ABSTRACT

DONOHUE, KEVIN VINCENT. Analysis of the Effects of a Dielectric Barrier Discharge on Arthropod Pests. (Under the direction of R. Michael Roe).

Atmospheric pressure plasma discharge (APPD) has been intensely studied in the past fifteen years as a method of sterilization. The effects of APPD on insects were previously unknown. The purpose of this study was to examine whether plasma could be useful for insect and mite control, determine the mode of action on arthropods, and optimize the discharge in order to achieve the shortest APPD treatment times. A positive correlation was found between exposure time to APPD and insect mortality with the level of mortality also increasing with time after treatment for western flower thrips, Frankliniella occidentalis (Pergande), tobacco thrips, Frankliniella fusca (Hinds), Asian tiger mosquito, Aedes albopictus (Skuse), two-spotted spider mite, Tetranychus urticae Koch, and German cockroach, Blattella germanica (L.). Generally, mortality was negatively correlated with the weight of the insect. Cockroaches exposed to APPD for 60, 90, 120 and 180s lost on average 7.5 ± 0.8, 8.1 ± 0.6, 8.7 ± 0.4, and 10.1 ± 1.1 (±1 SEM) mg of water weight, respectively, which was an increase over that of the controls. The metabolic rate of cockroaches exposed to plasma for 180 s increased from 0.80 ± 0.03 to 1.07 ± 0.04 ml of oxygen consumed mg-cockroach -1 h-1 at STP. The levels of cuticular hydrocarbons identified by EI GC-MS were not significantly affected by plasma exposure in the green peach aphid, Myzus persicae (Sulzer), German cockroach, and citrus mealybug Planococcus citri (Risso) except for a
reduction in n-tritriacontane only in the latter. However, changes in the behavior of cockroaches after plasma exposure including the loss of photo-, vibro-, and thigmotrophic responses, inability to right themselves, and hyperexcitatory symptoms, suggest that the site-of-action of APPD in insects is the nervous and/or neuromuscular system. The LT$_{50}$ of German cockroaches, western flower thrips, and citrus mealybugs 24 h after exposure to a 37°C helium discharge was 333.77, 28.72, and 999.95 s, respectively. The LT$_{50}$’s 24 h after exposure to a 37°C, 0.5% oxygen/99.5% helium discharge was 232.67, 19.99, <13.83 s, respectively. The LT$_{50}$ of German cockroaches, western flower thrips, and citrus mealybugs 24 h after exposure to the 50 ± 2°C, helium discharge was 117.80, 13.83, and 26.62 s, respectively. No mortality resulted in German cockroaches or citrus mealybugs after exposure to a helium atmosphere followed by 50°C air, however the LT$_{50}$ of western flower thrips was 29.04 s, based on the mortality recorded 24 h after treatment. The addition of oxygen or heat to the discharge resulted in higher mortality in cockroaches and mealybugs but not thrips.
ANALYSIS OF THE EFFECTS OF A DIELECTRIC BARRIER DISCHARGE ON ARTHROPOD PESTS

by
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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

ENTOMOLOGY

Raleigh

2005

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Dedication

I dedicate this work to Patrick and Frances Donohue, my parents who continue to provide unconditional support; to Callie Barber for giving me the drive to do anything; and to John Winsch who always thought being a scientist and a musician was a good idea.
Biography

Kevin Vincent Donohue was born in Albany, New York in 1974. At the age of 10 he decided to become an entomologist. He received a Bachelors of Science in Resource Management in 1996 from the State University of New York at Syracuse, College of Environmental Science and Forestry.

His interests during college shifted to music and he became a professional guitarist, touring the eastern US, releasing several recordings, and teaching lessons. During this time he also pursued work in the culinary arts, training with Certified Master Chef Dale L. Miller in New York. In the Spring of 2001 his girlfriend and he traveled throughout Western and Eastern Europe for three months. Upon returning to Chapel Hill, NC he decided to return to science and eventually joined the lab of Dr. R. Michael Roe in the Department of Entomology in 2003.

When not in the lab, his interests are traveling, playing guitar or mandolin, insect collecting, and cooking for his family and friends.
Acknowledgements

I would like to acknowledge the help and support of several individuals that played an integral role in this project, especially my committee members, Dr. R. Michael Roe, Dr. Mohamed A. Bourham, and Dr. Charles S. Apperson. Dr. Roe has been a source of support throughout my graduate career and has provided me with the ability to successfully complete my research. Not only learning how to properly conduct research, but how to apply it in ways that others may benefit from scientific discoveries is a lesson that I will continue to benefit from throughout my career. Dr. Bourham provided financial support for me from the beginning and gave me the chance to be a part of a fascinating interdisciplinary research area. Dr. Brian L. Bures, a collaborating nuclear engineering Ph.D. student, drove the engineering aspects of the project, and helped me to understand how to operate the device and understand the physics behind it. Students and staff of the Dearstyne Entomology Lab provided thoughtful review of the research.

This work was supported by a grant from USDA-APHIS.
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Review of Biologically Oriented Applications of High and Low Pressure Plasma

Description of Plasma

When molecules or atoms loose an electron, the particles become positively charged, or ionized. When the ions, electrons, and neutral particles exhibit collective behavior, the substance can be categorized as plasma, the fourth state of matter (Chen 1984). Greater than 99% of the universe is believed to be in the form of plasma. Common examples of plasmas are stars, fire, lightning, fluorescent lights, and neon signs. In a fluorescent light bulb, a gas at less than ambient pressure is supplied with voltage, which causes the collision of particles to occur and consequently ionization. As ions travel from the anode and strike the cathode, secondary electron emission occurs and light is produced. The cycle of ionization and secondary electron emission will continue as long as voltage is supplied. Without electricity the gas will return to its normal state and the plasma will be extinguished due to the lack of secondary emission of electrons. However, not all ionized gases can be considered plasma. Certain criteria must be met.

The requirements are that the plasma be dense enough so that the Debye length ($\lambda_D$) is much less than the dimensions of the device. Debye length, a measure of the distance over which an individual charged particle can exert an effect, is calculated with the formula

$$\lambda_D = (\varepsilon_0 K T_e / n e)^{1/2},$$

where $\varepsilon_0$ is the permittivity of free space ($8.85 \times 10^{-12}$ F/m), $K$ is Boltzmann’s constant ($1.38 \times 10^{-23}$ J/K), $T_e$ is the electron temperature in Kelvin (K), $n$ is the electron density ($m^{-3}$), and $e$ is the unit charge in coulombs ($1.602 \times 10^{-19}$ C). Secondly, there must be a sufficient number of particles ($N_D$) in a Debye sphere for Debye shielding to
be possible. A Debye sphere is the area around a charged particle with a radius of one Debye length. The number of particles can be calculated as $N_D = n(4\pi/3)\lambda_D^3$. Lastly, the product of the frequency of typical plasma oscillations ($\omega_p$) and the mean time between collisions with neutral atoms ($\tau$) must be greater than one (Chen 1984).

The use of the term “plasma” is commonly applied even when the third condition ($\omega_p \tau > 1$) is not met. A more accurate name would be a non-equilibrium discharge, with “non-equilibrium” referring to the fact that the gas temperature can be near ambient temperature, while $T_e$ is several thousands of Kelvin. Non-thermal plasmas are those in which the electron temperatures range from 0.1-5 eV (Napartovich 2001).

**Development of Plasma for Commercial Applications**

Plasma physics has been a widely studied field for over 100 years. The term “plasma” was first used in 1927 by Irving Langmuir to describe an ionized gas in the presence of an electric field. One of the first experiments leading to the understanding of plasma physics can be traced back as far as the year 1600 from the paper by William Gilbert entitled *De magnete, magneticisque corporibus, et de magno magnete tellure*. He found that a charged conductor loses its charge when brought near a flame, and an electroscope becomes charged when connected to a flame (von Engel 1955). Faraday (1844) discovered low pressure glow discharges and noted that the discharge contained luminous regions and dark regions, the latter eventually being given the name “Faraday Dark Space”. He also discovered that current could pass through a gas at low pressure without giving off any light at all, which he called a dark discharge. Major advancements in plasma physics and
characterizing glow discharges over the next 100 years were made by to Hittorf (1869), Crookes (1879a, b), Hertz (1887), Hallwachs (1888), Stoney (1891), Perrin (1896), and Aston (1933). During the first half of the 20th century, research areas of plasma physics dealt mainly with studies of the ionosphere, astrophysics, and thermonuclear fusion. Within the last 35 years applications and types of plasma have broadened widely and become commonly used in several commercial settings.


Non-equilibrium plasma generated at atmospheric pressure has broadened the field of applications to thermally sensitive objects, high throughput industries, and to objects that require surface modification without changing bulk properties. There are several atmospheric pressure plasma sources with various geometries and operating frequencies including the dielectric barrier discharge (DBD)(Pashaie et al. 1999), corona discharge (Abdel-Salam et al. 2003), glow discharge (Kanazawa et al. 1988), plasma jet (Spores and Pfender 1989), hollow-cathode discharge (Sturges and Oskam 1964), radio frequency and
microwave discharges (Napartovich 2001), and resistive barrier discharges (Laroussi et al. 2002a).

Several properties of plasmas are useful for characterization. The temperature of the plasma in terms of electron volts, direct or alternating current, and the frequency, if any, applied to the voltage. The frequency applied can range from a few kilohertz (kHz) to 10 gigahertz (GHz). The electron temperature can be 1 to 4 eV for a capacitive discharge, ~10 eV for inductive discharges, and 10-20 eV for wave-heated discharges.

**Use of Plasma for Sterilization**

The area of research involving plasma discharges for air filtration and sterilization has gained increased attention since the 1990’s. The ability of plasmas to deactivate spores such as *Bacillus anthracis* could be an extremely useful tool for anti-bioterrorist attacks. Birmingham (2004), has demonstrated a novel corona plasma device called a “plasma blanket” which can reduce the number of anthrax spores of the Ames strain by 99.9999% with a single 60 s exposure. The advantages of this device are high sterilization efficiency with short treatment times, flexibility and can therefore treat objects of various geometries, produces no waste heat or waste materials, and can be coupled with a detection system using matrix-assisted laser desorption ionization mass spectroscopy (MALDI-MS) and vapor sampling of ions by mass spectroscopy.

