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# USE OF ATMOSPHERIC PLASMA FOR INSECT CONTROL

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## Abstract

This paper presents the first evidence for the potential of non-thermal atmospheric plasma to control agriculturally important insects. Direct plasma exposure was effective in the control of neonates of the tobacco budworm on plastic surfaces, with 85% mortality in a 5 sec exposure. However, the treatment was significantly less effective when the insects were on artificial diet or cotton leaves, suggesting that additional studies are needed to fully understand the interaction between the insects and plasma. However, the atmospheric plasma under these standard conditions was highly effective in the control of aphids and thrips on plants. A 40 sec exposure of green peach aphids on tobacco leaves produced greater than 90% mortality. These studies showed that the plasma effect was selective, and mortality was not the result of changes in the leaf. Mortality of Western flower thrips on snap beans was greater than 95% in 5 sec. These studies suggest that atmospheric plasma may be used as a non-chemical and non-transgenic method of insect control in a variety of possible practical applications.

## Introduction

Traditional methods of insect control in agriculture include doing nothing until an economic threshold for damage is reached, cultural methods that minimize pest populations, the enhancement of insect biocontrol, transgenic plant technologies like Bt cotton, and chemical sprays, for example, organophoshates, pyrethroids, spinosyns and many others (Rose et al., 1999). Transgenic plants and chemical insecticides have become an essential and reliable part of most agricultural production systems and provide an important economic advantage to the farmer. With any control method, there is always the concern about insect resistance and the need for the development of new mechanisms of insect control. In addition, there have been concerns about the release of transgenic organisms and chemicals into the environment. Whether these concerns are valid or not, they exist in the general public. We have been interested in examining whether there are any other approaches to insect control than those listed to date.

There have been a number of studies on the use of atmospherically generated plasmas as a decontamination and sterilization method (Laroussi et al., 2000; Montie et al., 2000; Moisan et al., 2001). Plasma is a collection of ions, electrons and neutral species showing collective behavior (Chen, 1984). High-pressure plasma discharges have been used for many years, but the discharges are commonly thermal plasma or non-uniform corona discharges. The thermal plasmas are ideal for the metal industry, depositing of films, and coatings and waste incineration. In thermal plasma, the ions, the electrons and the neutrals species have similar temperatures. It is common for these plasmas to have temperatures in excess of 10,000 degrees K. In corona discharges, a few distinct filaments of plasma are generated from pointed surfaces. The electron temperature, the ion temperature, and the neutral species temperatures are ~10,000 degrees K, ~1000 degrees K, and ~300 degrees K, respectively (Roth, 1995). Since the density of ions and electrons is low, the neutral species mainly contribute to the thermal energy of these discharges.

Kanazawa et al. (1988) reported the development of a high-pressure plasma discharge that was non-thermal and uniform. Non-thermal atmospheric plasma has become a proven technique for high quality sterilization for many different types of surfaces (Herrmann et al., 1999; Kelly-Wintenberg et al., 2000; Laroussi et al., 2000; Montie et al., 2000; Roth et al., 2000). The two techniques available for using plasma are direct exposure and remote exposure. In direct exposure, the electrode gap where the plasma is generated must be sufficiently large to allow direct sample introduction. For most cases of interest in bacteria or virus sterilization, the samples have small dimensions and a few centimeters would be sufficient for samples exposure. A larger plasma volume with a greater distance between electrodes (> 10 cm) would be needed for many agricultural applications, especially those involving mature plants. For remote exposure, reactive gases are fed into the plasma and broken down into reactive chemical species ($O^+$, $O_3$, $O^-$, NO, etc). The reactive species are sprayed onto the substrates of interest (Hermann et al., 1999; Roth et al., 2000). For medical instruments and other metallic surfaces, remote exposure appears to be the best method for sterilization (Roth et. al., 2000). However, the reactive chemical species produced by remote exposure may be detrimental to living plants. For this reason, samples were directly exposed to the plasma in our studies.

Most non-thermal atmospheric pressure plasma is generated in narrow gaps (<3 cm) because the voltage requirements for these discharges are substantial (~ 30 kV/cm in air). In order to form atmospheric plasma at reduced voltages, inert gases are mixed with air to reduce the necessary breakdown voltage (~ 7 kV/cm in He/Air). Inert gases mixed with air also serve to reduce the concentration of undesirable reactivate species ($O_3$, $O^+$, etc) while providing high-energy metastable atoms. The metastable atoms have the ability to produce additional free electrons, which are a key component of the plasma. In this paper, we present the first use of non-thermal atmospheric plasma for the control of the tobacco budworm, the green peach aphid and Western flower thrips.

