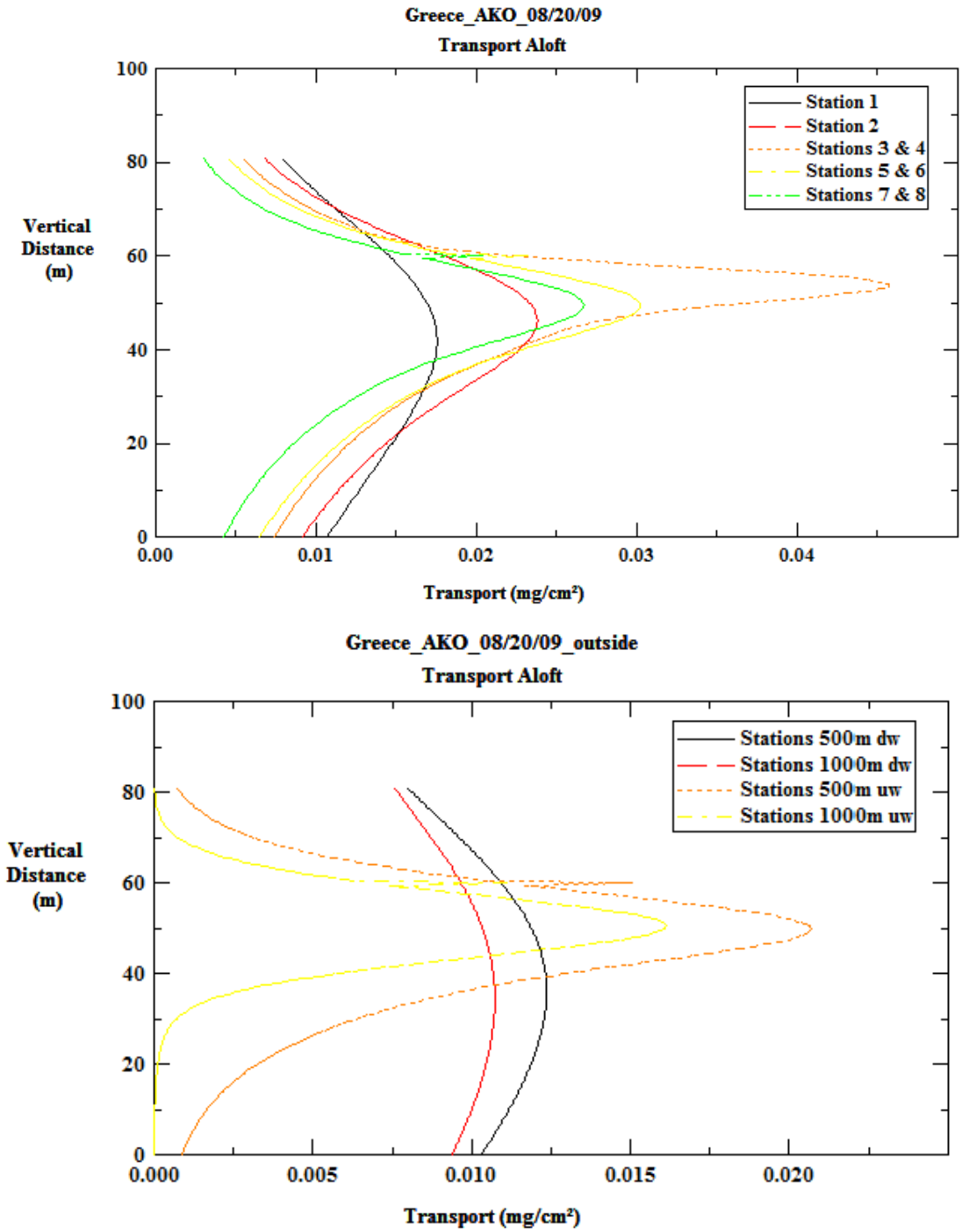


Figure 6-20. Trial with AKO on 08/20/09: Insecticide transport aloft values based on AGDISP predictions for all the station locations inside (graph at the top) and outside (downwind: dw, upwind: uw) the treatment area.