Non-thermal atmospheric pressure plasma discharges (APPD) operating with electron temperatures ranging 0.1 – 2.0 eV, have been shown to effectively kill bacteria (Efremov et al. 2000; Laroussi 2002; Laroussi et al. 1999; Laroussi et al. 2000; Laroussi et al.
The capability of using plasma for killing bacteria has fueled research on improving the current technology of surface sterilization, especially for those devices used in the medical field. Laroussi (1996) showed that a uniform glow discharge plasma could be a viable sterilization technique and an alternative to radiation, heat, and gases such as ethylene oxide. The use of plasma for sterilization has the ability to treat substances that would not otherwise be able to withstand high temperatures. Plasma treatments have the advantage of lower gas temperature, shorter treatment times, and use without toxic chemicals (Birmingham and Hammerstrom 2000). Devices have been commercialized for the sterilization of equipment, such as the “Sterrad” sterilization system, but the “plasma phase” of treatment is only a step in addition to the volatilization of hydrogen peroxide. The current commercialized devices use plasma primarily for the removal of harmful residues (Lerouge et al. 2001). The use of oxygen plasma has been shown to be just as effective for sterilization and has the advantage of functioning without hydrogen peroxide vapor (Moreira et al. 2004; Montie et al. 2000). Devices relying solely on oxygen plasma are still in development and are not yet available commercially.

**Mode of Action of Plasma on Bacteria**

The mode of action of APPDs on gram-negative bacteria such as *Eschericia coli* appears to be largely due to damage to the cell membrane that results in cell lysis (Mendis et al. 2000). After APPD exposure, cell lysis is believed to occur as a function of the irregularities of the outer membrane of the cell, with a smoother surface being less likely to undergo damage. The model from Mendis et al. (2000) based on the tensile strength of the
cell membrane calculates that an irregularity would increase the likelihood that the discharge could rupture the cell’s membrane. Laroussi et al. (2003) showed that with their equation based on a cell's tensile strength, membrane thickness, and surface potential, an electric field increases at the curvature of an irregularity on the cell membrane and increases the likelihood of rupturing the cell membrane. This idea is supported by the finding that cell lysis did not occur when *Bacillus subtilis*, which has a smooth outer membrane, was exposed to the discharge for the same duration as *E. coli* cells. Further support is provided by the fact that *B. subtilis* has been found to withstand ~20 atmospheres of pressure, while *E. coli* can support pressures of only 3-5 atmospheres (Boal 2002).

While gross structural damage of *B. subtilis* did not occur, a reduction in cell viability was observed suggesting the penetration of reactive species through the cell membrane may be possible (Laroussi et al. 2003). Alterations to the heterotrophic pathways of the plasma treated bacteria, measured by the increased utilization of L-fucose, D-sorbitol, D-galacturonic acid, and decreased utilization of methyl pyruvate, dextrin, and D,L-lactic acid, suggest that cell viability was compromised even though no visible damage to *B. subtilus* was observed (Laroussi et al. 2002b). They believed that in addition to morphological damage to a cell, the penetrative reactive species are an additional component of the discharge that may lead to cell death. Laroussi (1996) and Herrmann et al. (1999) had previously shown that UV radiation produced by the discharge had an insignificant effect on the plasma treated bacteria and was therefore dismissed as a key component of the mode of action.
Plasma Treatment of Multi-celled Organisms

Only limited research has been conducted in the treatment of multi-celled organisms with plasma. Dubinov et al. (2000) treated *Avena sativa* (cultivated oat) and *Hordeum vulgare* (barley) seeds with a glow discharge and found that the germination rates of the seeds increased significantly. They reported that a continuous discharge treatment was more effective in terms of increasing the germination rate over that of a pulsed plasma discharge. After germination, no change was found in the growth rate of the plants.

Studies by Mishenko et al. (2000) found that treating granary weevils, *Sitophilus granarius* L., using a combination of radiation and plasma discharge formed in a 50 millibar vacuum with air as the working gas resulted in 100% mortality. Morar et al. (1997), used a corona discharge at atmospheric pressure to treat *Phorodon humuli* (Schrank), and were able to achieve 100% mortality 24 h after a 30 min treatment, and 48 h after a 15 min treatment.

Keever et al. (2001) used a helium based atmospheric pressure plasma discharge (APPD) to treat cigarette beetles, *Lasioderma serricorne* (F.). They found that a 70 s exposure to the APPD resulted in 92% mortality of adults 2 d after treatment. The gas temperature of the plasma was >50°C and it was unknown whether there was a synergistic effect between heat coupled with plasma.

Bures et al. (2005a, 2005b) are the first publications to report mortality of insects exposed to a low temperature (0.1 – 2.0 eV) APPD formed in a dielectric barrier discharge with an actively cooled working gas (37 ± 2°C). Cooling the plasma allowed for a detailed study of the effects of plasma on insects. DBDs are unique in that a dielectric material covers one or both of the electrodes and allows for the homogenous treatment of a commodity and
prevents the transition to an arc. Tests with green peach aphids, human body lice, and citrus mealybugs revealed a significant increase in mortality with time after treatment in the discharge.

**Comparison of Plasma to Current Quarantine Technology for Insect Control**

Methyl bromide has served a critical role in the control of insect pests in post-harvest fumigation (Schneider et al. 2003). The Montreal Protocol and the US Clean Air Act began the process to phase out the use of methyl bromide in 2001 as a fumigant in an effort to reduce the use of ozone depleting substances. As of January 1, 2005 the use of methyl bromide has been completely banned with the exception of certain critical use exemptions for quarantine and pre-shipment purposes (EPA, 2004). In 1997, the Parties of the Montreal Protocol agreed that a critical use exemption must meet the following criteria: the lack of methyl bromide for the intended use would result in significant market disruption; no technically feasible alternatives are yet acceptable; all efforts must have been made to minimize the required amount and its resulting emission; and finally, a demonstration that alternatives are being sought to replace methyl bromide through research grants and development opportunities.

Methyl bromide alternatives have been widely studied, however long treatment times, effects on the commodity, cost, public acceptance of the technology, and ease of implementation has hindered their commercial introduction. Some of the alternatives proposed are high temperature, low oxygen pulse (Chervin et al. 1997), irradiation (Derr 1996; Giddings 1996; Loaharanu 1996; Hallman 1999) high temperature short time intervals
(Hallman 2000; Tang et al. 2000), 915 MHz microwaves (Ikediala et al. 1999), radio frequencies (Wang et al. 2001), low oxygen high carbon dioxide (Mitcham 1997; Epenhuijsen et al 2002; Yahia and Ortega-Zaleta 2000), and hot water treatment and insecticidal coatings (Smith and Lay-Yee 2000; Gould and McGuire 2000). Irradiation has been one of the more successful technologies and has already been approved for use by Texas and California for controlling the Caribbean fruit fly *Anastrepha suspensa* (Loew) on Florida guavas. Disadvantages of irradiation are that acute mortality is not always achieved, delicate commodities can be damaged by dose’s required for full insect control, and public acceptance has initially slowed its implementation (Hallman 1999).

Several characteristics of plasma could make this technology a favorable methyl bromide alternative. It has an advantage over traditional fumigants since residual chemical residues can be eliminated; a quality that makes this technology well suited for the sterilization of medical equipment (Montie et al. 2000). The advantage of leaving negligible residual effects after treatment is one of the factors that may make this technology publicly acceptable. Relatively short treatment times may be needed to reach 100% mortality using plasma. For example, hot water (49°C) treatments of citrus mealybugs, *Planococcus citri* (Risso) from the study by Gould and McGuire (2000) yielded 100% mortality of citrus mealybugs 2 d after a 15 min treatment. Our studies with citrus mealybugs found that a 60 s exposure to plasma (0.5% oxygen/99.49% helium working gas) yielded 100% mortality 1 h after treatment.

A disadvantage of using plasma for insect control is the damage it may cause if the insects being treated are on leafy plant material or vegetables. Depending on the insect being
treated, treatment times longer than 1 min can damage plant leaves such as tobacco (Bures et al. 2005a). We also found that the tobacco leaf detracted from the efficacy of the plasma for inducing mortality. Green peach aphid mortality was 40 ± 8.64% when the aphids were on the tobacco leaf compared to 88 ± 6.11% without the leaf. One hypothesis to explain this finding is the edges of the leaf may enhance the electric field causing the discharge to filament and therefore become less homogenous for treating all of the insects (Bures et al. 2005a). Further studies are needed to understand how treating insects on various organic materials will alter the efficacy of plasma for insect control and what damage or modification to the substrate will occur.

Another disadvantage of using plasma is cost. The geometry of the commodity being treated will dictate the cost of building a plasma device. Implementing a large-scale high throughput plasma device capable of operating in a quarantine facility may initially cost $100,000. While the initial cost may be high, if the treatment time of commodities could be shortened from weeks or days to under an hour, perishable items would pass through quarantine more quickly leading to increased savings or even previously inaccessible markets.

