ISJ 12: 158-165, 2015 ISSN 1824-307X

RESEARCH REPORT

Effect of silicon on the morphology of the midgut and mandible of tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) larvae

MC dos Santos[1], AM Resende Junqueira[1], VG Mendes de Sá[2], JC Zanúncio[3], JE Serrão[4]

[1]*Faculty of Agronomy and Veterinary Sciences, University of Brasilia, 70910-970, Brasília, Distrito Federal, Brazil*
[2]*Faculty of Engineering, University of the State of Minas Gerais, 35930-314, João Monlevade, Minas Gerais State, Brazil*
[3]*Department of Entomology, Federal University of Viçosa, 36.570-000, Viçosa, Minas Gerais State, Brazil*
[4]*Department of General Biology, Federal University of Viçosa, 36.570-000, Viçosa, Minas Gerais State, Brazil*

Accepted May 12, 2015

Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is an important insect pest causing serious losses to tomato plantations in Brazil. Some populations of *T. absolute* are reported to present insecticide resistance resulting in its control failure and the use of alternative control based on silicon, which is clean and sustainable, can reduce pesticide use, increasing fruit quality and protecting the environment. This study evaluated changes in the morphology of the midgut and the mandibles of *T. absoluta* larvae caused by feeding with compounds containing silicon. Larvae of *T. absoluta* were fed on tomato leaves with different compounds containing silicon and the histology of the midgut of fourth instar larvae was analyzed. The mandibles of all larval stages were dissected and analysed by scanning electron microscopy. There were no changes in the morphology of the mandibles of the larvae of *T. absoluta* fed on silicon compared to the control group. Larvae of *T. absoluta* from the control group and the treatments where the calcium silicate was applied to the soil had not differences in the morphology of the midgut epithelial cells, which had four cell types: digestive, goblet, regenerative and mycetocyte cells. In larvae of *T. absoluta* obtained from silicon-based treatments applied to the leaves, the midgut epithelium showed detachment of the basal membrane, which can characterize the possible effect of this toxic element to larvae of *T. absoluta*.

Key Words: *Lycopersicon esculetum*; fertilization; morphology; pest control

Introduction

The tomato (*Lycopersicon esculentum*) is widely cultivated in the world and it is one of the most produced vegetables in Brazil, which is currently among the top ten world producers (Vilela, 2009). The tomato crop in Brazil is expanding and modernizing, seeking greater productivity and quality to meet market demands, thereby generating more concern about pest control (Vivan *et al.,* 2002) that can cause substantial reductions in fruit quality (Miranda *et al.,* 1998; Kennedy, 2003; Mahanil *et al.,* 2008).

The tomato pinworm, *Tuta absoluta* (Lepidoptera: Gelechiidae), which attacks leaves and flowers causing mines and fruit drilling throughout the cycle of tomato, stands out as the most important insect pest of this crop and has usually been controlled by multiple applications of insecticides (Medeiros *et al.,* 2009). However, the frequent use of insecticides may be harmful to the natural biological control, generates the production of foods with high levels of toxic waste, environmental contamination, besides the risk of selecting resistant insects streams (Vianna *et al.,* 2009).

Many insecticides have the midgut cells of the insects as a first target (Forkpah *et al.,* 2014), which is the main organ where digestion and absorption take place (Lehane and Billingsley, 1996). The midgut wall has three layers: two muscular layers and an epithelial lining of the lumen, composed of a single layer of cells that, in most insects, has three cell types: columnar or digestive, regenerative, and endocrine, while a fourth cell type, goblet cells, occurs in Lepidoptera (Lehane and Billingsley, 1996). The epithelium surface is isolated from the food content by a non-cellular membrane, the peritrophic membrane or matrix (Lehane and Billingsley, 1996; Terra, 2001).

Corresponding author:
José Eduardo Serrão
Department of General Biology
Federal University of Viçosa
36.570-000, Viçosa, Minas Gerais State, Brazil
E-mail: jeserrao@ufv.br

Table 1 Treatments used to evaluate the effect of silicon on the morphology of the midgut and mandible of *Tuta absoluta* (Lepidoptera, Gelechiidae)

Treatments	Product	Amount (ton ha^{-1})
T1	Agrosilício™ Soil	0.45 (ton ha^{-1})
T2		0.9 (ton ha^{-1})
T3		1.35 (ton ha^{-1})
T4		1.8 (ton ha^{-1})
T5	Agrosilício™ leaves	2.0 (ton ha^{-1})
T6		4.0 (ton ha^{-1})
T7		6.0 (ton ha^{-1})
T8		8.0 (ton ha^{-1})
T9	Sili-K™ leaves	0.5 (l ha^{-1})
T10		1.0 (l ha^{-1})
T11		2.0 (l ha^{-1})
T12		3.0 (l ha^{-1})
T13	Silicic Acid leaves	0.25 %
T14		0.50 %
T15		0.75 %
T16		1.0 %
T17	Silicic Acid soil	0.25 %
T18		0.50 %
T19		0.75 %
T20		1.0 %

