2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

INVESTIGATION OF THE MODE OF ACTION OF A NON-EQUILIBRIUM DISCHARGE ON ARTHROPOD PESTS

Kevin V. Donohue, Brian L. Bures, Mohamed A. Bourham and R. Michael Roe
Raleigh, NC

Abstract

The use of a non-equilibrium atmospheric pressure plasma discharge (APPD) for controlling insects and mites has only recently been explored. Current research has been successful in showing that a low temperature (37° ± 2°C) discharge can kill arthropods. The mode of action is not understood. Cockroaches exposed to the discharge for 90 s lost significant motor control during the 24 h study, while those exposed only to the helium atmosphere required to operate the DBD (Dielectric Barrier Discharge) returned to normal behavior within 15 min. The oxygen consumption of cockroaches exposed to a 3 min discharge was greater than those only exposed to the helium atmosphere suggesting an increase in metabolic rate. Cockroaches exposed to APPD lost a significantly greater amount of water weight than the control. The cuticular hydrocarbons of citrus mealy bugs were unaffected by the discharge except a reduction in the abundance of n-tritriacontane. In green peach aphids, the trend was toward a reduction in hydrocarbon abundance, although this was not statistically significant for most of the compounds identified by GC-MS. Based on the aberrant behavior of the cockroaches after treatment in the discharge, it appears that the disruption of the nervous or neuromuscular system is a component of the mode of action of APPD.

Introduction

Atmospheric pressure plasma discharge (APPD) can be effective against a range of economically important pests such as *Myzus persicae* (Green peach aphid), *Tetranychus urticae* (two-spotted spider mite), *Planococcus citri* (citrus mealy bug), *Frankliniella occidentalis* (Western flower thrips), and *F. fusca* (tobacco thrips)(Bures et al., 2003, 2004, *in press*; Roe et al., 2003). This novel technology for the control of arthropods was first developed by our laboratory for quarantine applications as an alternative to methyl bromide (MeBr). The phase out of MeBr began with the Montreal Protocol on Substances that Deplete the Ozone Layer in 1987 (EPA, 2004). The use of APPD may also extend to green house applications, stored products such as seeds and grains, and packaging materials.

Low temperature (< 40°C) APPDs previously have been shown to effectively kill bacteria (Efremov et al., 2000; Laroussi, 2002; Laroussi et al., 1999, 2000, 2002, 2003; Montie et al., 2000). The mode of action of APPDs on gram-negative bacteria such as *E. coli* appears to be morphological damage to the cell membrane that results in cell lysis (Mendis et al., 2000). Cell lysis is partly a function of the irregularities of the outer membrane of the cell, with a smoother surface being less likely to undergo damage. *Bacillus subtilis* has a smooth outer membrane and was not damaged by the discharge, but a reduction in cell viability was observed suggesting the penetration of reactive species through the cell membrane may be possible (Laroussi et al., 2003). Effects upon the heterotrophic pathways of the bacteria explain the reduction in cell viability even though no visible damage to *B. subtilus* was observed (Laroussi et al., 2002).

Recently APPD has been shown to cause mortality in several species of insects and mites (Bures et al., 2003, 2004; Keever et al., 2001; Roe et al. 2003). Initial studies found a general increase in mortality with time after treatment with cold plasma (37° ± 2°C), suggesting irreversible damage occurs as a result of the discharge. These prior studies show that the helium atmosphere required to form a stable discharge and the temperature of the plasma have little to no effect on the mortality of the insects treated (Bures et al., 2004).

Since dielectric barrier discharges can chemically modify surfaces and break C-C bonds at energies >4.5eV (Ricard et al., 1999) one possible explanation for the insect mortality produced by APPD is damage to the hydrophobic waterproofing layer of the cuticle. Damage to the cuticle could cause water loss and eventual death (Gibbs, 2002; Lockey, 1976, 1991; Ramsay, 1935). Since the discovery of the use of APPD is novel, our knowledge of the mode of action of this method of insect control is minimal. The objective of the current study is to examine possible effects APPD might be having on the insect system.