Understanding the effect that plasma has upon plants is critical to the broad implementation of this technology for quarantine purposes. Certain situations such as the treatment of packaging material and stored products (seeds, grains, etc.) may be well suited for plasma treatment to eliminate arthropod pests since they are less likely to undergo damage from short time treatments (< 10 min) than delicate plant material, and since certain plasmas have been shown to actually improve seed germination rates (Dubinov et al. 2000).
Devices designed for high throughput treatment of commodities that use air as the working gas or an inert gas coupled to a recycling system, could offer a rapid non-chemical approach to current technology.
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Helfritch, D. J., J. R. Roth, T. C. Montie, K. Kelly-Wintenberg, P. P. –Y. Tsai, and D. M. Sherman. 1999. Air filter enhancement and sterilization using a dc electric field and


Mode of Action of a Novel Non-Chemical Method of Insect Control: Atmospheric Pressure Plasma Discharge

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ABSTRACT  Atmospheric pressure plasma discharge (APPD) has been applied to a number of industrial applications including sterilization medical equipment of bacteria. APPD can also be used for insect control. A positive correlation was found between exposure time to APPD and insect mortality with the level of mortality also increasing with time after treatment for western flower thrips, *Frankliniella occidentalis* (Pergande), tobacco thrips, *Frankliniella fusca* (Hinds), Asian tiger mosquito, *Aedes albopictus* (Skuse), two-spotted spider mite, *Tetranychus urticae* Koch, and German cockroach, *Blattella germanica* (L.). Mortality was negatively correlated with the weight of the insect except for the citrus mealybug, *Planococcus citri* (Risso). Cockroaches exposed to APPD for 60, 90, 120 and 180s lost on average 7.5 ± 0.8, 8.1 ± 0.6, 8.7 ± 0.4, and 10.1 ± 1.1 (±1 SEM) mg of water weight, respectively, which was an increase over that of the controls. The metabolic rate of cockroaches exposed to plasma for 180 s increased from 0.80 ± 0.03 to 1.07 ± 0.04 ml of oxygen consumed mg-cockroach⁻¹ h⁻¹ at STP. The level of cuticular hydrocarbons identified by EI GC-MS were not significantly affected by plasma exposure in the green peach aphid, *Myzus persicae* (Sulzer), German cockroach, and citrus mealybug except for a reduction in n-tritriacontane only in the latter. However, changes in the behavior of cockroaches after plasma exposure including the loss of photo-, vibro-, and thigmotrophic responses, inability to right themselves, and hyperexcitatory symptoms, suggests that the site-of-action of APPD in insects is the nervous and/or neuromuscular system.

KEY WORDS  Atmospheric Pressure Plasma, Insect Control, Dielectric Barrier Discharge, Quarantine
**Introduction**

With the general phase out of methyl bromide, which was completed in Jan 2005 (EPA 2004), the need for safe, environmentally friendly and effective alternatives to methyl bromide are greatly needed. Several technologies have been proposed as alternatives, but shortcomings generally result from unreasonably long treatment times (2-3 d), high cost, temperature limits for application, changes to the aesthetics of the product, negative environmental effects and acceptance by the public. One alternative, which has been of interest to our laboratories, has been the use of atmospheric pressure plasma discharge (APPD) (Bures 2004; Bures et al. 2005).

Gases treated with high energy lose electrons and become driven by electromagnetic interactions instead of hydrodynamic forces, producing plasma. Two important parameters, temperature and density, are used to characterize the neutral species, electrons and ions found in the plasma. The temperature of the particles, usually expressed in eV (1eV = 11,600 K) ranges from room temperature to million of degrees Celsius, and the density from a few parts per billion to greater than solid density. A common commercial application of plasma is fluorescent light bulbs. In this application, the temperature of the neutral particles and ions is low (~ 500 K), but the electron temperature is high (~10,000 K). The electron density and ion density is only a small fraction (<<1%) of the neutral particle density. In general, plasma can be derived from the rapid compression of materials, extreme heat, electrical energy, and intense radiation (Eliezer and Eliezer 2001; Parks 2003).

Various types of plasma are useful in industrial applications such as fusion reactions (Alexeff 2004), chemical and physical modification of surfaces (Yokoyama et al. 1990),
efficient light production, treatment of hazardous waste (Eliasson and Kogelschatz 1991), radiation sources (alpha, neutron, X-ray) (Moselhy et al. 2002), and biological and chemical decontamination (Birmingham and Hammerstrom 2000; Helfritch et al. 1999; Kelly-Wintenberg et al. 2000; Laroussi 2002; Laroussi et al. 1999; Laroussi et al. 2000; Massines et al. 1992; Montie et al. 2000). Plasma generated at atmospheric pressure has broadened the field of applications to thermally sensitive objects, high throughput industries and to objects that require surface modification without changing bulk properties (Herbert and Bourdin 1998; Laroussi et al. 2003). There are several atmospheric pressure plasma sources with various geometries and operating frequencies including the dielectric barrier discharge (DBD)(Pashaie et al. 1999), corona discharge (Abdel-Salam et al. 2003), glow discharge (Kanazawa et al. 1988), plasma jet (Spores and Pfender 1989), hollow-cathode discharge (Sturges and Oskam 1964), radio frequency and microwave discharges (Napartovich 2001), and resistive barrier discharges (Laroussi et al. 2002a). Non-thermal (0.1 – 2.0 eV) atmospheric pressure plasma discharges (APPD) previously have been shown to effectively kill bacteria (Efremov et al. 2000; Laroussi 2002; Laroussi et al. 2000; Laroussi et al. 2002b). The mode of action of APPDs on gram-negative bacteria such as *Escherichia coli* Castellani and Chalmers, appears to be damage to the cell membrane resulting in cell lysis (Mendis et al. 2000).

The authors have been interested in the possible use of plasma for insect control in general and as an alternative to methy bromide in quarantine applications. Bures (2004) and Bures et al. (2005) found that a low temperature (0.1 – 2.0 eV) APPD produced mortality in green peach aphids, *Myzus persicae* (Sulzer), human body lice, *Pediculus humanus humanus*
L., and citrus mealybugs, *Planococcus citri* (Risso) on a variety of substrates including living plant material. Green peach aphid mortality was 87%, 24 h after a 120 s exposure, human body lice mortality was 95%, 24 h after a 60 s exposure while the citrus mealybug was naturally resistant with only 24% mortality 24 h after a 240 s exposure. In these studies, a detailed physical and chemical analysis of the plasma was conducted. Studies by Mishenko et al. (2000) also found that a combination of radiation and plasma was lethal to the granary weevil, *Sitophilus granarius* L., and Morar et al. (1997) found that corona discharges at atmospheric pressure were lethal to the hop aphid, *Phorodon humuli* (Schrank). The advantage of APPD is that plasma can be produced at ambient temperatures with reasonable electrical power requirements, the chemical reactive species produced by the plasma were not excessive, there was no ionizing radiation and the plasma was effective against insects but safe to the end-user and the environment. Although there is some information on the mode-of-action of APPD on bacteria, no mechanistic studies have been conducted on insects. The objective of the current paper is to determine the effect of APPD on mortality, cuticular hydrocarbons, water balance, metabolism and behavior to determine a possible mode-of-action. Western flower thrips, *Frankliniella occidentalis* (Pergande), tobacco thrips, *Frankliniella fusca* (Hinds), Asian tiger mosquitoes, *Aedes albopictus* (Skuse), two-spotted spider mites, *Tetranychus urticae* Koch, German cockroaches, *Blattella germanica* (L.), green peach aphids, and citrus mealybugs were used in the study.
Materials and Methods

Dielectric Barrier Discharge Device. Atmospheric pressure plasma discharge was generated between parallel electrodes (each 16 cm x 20 cm) in a ≥ 99.9% helium atmosphere using the device in Fig. 1. The electrodes are composed of copper covered with a thin layer (0.793 mm) of Garolite Grade 7 (McMaster-Carr, Atlanta, GA), a woven glass fabric laminate with a silicon resin bonded to the electrodes (Fig. 1A). Garolite is a superior insulator for DBD devices to produce a uniform discharge at ambient pressure and prevent high temperature arcing. Two adjustable pedestals support the electrodes. Although the pedestals allow the electrodes to be separated by variable distances (1.5-10.0 cm) to accommodate different sample sizes, the electrode gap was fixed at 5.0 cm for the studies reported. Heat generated by the electrodes was removed by circulating cooled distilled water through channels over the electrode surface. Distilled water was used to prevent the conduction of current from the electrode through the water to the cooling system. High voltage (approximately 2.7 kV) was supplied to the electrodes through a pair of high voltage transformers (Del Electronics Corporation, Valhalla, NY) and measured with two P6015A high voltage probes (Tektronix, Richardson, TX). The current (approximately 24.2 mA) to each electrode was measured through a 100 Ω resistor in series between the transformer and ground. For all treatments, the power ranged between 85-95 W.

The DBD device was housed in an acrylic chamber (48.26 cm height x 102.87 cm width x 58.42 cm depth, Fig. 1B), which was filled with helium. The electrode pedestal supports pass through the acrylic chamber on the top and bottom. The chamber has an internal volume of 259 liters. A rubber glove was attached to the front panel of the chamber.
to move samples in and out of the APPD. Helium (HE291, Machine and Welding Supply, Inc., Dunn, NC) was supplied to the chamber at 34 standard liters per minute (slpm) through a Type 1179A general-purpose mass flow controller and a Type 247D four channel power supply readout (MKS Instruments, Wilmington, MA). The effluent of the chamber was measured using a helium gas monitor (Model 334WP, Nova Analytical Systems, Niagara Falls, NY) to determine when the atmosphere was at least 99.9% helium (approximately 45 min). Insects were housed in the chamber during the He equilibration for the experimental treatments described later.

Once the atmosphere in the chamber reached 99.9% helium, voltage was applied from an HTA-250A power supply (Bogen, Ramsey, NJ) with an acoustic frequency of 4 kHz supplied from a 5 MHz function generator (BK Precision, Yorba Linda, CA) to form the APPD. The ambient gas temperature of the discharge was measured in situ with an electrically insulated K-type contact thermocouple (Omega Engineering, Stamford, CT). The thermocouple was insulated to prevent plasma filaments from striking the thermocouple and producing an inaccurate temperature measurement of the discharge.

Insects. Green peach aphids were obtained from a lab-reared strain maintained for >10 yr on red pepper, *Capsicum annuum* var. *annuum* L., at BASF Chemical Corporation, Research Triangle Park, NC. The remaining insects were laboratory reared in the Department of Entomology at NC State University, Raleigh, NC. Citrus mealybugs, were maintained on crooked neck squash, *Cucurbita moschata* Duchesne; the strain was originally established in 2002 from a greenhouse located on campus. Adult male German cockroaches were fed Purina Rat Chow #5012 (Purina Mills, St. Louis, MO) and originated from an
American Cyanamid insecticide-susceptible strain. The adult male and non-blood fed female Asian tiger mosquitoes were from the RalAl strain established in 2004 from a field collection in Raleigh. Adult male and female two-spotted spider mites were maintained on several varieties of beans, *Phaseolus spp.* The strain was established from a population on common tobacco, *Nicotiana tabacum* L. at NCSU. Adult male and female Western flower thrips were reared on pole beans, *Phaseolus vulgaris* L., and were originally found on *C. annum* in Sampson County, NC. Adult male and female tobacco thrips were grown on *P. vulgaris*. The colony was established in 1997 from specimens collected from peanut, *Arachis hypogaea* L., in Chowan County, NC.