## Materials and Methods

### Plasma Device

The devices that generate non-thermal atmospheric plasma come in a variety of sizes, electrode configurations and operating frequencies (Kanazawa et al., 1988; Herrmann et al., 1999; Kelly-Winteberg et al., 2000; Laroussi et al., 2000; Montie et al., 2000; Roth et al., 2000; Moisan et al., 2001). For the experimental setup in this paper, a parallel plate configuration was chosen with 5.0 cm electrode separation and an applied potential greater than 10 kV at acoustic frequencies (Figure 1). The instrument is housed in an acrylic enclosure in order to contain the carrier gas. Helium gas was injected between the electrodes, at a constant flow rate, to reduce the breakdown requirements and formulate the desired plasma. The helium displaces some of the air, but does not completely eliminate the air.

The electrodes (16 cm by 20 cm) are pressed against acrylic sheets (5 mm thick). The acrylic acts to isolate the electrodes from the plasma and create a dielectric barrier discharge. Since acrylic is a dielectric, it prohibits the transition from a non-thermal discharge to a thermal arc (Chen, 1984; Roth, 1995). The transition to arc focuses the plasma from a diffuse medium that fills the volume between the electrodes into a localized arc between the electrodes (Chen, 1984; Roth, 1995). The localized arc is thermal plasma so it is capable of damaging plants. The specifications for the instrument and the actual conditions used for the generation of atmospheric plasma are provided in Table 1.

### Tests with the Tobacco Budworm

Neonates of the tobacco budworm, *Heliothis virescens* (Hv97 strain), were obtained from the insectary in the Department of Entomology at North Carolina State University. Ten-1 day old (from egg hatch) neonates were placed onto standard budworm artificial diet (Burton, 1970) in a 29.6 ml Solo plastic rearing cup (Solo Cup Company, Urbana, IL), into a Solo cup without diet or on a leaf of the cotton plant in a plastic petri dish (4 cm diameter); and then exposed to a plasma field for 0, 5, 10, 20, 40 or 60 sec. Three replicates were conducted per treatment. After exposure, insects were transferred to standard artificial diet in 29.6 ml Solo plastic rearing cups and reared at 27 degrees C, 50% relative humidity and a photoperiod of 14h light: 10 h dark. Mortality (no movement when touched with a blunt probe) was assessed at 24 h after treatment and survivors were reared to adulthood.

Experiments were also conducted with older budworms. Ten-third instar tobacco budworms were transferred to a 29.6 ml Solo plastic cup, which was then covered with a matching plastic lid containing 50-60 pinholes. The perforated lid did not appear to hinder the filling of the diet cup with plasma. Three replicates were conducted per treatment. After exposure to the plasma field for 0, 10, 20, 40 and 60 sec, the insects were transferred onto standard artificial diet in 29.6 ml Solo cups (1 insect per cup) and reared at 27 degrees C, 50% relative humidity, and a photoperiod of 14 h light: 10 h dark. Mortality was assessed at 48 h after treatment, and survivors were reared to adulthood.

### Tests with the Green Peach Aphid

Green peach aphids, *Myzus persicae*, were obtained from the Department of Entomology at North Carolina State University. These aphids are routinely maintained on tobacco in the green house.

*Insect-Leaf Treatment (ILT)*. A tobacco leaf containing green peach aphids was cut from the whole plant, the end of the stem cut into a point, and the stem inserted into a cylindrical foam pad (7 cm diameter, 4.5 cm height, with a small hole in the center) in a container (9 cm bottom diameter, 11 cm top diameter, 4.5 cm height) containing tap water. Each leaf contained 40 to 320 aphids. When the aphids on the tobacco leaf were placed into the plasma field, the leaves were transferred into holes on a square cardboard sheet (8 cm by 10 cm). This method held the leaves in an upright position in the plasma field. After exposure, the leaves were returned to the wetted foam pad for incubation.

*Leaf Treatment (LT)*. In this assay, a leaf without aphids was exposed to the plasma then insects were placed onto this leaf and incubated.

*Insect Treatment (IT)*. For this assay, the insect is exposed to the plasma on a leaf, a fragment of this leaf containing aphids placed on a leaf not exposed to the plasma, and then incubated.

After treatment under the above described conditions (*ILT*, *LT* and *IT*), the insects were incubated at 27°C, 50% relative humidity and a photoperiod of 14 hours light: 10 hours dark. Three replicates were conducted for each treatment. The plasma exposure times are given in the Results and Discussion. Mortality was assessed at 24 hours. Mortality in these experiments was defined as lack of movement when touched with a camelhair brush.

