RESEARCH ARTICLE

EFFECT OF JASMONIC ACID ON THE HERBIVORY AND OVIPOSITION OF Neoleucinodeselegantalis (Guenée) IN Solanumquitoense (Lam) PLANTS.

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Abstract

Plants can respond to exogenous natural factors that induce resistance to herbivory. To use the agriculture products that can generate responses in plants we must know the effects they produce. The foliar application of jasmonic acid (JA) increased the levels of polyphenol oxidase, an enzyme that occurs in the defense against several herbivorous insects. The plants in which it was used (AJ) as an inducer had 60% less leaf damage than the control treatment plants. The effects of this induction on seedling survival, leaf damage, presence or absence of herbivores and oviposition were measured. The plants with application of JA presented lower oviposition than those of the control. The JA induces resistance in S. quitoense plants. Products like him JA, which generate this type of responses in the plants, can be a valuable tool for use by the farmer, especially when there are high densities of herbivores that could reduce crop yields.

Introduction:

Various plants show induced responses to insect damage. Induced responses are changes that occur in the plant against abiotic and biotic factors. From the decade of the eighties, other effects of Jasmonic Acid (JA) were shown in plants, such as: increase in agricultural yields in strawberry, soybean and sugarcane; the stimulation of tuber formation in Dioscorea spp., Helianthus tuberosum and Solanumtuberosum (Koda, 1997); ripening of fruits in tomato and apple (Sembdner and Parthier, 1993) and a special role in the defense mechanisms of plants (Edward, 1994). However, many studies have documented negative effects of induced responses of herbivores on performance (Stout and Duffey 1996, Paul and Tumlinson, 1999, Karbanet al., 2003). Inducers are natural or synthetic compounds that produce herbivory resistance in the plant when applied to foliage and roots. The use of inducers is an alternative approach that potentially avoids damage by herbivores and can reduce the colonization of a pest in agricultural crops (Karbanet al., 1997).

To use induced responses as an effective tool of pest management should assess the effects on the crop and its agricultural environment. Manipulating plants to induced responses will only be an effective tool if the plant benefits by reducing herbivory. A larva of Opsiphanestamarindi can consume a total of 1606.7 cm$^2$ of leaf area throughout its larval stage. During its first two instar the damage it causes does not exceed 17% of the total damage, which shows that this pest causes greater damage (83%) in its last three instar, especially in the fifth that consumes an average of 628.3 cm$^2$ of leaf banana, which represents 39.1% of the total consumed (Uquillas, 2002). JA is found in
many plant species and is involved in the regulation of different functions, including plant resistance and senescence (Creelman and Mullet, 1997). JA is produced by the plant after larval damage and results in increased production of compounds involved in the resistance (Conxabelet et al., 1995; Thaler et al., 1996).

In potato, the application of JA expressed results with the induction of protein inhibitors and polyphenol oxidase, in the decrease in preference, yield and abundance of many common herbivores including Frankliniella occidentalis, Spodoptera exigua, Trichoplusia niHubner, Epitrix hirtipennis Melsheimer, Macrosiphum euphorbiae Thomas, and Myzus persicae Sulzer (Thaler et al., 1996). The exogenous application JA increases the rate of photosynthesis of plants (Metodiev et al., 1996), causes an increase in the production of ethylene, a hormone involved in the ripening of the fruit (Saniewski et al., 1987).