**APPD Treatments.** APPD treatments of green peach aphids, citrus mealybugs, German cockroaches and Asian tiger mosquitoes were conducted in a plastic container (3 cm high) with a BugBed 123 (Green Thumb Group, Downers Grove, IL) mesh top (11.5 cm diameter) and bottom (10.0 cm diameter). For bioassays with mites and thrips, the insects were transferred into a plastic container (2.5 cm high) with a BugBed 123 mesh top and bottom (2 cm diameter). The mesh used does not interfere with the penetration of APPD into the container. The insects in containers were placed into the DBD chamber and the chamber purged with helium at 34 slpm for 45 min prior to the start of plasma treatments. Control exposures were of two types: insects held outside of the DBD chamber (not exposed to the helium atmosphere or plasma discharge), herein referred to as the “non-plasma, non-helium control” and insects held inside the DBD chamber (exposed to the helium atmosphere but not placed into the APPD) herein referred to as the “non-plasma helium control”. Afterwards, handling and/or incubation of the controls were identical to the treated samples.
Mortality Assays. Thirty to 110 adult western flower thrips, 30-110 tobacco thrips, 11-28 two-spotted spider mites, or 25 German cockroaches were treated per replicate with APPD for varying durations and then mortality recorded at 1, 3, 5 and 24 h after exposure. Twenty-three to 37 mosquitoes were exposed per replicate to the discharge for 5, 10, or 20 s; mortality was recorded at 1 and 2 h after treatment. The insects were incubated post-treatment at 26 ± 2°C, 65 ± 4% relative humidity and a 14:10 h (light: dark) cycle. None of the insects used in the experiments were given access to food or water for the duration of the test except for thrips, which were unable to survive without food during the 24 h assay. All experiments were replicated three times and mortality data corrected using Abbott’s formula (Abbott, 1925). Abbot corrections were conducted using the non-plasma helium controls, which produced the highest level of control morality. Control mortality ranged from 0-10% but in most cases was less than 5% and often was 0%.

Measurement of Water Loss. Twenty-five male cockroaches per replicate were anesthetized with carbon dioxide gas for 10 s and then transferred to plastic containers. The insects were placed into the DBD chamber 30 min later and treated with plasma for 60, 90, 120 and 180 s. The controls were non-plasma non-helium and non-plasma helium as previously described. The cockroaches were not provided food or water at any time during the course of the experiment. The total weight of the cockroaches and any fecal material was measured immediately before the containers were put into the DBD chamber and 24 h after treatment. Post treatment the insects were incubated at 26 ± 2°C, 65 ± 4% relative humidity and 14:10 h light: dark. Following the 24 h weight measurement, the cockroaches and all fecal material were stored at -80°C. An additional 25 cockroaches per replicate (untreated
and not used as controls) were weighed and frozen at the start of the experiment. The dry weight of all roaches studied was determined after lyophilization (Bench Top 6, Virtis, Gardiner, NY; cold trap = -70°C, ≈200 mTorr, ambient temperature = 23°C). All experiments were repeated three times.

The weight of the cockroaches before and after lyophilization was used to calculate the average percent water and dry weight at 0 and 24 h. The wet weight loss was calculated as the wet weight in mg at the beginning of the experiment minus the wet weight at 24 h. Water loss was calculated as the mg wet weight at 0 h times the average percent water at 0 h minus the mg wet weight at 24 h times the average percent water 24 h after treatment. The dry weight loss was calculated as the mg wet weight at 0 h multiplied by the average percent dry weight at 0 h minus the mg wet weight at 24 h times the average percent dry weight 24 h after treatment.

**GC MS Analysis of Cuticular Hydrocarbons.** Hydrocarbons were analyzed with a Hewlett-Packard (San Fernando, CA) model 6890 GC coupled to a model 5973A mass selective detector with an electron impact ion source. The GC was equipped with a HP-5ms (5% diphenyl-95% dimethylsiloxane) capillary column (30 m length, 0.25 µm film thickness and 0.25 mm inside diameter) (Agilent Technologies, Palo Alto, CA). The injector temperature was 300°C in splitless mode with a helium carrier gas flow of 1.5 ml min⁻¹. All data were recorded in scan mode (25-550 m/z). Each experiment was repeated three times.

Cuticular analyses were conducted on 10 apterous-mixed sex aphids, 10 adult female citrus mealybugs or 1 adult male cockroach per sample 24 h after a 120 sec, 240 s and 180 sec, respectively, exposure to plasma. The plasma exposure times used in these studies
produced the greatest mortality recorded in the experiments previously described. All results were compared to non-plasma non-helium controls. To extract the cuticular hydrocarbons, the insects in each sample were gently washed for 5 min with 1, 1, and 3 ml, respectively, of optima hexane (Fisher Scientific, Pittsburgh, PA) containing 0.6, 1, or 10 µg, respectively, of n-tetratriacontane (≥98% purity, Sigma, St. Louis, MO) internal standard in a 1 dram glass vial (Fisherbrand, Pittsburgh, PA) fitted with a Teflon lined cap. The cockroach only was washed two additional times with 1 ml each of hexane; the extracts were then pooled, reduced just to dryness under low heat and a stream of nitrogen gas, and re-suspended in 500 µl of hexane. The resulting extracts from the three different insects studied for each sample analyzed were separately transferred to the top of a disposable Pasteur pipette (7 cm x 0.5 cm internal diameter) packed with 100-200 mesh silica gel (Matheson Coleman & Bell, Norwood, Ohio). Prior to the extraction procedure the silica gel was washed in optima chloroform (Fisher Scientific, Pittsburgh, PA) then heat activated for at least 1 h at 100°C. The cuticular hydrocarbons were eluted with 7 ml of hexane and reduced just to dryness by low heat under a slow stream of nitrogen gas; the sample was then resuspended immediately in 100, 100, and 200 µl, respectively, of hexane. Aliquots of 1 µl were injected into the GC-MS and eluted with the following temperature program: (aphid) 50 to 180° at 40°C per min, increase to 320° at 8°C per min, and hold for 5 min; (mealybug) 50 to 320°C at 10° per minute, and hold for 5 min; and (cockroach) 150°C and hold for 2 min, to 245° at 10°C per min, to 260° at 1°C per min, to 280° at 10°C per min and hold for 3 min, respectively. The data were normalized by dividing the peak area of each hydrocarbon by the peak area of the
internal n-tetratriacontane standard and the normalized data represented as relative abundance.

Immediately following the extraction of cuticular hydrocarbons, the same samples were analyzed for total protein content using the Bradford protein assay according to the manufacturer’s instructions (Bio-Rad, Hercules, CA) and relative abundance expressed per mg protein. The protein concentration was standardized using bovine serum albumin (fraction 5, biotech grade, Fisher, Fairlawn, NJ). No differences in cuticular hydrocarbons were found between the non-helium, non-plasma controls as compared to the helium, non-plasma control. Therefore, only the non-helium control was used in our analysis.

**Metabolic activity.** The rate of oxygen consumption at STP was determined using a Gilson differential respirometer (Middletown, WI) for adult male cockroaches 1 h after exposure to plasma for 180 s and as compared to non-plasma helium controls. Two roaches were assayed per reaction vessel replicated at least 12 times each for the treatment and control. The insects were not provided food or water during the assay.

**German Cockroach Behavior Studies.** Twenty-five male cockroaches per replicate were anesthetized with carbon dioxide gas for 10 s, transferred to plastic containers, and treated for 90 s with plasma. A treatment of 90 s affected the behavior of the insect but did not produce any mortality. The controls conducted were described previously. Immediately following treatment, cockroach behavior was monitored continuously for the first 80 min and then at 2, 3, 4, 5 and 24 h post treatment. All cockroaches remained in plastic containers for the duration of the test. Incubation conditions were 26 ± 2°C, 65 ± 4% relative humidity and
14:10 h (light: dark). The roaches were not provided with food or water during the bioassay. The experiment was repeated three times and each replicate yielded identical results.

**Data Analysis.** Hydrocarbon abundances were individually compared between non-plasma non-helium controls and plasma treated insects using Student’s *t*-test (PROC TTEST, SAS Institute Inc.). Twenty-four hour mortality data were transformed to LT$_{50}$ values (defined here as the lethal plasma exposure time to kill half the population) using proc probit, to allow for the direct comparison of insect mortality by species since different species were exposed to the plasma for differing lengths of time due to differences in susceptible to the discharge. Pearson product moment correlation analyses of weight and LT$_{50}$ were carried out (PROC CORR, SAS Institute Inc.).

**Results and Discussion**

**Plasma Production.** The device used to generate the atmospheric pressure plasma discharge (APPD) in the current study is shown in Fig. 1. When the acrylic chamber was purged with helium at the rate of 34 slpm, the atmosphere in the chamber reach 99.9% helium in approximately 40 min. Therefore, a 45 min equilibration time was used to ensure at least 99.9% helium for the studies that follow. A predominant helium atmosphere was used as opposed to air, since an APPD can be generated at a low voltage and power requirement (85-95 W) at 4 kHz in He. The low power requirement coupled with circulating chilled water across the electrodes allowed for the production of plasma at a temperature of 37 ± 2°C; this temperature is not lethal to most insects at the short exposure times used in our studies. The use of an inert gas also reduced the production of chemically reactive species in
the plasma, which could be toxic to insects. In addition, the dielectric Garolite around the electrodes (Fig. 1A) made it possible to produce a uniform discharge without arcing, permitting an even treatment of all insect surfaces exposed to the He atmosphere.