**Tests with the Western Flower Thrips**

Western flower thrips, *Frankliniella occcidentalis*, were supplied by the Department of Entomology at North Carolina State University. Thrips on snap beans (90-120 larvae per bean) were wrapped with nylon organdy cloth and then exposed for 0, 5, 10, 20, and 40 sec to plasma. Two replicates were conducted for each treatment. After exposure, each bean was separated from the organdy cloth and placed into the bottom of a plastic container (21 cm top diameter, 18 cm bottom diameter, 16 cm in height); about half of the surface area of the container bottom had been previously replaced with organdy cloth for increased ventilation. The top of the container is sealed with the nylon organdy cloth that was used to wrap the bean. The insects were incubated at room temperature, and mortality determined at 24 hours. Insects were considered dead if they did not move in 2-5 sec.

## Results and Discussion

The control data reported in this paper represent insects that were placed inside the acrylic enclosure but not into the plasma field. No differences in percent mortality were noted for insects placed in the acrylic enclosure (but not in the plasma) and those held just outside the chamber (data not shown). All mortality data reported except for that of the control (zero time exposed) were corrected using Abbot's formula.

The plasma was most effective when neonates were treated on a plastic surface. Mortality was 85% at 5 sec and reached 100% at 20 sec (Figure 2). Insects on artificial diet were less susceptible, with mortality at 5 sec of 35%, 45% at 10 sec and 90% at 20 sec (Figure 3). Neonates on cotton leaves were the least susceptible to atmospheric plasma; mortality was about 50% for a 20 sec exposure (Figure 4). All surviving insects in these experiments (24 h after plasma exposure) successfully pupated and became adults. When insects were placed on artificial diet in plastic cups or on a cotton leaf in a plastic petri dish, it was not possible to limit the insect to the diet or leaf surface. Therefore, it is possible that the mortality observed in these treatments (Figures 3 and 4) may be the result of the insects moving to the plastic surface. Although it was clear that the plasma is effective causing high mortality when neonates are on plastic, further studies will be needed relative to the mechanism of action and field characteristics to understand the reason for reduced mortality on the other surfaces tested. This is critical to the usefulness of atmospheric plasma in practical applications for the control of budworm neonates. The results of plasma treatments on third instars of the tobacco budworm on plastic are shown in Figure 5. Mortality was only 13% at 20 sec and 33% at 60 sec.

It was clear from the studies conducted that atmospheric plasma was highly effective producing 92% mortally in 40 sec on green peach aphids feeding on tobacco leaves (Figure 6). This mortality is apparently not the result of the leaf's exposure to the plasma. When leaves were exposed to the plasma and then aphids transferred to the exposed leaf, mortality was low (LT-40 and LT-60, Figure 7). When aphids were exposed on leaves to atmospheric plasma and then transferred to unexposed leaves, mortality was high on the unexposed leaves (IT-60, Figure 7). These experiments (Figures 6 and 7) indicate that atmospheric plasma was an effective method for the control of the green peach aphid on tobacco under the experimental conditions of our tests.

The non-thermal atmospheric plasma was highly effective in the control of Western flower thrips on snap beans under the conditions of our assay. Greater than 95% mortality was noted in 5 sec (Figure 8).

## Acknowledgments

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Table 1. Specifications and experimental conditions for the device used for the generation of non-thermal atmospheric plasma.

| Parameter | Device Specifications | Actual Experimental |
|---|---|---|
| Peak Voltage | 0-25 kV | 16-20 kV |
| Peak Current | 0-120 mA | 20-40 mA |
| Peak Power | 0-500W | ~500W |
| Frequency Range | 2-15 kHz | 8-10 kHz |
| Electrode Area | 320 $cm^2$ (16 cm by 20 cm) | 320 $cm^2$ (16 cm by 20 cm) |
| Electrode Gap | 2.0-8.0 cm | 5.0 cm |
| Carrier Gases | He, Ar | He |
| Other Gases | Air, $N_2$, $H_2$, $O_2$ | Air |
| Barrier Material | acrylic (5 mm), Garolite G-7 (0.79 mm) | acrylic (5 mm) |
| Electrode Material | Cu | Cu |