2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

Methods and Materials

Device setup

Figure 1 shows a generalized setup of the Dielectric Barrier Discharge (DBD) device. The atmospheric pressure plasma discharge is generated between parallel electrodes in a helium atmosphere. The electrodes are composed of copper with a thin layer (0.793 mm) of Garolite Grade 7, a dielectric composite material, bonded to the electrodes. The Garolite is required to produce a uniform discharge at ambient pressure. Two adjustable pedestals support the electrodes. The pedestals allow the electrodes to be separated by variable distances (1.5-10.0 cm). For the experiments presented herein, the electrode gap was fixed at 5.0 cm. Heat generated by the electrodes during our studies is removed by circulating cooled distilled water through channels over the electrode surface. Distilled water is used to prevent the conductance of current from the electrode through the water to the cooling system used. The high voltage is supplied to the electrodes through a pair of high voltage transformers and is measured with two Tektronix P6015A high voltage probes. The current flowing to each electrode is measured through a 100 Ω resistor in series between the transformer and ground.

Since the discharge is generated in a helium atmosphere, an acrylic chamber is required to contain the helium around the parallel electrodes. The electrode pedestal supports pass through the acrylic chamber on the top and bottom. The chamber has an internal volume of 259 liters. A thick rubber glove is attached to the front panel of the chamber to allow the manipulation of samples. In addition to the glove and pedestal supports, high voltage connections, coolant lines, gas lines and other diagnostic ports penetrate the chamber. Commercial grade helium (HE291, Machine and Welding Supply, Inc) is supplied to the chamber at 34 slpm (standard liters per minute). The effluent of the chamber is measured using a helium gas monitor to determine when the atmosphere is composed primarily of helium. The helium gas monitor has a range of 0-100% helium in air with a resolution of 0.1%.

Once the atmosphere in the chamber is nearly 100% helium, the high voltage is applied which forms the APPD. The ambient gas temperature of the discharge is measured *in situ* with an electrically insulated K-type contact thermocouple. The insulation prevents plasma filaments, small lightning like features, from striking the thermocouple. The insulation can prevent plasma filaments from striking the thermocouple, but high voltage can still be conducted to the thermocouple readout so the readout electrically floats to ensure an accurate measurement of the discharge temperature.

For all treatments the frequency applied was 4 kHz and the power was 90 W.

Insects

Green peach aphids
For studies recording mortality after plasma treatment, green peach aphids, *Myzus persicae* (Sulzer), were obtained from a strain maintained on *Nicotiana tabacum* (tobacco) in a greenhouse at the Department of Entomology at NCSU (North Carolina State University), Raleigh, NC. This strain originated from a field caught population on tobacco in Clayton, NC. For cuticular hydrocarbon extraction, purification, and gas chromatography/mass spectroscopy, green peach aphids were obtained from a lab-reared strain maintained for >10 years on *Capsicum annuum* var. *annuum* (California Wonder bell pepper) at BASF Chemical Corporation, Research Triangle Park, NC.

Citrus mealy bugs
All citrus mealy bugs, *Planococcus citri* (Risso), were obtained from a lab-reared strain maintained on *Cucurbita moschata* (butternut squash) at the Department of Entomology at NCSU, Raleigh, NC. The strain was originally collected in 2002 from a green house located in Raleigh, NC.

German cockroaches
Adult male German cockroaches, *Blatella germanica* (L.), were obtained from a lab-reared strain maintained in the Department of Entomology at NCSU, Raleigh, NC. They 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*.

Plasma treatment

For all bioassays conducted in APPD, 25 insects were placed 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). This mesh does not interfere with the APPD. After the APPD treatments the insects were incubated in the plastic containers at 26° ± 2°C, 65 ± 4% relative humidity, and 14:10 (light:dark) photocycle. None of the insects used in the experiments were given access to food or water for the duration of the test. The insects were placed into the DBD chamber and the chamber was filled with helium at 34 slpm for 1 h. The control exposures were of two types: insects in the same containers held outside of the DBD chamber (not exposed to the 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". Afterwards, incubation of the controls was identical to the treatments. All experiments were conducted three times.