Insects to be treated with plasma were placed in the chamber during the helium equilibrium period and eventually became immobile due to anoxia. Once the insects were returned to air, their behavior appeared normal in approximately 5-10 min. The helium exposure did not cause mortality in mosquitoes, cockroaches or mites. In thrips, percent mortality in the non-plasma helium controls as compared to the non-plasma non-helium controls was only slightly higher, on the average by a factor of 1.5-fold and never exceeded 10% (usually <5%). Although it is clear that the He treatment had a minimal and in most cases no effect on control mortality, we cannot rule out possible He-plasma interactions in our assessment of the mode-of-action of APPD. However, as discussed previously, the He was necessary to eliminate reactive chemistries and to reduce the temperature of the plasma to a non-lethal level.

The make-up of the plasma generated by the device in Fig. 1 was previously characterized in our laboratories (Bures 2004). Using the neutral bremsstrahlung technique (NB), the electron temperature and density were calculated as 1.1 eV (12,760 K) and ~10^8 cm^-3, respectively. Use of the NB technique was valid in this analysis since the electron-neutral collision frequency of ~1 THz dominates the bremsstrahlung spectrum in comparison to the electron-ion collision frequency of ~100 Hz (Park et al. 2000). The justification for the use of the neutral bremsstrahlung continuum to characterize APPD was described earlier (Bures 2004). The target discharge power for our studies was 90 W, but fluctuated between
85 and 95 W. Keeping the power below 100 W was critical to maintaining a gas temperature of 37 ± 2°C.

Emission spectroscopy was used in the absence of any insect samples to identify the chemical species in the discharge (Bures 2004). As expected helium neutral lines dominated the spectrum, however molecular nitrogen, $\text{H}_\alpha$ and $\text{H}_\beta$ (which suggest the presence of atomic hydrogen), and atomic oxygen were also present. The source of the nitrogen, hydrogen, and oxygen could be from either etching of the dielectric material, the 0.1% of air that remains in the chamber, or impurities in the helium gas tank. Since molecular oxygen was absent from the emission spectrum and the electron temperature was below the dissociation energy, the most likely source of the atomic oxygen was etching of the dielectric barrier. In order to test this theory, oxygen was introduced at a volume ratio of 0.5% oxygen/99.5% helium to the chamber, and the emission spectrum recorded. The result revealed that the helium, nitrogen, and hydrogen lines were still present, however two ionic molecular oxygen bands were also present (in addition to the atomic oxygen bands previously found). Since the addition of oxygen gas did not change the results in terms of the atomic oxygen, but rather only appeared in the spectrum as ionic molecular oxygen, the atomic oxygen in the 99.9% helium discharge is likely a result of etching of the dielectric material. Creating a discharge that is completely devoid of any non-helium impurities was not possible. The separate role of these low level impurities in insect mortality also could not be evaluated.

**Effects of Plasma on Insect Mortality.** The mortality of German cockroaches, western flower thrips, tobacco thrips, Asian tiger mosquitoes and two-spotted spider mites was recorded after plasma exposure (Fig. 2). Data were corrected using Abbott’s formula
(Abbott, 1925), using the non-plasma, helium control. The mortality of thrips did not increase significantly with time after treatment in the discharge ($P > 0.05$, $t$-test) with the exception of the 10 s APPD treatment of tobacco thrips ($P < 0.02$); however, there was a clear trend in both thrips species examined of an increase in mortality post treatment (Fig. 2A and 2B). In both species of thrips, there was also a significant plasma dose effect ($P < 0.05$) with the exception of the 1 h mortality measurement for Western flower thrips ($P < 0.09$) and the 3 h measurement for tobacco thrips ($P < 0.06$). German cockroach mortality (Fig. 2E) increased with time after treatment as a result of the 180 s exposure time only ($P < 0.0001$), and a significant dose effect occurred but was only significant at 5 ($P < 0.02$) and 24 h post-treatment ($P < 0.0001$). Mortality in two-spotted spider mites (Fig. 2D) increased significantly with time after treatment and as a result of longer treatment times ($P < 0.05$). Asian tiger mosquitoes (Fig. 2C) were highly susceptible to the discharge with all treatment times leading to 100% mortality 2 h after treatment. Only at the 5 s treatment time did mortality significantly differ between the 1 and 2 h time points after APPD exposure ($P < 0.038$). In general, mortality increased with plasma dose and time post-treatment for the insects studied.

Susceptibility to plasma appeared to be related to the weight of insects for most of the insects that have been studied. A correlation was made between the average insect weight and LT$_{50}$ of German cockroaches, western flower thrips, tobacco thrips, Asian tiger mosquitoes and two-spotted spider mites for the data in the current paper (Fig. 2). Added to this analysis were results from the green peach aphid, human body louse, and citrus mealybug obtained in our earlier work (Bures 2004). The correlation between weight and the
LT$_{50}$ was not significant (Pearson’s Correlation Coefficient = 0.21, $P < 0.617$). However, when the results for mealybugs were removed from the analysis, a strong positive correlation was obtained (Pearson’s Correlation Coefficient = 0.99, $P < 0.001$) between weight and the LT$_{50}$, meaning that larger insects are more resistant of the discharge. With the exception of mealybugs, smaller insects appear to be more susceptible to APPD.

It appeared that mealybugs with their small size were not susceptible to APPD exposure, suggesting that other factors may be important in the activity of plasma on insects. Citrus mealybugs, unlike the other species used in this study, produce copious wax secretions from the trilocular pores on their dorsal and ventral surfaces and from the less abundant multilocular pores and tubular ducts (Cox and Pearce 1983). This loose filamentous wax layer may serve as a protective barrier around the insect and account for their natural tolerance to APPD.

**Effect of Plasma on Water Loss.** The wet weight, water weight, and dry weight of German cockroaches exposed to APPD were measured 24 h after treatment (Fig. 3). During this time, the insects were not provided food or water. The mean non-plasma helium control water loss of $4.91 \pm 0.59$ mg ($\pm 1$ SEM) was greater than that of the non-plasma non-helium control, $3.12 \pm 0.60$ mg ($P < 0.055$, $t$-test). The water loss of cockroaches exposed to the discharge for 60, 90, 120 and 180 s was $7.53 \pm 0.77$, $8.08 \pm 0.57$, $8.69 \pm 0.40$ and $10.14 \pm 1.06$ mg, respectively, all of which were significantly greater than the non-plasma helium control ($P < 0.009$, $t$-test). Dry weight loss of the controls and the APPD treated roaches after 24 h was not significantly different ($P > 0.05$). While APPD clearly increased the rate of water loss over that of the non-plasma helium control, the latter also increased water loss.
over that of the non-plasma, non-helium control. The importance of the helium effect alone on APPD susceptibility could not be determined for reasons previously discussed.

**Effects of Plasma on Cuticular Hydrocarbons.** It was shown previously that DBDs with an electron plasma temperature above 4.5 eV could break the C-C bonds of polymers (Ricard et al., 1999). The plasma used in our studies had an electron temperature of only 1-2 eV but the effect of APPD on long chain hydrocarbons in the insect cuticle had not been previously studied. Considering the finding that insect plasma treatments increased water loss (discussed earlier), one possible mechanism of action of APPD might be disruption of the cuticular wax layer. Several studies have shown that damage of the wax layer can result in insect death (Gibbs, 2002; Lockey, 1976, 1991).

The identity and abundance of the cuticular hydrocarbons in plasma treated green peach aphids, citrus mealybugs and German cockroaches were determined by EI GC-MS (Figs. 4-6). The hydrocarbons of German cockroaches were predominantly methyl branched. Mealybugs had three methyl branched alkanes with the remaining twenty six being straight chained while the aphid cuticle contained only straight chain hydrocarbons. Young et al. (2000) reported that 95% of the cuticular hydrocarbons in the German cockroach melted at 42.25 ± 0.89°C. If the temperature of the APPD was a factor in affecting water loss or mortality in the current study, it would have likely affected cockroaches first, since branched alkanes tend to have a lower melting point than straight chained hydrocarbons. Recall that the German cockroach was naturally resistant to APPD exposure. The only statistically significant change in hydrocarbon abundance normalized per mg protein for the three insects examined was n-tritriacontane in mealybugs ($P < 0.0176$, $t$-test). This difference is probably
not important in terms of the APPD mode of action, since the mealybug mortality was only 24%, 24 h after a 240 s treatment with plasma. It was apparent from these results that the APPD did not degrade the cuticular hydrocarbons of the insects studied (with one exception, n-tritriacontane in mealybugs) and is probably not responsible for the increased water loss and mortality observed after plasma treatments. However, changes in the organization of the lipid layers were not investigated.

**Effects of Plasma on Insect Behavior.** The behavior of German cockroaches exposed to APPD for 90 s was monitored after treatment (Table 1). The 90 s time point was chosen in the current study because the insects were clearly affected behaviorally by this treatment duration and no cockroach mortality occurred during the observation period. The bioassay was repeated three times and the results were identical.

During the helium equilibration period discussed earlier, the German cockroaches became fully immobilized, unreactive to physical stimuli like touch with a blunt probe or movement from one location to another within the acrylic chamber of the DBD device. However, when the cockroaches were placed into the discharge, their wings immediately moved to a perpendicular position to the anterior-posterior axis of the body, and their legs twitched with without consistent rhythm. Upon removal from the discharge, the legs ceased to move, and the hind legs were fully extended. The wing position returned to normal within 5-10 min after removal from the discharge. The distal 40-50% of the antennae of half of the APPD treated cockroaches were curled over and did not return to their normal shape 24 h after treatment, suggesting that the plasma caused permanent physical damage. This phenomenon was not witnessed in the other species used in this study. Twenty-four h after
treatment the insects had neither regained full motor control nor were thigmotactic, although they were able to right themselves. Their response to light and vibration was limited to movement in a forward direction of approximately 2-3 cm and did not elicit the typical running behavior witnessed in the controls. The symptoms of cockroaches exposed to APPD shares similarities to classical behavioral changes recorded after the application of nerve toxins (Salgado 1998). Our findings suggest that the mode of action of APPD is partly an effect on the nervous or neuromuscular system.

**Effect of Plasma on Metabolic Rate.** The oxygen consumption rate of cockroaches 1 h after a 180 s exposure to APPD was 1.07 ± 0.04 ml (±1 SEM) of oxygen consumed mg-wet weight^-1 h^-1 at STP, while the non-plasma helium control was 0.79 ± 0.03 ml of oxygen consumed mg-wet weight^-1 h^-1 at STP. The significantly higher metabolic rate for the APPD treated roaches (*P* < 0.05, *t*-test) might be expected for insects demonstrating obvious hyperexcitatory symptoms (Prosser, 1973). Increased water loss associated with increased levels of respiration might also be expected, although it is impossible to determine this with certainty.