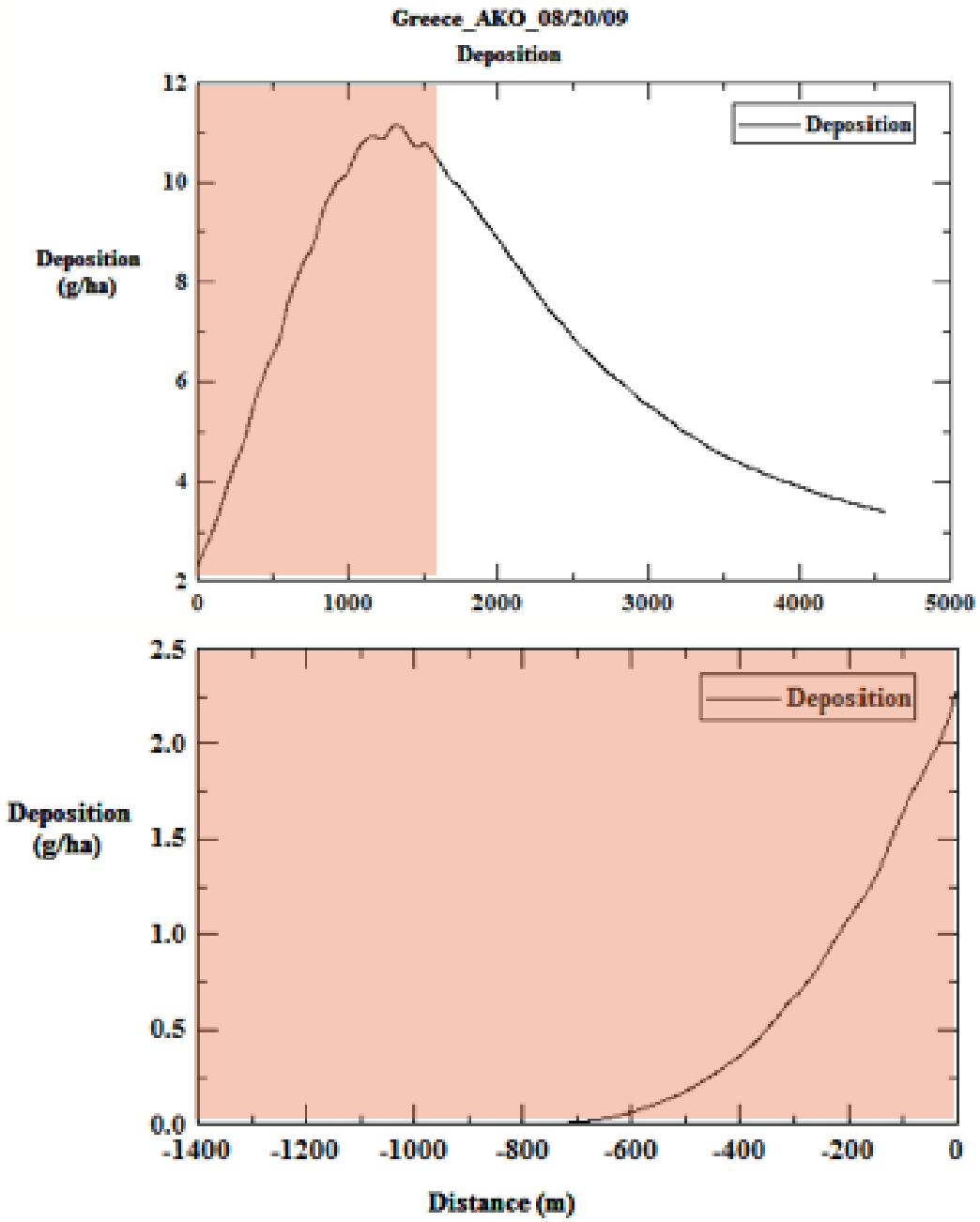


Figure 6-21. Trial with AKO on 08/20/09: Insecticide deposition values based on AGDISP predictions inside and outside the treatment area. The shadowed area corresponds to values inside the treatment area.

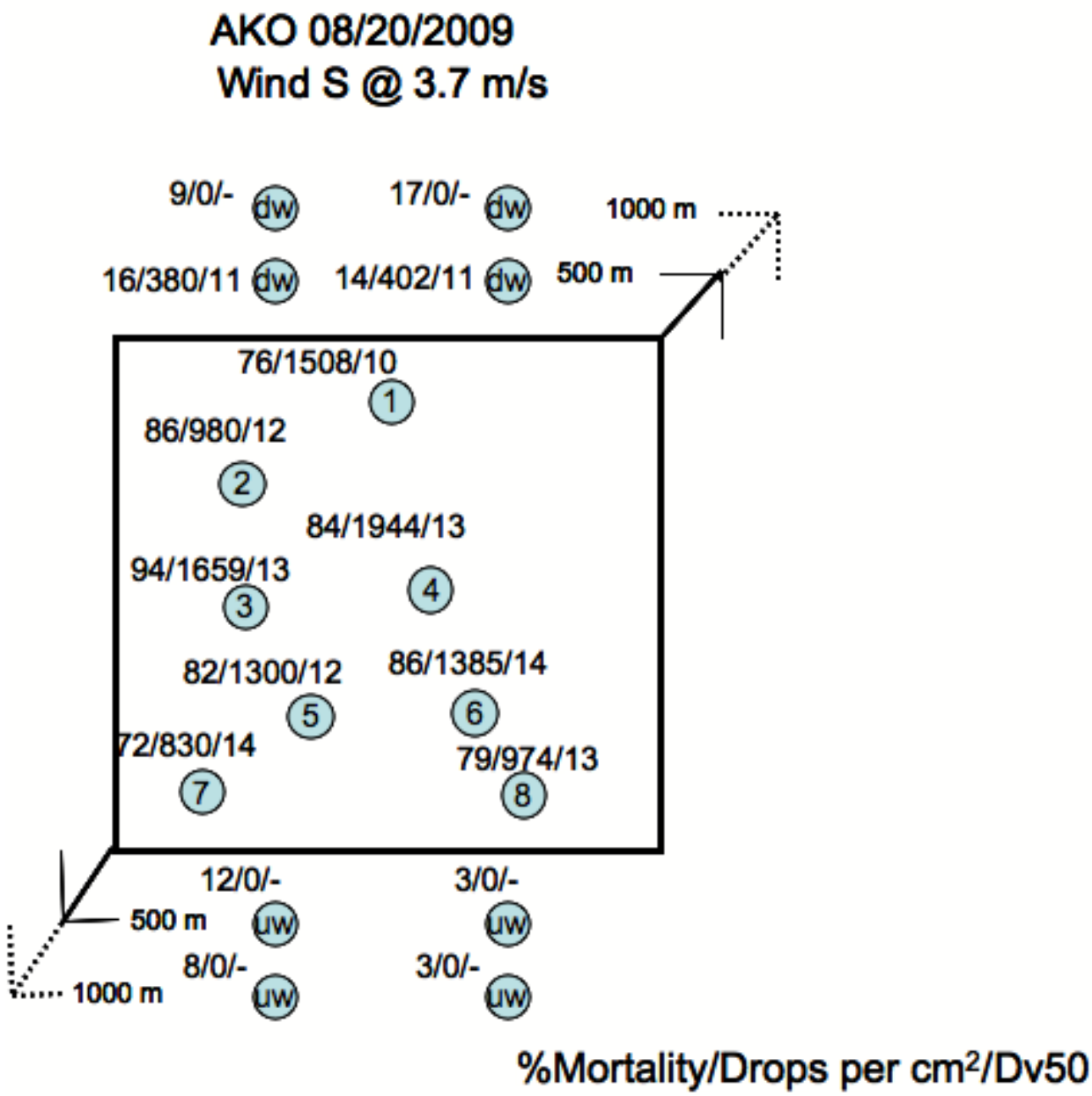


Figure 6-22. Trial with AKO on 08/20/09: Caged-mosquito mortalities, droplet densities along with the corresponding Dv_{50} for all the stations located inside and outside (downwind: dw, upwind: uw) the treatment area. Slides from sampling stations with not enough droplets to determine Dv_{50} (<100 drops/cm^2) or slides that were destroyed because of dust from the field are represented with - .

CHAPTER 7
OPERATIONAL AERIAL ULV ADULTICIDING IN RESPONSE TO THE FIRST WEST NILE VIRUS EPIDEMIC IN GREECE

West Nile Virus in Europe

West Nile virus (WNV) (Family: Flaviviridae) was isolated for the first time in 1937 in Uganda. The virus is primarily transmitted to birds through mosquito bites while humans are incidental hosts. Incidental infection may also occur in other mammals including horses, cats, and domestic mammals. The virus is widely distributed throughout Africa, the Middle East, Europe, parts of the Soviet Union, India and Indonesia (Mullen and Durden 2002). It was introduced in 1999 to the United States and was linked to human encephalitis with 61 confirmed cases. In Europe one of the largest and most recent outbreaks occurred in the summer of 1996 in Bucharest, Romania with more than 500 clinical cases and a case fatality rating approaching 10% (Hubalek and Halouzka 1999). Mosquito vectors of WNV belong primarily in the genus *Culex*, with *Culex pipiens* L. and *Culex modestus* Ficalbi being the most important vectors in Europe (Balenghien et al. 2006, 2007, 2008).