Green Peach Aphids
Adult apterous mixed sex aphids were transferred from the plant to the plastic containers with fine forceps or a camelhair brush. Aphids were exposed to the discharge for 10, 20, 40, 60, 90, and 120 s. Mortality was recorded 1, 3, 5, and 24 h after exposure to the discharge.

Citrus Mealy Bugs
Adult female citrus mealy bugs were transferred from the plant to the plastic containers with fine forceps. They were exposed to the discharge for 1, 2, 3, and 4 min. Mortality was recorded at 1, 3, 5, and 24 h after treatment in the discharge.

German Cockroaches
Adult male cockroaches were anesthetized with 20-30 s of carbon dioxide at atmospheric pressure and room temperature and then transferred to the plastic containers with fine forceps. They were exposed to the discharge for 60, 90, 120, and 180 s. Mortality was recorded at 1, 3, 5, and 24 h after treatment in the discharge.

Behavioral study with German cockroaches

Cockroaches were anesthetized with 20-30 s of carbon dioxide gas at atmospheric pressure and room temperature and then transferred to the plastic containers described earlier. Three treatments were studied: non-plasma non-helium controls, non-plasma helium controls, and 90 s plasma exposure. A treatment time of 90 s in the discharge was chosen because with this treatment the mortality was low and yet the behavior is still clearly affected. Immediately following treatment, their behavior was constantly monitored for the first 80 min and then at 2, 3, 4, 5 and 24 h post treatment. All cockroaches remained in the plastic containers for the duration of the test. Incubation conditions were 26° ± 2°C, 65 ± 4% relative humidity, 14:10 (light:dark) photophase. The roaches were not provided with food or water for during the bioassay. The experiment was repeated three times and in each case the results were identical.

Measurement of water loss

Cockroaches were anesthetized with 20-30 s of carbon dioxide as described before and twenty-five adult male cockroaches per sample were added to the plastic containers previously described. The insects were placed into the DBD chamber 30 min later and the chamber was filled with helium at 34 slpm for 1 h. Samples consisted of non-plasma non-helium controls, non-plasma helium controls, and cockroaches exposed to plasma for 60, 90, 120, and 180 s. 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 recorded on a Mettler scale (Toledo, Ohio) immediately before the containers were put into the DBD chamber and 24 h after treatment. During the 24 h after treatment the insects were incubated in the plastic containers at 26° ± 2°C, 65 ± 4% relative humidity, and 14:10 (light:dark) photocycle. Following the 24 h measurement the cockroaches and all fecal material were immediately stored at -80°C. An additional sample of 25 cockroaches used to calculate the water content of cockroaches at the beginning of the experiment was collected, weighed, and then stored at –80°C. The next day, the water was removed by lyophilization (Bench Top 6, Virtis, Gardiner, NY; cold trap = -70°C, ≈200 mTorr, ambient temperature = 23°C) for a minimum of 24 h (Bailey et al., 2001). The weight of the freeze-dried insects and fecal material was recorded. 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 composition of cockroaches at 0 h 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. The water weight loss was calculated as the wet weight in mg at 0 h multiplied by the average percent water composition of cockroaches at 0 h, minus the wet weight in mg at 24 h multiplied by the average percent water composition of the cockroaches 24 h after treatment. The dry weight loss was calculated as the wet weight in mg at 0 h multiplied by the average percent dry composition of cockroaches at 0 h, minus the wet weight in mg at 24 h multiplied by the average percent dry composition of the cockroaches 24 h after treatment.

Cuticular hydrocarbon extraction, purification, and gas chromatography/mass spectroscopy

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.

Green Peach Aphids
Non-plasma, non-helium controls and 120 s plasma exposed aphids were used for hydrocarbon analysis 24 hours after treatment. We chose to analyze the hydrocarbons of aphids that were exposed to the discharge for 120 s because this was the longest treatment time in APPD. Ten live adult apterous mixed sex aphids per sample were gently washed for 5 min with 1 ml of optima hexane (Fisher Scientific, Pittsburgh, PA) containing 0.6 µg 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 resulting 1 ml extracts from each sample was transferred to the top of a disposable Pasteur pipette (7cm x 0.5cm 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 µl of hexane. Aliquots of 1 µl were injected into the GC-MS and eluted with the following temperature program: 50 to 180° at 40°C per min, increase to 320° at 8°C per min, and hold for 5 min. The method was adapted from Clements et al. (2000). Exposure to the helium atmosphere was previously found to have no effect on the cuticular hydrocarbons.