The induction of plant defense could affect herbivorous insects directly through the production of toxic compounds or indirectly through the production of signals indicating intra- or interspecific competition (Schoonhoven et al., 2005). Herbivores can detect the defense induced by volatile compounds and respond by avoiding these plants (De Moraes et al., 2001, Kessler and Baldwin, 2001, Meiners et al., 2005, Snoren et al., 2007). For parasitoids and predators, induced defense of plants may indicate the presence of their host (Turlings et al., 1990). Phenotypic changes in plants, therefore, affect insects at different trophic levels, in the composition of the insect community and the food chain associated with the plant (Takabayashit et al., 1994, Van Zandt and Agrawal, 2004). Manduca quinquemaculata, one of the most important pests of wild tobacco, knows how to take advantage of the odoriferous signals of the plants when looking for a suitable place to oviposition, the adult moth avoids the tobacco plants that were already damaged by caterpillars of the same species (Kessler and Baldwin, 2002). Due to the diversity of effects of the JA, the application with aerosol in S. quitoense plants, in the growth stages, will reduce the herbivory and oviposition of N. elegantalis.

For the present study the following questions are posed. The application of JA induces phytochemical changes in S. quitoense plants?; Is there an induced response in the survival of seedlings in the presence of herbivores ?; The foliar application of JA influences the oviposition of the lepidopteran?

Materials and Methods:

Plants of S. quitoense Lam. cv. "quitoense" with thorns (Sq). Originated from sexual seeds. These plants have the characteristics of bushy growth (1.8 - 3 m.), quite branched, with large leaves, wavy edges, actinomorphic flowers arranged in the armpits of the branches forming small corymbs, large spherical fruits covered with fluff, roots pivoting and secondary well developed and ligneous.

Individuals of N. elegantalis from fifth-generation endogamy populations coming of mass rearing was employed in this experiment.

Establishment of the assay:

A seed was planted per pot of 23 x 23 x 20 cm (300 pots), which contained a combination of soil: sand (3:1); 23 °C; photoperiod (15:9) light: darkness; 40 - 80% relative humidity. Fertilization in relation to 8 - 24 - 6 (N-P-K). No chemical pesticides were used.

Jasmonic acid:

AJ was synthesized from linolenic acid, according to methods described by Farmer et al., (1992). The JA that was used was dissolved in 1 mL of acetone and dispersed in 8 liters of water. The control solution was composed of 1 mL of acetone per 8 liters of water. The solution of JA was applied with a backpack sprinkler, to plants with the presence of 4 leaves and the second time four weeks later.

The plants were randomly divided by 3 treatment groups: control (water and acetone), JA low (0.5 mMjasmonic acid per plant) and high JA (1.5 mMjasmonic acid per plant). These doses are lower than the toxic levels in plants (Thaler et al., 1996). The dose low of JA simulates the level of resistance induced after 24 hours of feeding in a fourth instar by Helicoverpazea (Boddie) larva grown in the greenhouse (Thaler et al., 1996).

The high dose of JA was chosen based on greenhouse studies in which plants applied with high doses of JA produced approximately twice the level of polyphenol oxidase and proteinase. Individual plants of each treatment
were arranged in a completely randomized design. One system was used to measure survival and the other to determine the effects of induction in the presence and absence of the herbivory.

**Chemical Induction:-**
The treatments with JA were applied twice during the growth stage. The polyphenol oxidase was measured after each spraying to verify that the plants were induced. The activity of polyphenol oxidase was determined by spectrophotometry (digital spectrophotometer: UV - VHI, HAZ Double LABOVS2700) according to the methods of Thaler et al., (1996), with 15 repetitions for each treatment. Effects of induction of polyphenol oxidase activity were analyzed using one-way variance analysis (ANOVA) with induction as the main effect and activity of polyphenol oxidase as response variable.

**Survival of the seedling:-**
*S. quitoe* seedlings with 2 fully extended leaves were divided at random in the two treatment groups, the dose with low JA and the control. A week later the number of surviving plants was counted; fourth instar larvae of *N. elegantalis* and higher consumption rate per unit area. Induction effects on seedling survival were analyzed by chi-square tests.

**Foliar damage:-**
One month after the first application of the plants with treatments of JA low dose and those of control. Complete plants were introduced in the laboratory and an exhaustive sample of damage by herbivory was carried out. The number of leaves per plant with visible foliar herbivory was determined.