Currently, in 2010, there is an ongoing outbreak of WNV for the first time in Greece (more details are provided below). In addition to the WNV outbreak in Greece several other European member states, as well as some neighboring countries are reporting WNV transmission. The Romanian health authorities have reported a total of thirteen confirmed cases of WNV infection during the months of July and August. Two infected persons have died, both above 75 years old. The cases have a median age of 50 years (18-79 years of age) and are spread over 11 different districts in the country. Three confirmed case were reported by the Hungarian health authorities on the 2nd of September, one of whom lives close to the Romanian borders. Finally, one confirmed case of WNV infection was reported from the Veneto region of Italy, in the frame of an enhanced surveillance system for summer fevers during the month of August and one

probable case from Portugal in July (ECDC 2010, Figure 7-1). Outside the EU, during July and August 2010, a total of 231 cases of WNV have been reported in Russia, among them 6 fatal cases. On August 10, 24 confirmed cases of WNV were reported from Israel. Lastly in Morocco, while no human cases have been reported up to now, WNV infection was confirmed in 18 horses.

West Nile Virus Epidemic in Greece, 2010

A West Nile virus epidemic occurred for the first time in Northern Greece in 2010 in the prefecture of central Macedonia (population of 2 million people) that includes Thessaloniki, the second largest city of Greece (population of 1 million). From the beginning of the outbreak (7/6/2010) until the present (10/29/2010) 261 confirmed cases have been reported (Figure 7-2) with thirty-four deaths (predominantly in persons over 70 years of age). Even though the cases were widespread the epicenter of the outbreak could be identified as the larger area surrounding the Axios and Aliakmonas rivers. This area is comprised mainly of rice fields (primary mosquito breeding sites) and other natural wetlands. The main WNV vector species in the area are presumed to be *Culex pipiens* L. and *Culex modestus* Ficalbi.

Prior to the WNV outbreak the only available mosquito control technique approved for use in Greece was larviciding and there were no products available for adulticiding. After the onset of the disease, and as a direct result of our study, 2 vector control products received registrations for adulticiding and emergency spraying was conducted by air in an attempt to interrupt the disease transmission cycle. When a mosquito-borne disease epidemic is in progress, vector-control tools that can provide immediate, effective, and environmentally acceptable control practices should be available both as a proactive measure and an emergency response tool for mosquito control.

The First Operational Aerial ULV Adulticiding in Greece

Aerial Adulticiding Mission: 9/2/2010, Area of Chalastra-Kaloxwri

The product used was Pesguard (d-phenothrin 10%) and the dose applied corresponded to 0.75 g AI/stremma (7.5 g AI/hectare). The total area sprayed was 3,700 hectares (Figure 7-3). The spray block, based on the regulations issued by the Greek Ministries of Agriculture and Public Health, had to be located 2 km away from any residential areas. The efficacy of the spray was assessed with the use of CDC miniature light traps. The traps were deployed 24 hrs before and 24 hrs after the treatment. Based on pre-and post-spray trapping the spraying caused approximately 82% wild mosquito population decrease (Figure 7-3). The traps deployed 24 hrs before spraying caught an average of 90,000 adult mosquitoes. The mosquitoes caught in the traps belonged mainly to the genus *Culex* (22%) and *Anopheles* (75%).

Aerial Adulticiding Mission: 9/3/2010, Area of Malgara-Kimina-Klidi

The product used was Pesguard (d-phenothrin 10%) and the dose applied corresponded to 0.75 g AI/stremma (7.5 g AI/hectare). The total area sprayed was 3,500 hectares (Figure 7-4). The spray block, based on the regulations issued by the Greek Ministries of Agriculture and Public Health, had to be located 2 km away from any residential areas. The efficacy of the spray was assessed with the use of CDC miniature light traps. The traps were deployed 24 hrs before and 24 hrs after the treatment. Based on pre-and post-spray trapping the spraying caused approximately 91% wild mosquito population decrease (Figure 7-4). The traps deployed 24 hrs before spraying caught an average of 35,000 adult mosquitoes. The mosquitoes caught in the traps belonged mainly to the genus *Culex* (26%) and *Anopheles* (70%).

Aerial Adulticiding Mission: 9/6/2010, Area of Aiginio-Klidi

The product used was Pesguard (d-phenothrin 10%) and the dose applied corresponded to 0.75 g AI/stremma (7.5 g AI/hectare). The total area sprayed was 2,600 hectares (Figure 7-5).

The spray block, based on the regulations issued by the Greek Ministries of Agriculture and Public Health, had to be located 2 km away from any residential areas. The efficacy of the spray was assessed with the use of CDC miniature light traps. The traps were deployed 24 hrs before and 24 hrs after the treatment. Based on pre-and post-spray trapping the spraying caused approximately 90% wild mosquito population decrease (Figure 7-5). The traps deployed 24 hrs before spraying caught an average of 40,000 adult mosquitoes.

Aerial Adulticiding Mission: 9/7/2010, Area of Vraxia-Adendro-Klidi

The product used was Pesguard (d-phenothrin 10%) and the dose applied corresponded to 0.75 g AI/stremma (7.5 g AI/hectare). The total area sprayed with Pesguard was 1,000 hectares (Figure 7-6). Also, the product Aqua-K-Othrine was used (2% deltamethrin) and the dose applied corresponded to 0.1 g AI/stremma (1 g AI/hectare). The total area sprayed with Aqua-K-Othrine was 2,500 hectares. The spray block, based on the regulations issued by the Greek Ministries of Agriculture and Public Health, had to be located 2 km away from any residential areas. The efficacy of the spray was assessed with the use of CDC miniature light traps. The traps were deployed 24 hrs before and 24 hrs after the treatment. Based on pre-and post-spray trapping the spraying caused approximately 98% wild mosquito population decrease (Figure 7-6). The traps deployed 24 hrs before spraying caught an average of 42,000 adult mosquitoes.

Aerial Adulticiding Mission: 9/9/2010, Area of Chalastra-Kaloxwri

The product used was Pesguard (d-phenothrin 10%) and the dose applied corresponded to 0.75 g AI/stremma (7.5 g AI/hectare). The total area sprayed was 2,500 hectares (Figure 7-7). The spray block, based on the regulations issued by the Greek Ministries of Agriculture and Public Health, had to be located 2 km away from any residential areas. The efficacy of the spray was assessed with the use of CDC miniature light traps. The traps were deployed 24 hrs before and 24 hrs after the treatment. Based on pre-and post-spray trapping the spraying caused

approximately 98% wild mosquito population decrease (Figure 7-7). The traps deployed 24 hrs before spraying caught an average of 16,000 adult mosquitoes.