Citrus Mealy Bugs
Non-plasma, non-helium controls and 4 min plasma exposed adult female citrus mealy bugs were used for hydrocarbon analysis. We chose to analyze the hydrocarbons of mealy bugs that were exposed to the discharge for 4 min because this was the longest treatment time in APPD. After treatment in the discharge and prior to hydrocarbon extraction, the insects were stored overnight at –20°C for 24 h. Freezing the insects prior to hydrocarbon extraction has no effect on their abundances (Young and Schal, 1997). Ten adult female mealy bugs per sample were gently washed for five min in 1 ml of optima hexane containing 1 µg of n-tetratriacontane internal standard in a 1 dram glass vial fitted with a Teflon lined cap. The resulting 1 ml extracts from each sample was transferred to the top of a disposable Pasteur pipette, packed with 100-200 mesh heat activated silica gel. The cuticular hydrocarbons were eluted with 7 ml of hexane and then reduced just to dryness by low heat under a slow stream of nitrogen gas; the sample was then resuspended immediately in 100 µl of hexane. Aliquots of 1 µl were injected into the GC-MS with the following temperature program: 50 to 320°C at 10° per minute, and hold for 5 minutes.

The peak area of each individual hydrocarbon in each of the samples of green peach aphids and citrus mealy bugs was recorded. In each sample, dividing the peak area of each individual hydrocarbon by the peak area of the internal standard (n-tetratriacontane) normalized the data, herein referred to as relative abundance.

Protein analysis

Immediately following the extraction of cuticular hydrocarbons from green peach aphids and citrus mealy bugs, the same samples were analyzed for total protein content. The results were used to calculate the relative abundance of

each cuticular hydrocarbon per mg of total protein in order to compensate for variations in insects mass. Ten green peach aphids per sample or 10 mealy bugs per sample were each homogenized separately after hydrocarbon extraction, in 100 µl of distilled water. Appropriate dilutions (10 µl total volume) of each homogenate were added to individual wells of a flat-bottom (96 well) microtiterplate (Becton Dickinson, Franklin Lakes, NJ) followed by 200 µl of 1:4 diluted Bio-Rad dye reagent (Hercules, CA). Optical density was measured with a SpectraMax 384 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) and the protein concentration in each sample determined by comparison to a bovine serum albumin (fraction 5, biotech grade, Fisher, Fairlawn, NJ) standard curve.

Metabolic activity

The rate of oxygen consumption at STP was used as a measure of overall insect metabolic activity. Cockroaches were either non-plasma helium controls or plasma exposed for 180 s. Fourteen insects were used in the controls and 12 were used in the treated samples. The treatment time of 180 s was chosen because it was the longest treatment time used in the mortality bioassays. None of the insects died during the course of the experiment. Food and water was not provided during the experiment. Oxygen consumption of the non-plasma helium controls and 180 s plasma exposed roaches was measured 1h after APPD exposure in a Gilson differential respirometer (Middleton, WI). Each channel (respirometer flask) contained two roaches. Immediately after the measurement of oxygen consumption, the weight of the two roaches was measured, and the results were normalized to ml of oxygen consumed at STP per mg animal per h. The assay was repeated twice.

Results and Discussion

Device setup

Figure 1 shows the basic setup of the DBD. The APPD is formed in the location labeled “plasma bulk”. Once a stable discharge is formed, the plastic containers are loaded into the APPD on a sliding drawer. During treatment the samples are completely immersed in the APPD.