Susceptibility to APPD treatments varied significantly among the insects tested with the smaller insects being most susceptible and demonstrating the greatest mortality. Mealybugs were an exception and demonstrated natural resistance to APPD exposure, suggesting that other factors may interfere with plasma mode of action. Overall, it was found that mortality increased with increased treatment times and with time after treatment in the discharge. The increased water loss associated with plasma exposure could not be explained by changes in the relative abundance of cuticular hydrocarbons in the green peach aphid,
citrus mealybug and German cockroach. However, hyperexcitation and other changes in behavior along with an increase in the metabolic activity might result in increased water loss and suggests that the primary mode of action of APPD might be the insect nervous or neuromuscular system. A better understanding of the mode of action of plasma will be important in the application of this novel technology to insect control.

Acknowledgements

The authors thank Dr. Clyde Sorenson of NCSU Entomology and William R. Fisher of BASF Chemical Corporation for supplying green peach aphids, Dr. Christine Casey and Ellen Reeves of NCSU Entomology for supplying the citrus mealy bugs, Dr. Coby Schal and Rick Santangelo for supplying German cockroaches, Dr. Charles Apperson for supplying Asian tiger mosquitoes, and Dr. Robin Todd and Jennifer Russell at ICR Labs for generously supplying human body lice. Thanks to Matthew M. Lyndon of NCSU Mass Spectrometry Facility for assistance with GC/MS, and Dr. Christof Stumpf for assistance with statistical analysis. Additionally, members of the Dearstyne Entomology Lab at NCSU provided thoughtful ideas and discussion of the research. This work is supported by a grant from the USDA-APHIS under cooperative agreement No. 01/02/03-8100-0783-CA and the North Carolina Agricultural Research Service.
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<th>Time After Treatment</th>
<th>Exposed to APPD, 90 s</th>
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<tr>
<td>During Treatment</td>
<td>Legs twitch</td>
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<td>Wings perpendicular to anterior-posterior axis</td>
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<td>Distal 40-50% of the antennae curled over</td>
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<td>Ataxia and only able to move mouthparts</td>
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<td>15-20 min</td>
<td>Ataxia and no posterior leg control</td>
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<td>Abdominal muscles contracting</td>
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Figure legends

Figure 1. A. Schematic of the DBD device. The plasma bulk is the area in which the APPD forms. The frequency applied for these studies was 4 kHz and the power ranged from 85-95 W. The dielectric (an electrical insulating material constructed from a woven glass fiber laminate) keeps the plasma from forming an arc. B. Photo of DBD device.

Figure 2. Mortality of *F. occidentalis* (A), *F. fusca* (B), *A. albopictus* (C), *T. urticae* (D), and *B. germanica* (E), exposed to APPD. The legend refers to the time after treatment in the discharge in which mortality was recorded. Error bars represent ± 1 standard error of the mean, which in some cases were too small to illustrate. All data are corrected using Abbott’s formula (Abbott, 1925).

Figure 3. Wet weight loss, water weight loss and dry weight loss of *B. germanica* exposed to APPD. Error bars represent ± 1 standard error of the mean. The water weight loss differed significantly from the non-plasma helium control for all treatment times (*P* < 0.05, *t*-test) while the dry weight did not differ (*P* > 0.05). The non-plasma helium control also differed significantly from the non-plasma non-helium control (*P* < 0.055).

Figure 4. Cuticular hydrocarbons identified by GC-MS of *M. persicae* exposed to APPD for 120 s. Error bars represent ± 1 SEM. The relative abundance was calculated as the peak area per compound divided by the area for the internal standard, n-tetratriacontane. Relative
abundance was expressed per mg total body protein to compensate for differences in insect mass. The abundance of each hydrocarbon for the APPD exposed aphids did not differ significantly from the controls ($P > 0.05$, $t$-test)

Figure 5. Cuticular hydrocarbons identified by GC-MS of *P. citri* exposed to APPD for 240 s. Error bars represent ± 1 SEM. The relative abundance was calculated as the peak area per compound divided by the area of the internal standard, n-tetratriacontane. Relative abundance was expressed per mg total body protein to compensate for differences in insect mass. The abundance of each hydrocarbon for the APPD exposed mealybugs did not differ significantly from the controls ($P > 0.05$, $t$-test), except for n-tritriacontane ($P < 0.0176$).

Figure 6. Cuticular hydrocarbons identified by GC-MS of *B. germanica* exposed to APPD for 180 s. Error bars represent ± 1 SEM. The relative abundance was calculated as the peak area per compound divided by the area of the internal standard, n-tetratriacontane. Relative abundance was expressed per mg total body protein to compensate for differences in insect mass. The abundance of each hydrocarbon for the APPD exposed cockroaches did not differ significantly from the controls ($P > 0.05$, $t$-test).
Figures

A

Electrode

Dielectric

Plasma Bulk

RF power supply

B

Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6.
Enhancement of Atmospheric Pressure Plasma for Insect Control

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ABSTRACT. Exposure of insects and mites to a low temperature (37 ± 2°C), non-thermal (1-2 eV) atmospheric pressure plasma discharge (APPD) has recently been shown to result in insect mortality. The LT$_{50}$ of German cockroaches, *Blattella germanica* L., western flower thrips, *Frankliniella occidentalis* (Pergande), and citrus mealybugs, *Planococcus citri* (Risso) 24 h after exposure to a 37°C helium discharge was 333.77, 28.72, and 999.95 s, respectively. The LT$_{50}$’s 24 h after exposure to a 37°C, 0.5% oxygen/99.5% helium discharge were 232.67, 19.99, <13.83 s, respectively. The LT$_{50}$ of German cockroaches, western flower thrips, and citrus mealybugs 24 h after exposure to the 50 ± 2°C, helium discharge were 117.80, 13.83, and 26.62 s, respectively. No mortality resulted in German cockroaches or citrus mealybugs after exposure to a helium atmosphere followed by 50°C air, however the LT$_{50}$ of western flower thrips was 29.04 s, based on the mortality recorded 24 h after treatment. The addition of oxygen or heat to the discharge resulted in higher mortality in cockroaches and mealybugs but not thrips.
Introduction

METHYL BROMIDE has been widely used for pre- and post-harvest fumigation in addition to quarantine applications (Schneider et al. 2003). In 1987 the Montreal Protocol on Substances that deplete the ozone layer as well as the Clean Air Act began a general phase out of methyl bromide as a fumigant due to its ozone depleting properties. As of January 1, 2005, methyl bromide fumigation has been banned except for critical use exemptions that were granted by the Parties of the Protocol (EPA 2004). The exemptions represent situations where no other alternative technology has become available that are as efficacious as methyl bromide, do not significantly damage the commodity, is effective in a reasonable time frame, or has been deemed acceptable by the general public as safe for use. Competing new technologies must be as effective as methyl bromide and implementation of the alternative must be relatively easy to adopt without prohibitive cost or training time. In an effort to develop an alternative method for insect control we have studied the use of an atmospheric pressure plasma discharge (APPD) for insect control (Bures et al. 2005, Donohue et al. 2005).

Plasma is created when gases become ionized due the addition of energy such as extreme heat, electrical energy, or intense radiation (Eliezer and Eliezer 2001) and electrodynamic rather than hydrodynamic forces cause the charged and neutral particles to exhibit collective behavior (Chen 1984). Plasmas are commonly used for the modification of surfaces (Rehn and Viöl 2004), thin film deposition (Yoshizawa et al. 1993), manufacturing computer processors (Sarajlić et al. 2004), air filtration (Daniels 2002), treatment of hazardous waste (Eliasson and Kogelschatz 1991), and biological and chemical decontamination (Birmingham and Hammerstrom 2000; Kelly-Wintenberg et al. 2000;
Using plasma for sterilization has been shown to be a very successful method for destroying bacteria (Laroussi 2002; Vleugels et al. 2005). Birmingham (2004), for example has developed a “plasma blanket” that produces a discharge shown to deactivate 99.9999% of the anthrax spores treated in 60 s or less. This and other non-thermal plasmas operate in a range of 0.1-2 eV, where the gas temperature in relatively low (<60°C) while the electron temperature is high (1500-23,000 K). Since the gas temperature of the discharge is weakly affected by the electron temperature, the gas temperature can be kept low and allow for the treatment of thermally sensitive objects. Additionally, using plasma for sterilization instead of traditional chemical approaches, such as ethylene oxide, makes this approach safer for the operator.

Our previous studies (Bures et al. 2005; Donohue et al. 2005) extended the use of plasma to treat insects to study whether this emerging technology could be adapted for use on insect pests and to assess the mode of action of plasma on several insect species. We have shown that a non-chemical, non-thermal (1-2 eV) atmospheric pressure plasma discharge (APPD) can control insects on synthetic substrates as well as on living plant material. The mode of action on insects appears to be an effect on the nervous or neuromuscular system, as well as increased water loss, and an increase in metabolic rate, but did not reduce the abundance of cuticular hydrocarbons. A significant negative correlation between mortality and weight was found using the results of western flower thrips, *Frankliniella occidentalis* (Pergande), tobacco thrips, *Frankliniella fusca* (Hinds), Asian tiger mosquitoes, *Aedes albopictus* (Skuse), two-spotted spider mites, *Tetranychus urticae* Koch, German cockroaches, *Blattella germanica* (L.), green peach aphids, *Myzus persicae* (Sulzer), and
human body lice, *Pediculus humanus humanus* L., with the exception of citrus mealybugs, *Planococcus citri* (Risso).