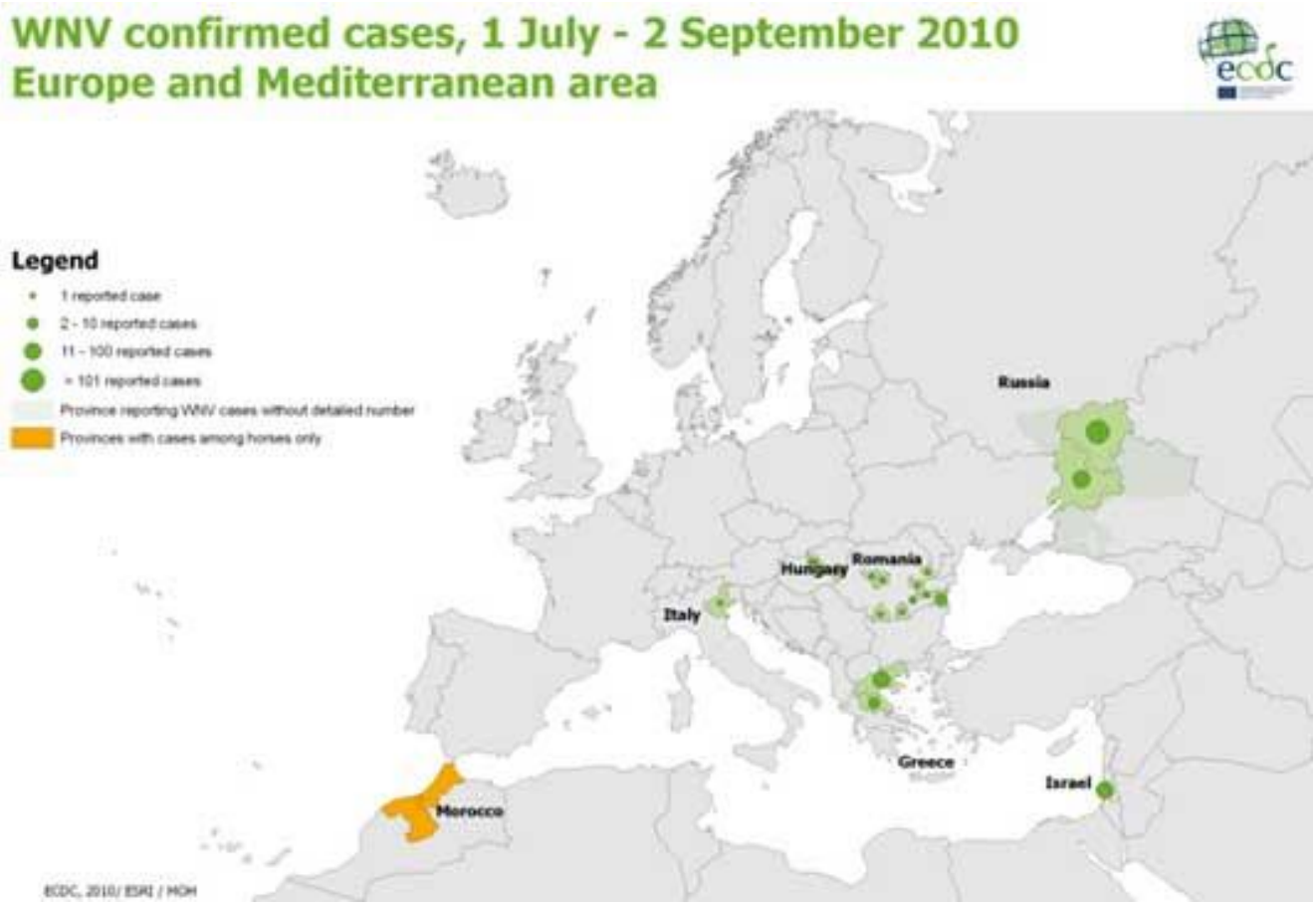


Figure 7-1. WNV confirmed cases in Europe and Mediterranean area, 2010.

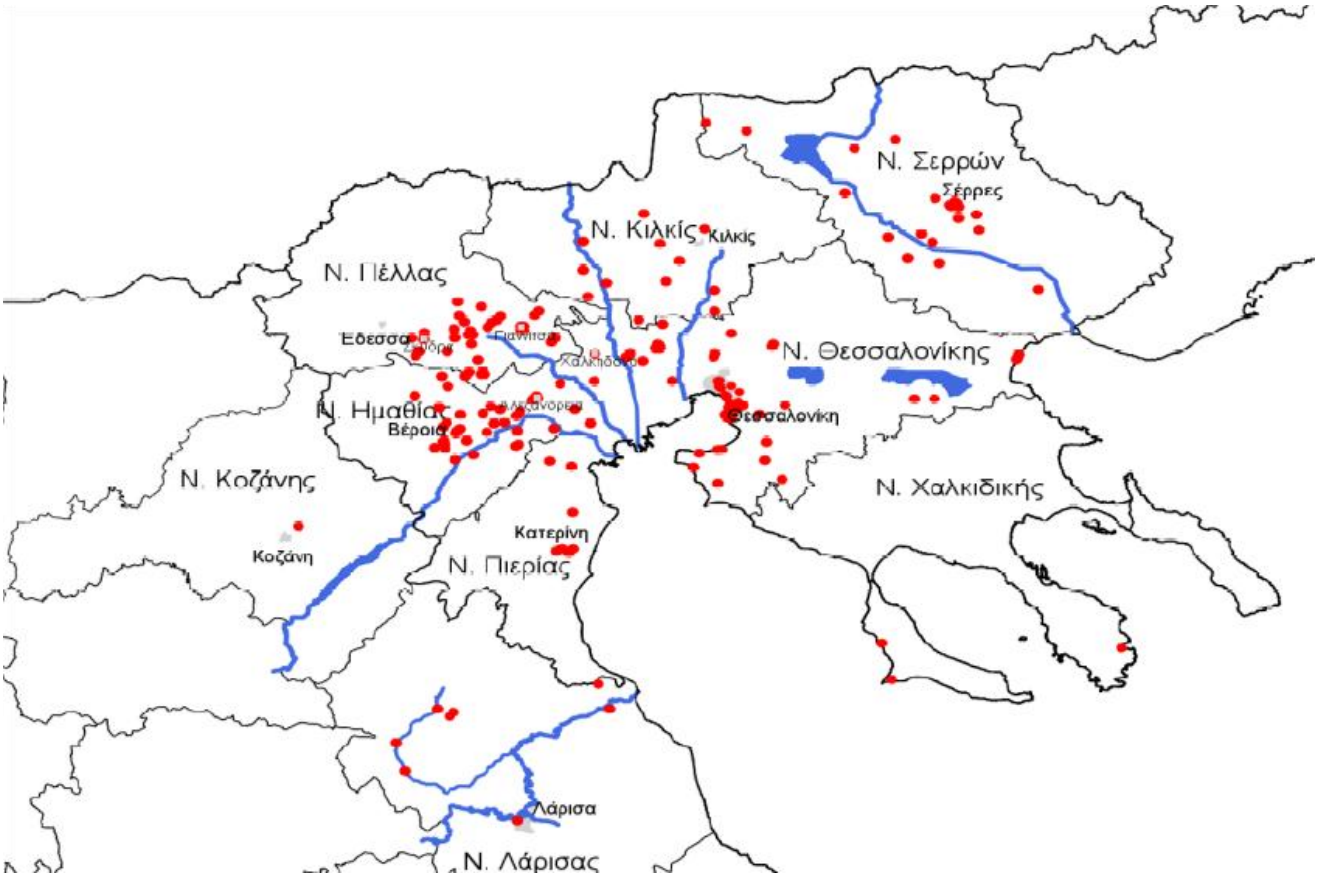


Figure 7-2. Human neuro-invasive WNV confirmed cases in Greece as of 10/29/2010.