Damages were measured as the presence or absence of herbivory on a given leaf. Effects of induction on foliar damage were analyzed by 2-way ANOVA with induction and block as the main effects and the number of leaves per plant with damage as the response variable.

**Cumulative Oviposition:-**
One month after the first application in the plants with low dose JA and control treatment were placed two female adults of *N. elengantalis*, reared in the laboratory, per plant. Continuous individual observations were made. Within each observation period, the number of eggs per plant was determined. Experiments were handled at 25 °C. The response of this variable was tested in a general linear model.

**Results:-**
**Chemical Induction:-**
Treatments with JA significantly increased the levels of the enzyme polyphenol oxidase eight days after the initial application with aerosol (mean ± SE, Δ optical density.min.gm⁻¹ tissue of the control sheet = 7.69 ± 1.07; JA low = 30.43 ± 3.38 and JA high = 37.35 ± 4.56) (Table 1).

Five weeks later, after the second application of JA, the differences between the chemical activity were significant in the removal treatment of the larvae and not detectable in the treatments under herbivory (mean ± SE, Δ optical density.min.gm⁻¹ tissue. Herbivory reduction: control: 15.24 ± 1.63, JA low: 19.09 ± 3.08 and JA high: 21.65 ± 3.72 (Table 1).

**Table 1:-** ANOVA of polyphenol oxidase activity in control plants and plants applied Jasmonic Acid.

<table>
<thead>
<tr>
<th>Date</th>
<th>Source</th>
<th>df</th>
<th>sm</th>
<th>F cal</th>
<th>F tab 5 %</th>
<th>F tab 1 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eight days after the first application</td>
<td>Treatment</td>
<td>2</td>
<td>3583.41</td>
<td>19.85</td>
<td>4.055</td>
<td>6.075</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>42</td>
<td>180.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second application</td>
<td>Treatment</td>
<td>2</td>
<td>158.10</td>
<td>2.23</td>
<td>4.055</td>
<td>6.075</td>
</tr>
<tr>
<td>Herbivory reduction</td>
<td>Error</td>
<td>42</td>
<td>70.91</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Survival of the seedling:-**
The survival of the seedlings was high and is not affected by the application of JA. Of 280 seedlings that were evaluated up, 16 died, of which 10 are from the control and 6 by the application of JA ($X^2 = 2.022, P = 0.364$).

**Foliar damage:-**
The plants induced with the JA received 60% less damage than the control plants (Table 2, Figure 1). The larval stage could have contributed to this difference in damage (Karban *et al.*, 2003).

**Table 2:** ANOVA of the number of leaves per plant damaged by *N. elegantalis* in control plants and those applied Jasmonic Acid.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>sm</th>
<th>$F_{cal}$</th>
<th>$F_{tab}$5 %</th>
<th>$F_{tab}$1 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>69.68</td>
<td>14.52</td>
<td>5.72</td>
<td>9.56</td>
</tr>
<tr>
<td>Blocks</td>
<td>1</td>
<td>149.42</td>
<td>31.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trat. x Bloc.</td>
<td>1</td>
<td>5.68</td>
<td>1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>26</td>
<td>4.80</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Accumulated oviposition:-**
The plants of the control treatment at the end of the 6 days, in which the adults of *N. elegantalis* survived, had 76.3 ± 1.8 eggs accumulated while, that the plants with induced treatment had 42.5 ± 1.2 eggs accumulated. Plants induced with JA received 44% less oviposition than control plants (Figure 2). The presence of less oviposition may be due to the fact that the extra eggs could be reassigned to the longevity through the reabsorption of them, when presented an adverse environment - host (Jervis *et al.*, 2001) or that the insect could perceive the presence of methyl jasmonatos reducing its oviposition. This would be useful only in the extrinsic mortality rate; this is not unique determination of the adult pest in its life expectancy (Rosenheim, 1998). The postures they are gregarious and consist of 18 to 57 eggs, so it can be deduced that a single female can place this number of eggs.