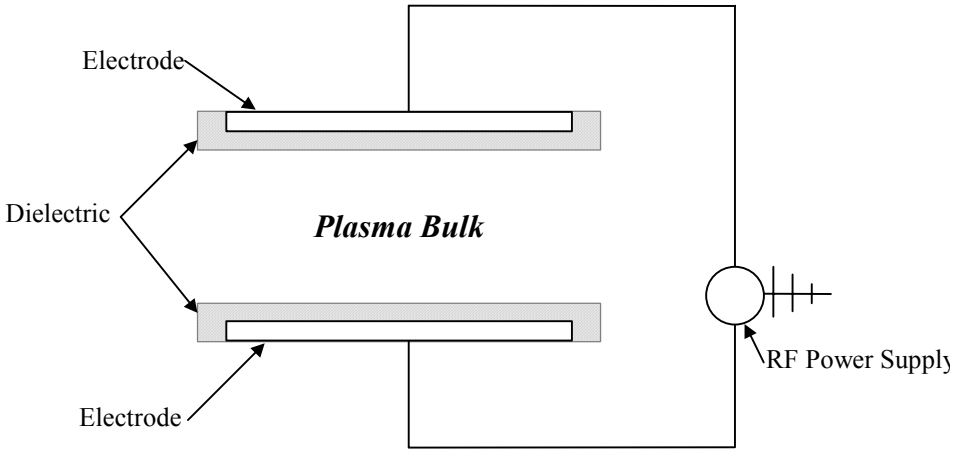


Figure 1. Schematic of device for the generation of atmospheric plasma.

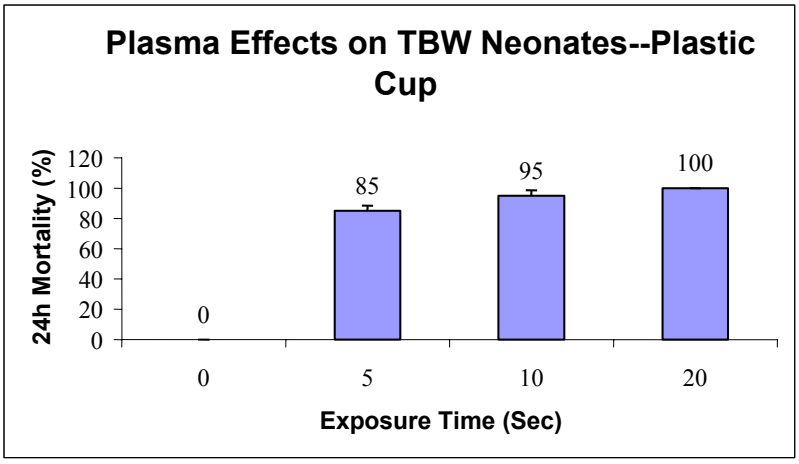


Figure 2. Effect of low temperature, atmospheric plasma on mortality of neonates of the tobacco budworm (TBW) on plastic.

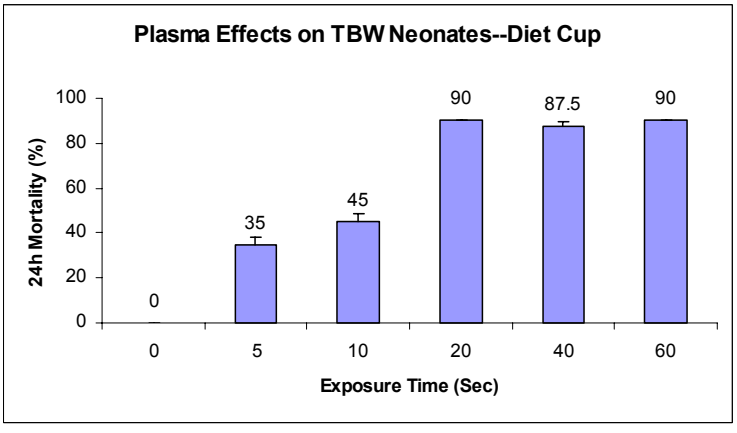


Figure 3. Effect of low temperature, atmospheric plasma on mortality of the tobacco budworm (TBW) on artificial diet.

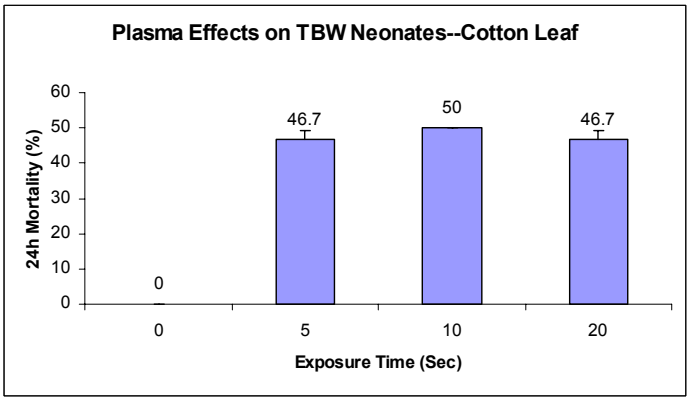


Figure 4. Effect of low temperature, atmospheric plasma on mortality of the tobacco budworm (TBW) on cotton leaves.