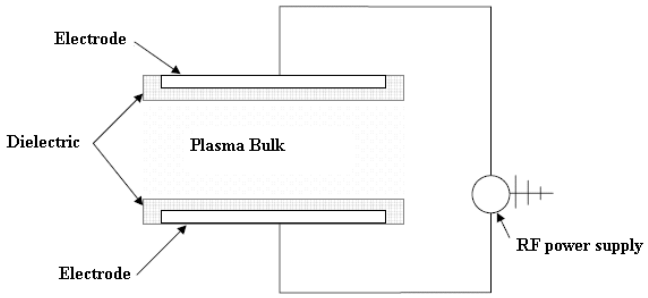

Figure 1. Diagram of DBD device.

Mortality of German cockroaches, citrus mealy bugs, and green peach aphids exposed to APPD

Figure 2 shows the percent mortality (± 1 standard error of the mean, SEM) of cockroaches, mealy bugs, and aphids after a 120 s exposure to APPD. Aphids were most susceptible to the discharge while no differences (t-test, α=0.05) were found between mealy bugs and cockroaches. Increasing the exposure time to APPD increases percent mortality in insects (Bures et al., 2004).

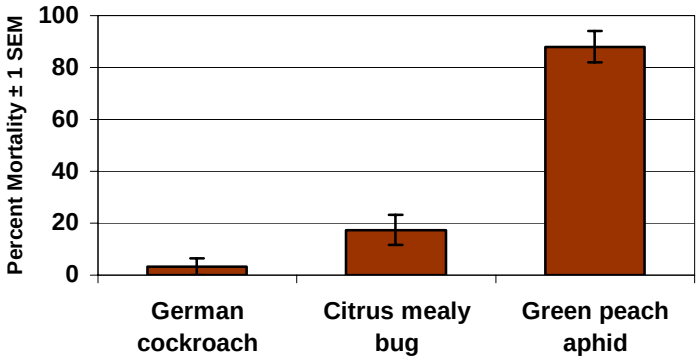

Figure 2. Percent mortality at 24 h of German cockroaches, citrus mealy bugs, and green peach aphids exposed to the APPD for 120 s. There was no mortality in the controls of cockroaches or mealy bugs; in aphids there was 1.33 ± 1.33% (1 SEM) mortality in non-plasma, non-helium controls and 2.67 ± 1.33% mortality in non-plasma helium controls. Abbot's correction was not used for this data.

Effects of APPD on the behavior of German cockroaches

The behavior of German cockroaches was monitored after exposure to APPD. The symptoms of neuromuscular disruption in German cockroaches are easily characterized making them a classical model used for behavioral studies. Table 1 shows that cockroaches exposed to the discharge for 90 s have altered behavior. Twenty-four h after exposure they continued to show a lack of full motor control suggesting that APPD can disrupt normal function of the neuromuscular system.

Table 1. Characterization of the behavior of *Blatella germanica* exposed to APPD

Time After Treatment	Non-plasma, non-helium control	Non-plasma, helium control	Plasma, 90 s
During Treatment	Normal behavior	Anesthetized	Legs twitch while in discharge, streamers collect on tips of appendages, wings of two roaches immediately point perpendicular to and above the thorax, antennae disfigured on 1/3 of the roaches
10-15 min	Normal behavior	All roaches able to right themselves, respond to tactile stimuli	Only move mouthparts when probed on ventral side of the thorax, no response to tactile stimulus of legs or antennae
15-20 min	Normal behavior	Normal behavior	Ataxia, abdominal and thoracic muscles contracting
20-30 min	Normal behavior	Normal behavior	Constant grooming of antennae, unable to walk, cannot move hind legs, no response to vibration or light, not thigmotactic, 50% cannot right themselves, responsive to general tactile stimuli
30-80 min	Normal behavior	Normal behavior	Partial response to vibration and light, no control of hind legs, hind legs extended at ~45° angles to the body
5 h	Normal behavior	Normal behavior	No change in behavior from 80 minute time point, other than all were able to right themselves
24 h	Normal behavior	Normal behavior	Uncoordinated leg movement, none regained full motor control of appendages, not thigmotactic
Normal behavior is defined as responsive to tactile stimuli, vibrations, and light; normal thigmotaxis; ablility to right themselves; full motor control of legs, antennae, and mouthparts.			