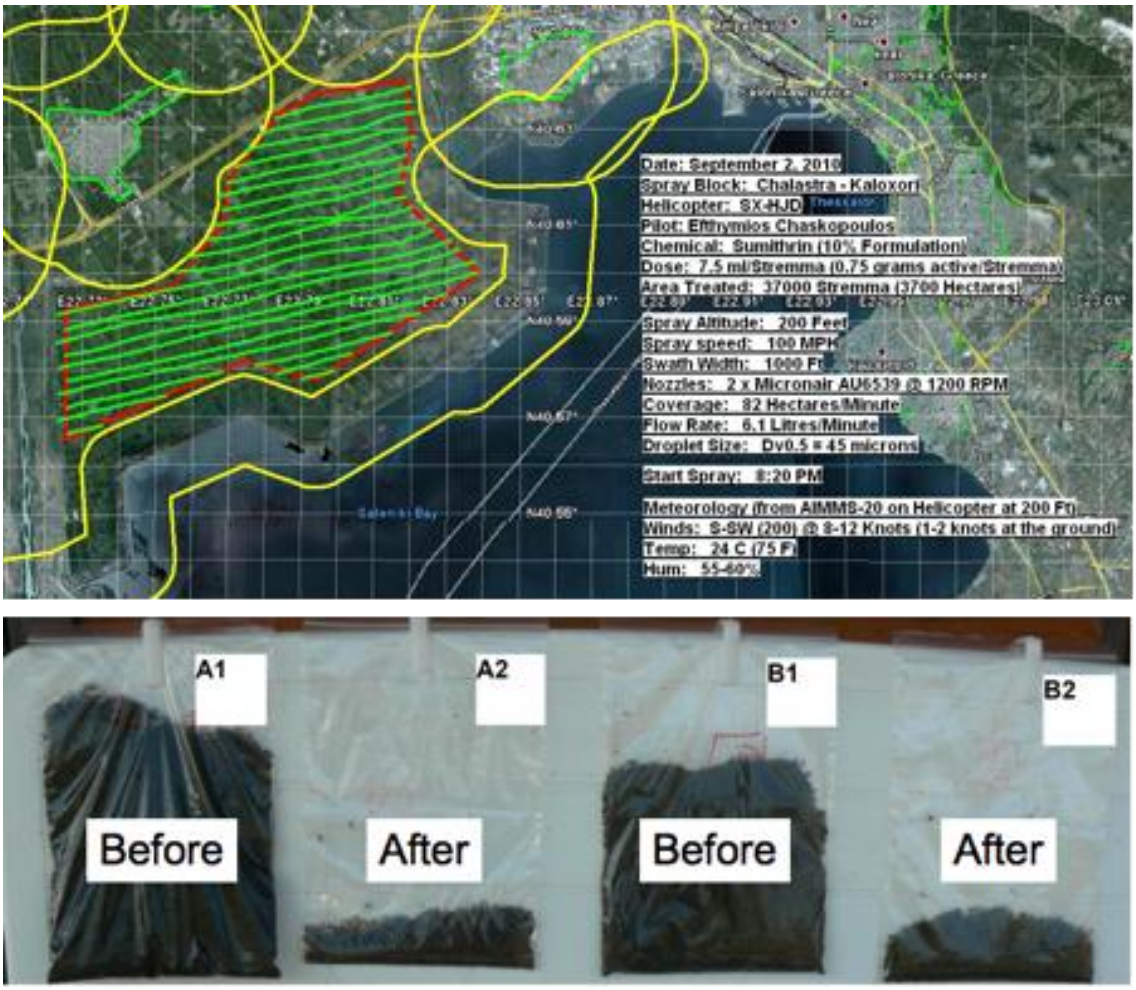


Figure 7-3. Aerial Map Mission 9/2/2010. A) Map with spraying lines. B) Mosquitoes caught in CDC light traps (trapping location A and B).