In the previous studies we maintained a gas temperature <40°C and used helium as the working gas to suppress the formation of chemically reactive species in order to understand the mode of action and measure mortality of the discharge alone. Several modifications such as increasing the gas temperature and using different working gases, changes the properties of the discharge and may increase or decrease the effectiveness of APPD on insects. Our objective in this study was to shorten the treatment times required to induce mortality in insects by adding oxygen to the helium working gas or by increasing the gas temperature. The addition of oxygen to plasma results in several types of oxygen species being created (Leiberman and Leichtenberg 1994) and has shown improved efficacy of plasma for sterilization purposes (Moreira et al. 2004). Experiments were also designed to understand the effect of exposing insects to helium followed by heat to study whether increased gas temperature plasma has an additive or synergistic effect on insect mortality. By reducing the treatment times to less than a few minutes, APPD could be a competitive technology for insect control.

**Materials and Methods**

**Dielectric Barrier Discharge Device.** Atmospheric pressure plasma discharge was generated between 16 x 20 cm parallel electrodes in either ≥ 99.9% helium atmosphere (HE291, Machine and Welding Supply, Inc., Dunn, NC) or a ≥ 99.9% mixture of oxygen (HP, Machine and Welding Supply) and helium in the ratio of 0.5% oxygen/99.5% helium by
volume. The electrodes are composed of copper covered with a thin layer (0.793 mm) of Garolite Grade 7 (McMaster-Carr, Atlanta, GA), a woven glass fabric laminate with a silicon resin bonded to the electrodes. Garolite is a good insulator for dielectric barrier discharge (DBD) devices and aids in the formation of a uniform discharge at ambient pressure and prevents the transition of the discharge to an arc. Two adjustable pedestals support the electrodes. Although the pedestals allow the electrodes to be separated by variable distances (1.5-10.0 cm) to accommodate different sample sizes, the electrode gap was fixed at 5.0 cm during the current study. Heat generated by the electrodes can be removed by circulating cooled distilled water through channels (Fig. 1) over the electrode surface. Distilled water was used to prevent the conduction of current from the electrode through the water to the cooling system. During active cooling the discharge temperature was 37 ± 2°C, and without cooling the temperature rose to 50 ± 2°C. High voltage (approximately 2.7 kV) was supplied to the electrodes through a pair of high voltage transformers (Del Electronics Corporation, Valhalla, NY) and measured with two P6015A high voltage probes (Tektronix, Richardson, TX). The current (approximately 24.2 mA) to each electrode was measured through a 100 Ω resistor in series between the transformer and ground. For all treatments, the power ranged between 85-95 W.

The DBD device was housed in an 259 L acrylic chamber (48.26 cm height x 102.87 cm width x 58.42 cm depth), which was filled with either helium, or 0.5% oxygen/99.5% helium at 34 standard liters per minute (slpm) through a Type 1179A general-purpose mass flow controller and a Type 247D four channel power supply readout (MKS Instruments, Wilmington, MA). The electrode pedestal supports pass through the acrylic chamber on the
top and bottom. A rubber glove was attached to the front panel of the chamber to move samples in and out of the APPD. The effluent of the chamber was measured using a helium gas monitor (Model 334WP, Nova Analytical Systems, Niagara Falls, NY) to determine the helium composition inside the chamber. A fill time of 45 minutes was used to ensure a working gas composition of at least 99.9% purity. Use of a predominantly helium atmosphere or similar inert gas offers two advantages; the formation of potentially harmful reactive species to the operator can be suppressed, and the power input needed to form the APPD is less than 100W which allows the operator to control the thermal component of the discharge. Insects were housed in the chamber during the helium equilibration for the experimental treatments described later.

Once the atmosphere in the chamber equilibrated, voltage was applied from an HTA-250A power supply (Bogen, Ramsey, NJ) with an acoustic frequency of 4 kHz supplied from a 5 MHz function generator (BK Precision, Yorba Linda, CA) to form the APPD. The ambient gas temperature of the discharge was measured in situ with an electrically insulated K-type contact thermocouple (Omega Engineering, Stamford, CT). The thermocouple was insulated to prevent plasma filaments from striking the thermocouple and producing an inaccurate temperature measurement of the discharge.

**Insects.** All insects used in the study were reared in the Department of Entomology at NC State University, Raleigh, NC. Citrus mealybugs were maintained on crooked neck squash, *Cucurbita moschata* Duchesne. The strain was established in 2002 from a greenhouse located at NCSU. Adult male German cockroaches originated from an American Cyanamid insecticide-susceptible strain and were fed Purina Rat Chow #5012 (Purina Mills,
St. Louis, MO) and given water ad libitum. Western flower thrips were maintained on pole beans, *Phaseolus vulgaris* L., and the strain was established from a population on red pepper, *Capsicum annum* L. in Sampson County, NC.

**APPD Treatments.** Citrus mealybugs and German cockroaches were housed in plastic containers (3 cm high) with a BugBed 123 (Green Thumb Group, Downers Grove, IL) mesh top (11.5 cm diameter) and bottom (10.0 cm diameter). Thrips were placed into plastic containers (2.5 cm high) with a BugBed 123 mesh top and bottom (2 cm diameter). The mesh did not interfere with the APPD. Mealybugs and cockroaches were not given access to food or water for the duration of the test but thrips were supplied with pole beans after treatment since they were unable to survive 24 h without a food source.

Control exposures were of two types: insects in the same containers held outside of the DBD chamber (not exposed to the predominantly helium atmosphere or discharge), herein referred to as “non-plasma, non-helium control”, and insects in the same containers held inside the DBD chamber (exposed to the helium atmosphere but not placed into the APPD), herein referred to as “non-plasma helium control”. In all experiments the non-plasma helium control mortality was under 10%, in most cases less than 5%, and often zero. After APPD treatments, the insects were incubated in the plastic containers at 26 ± 2°C, 65 ± 4% relative humidity, and 14:10 (light: dark) photocycle. Incubation of the controls was identical to the treated samples.

**Mortality assays.** Two separate bioassays with APPD were conducted. The first involved a 99.9% helium atmosphere and operation of the device without active cooling of the electrodes to increase the gas temperature of the discharge to 50 ± 2°C. In the second
bioassay the gas temperature was maintained at 37 ± 2°C by actively cooling the electrodes, but the chamber was filled with 0.5% oxygen/99.5% helium by volume. In both cases the insects were loaded into the acrylic enclosure followed by a 45 min fill time with the working gas before APPD operation. Adult female citrus mealybugs, adult male cockroaches, and adult western flower thrips were used in each bioassay and exposed to the discharge for the following durations: 60, 120, 180, or 240 s; 10, 30, 60, 90, 120, or 180 s; and 10, 30, 60, or 120 s, respectively. Mortality was recorded at 1, 3, 5, and 24 h after exposure to the discharge. Mortality data were corrected with Abbott’s formula (1925), using the results from the non-plasma helium control since the mortality was always higher than the non-plasma non-helium control.

**Exposure to helium and 50°C air.** Twenty-five mealybugs, 25 cockroaches, and 40-116 thrips were each placed into the plastic containers described earlier, then placed in a 2.8 L white plastic bucket with dimensions 12.5 cm diameter top, 17 cm diameter bottom, and 15.5 cm height (Plastic Packaging Company, West Springfield, Massachusetts). The bucket was filled with helium at 0.368 slpm regulated with a micrometering valve (Part #SS-4BMG, Nupro Company, Willoughby, OH) for approximately 45 min to exactly mimic the fill rate and fill time with the larger acrylic chamber used for plasma treatments. The insects were exposed to the 99.9% helium atmosphere then immediately transferred to a Thelco Model 28 oven (Thermo Electron Corporation, Marietta, OH) at 50 ± 1°C. Exposure times to the heat were identical to exposure times during APPD mortality bioassays. Two controls were used during the bioassay; one that was neither exposed to the helium atmosphere or hot
air and a control that was exposed to helium and no hot air. Incubation of the insects was identical to those during the mortality bioassays.

**Statistical Analysis.** LT$_{50}$ data (lethal APPD exposure time to kill half the population) were generated from mortality data collected 24 h after treatment in APPD (PROC PROBIT, SAS Institute Inc.). LT$_{50}$’s for 37°C, 99.9% helium APPD were calculated using mortality data recorded 24 h after APPD treatment during our previous studies, for comparison with the current results (Bures 2004; Donohue et al. 2005). The non-plasma helium control was used as the “zero dose” for the probit calculations since the mortality was always equal to or greater than the non-plasma non-helium control. Mortality data were analyzed using Student’s $t$-test and analysis of variance (PROC TTEST, PROC REG, PROC GLM, SAS Institute Inc.).

**Results and Discussion**

**Plasma.** Using a predominantly helium atmosphere limits the chemical species in APPD, allows the temperature to be easily controlled with cooled distilled water, limits etching of the dielectric barrier, and is safe for the operator. We had previously found that purging the chamber for approximately 30 min with helium resulted in a 99.9% helium atmosphere, but a conservative equilibration time of 45 min was used to ensure a 99.9% helium atmosphere.

Our previous studies with APPD were conducted with a 99.9% helium atmosphere and a discharge temperature of 37 ± 2°C in an effort to study the effects of plasma alone on...
insect mortality and to determine the mode of action (Donohue et al. 2005). By using an inert gas such as helium, reactive chemical species were suppressed. Additionally, keeping the power supplied to the system below 100 W and active cooling of the electrodes limited the thermal component and maintained a gas temperature of 37 ± 2°C.

In the current study the APPD was modified relative to previous work by the addition of either heat or oxygen to the discharge in order to study the effect on insect mortality and whether the efficacy of APPD could be improved for insect control. Without the supply of cooled distilled water to the electrodes (Fig. 1) the APPD can be operated at 50 ± 2°C when the applied power is 85-95W. During assays with oxygen added to the discharge, 0.5% oxygen by volume was added to the helium flow to the chamber, otherwise the fill rate and time were identical to assays in which only helium was added to the chamber. Adding oxygen to plasma can result in several forms of reactive oxygen species (Jeong et al. 2000; Lieberman and Lichtenberg 1994) that have been shown to be an effective method for sterilization due to the synergism of ion bombardment and surface etching (Moreira et al. 2004).