Water loss of German cockroaches after APPD exposure

Table 2 shows the wet weight, water weight, and dry weight loss of cockroaches after exposure to APPD. The 120 s and 180 s plasma exposed cockroaches lost a significantly greater amount of wet and water weight than non-plasma, non-helium controls and non-plasma helium controls (p-value < 0.05, t-test). Dry weight loss was not significantly different. It should be noted that all insects were first anesthetized with a 20-30 s exposure to carbon dioxide, one h prior to the beginning of the test in order to place the cockroaches into each plastic container previously described. While exposure to carbon dioxide is known to reduce the metabolic rate of insects, Woodring et al. (1978) found that exposures to CO_2 less than 1 min did not decrease the metabolic rate of larval house crickets.

Table 2. Average weight loss (mg) in German cockroaches, 24 h after exposure to APPD (± 1 SEM). The 120 and 180 s treatments differ significantly (p-value < 0.05, t-test) from non-plasma, non-helium controls and non-plasma helium controls.

Treatment	Wet Weight Loss (mg)	Water Weight Loss (mg)	Dry Weight Loss (mg)
Non-plasma, non-helium control	4.50 ± 0.86	3.12 ± 0.86	1.38 ± 0.86
Non-plasma helium control	6.59 ± 0.86	4.91 ± 0.86	1.68 ± 0.86
60 s	9.30 ± 1.19	7.53 ± 1.19	1.77 ± 1.19
90 s	9.68 ± 0.91	8.08 ± 0.91	1.60 ± 0.91
120 s	10.64 ± 0.66	8.69 ± 0.66	1.96 ± 0.66
180 s	12.75 ± 1.68	10.14 ± 1.68	2.62 ± 1.68

Interaction of APPD with cuticular hydrocarbons

Tables 3 and 4 show the hydrocarbon abundances of green peach aphids and citrus mealy bugs, respectively. The abundance of cuticular hydrocarbons in green peach aphids and citrus mealy bugs was not significantly affected by exposure to APPD (p-value > 0.05, t-test) except for n-tritriacontane in mealy bugs. The cuticular hydrocarbons of German cockroaches were also found to have no significant differences in their abundances a result of a 120 s APPD exposure (data not shown).

The average of each of the individual hydrocarbon abundances of green peach aphids was reduced by at least 35%, and aphid mortality was 88 ± 6.18% (1 SEM). In mealy bugs, average hydrocarbon abundances were unchanged except for n-dotriacontane and n-tritriacontane which were reduced by 40% and n-heptatriacontane by 25%. The mealy bug mortality was 17.33 ± 5.81% (1 SEM). While these reductions were not statistically significant (except for n-tritriacontane in mealy bugs), they may have biological significance in terms of inducing mortality.

Table 3. Relative abundance ± 1 SEM of cuticular hydrocarbons per mg of total protein of green peach aphids exposed to APPD for 120 s.

Equivalent Chain Length	Compound Name	Non-plasma, non-helium control Relative abundance ± 1 standard error	120 s APPD exposure Relative abundance ± 1 standard error
25	n-Pentacosane	35.35 ± 5.90	19.31 ± 5.43
26	n-Hexaconsane	43.96 ± 11.51	24.43 ± 6.99
27	n-Heptacosane	49.33 ± 6.99	31.02 ± 7.36
28	n-Octacosane	25.52 ± 11.86	12.50 ± 2.99
29	n-Nonacosane	63.09 ± 12.01	40.83 ± 4.01
30	n-Triacontane	27.18 ± 7.63	17.29 ± 4.13
31	n-Hentriacontane	132.74 ± 23.69	73.93 ± 6.96
33	n-Tritriacontane	36.20 ± 6.57	23.30 ± 5.31

Table 4. Relative abundance ± 1 SEM of cuticular hydrocarbons per mg of total protein of citrus mealy bugs exposed to APPD for 4 min.