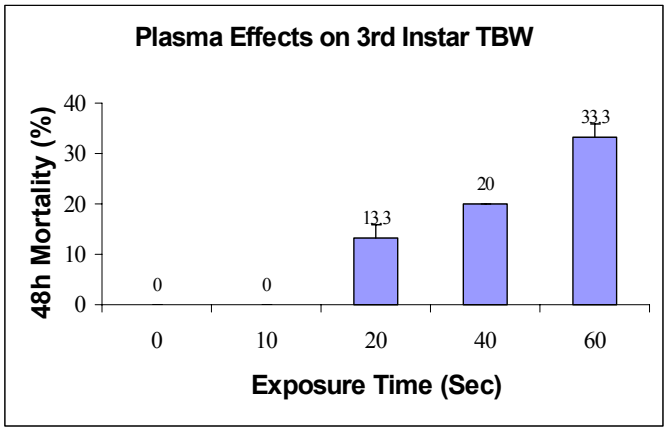


Figure 5. Effect of low temperature, atmospheric plasma on mortality of the tobacco budworm (TBW)(3rd instars).

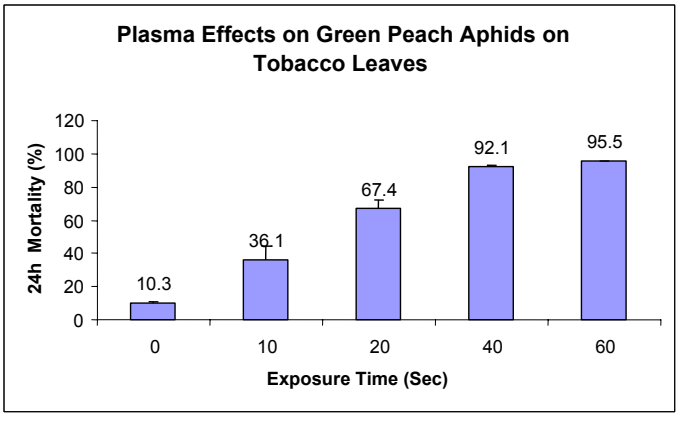


Figure 6. Effect of low temperature, atmospheric plasma on green peach aphids on tobacco leaves.

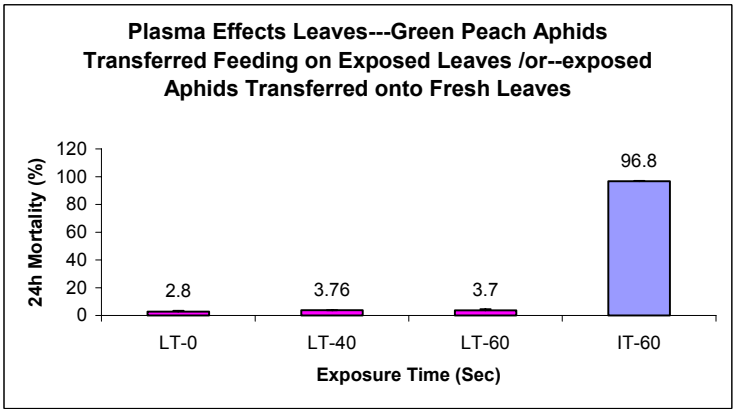


Figure 7. Effect of low temperature, atmospheric plasma on the green peach aphid versus the host plant. LT, leaf treated; IT, insect treated.

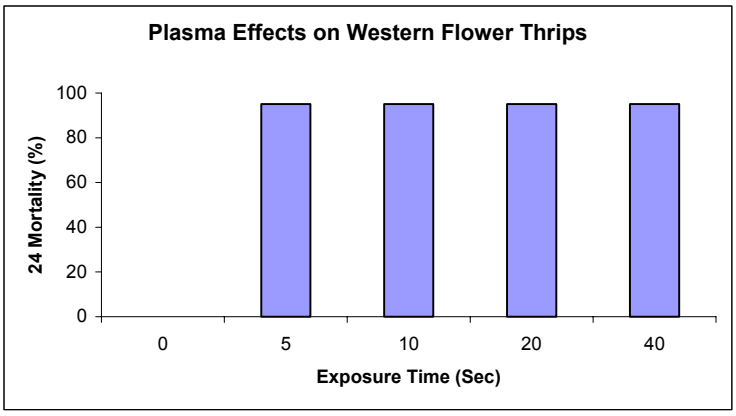


Figure 8. Effect of low temperature, atmospheric plasma on Western flower thrips on snap beans.