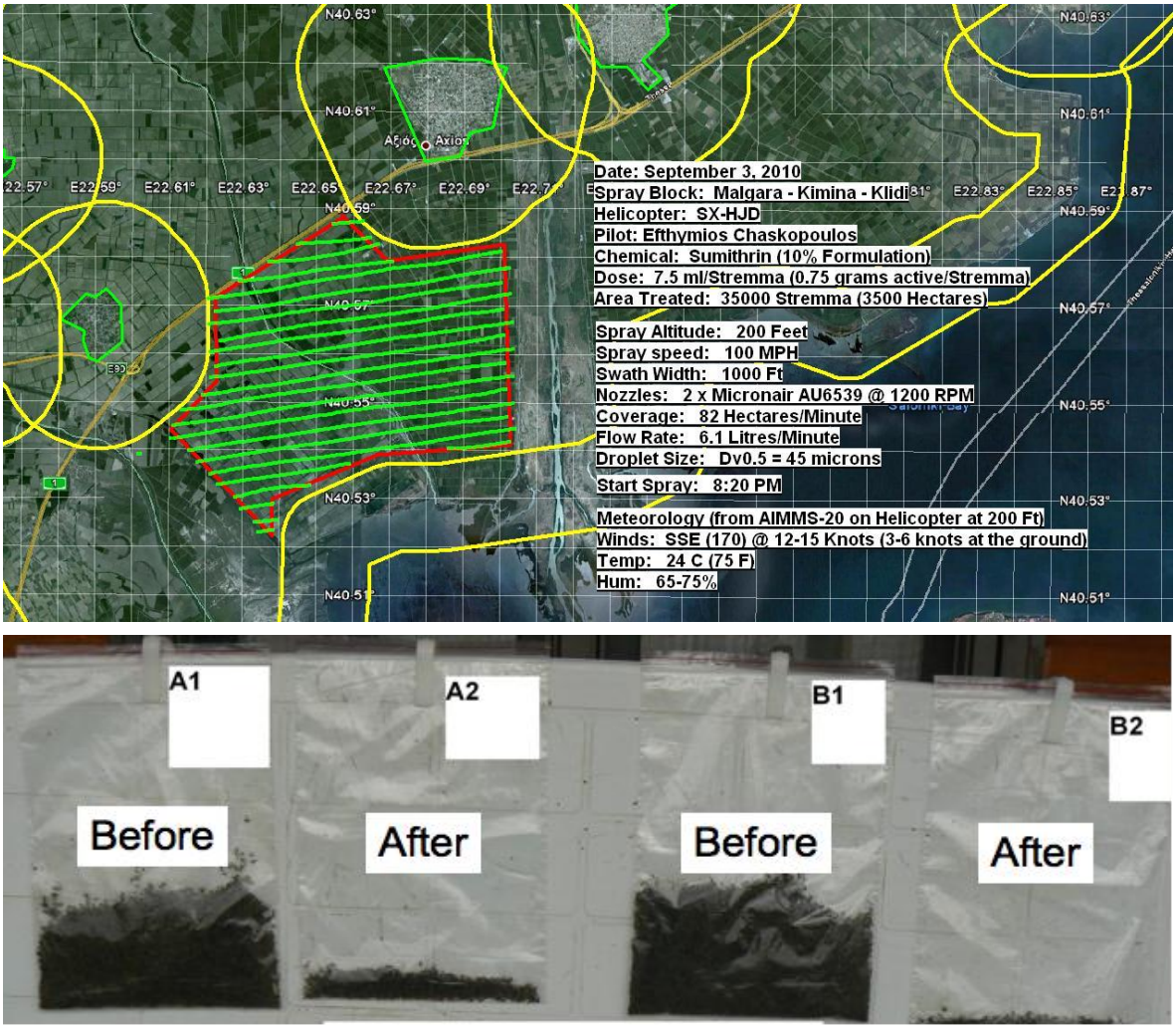
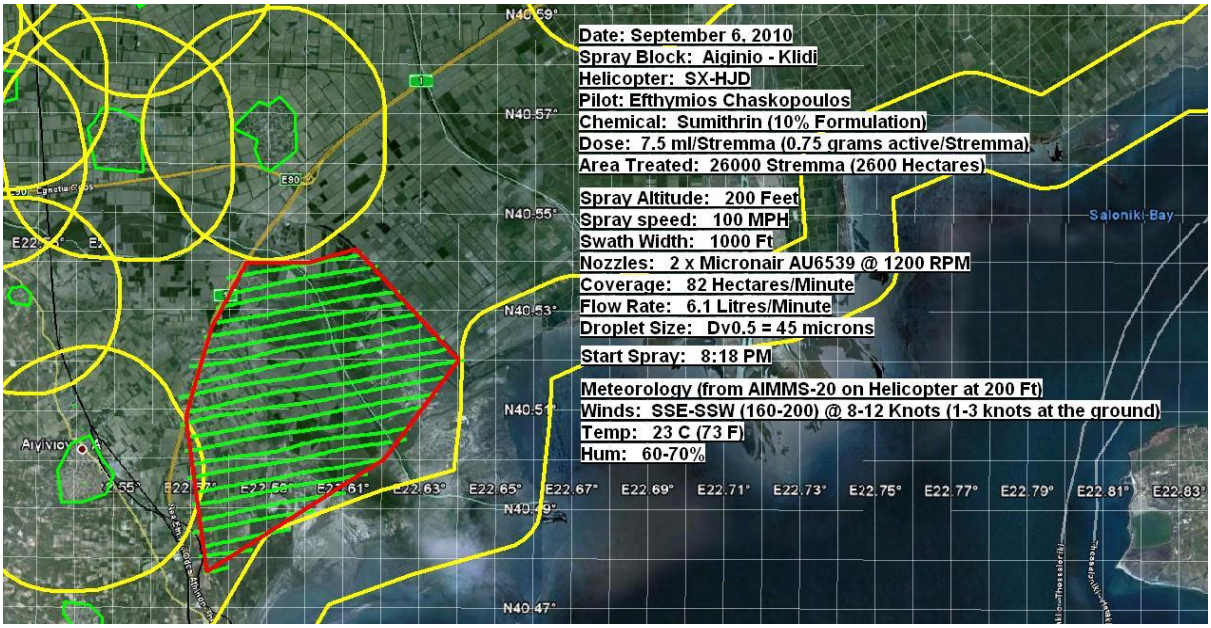
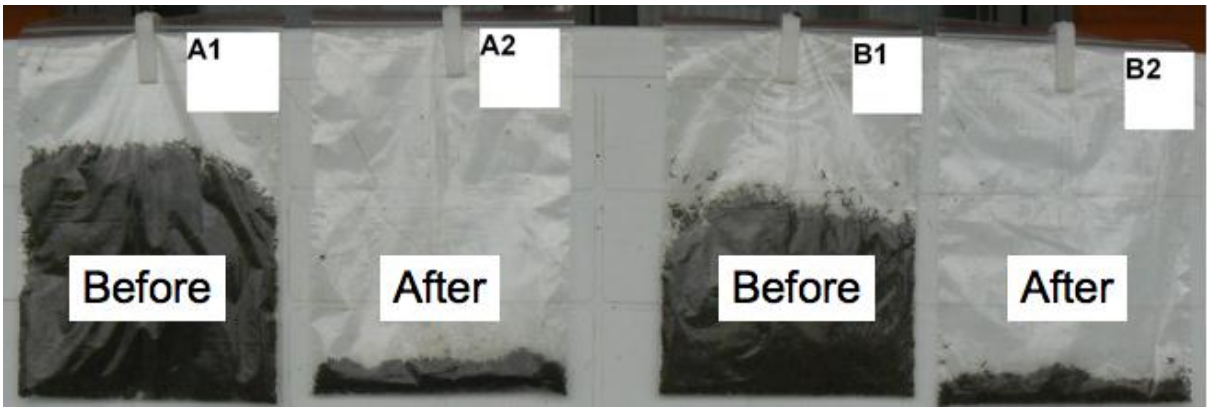


Figure 7-4. Aerial Map Mission 9/3/2010. A) Map with spraying lines. B) Mosquitoes caught in CDC light traps (trapping location A and B).