Equivalent	Compound Name	Non-plasma, non-helium control	4 min APPD exposure

Chain Length		Relative abundance ± 1 standard error	Relative abundance ± 1 standard error
24	n-Tetracosane	3.27 ± 0.59	3.48 ± 0.47
25	n-Pentacosane	1.85 ± 0.40	1.95 ± 0.28
26	n-Hexaconsane	1.52 ± 0.16	1.69 ± 0.43
29	n-Nonacosane	9.23 ± 1.41	12.96 ± 0.98
30	n-Triacontane	2.38 ± 0.80	3.28 ± 0.79
31	n-Hentriacontane	6.07 ± 0.19	6.80 ± 0.68
32	n-Dotriacontane	4.37 ± 2.11	2.42 ± 1.06
33	n-Tritriacontane	14.11 ± 1.46	8.13 ± 0.49
35	n-Pentatriacontane	11.34 ± 0.88	11.71 ± 0.38
37	n-Heptatriacontane	15.73 ± 1.04	11.78 ± 1.13

Change in the metabolic rate

The metabolic rate of cockroaches exposed to APPD for 180 s was 1.07 ± 0.04 ml (1 SEM) of oxygen consumed per mg insect per h. This was significantly greater than the non-plasma helium control, which was 0.79 ± 0.3 ml of oxygen consumed per mg insect per h (p-value < 0.05, t-test). It is unclear whether the changes in behavior presumed to be from an interaction of the neuromuscular system and APPD is related to the increased oxygen consumption in cockroaches.

Summary

The use of APPD for insect control could be a viable alternative to chemical insecticides or methyl bromide fumigation, useful in greenhouse agricultural production, quarantine, and treatments of stored products or packaging materials. Behavioral changes after APPD exposure in cockroaches suggest that the plasma mode of action may be disruption of the nervous or neuromuscular system. There was a trend toward the individual reduction of all cuticular hydrocarbon abundances in aphids but not mealy bugs. In cockroaches the APPD caused a significant amount of water loss and an increase in the metabolic rate.

Acknowledgments

The authors would like to 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, and Dr. Coby Schal and Rick Santangelo for supplying German cockroaches. Additionally, Dr. Deborah Thompson, Dr. Charles S. Apperson, and Dr. Sayed M. S. Khalil 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

Bailey, W. D., C. Brownie, J. S. Bacheler, F. Gould, G. G. Kennedy, C. E. Sorenson, and R. M. Roe. 2001. Species diagnosis and *Bacillus thuringiensis* resistance monitoring of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) field strains from the Southern United States using feeding disruption bioassays. J. Econ. Entomol. 94(1): 76-85.

Bergman, D. K., J. W. Dillwith, R. K. Campbell, and R. D. Eikenbary. 1990. Cuticular hydrocarbons of the Russian wheat aphid. Southwest. Entomol. 15(2): 91-100.

Buehlmann, G. 1975. The effect of carbon dioxide anesthesia and cold exposure on the ovarian growth of the cockroach *Nauphoeta cinerea*. Rev. Suisse Zoologie 82(4): 676-679.

Bures, B., K. V. Donohue, S. Long, M. A. Bourham, and R. M. Roe. 2004. Mortality of Insects on the Surface of Plants Using an Atmospheric Pressure Plasma Discharge. *In* Proceedings Beltwide Cotton Conferences. National Cotton Council, San Antonio, TX. pp. 1518-1523.

Bures, B., T. Gray, M. A. Bourham, R. M. Roe, S. Long, and K. V. Donohue. 2003. Reaction of Small Insects Exposed to an Ambient Pressure Dielectric Barrier Discharge. *In* Proceedings of APS-Division of Plasma Physics Conference. Bull. Am. Phys. Soc. Vol. 48, No. 7, Paper LP1 116 Albuquerque, NM Oct 27-31, 2003. pp. 239.

Clements, K. M., C. E. Sorenson, B. M. Wiegmann, P. A. Neese, and R. M. Roe. 2000. Genetic, biochemical, and behavioral uniformity among populations of *Myzus nictonianae* and *Myzus persicae*. Entomol. Exper. Appl. 95: 269-281.

Efremov, N. M., B. Y. Adamiak, V. I. Blochin, S. J. Dadshev, K. I. Dmitriev, O. P. Gryaznova, and V. F. Jusbashev. 2000. Action of a self-sustained glow discharge in atmospheric pressure air on biological objects. IEEE Trans. Plasma Sci. 28(1): 238-241.