![Figure 1: Damage to leaves of *S. quitoense* by larvae of *N. elegantalis* in control plants and applied with low concentration of Jasmonic Acid.](image-url)
Figure 2: Accumulated number of eggs in control plants and those treated with low concentration of Jasmonic Acid.

Discussion:
Resistance induced to phytophagous is observed in many crop plants and has been intensively studied in several agricultural systems, soybean, tomato, potato, wheat and cotton (Kogan and Paxton 1983, Creelman et al., 1992, Stotz et al., 2000). In response to treatments of JA or methyl jasmonate, increased concentrations of several compounds that have been documented in a number of plant species, for example, the proteinase inhibitors (Yamagashiet al., 1993), polyphenol oxidases (Thaler et al., 1996), nicotine (Baldwin et al., 1996), trypsin inhibitors (Cipollini and Sipe, 2001), glucosinolates (Cipollini and Sipe, 2001; Van Dam et al., 2004; Mewis et al., 2005), and the increase in volatile emissions (Boland et al., 1995, Dicke et al., 1999, Michelenaet al., 2001). Tomato plants can be induced in the field and this induction has negative effects on the number of pests insect, including caterpillars of Lepidoptera, beetles, thrips and aphids (Feng et al., 2007). By understanding the chemical mechanisms of induction, we can predict that herbivores will be affected through induced responses. This prediction is important if it can be used in agriculture. However, for agriculture, these effects of induction of plants on herbivores can only be important if they increase the productivity of the plants or their value (Koda, 1992). Chemical inducers have been identified by triggering responses of plants to abiotic and biotic factors, a resistance to herbivores in the field and laboratory (Thaler et al., 1996, Inbaret al., 1998).

Before elicitors can be used effectively in agricultural systems, we must understand the relationship between the chemicals they induce, the effects that these substances have on herbivores in the field, and the effects on the value of plants. Due to the negative effects of the induction of resistance of herbivores in some cases these do not necessarily translate into net benefits for the farmer. Nicotinic acid, for example, is an inducer that produces strong negative effects on plant pathogens; this is considered as a management tool for a pest due to its high phytotoxicity (Schneider et al., 1996). Plants that produce chemicals induced by herbivory defense may have less energy to allocate to others functions. Brown (1988), studied the costs of allocation of proteinase inhibitors, induced by the injection of chitin in tomato plants under various nitrogen levels. He found no costs for induction in both low and high nitrogen levels. The application of JA in tomatoes and potato tubers has caused the accumulation of the protease inhibitor (Yamagashiet al., 1993) and in rice plants it inhibits spore germination of Pyriculariaorzyae fungus that causes the disease known as late blight of rice. The jasmonatios with greater biological activity are (+) 7 iso JA and its methyl ester, although these are also rapidly isomerized (Anurag and Grawal, 2005). According to these studies, it could be expected that plants have a higher endogenous induced resistance but their energy expenditure or compensation of their biological processes should be studied. In other investigations, in eggplant, it has been found that plants do not alter the costs of foliar herbivory unless the levels of damage are high. Welte and Steggall (1993), found that tomato plants, could receive from 15 - 30% leaf damage without producing negative...
effect on the fruit. Gertz and Bach (1994), determined that tomato plants were able to compensate the negative effects of herbivory in low light and low levels of nutrients compared to plants in high resource conditions. In crops where herbivory damage occurs in the early stages, they are candidates for the successful use of induced defenses because leaf damage is often stronger in young plants (Sengottayanet al., 2009). Photochemical induction can protect plants in a particularly vulnerable stage and slow accumulation of phytophagous pests. The instars larval of N. elegantalis are gregarious on the leaf and fruit. They consume young leaves. Kirby and Spence (1863), observed that the females of Pieris brassicae preferred to deposit their eggs in the plants without eggs. Later, this was confirmed in more controlled conditions by Rothschild and Schoonhoven (1977), both for P. brassicae and P. rapae. This avoidance of infested plants is due to a physiological response of the plant to oviposition, instead of the compounds excreted by the insects themselves (Blaakmeerset al., 1994). It has been postulated that butterflies avoid laying eggs on plants infested by an herbivore, because the attack of herbivores induces defense compounds in plants that can influence the growth of their offspring and to reduce the risk of inter or intraspecific competition and parasitism (Thompson and Pellmyr, 1991; Shiojiri et al., 2002). In the presence of an oviposition, chemical changes occur in plants of Brassica oleracea, it is known that it is a specific signal for parasitoids Trichogramma spp., to parasitize the eggs of P. rapae (Fatouros et al., 2005). Oviposition selection in situ is an important decision of the behavior of the life cycle of an herbivorous insect, because hatching larvae have a limited dispersal capacity (Kleunenet al., 2004). The selection of the oviposition site is carried out in successive stages of the search and contact evaluation. Adults of P. rapae use visual, olfactory and tactile cues during these phases (Black et al., 2003; Arimura et al., 2005).