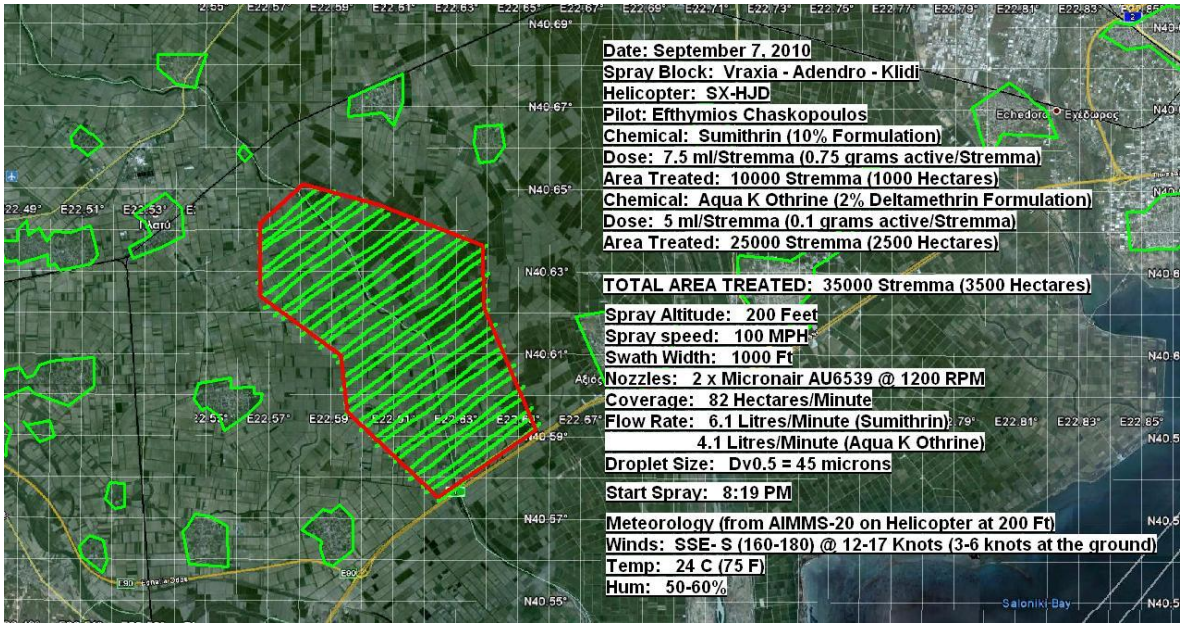


A

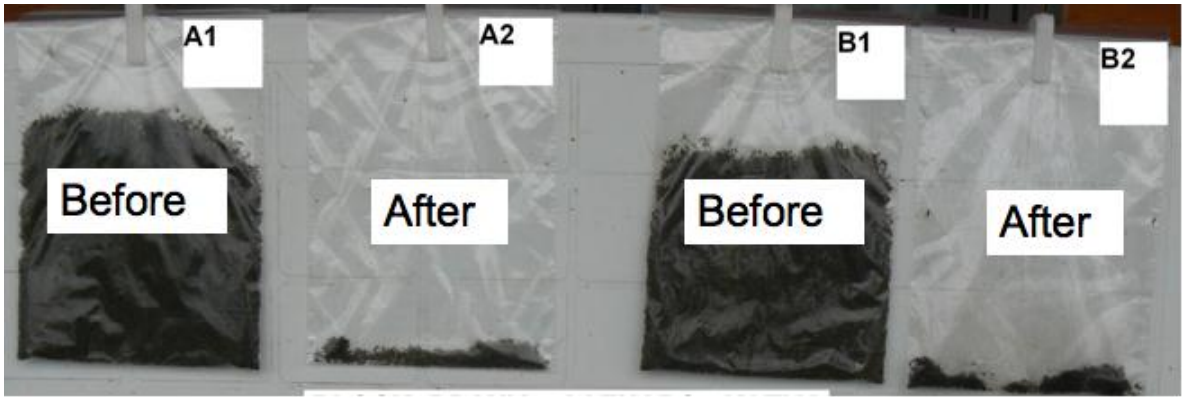


B

Figure 7-5. Aerial Map Mission 9/6/2010. A) Map with spraying lines. B) Mosquitoes caught in CDC light traps (trapping location A and B).

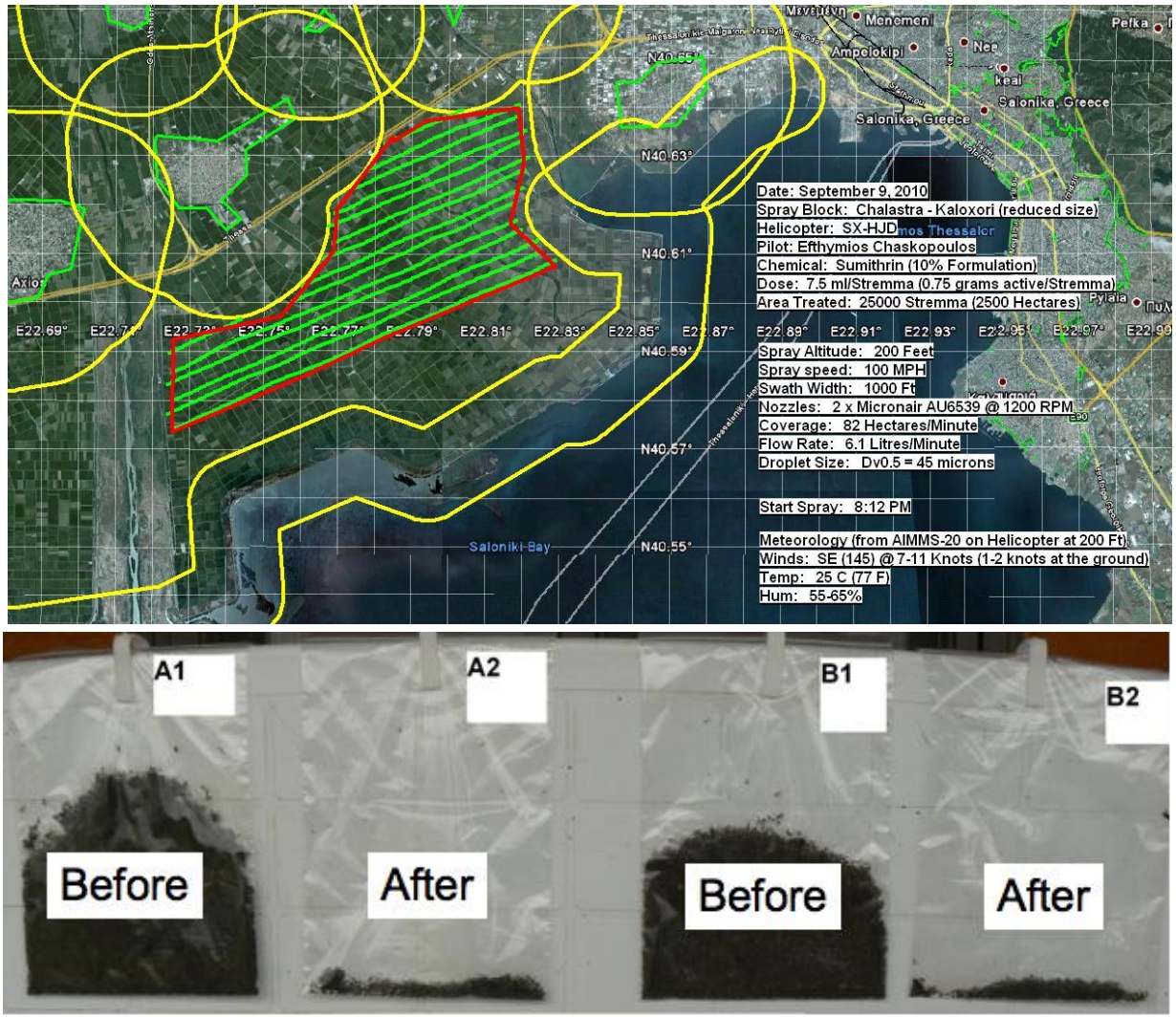


A



B

Figure 7-6. Aerial Map Mission 9/7/2010. A) Map with spraying lines. B) Mosquitoes caught in CDC light traps (trapping location A and B).