Environmental Protection Agency. 2004. Protection of Stratospheric Ozone: Process for Exempting Critical Uses from the Phaseout of Methyl Bromide; Final Rule. *In* Federal Register [December 23, 2004], Part II, Environmental Protection Agency, 40 CFR Part 82. Fed. Register. 69(246): 76981-77009.

Gibbs, A. G. 2002. Lipid melting and cuticular permeability: new insights into an old problem. J. Ins. Physiol. 48:391-400.

Hebanowska, E., E. Maliński, J. Nawrot, M. Ruszkowska, K. Pihlaja, and J. Szafranek. 1989. The composition of cuticular hydrocarbons of the cereal aphid *Sitobion avenae* F. (Homoptera, Aphididae). Comp. Biochem. Physiol. 94B(4): 723-727.

Keever, D. W., A. K. Dowdy; B. L. Bures, O. E. Hankins, and M. A. Bourham. 2001. Mortality and sterility of the cigarette beetle, *Lasioderma serricorne* (F.), due to exposure to atmospheric plasma. Proc. Methyl Bromide International Conference, San Diego, CA, Paper 128. November 2001.

Laroussi, M. 2002. Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects. IEEE Trans. Plasma Sci. 30(4): 1409-1415.

Laroussi, M. I. Alexeff, and W. L. Kang. 2000. Biological decontamination by nonthermal plasmas. IEEE Trans. Plasma Sci. 28(1): 184-188.

Laroussi, M, D. A. Mendis, and M. Rosenberg. 2003. Plasma interaction with microbes. New J. Phys. 5:41.1-41.10.

Laroussi, M., J. P. Richardson, and F. C. Dobbs. 2002. Effects of non-equilibrium atmospheric pressure plasmas on the heterotrophic pathways of bacteria and on their cell morphology. Appl. Phys. Lett. 81(4):772-774.

Laroussi, M., G. S. Sayler, B. B. Glascock, B. McCurdy, M. E. Pearce, and C. M. Malott. 1999. Images of biological samples undergoing sterilization by a glow discharge at atmospheric pressure. IEEE Trans. Plasma Sci. 27(1): 34-35.

Lockey, K. H. 1976. Cuticular hydrocarbons of *Locusta*, *Schistocera*, and *Periplaneta*, and their role in waterproofing. Ins. Biochem. 6: 457-472.

Lockey, K. H. 1991. Insect hydrocarbon classes: implications for chemotaxonomy. Insect Biochem. 21(1): 91-97.

Mendis, D. A., M. Rosenberg, and F. Azam. 2000. A note on the possible electrostatic disruption of bacteria. IEEE Trans. Plasma Sci. 28(4): 1304-1306.

Montie, T. C., K. Kelley-Winterberg, and J. R. Roth. 2000. An overview of research using the one atmosphere uniform glow discharge plasma (OAUGDP) for sterilization of surfaces and materials. IEEE Trans. Plasma Sci. 28(1):41-50.

Ramsay, J. A. 1935. The evaporation of water from the cockroach. J. Exp. Biol. 112:373-383.

Roe, R. M., M. A. Bourham, S. Long, and B. L. Bures. 2003. Use of atmospheric plasma for insect control. Proc. Beltwide Cotton Conference. Nashville, TN, Jan 6-8, 2003. pp. 1150-1156.

Ricard, A., P. Décomps, and F. Massines. 1999. Kinetics of radiative species in helium pulsed discharge at atmospheric pressure. Surf. Coat. Tech. 112:1-4.

Woodring, J. P., C. W. Clifford, R. M. Roe, and B. R. Beckman. 1978. Effects of CO_2 and anoxia on feeding, growth, metabolism, water balance, and blood composition in larval female house crickets, *Acheta domesticus.* J. Insect Physiol. 24: 499-509.

Young, H. P. and C. Schal. 1997. Cuticular hydrocarbon synthesis in relation to feeding and developmental stage in nymphs of *Blatella germanica* (Dictyoptera: Blatellidae). Ann. Entomol. Soc. Amer. 90(5): 655-663.