A single caterpillar of the genus M. quinquemaculata has to eat two to three tobacco plants to reach the state of pupa. Oviposition on a plant already occupied by another caterpillar would be fatal to newly laid eggs. The neonatal larvae not would find enough food to reach a size that allows them to reach a neighboring plant. This behavior not only reduces the pressure of predation on the offspring, but also the strong intraspecific competition. Anyway, for the tobacco plant it is useful. The odoriferous signal serves as a double weapon: it attracts predators and discourages the oviposition of the pest, serving the "top-down" and "bottom-up" type control of the plague population (Kessler and Baldwin, 2001). Ellerset al., (2000) demonstrated stochasticity in the type of host encounter; this can lead to an optimal egg load, higher than the expected number of unwanted host encounters. Application of JA in plants generates synomonas emissions that attract natural enemies such as predatory mites and parasitoids (Maleck and Lawton, 1998, Hilker and Meiners, 2002, Van Poecke and Dicke, 2002, Hilker and Meiners, 2006); this results in the attraction in a higher density of natural enemies around the damaged plants, and therefore, reduces the interest of the herbivores to avoid oviposition in the induced plants. Studies on feeding - host, behavior centered on ecology and evolutionary aspects; this on the host feeding function in terms of fecundity and insect longevity and on when host-feeding compensates for the loss of an oviposition opportunity (Heimpel and Collier, 1996, Masayoshi et al., 2010). Progress in the study of biochemistry and physiological aspects of host - food behavior, that is, the host's hemolymph composition and how nutrients are assigned (Rivero and Casas, 1999, Olson et al., 2000). Other stimulated chemicals were not quantified against herbivory, deterrence or precursors that could mediate the behavior of preferences, such as isothiocyanates, terpenoids, glycosides, glucosinolates and amino acids.

In conclusion, S. quitoense plants can be induced chemically by elicitors, Jasmonic acid, which considerably reduce herbivory levels and therefore increase crop yield. The specificity of the phytochemical induction depends without doubt on the type of plant that is being attacked by the pest, therefore the age of the plant and biotic factors such as temperature, wind, relative humidity, among others, must be taken into account. With the use of chemical plant defense inducers, we may be able to adapt the resistance of the plants to manage the characteristics of the pest in a particular region. The effectiveness of the application of JA as a treatment on herbivory depends largely on environmental conditions. Inducers are a practical way to promote plant responses, since they can be patented, manufactured and applied to a large number of plants by conventional spraying technologies. It should be analyzed if the persistence of volatiles continues over time. In the complementation of the phytochemical reactions, it should be analyzed if the synergism between methyl jasmonate and ethylene contributes in induced defense mechanisms and at what cost?

Acknowledgments:-
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ic acid and coronatin induce odor (2001). Bacillus thuringiensis. Life found

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