A prior study by Bures (2004) to characterize the discharge found that during the operation of the device with helium only as the working gas, a small amount of atomic oxygen was detected in the discharge by emission spectroscopy at 777.1 and 844.6 nm. The presence of atomic oxygen was believed due to impurities in the helium source, etching of the dielectric barrier, or the 0.1% air (which can be assumed to be ~0.02% oxygen) remaining in the chamber during operation. The emission spectrum during the addition of 0.5% oxygen by volume indicated the only changes in the spectrum were the presence of two
ionic molecular oxygen bands at 559.7 and 597.3 nm. The atomic oxygen bands did not change suggesting that the atomic oxygen in the discharge was a result of etching of the dielectric. Other oxygen species may have been present in the oxygen/helium discharge, but they were not determined in the current study.

**Effect of APPD with elevated gas temperature on insect mortality.** Citrus mealybugs, German cockroaches, and western flower thrips were exposed to APPD without active cooling of the electrodes which caused the gas temperature to rise to 50 ± 2°C during operation (Fig. 2). Our goal was to lessen the exposure time needed to induce mortality in comparison to our earlier studies with APPD in which the electrodes were cooled to 37 ± 2°C. The predominantly helium atmosphere required to operate the discharge resulted in non-plasma helium control mortality that was <10% in all cases and often zero.

Mortality of cockroaches only increased significantly with time after the 60 s APPD treatment ($P < 0.03$, *t*-test). Increasing APPD treatment time significantly increased the mortality when analyzed at the 1, 3, 5, and 24 h time points post treatment ($P < 0.05$). Citrus mealybug mortality only increased significantly with time after the 120 s APPD exposure ($P < 0.04$), and a dose effect (increased mortality with longer APPD treatment times) was significant at the 1, 3, 5, and 24 h treatment times ($P < 0.045$). Western flower thrips mortality increased significantly with time post treatment ($P < 0.02$) except for the 60 s APPD exposure ($P < 0.25$). Thrips mortality increased significantly with increasing APPD exposure times when analyzed at 1, 3, 5, and 24 h post treatment ($P < 0.0001$). A separate bioassay was designed to measure the mortality of German cockroaches, citrus mealybugs, and western flower thrips that were exposed only to helium for approximately 45 min, then
50°C air for the same APPD treatment durations. No mortality in cockroaches or mealybugs occurred as a result of the experiment, however the thrips were affected (Fig. 3). A comparison of the thrips data in Fig. 2B and Fig. 3 revealed that the 50 ± 2°C APPD treatments resulted in significantly higher mortality 1, 3, 5, and 24 h after the 60 s exposure time, and 1, 3, and 5 h after the 120 s treatment ($P < 0.05$).

Increasing the gas temperature of APPD appears to have a synergistic effect in terms of inducing mortality in German cockroaches and citrus mealybugs. The results after treatment of western flower thrips show the increased gas temperature APPD offered only a comparatively minor advantage. The exposure to helium followed by heat may stress the insects but not necessarily always result in mortality, and therefore the insects may become more susceptible to the discharge. Another possible reason for the increase in insect mortality due to the increased APPD gas temperature is that since the pressure remains constant and the temperature increases, the ionization in the discharge would increase and ion bombardment of the insects may increase. Increasing the temperature with constant pressure reduces the bulk density of the discharge, which reduces the collision frequency of the particles, thereby allowing electrons more time to gain energy and increase the rate of ionization. Without further investigation we cannot be certain that if the ionization rate increased significantly, insect mortality would also increase.

**Effect of the addition of oxygen to APPD on insect mortality.** An alternative strategy to improve the efficacy of plasma was to use a 0.5% oxygen/99.5% helium working gas required to form the discharge since the introduction of oxygen into plasma results in reactive oxygen species (Leiberman and Leichtenberg 1994). The mortality of German
cockroaches (Fig. 4A) increased with time after the 60, 90, 120, and 180 s treatment times ($P < 0.05$, $t$-test). Mortality did not increase as a result of longer exposure times ($P > 0.05$).

Western flower thrips mortality (Fig. 4B) increased significantly with time post treatment for the 30 and 60 s treatment times. Mortality increased as a result of longer APPD exposure times at the 1, 3, and 5 h time intervals ($P < 0.005$), but not at the 24 h time post treatment measurement ($P < 0.092$). Citrus mealybugs were the most susceptible to the oxygen/helium APPD treatments (Fig. 4C) with mortality reaching 100%, 1 h after treatment for the 60 and 240 s treatment times. Mortality as a result of the 120 and 180 s treatments did not increase significantly with time after treatment ($P < 0.05$).

**Comparison of the three plasma regimes.** The data from the mortality bioassays were used to calculate LT$_{50}$’s, herein defined as the median lethal exposure time in seconds to kill half the population (Table 1.). In our previous studies (Donohue et al. 2005), German cockroaches, citrus mealybugs, and western flower thrips were exposed to APPD with 99.9% helium working gas and $37 \pm 2^\circ$C gas temperature. Those data were transformed to LT$_{50}$’s for comparison with the higher gas temperature plasma and the 0.5% oxygen/99.5% helium plasma.

Citrus mealybugs were the most susceptible to the oxygen/helium APPD. The LT$_{50}$ could not be calculated since the shortest treatment time yielded 100% mortality. The 50°C APPD also provided an advantage over the 37°C APPD treatments, decreasing the LT$_{50}$ of mealybugs from 999.95 s to 13.83 s. The most successful treatment for German cockroach control was the 50°C APPD with an LT$_{50}$ of 117.80 s. Results from the treatment of western
flower thrips revealed the only significant improvement in terms of mortality was with the 0.5% oxygen/99.5% helium APPD with an LT$_{50}$ of 19.99 s.

**Insect-Substrate Interactions.** The efficacy of APPD for insect control may change depending upon the substrate on which the insects reside during treatment. We have previously found that the mortality of green peach aphids exposed to APPD for 120 s on tobacco leaves was 40 ± 8.64% (± 1 SEM), compared to 88 ± 6.11% while on the plastic BugBed 123 fabric (Bures et al. 2005). During treatment, it appeared that the edges of the tobacco leaf locally enhanced the electric field, thereby causing the discharge to become less uniform and result in lower aphid mortality. Damage to the tobacco leaf appeared 24 h after exposure as several 0.5-1.0 cm diameter areas that appeared to be completely desiccated. A detailed study of the effects of APPD on several species of living plants will be necessary to optimize the discharge for insect control and minimize plant damage to economically acceptable levels.

Situations other than the treatment of leafy plant material may be well suited for insect control with plasma using the current technology. Dubinov et al. (2000) found that the seed germination rate of oat, *Avena sativa* L., and barley, *Hordeum vulgare* L. seeds increased as a result of exposure to plasma for 2-4 min. After germination the sprouts grew normally. Additionally, since plasma discharges have been studied extensively for decontamination of materials of bacteria and spores, it has been shown that a device similar to the one used in the current study will not result in modification to paper, plastics, and packaging material (Thiyagarajan et al. 2005). The plastic mesh substrate of the containers
housing the insects used in the current study was continually reused for APPD treatments, and no physical damage was apparent to the naked eye after multiple APPD exposures.

We have found that increasing the temperature of the discharge increases the mortality of German cockroaches and citrus mealybugs and adding oxygen to the discharge while maintaining a low gas temperature (37 ± 2°C) increased the mortality of German cockroaches, citrus mealybugs, and western flower thrips. Exposing cockroaches and mealybugs to helium then 50°C air for the same durations as APPD exposure resulted in no mortality, revealing that the 50°C discharge had a synergistic effect. The ability to significantly increase the mortality of insects exposed to APPD by simply adding heat or oxygen to the discharge may result in significantly decreased treatment times to achieve insect control levels suitable to quarantine and post-harvest applications.

Acknowledgements

The authors would like to thank Dr. Christine Casey and Ellen Reeves of NCSU Entomology for supplying the citrus mealy bugs and western flower thrips, and Dr. Coby Schal and Rick Santangelo for supplying German cockroaches. Thanks to Dr. Christof Stumpf assistance with statistical analysis of the data. Additionally, members of the Dearstyne Entomology Lab at NCSU provided thoughtful ideas and discussion of the research. This work is supported by a grant from the USDA-APHIS under cooperative agreement No. 01/02/03-8100-0783-CA and the North Carolina Agricultural Research Service.
References Cited


### Table 1. Comparison of the effects of APPD with either increased gas temperature or the addition of oxygen on *Blattella germanica*, *Planococcus citri*, and *Frankliniella occidentalis*. LT$_{50}$ values are expressed in seconds and are based on the mortality recorded 24 h after APPD exposure.

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<td>89.37</td>
</tr>
</tbody>
</table>

$^a$LT$_{50}$ is defined as the APPD exposure time in seconds to kill 50% of the population within 24 h.

$^b$Values calculated using probit analysis (SAS, 2001).

$^c$Gas composition: 99.9% helium.

$^d$Calculated using data from Donohue et al. 2005.

$^e$Gas composition: 0.5% oxygen, 99.5% helium by volume.

$^f$Calculated using data from Bures et al. 2005b.

$^g$Probit model could not be applied since the shortest treatment time (60 s) yielded 100% mortality.
Figure Legends

Fig. 1. Simplified drawing of the dielectric barrier discharge device. Cooled distilled water can be pumped through the cooling channels to cool the copper electrodes to maintain a gas temperature of 37 ± 2°C for a 85-95W discharge. Deactivating the coolant allows the temperature to increase to 50 ± 2°C under the same conditions.

Fig. 2. Exposure of *Blattella germanica* (A), *Frankliniella occidentalis* (B), and *Planococcus citri* (C) to APPD with 99.99% helium atmosphere. The gas temperature of the plasma was 50 ± 2°C. Error bars represent ± 1 SEM. Time points that lack error bars signify no variation in the measurement.

Fig. 3. Mortality of *Frankliniella occidentalis* due to a 45 min helium exposure followed by exposure to 50 ± 2°C air. The gas equilibration time of 45 min, and the exposure times to the 50 ± 2°C were identical to the APPD treatments.

Fig. 4. Exposure of *Blattella germanica* (A), *Frankliniella occidentalis* (B), and *Planococcus citri* (C) to APPD with a 0.5% oxygen / 99.5% helium atmosphere. The gas temperature was 37 ± 2°C. Error bars represent ± 1 SEM. Time points that lack error bars signify no variation in the measurement.
Fig. 1.
Figure 2.
Figure 3.
Figure 4.