A

B

Figure 7-7. Aerial Map Mission 9/9/2010. A) Map with spraying lines. B) Mosquitoes caught in CDC light traps (trapping location A and B).

CHAPTER 8
CONCLUSIONS

This is the first report of efficacy of 2 unsynergized, water-based pyrethroids, Aqua-K-Othrine (2% deltamethrin) and Pesguard S102 (10% d-phenothrin), applied aerially for control of adult mosquitoes. Two application rates were tested per formulation that corresponded to 0.75 and 1.00 g AI/ha of deltamethrin and 7.50 and 10.00 g AI/ha of d-phenothrin. The mosquitoes used for the trials were the main nuisance species found in the rice field areas of Thessaloniki, which were primarily *Aedes caspius* Pallas, *Culex modestus* Ficalbi and *Anopheles sacharovi* Favre. Both products provided similar level of control, however, the amount of AI/ha of d-phenothrin was 10-fold higher than the one for deltamethrin. Specifically, the mean mortality observed in caged mosquitoes was ~69.2% and ~64.8% for deltamethrin and d-phenothrin, respectively. Wild mosquito populations decreased up to ~90 % for both products tested. The control mean caged-mosquito mortalities remained low (<20%) during all trials.

Our study provided direct evidence that Teflon®-coated slides are not the proper sampling technique for FFAST® anti-evaporant droplets and MgO slides should be preferred over Teflon®. High droplet densities (400-4,000 drops/cm^2) as assessed by the MgO spinning slides, were observed throughout the treatment areas. The application technologies along with the spray dispersal modeling enabled the majority of the treatment areas to receive homogeneous coverage and high droplet densities that resulted in uniform caged-mosquito mortalities

In our study of non-target organisms, very low mortalities post-treatment were observed for all non-target insects, *Aedes* sp. larvae, *Ch. carnae* larvae, *H. geminus* and *Cr. montrouzieri* adult beetles exposed to both products tested. These results suggest that very low ground deposition of insecticides occurred during our trials. Mosquito larvae in particular are known to

be very susceptible to pyrethroid insecticides, with LC_{50}s for *Aedes* species as low as 0.07 and 0.56 µg AI/L of water for deltamethrin and d-phenothrin, respectively (U.S. EPA 2009).

Very low, not significant bee mortalities were observed in our experimental beehives. Honeybees are highly susceptible to both products tested, with LD_{50s} 0.05 (24 h mortality) and 0.005 µg/bee (48 h mortality) for deltamethrin and d-phenothrin, respectively (NPIC 2010). Studies have shown that the timing of an insecticide application (diurnal versus nocturnal) has significant effects on bee mortality and subsequent hive performance (Anderson and Atkins 1968, Byrne and Waller 1990). It was shown that pesticides caused higher losses to bees when applied during day (Byrne and Waller 1990). Because our spraying trials occurred during late evening hours, when the vast majority of the bees are not actively foraging and are sheltered inside their beehives (Seeley 1995), the bees were minimally exposed to the insecticide. This is probably one of the main reasons why no significant adult bee mortalities were observed the morning following each treatment.

Insecticide residues on water, or any nectar and pollen source for bees could contaminate the hive and stress or kill the bees. For example, sublethal doses of parathion were shown to alter bee foraging activity by slowing flight speed and affecting the time sense of foraging bees (Desneux et al. 2007). These effects would significantly reduce beehive productivity. In our study, the beehives in the treated areas performed as well as those in the untreated control area and increased in adult bee population, brood, and weight. This indicates that insecticide deposits from our treatments were very low and did not have any biologically significant effect on the experimental bees foraging within the treatment areas.

Typically bees do not prefer to collect pollen from anemophilous plants, such as rice pollen, probably because of its unpalatability and unsuitability for normal hive development

(Pernal and Currie 2002). Approximately, 90% of our treatment areas consisted of rice cultivation. Interestingly, the experimental bees collected mainly rice pollen and were able to maintain healthy and productive hives.

Overall, the aerial adulticiding treatments conducted over the rice field area of west Thessaloniki provided high mosquito mortalities without causing any significant mortality to the non-target insects exposed to the treatments. The application technologies along with the spray dispersal modeling resulted for the majority of the trials in a homogeneous coverage of the treatment area with high droplet densities that resulted in uniform caged-mosquito mortalities. The results of this study provide evidence that aerial application of water-based, unsynergized pyrethroids, when applied properly, can result in targeted treatments with no significant non-target mortalities and high mosquito control levels, even in a heavily infested mosquito environment such as the rice fields.

With the increasing prevalence of mosquito borne pathogens in the European community, such as the very recent West Nile virus epidemic in North Greece, with approximately 261 human cases and 34 deaths, pyrethroids, such as d-phenothrin and deltamethrin, will be used more widely to manage/control adult mosquitoes. Associated with the increasing use of pyrethroids are public concerns about the environmental effects of their usage. It is therefore crucial to utilize proper insecticide application techniques with knowledge of the mosquito biology to result in targeted, efficacious insecticide treatments while protecting the environment and the life within it.

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BIOGRAPHICAL SKETCH

Alexandra Chaskopoulou was born in Thessaloniki, Greece, to Efthimios and Kalliopi Chaskopoulos. She has one sister and one brother. She and her family have spent most of their lives in Greece. Upon completion of her high school education in Greece, she decided to come to the United States in order to pursue her college education as an entomologist. She arrived at the United States in 2003, and within a year she earned her minor in biology from St. Andrews University of Michigan. In 2004 she moved in Gainesville, Florida where she earned her Bachelor of Science degree in entomology and nematology from the University of Florida and graduated in 2005. She remained at the University of Florida since 2010, during which time she earned a Master of Science and a Doctor of Philosophy degrees in entomology and nematology. In 2010 she accepted a post doc research position with University of Florida and USDA European Biological Control Laboratories. Her work will involve research in the field of medical entomology and vector control.