In Lepidoptera larvae, the peritrophic membrane is secreted by the midgut digestive cells (De Priester, 1971; Terra, 1988). This membrane, although it prevents direct contact of food with the digestive cells, allows the passage of digestive enzymes in the direction of the midgut lumen and the absorption of the products of digestion which are then eliminated with the faeces (Terra, 1988) avoiding mechanical damage, impeding or preventing the entry of pathogens and partition the digestion process (Terra, 1988; 2001). The digestive cells are the most abundant and are responsible for producing enzymes and absorbing products of digestion (Terra, 1988). Goblet cells are involved in the homeostasis and absorption of metabolites, a function performed in conjunction with the digestive cells (Lehane and Billingsley, 1996; Terra *et al.,* 2006). The regenerative cells are seen alone, paired or in nidi at the base of the epithelium and they play a role in cell renewal (Serrão and Cruz-Landim, 1996a; Martins *et al.,* 2006). The endocrine cells are located at the base of the epithelium and they are characterized by the presence of a large number of cytoplasmic granules, which produce peptide hormones (Serrão and Cruz-Landim, 1996b; Neves *et al.,* 2002).

Synthetic insecticides results in many ecological problems such as selection of resistance strains of insects, ecological unbalance and mammals intoxications. Thus, it is necessary the use of programs of integrated pest management, which aim the reduction of the use of pesticides compatible with natural enemies (Zanuncio *et al.,* 2003). Although silicon is not essential for most plants (Savant *et al.,* 1999), it is beneficial to improve plant resistance against some biotic and abiotic stresses (Do Gramaci *et al.,* 2013; Haynes, 2014). The application of silicon has increased plant resistance to insect pests (Almeida *et al.,* 2008; Cherry *et al.,* 2012; Sidhu *et al.,* 2013; Keeping *et al.,* 2014; Pinto *et al.,* 2014), mainly for its ability to accumulate in the plant cell wall (Costa and Moraes 2006), thereby increasing the synthesis of lignin and phenolic compounds (Chérif *et al.,* 1992; Ghanmi *et al.,* 2004), in addition to activating the endogenous chemical defenses of plants (Gomes *et al.,* 2005). The use of silicon in controlling defoliator insects occurs due to the action of the mechanical barrier provided by the deposition of this mineral in the cell wall of leaves (Goussain *et al.,* 2002; Massey *et al.,* 2006; Kvedaras *et al.,* 2009).

Tuta absoluta reared on tomato plants accumulating silicon show decrease in larvae and pupae survival and male and female weight (Santos *et al.,* 2012). The deleterious effects on insect larvae that feed on plants treated with calcium silicate have been suggested to occur due damages in the incisor teeth of the mandibles, affecting the insect nourishment and development (Goussain *et al.,* 2002; Kvedaras *et al.,* 2009) but data about its effect on midgut remains unknown.

This study evaluated whether treatment of tomato plants with silicon affects the morphology of the midgut and the mandibles of the tomato pinworm *T. absoluta.*

Materials and Methods

Planting, experimental design and treatments

The planting of seedlings of tomato, variety Tospodoro obtained at Embrapa Vegetables, Brasília, DF, was carried out a greenhouse at the Federal University of Viçosa, Viçosa, MG, Brazil. The experimental plots of tomato plants grown in 3 L polyethylene pots, containing one plant in each with planting nitrogen-containing fertilizer (600 kg ha^{-1} ammonium sulfate), phosphorus (3300 kg ha^{-1} superphosphate) and potassium (330 kg ha^{-1} potassium chloride), whose amounts were calculated on the basis of soil analysis. Three compounds used as sources of silicon were: Agrosilício™ (10.5 % Si), Sili-k™ (12.2 % Si) and silicic acid (100 % Si). The experimental design was randomized blocks with five replications, twenty treatments (Table 1) and control (without addition of any compound containing silicon). The Agrosilício™ was added to the soil together to fertilizer planting, aiming to increase alcaline saturation to 70 % (Ribeiro and Guimarães 1999) in the treatments T1 to T4, and applied weekly by foliar sprays in treatments T5 to T8. The Sili-K™ product was applied weekly only to the leaves, in the treatments T9 to T12, due to the fact that compound is not available for soil application. The silicic acid solution was applied weekly, in foliar application, treatments T13 to T16 and in the soil around the stems of plants, treatments T17 to T20. The first foliar application of the products was made 30 days after planting of tomatoes, a total of three applications at weekly intervals in treatments where Agrosilício™ was applied to the soil, since this product has a soil corrective effect (Sommer *et al.,* 2006). In all treatments with Agrosilício™, Sili-K™ and silicic acid soil were corrected with dolomitic lime (0.8 t ha^{-1}) in order to raise the alkaline saturation to 70 %.

Release of T. absoluta eggs

Tomato leaves with *T. absoluta* eggs of the same age, from the mass rearing of the Laboratory of Agricultural Entomology, Federal University of Viçosa, were sectioned in order to contain about 30 eggs approximately. Each of these sectioned areas was fixed with the aid of a pin in the branches of the tomato plant caged in organza bags 40x70 cm involving the entire plant, seven days after the last foliar application of products containing silicon.

Light Microscopy

To evaluate the silicon effect in the midgut of *T. absoluta*, fourth instar larvae obtained from the treatments were collected and transferred to flasks containing 2 mL of Zamboni fixative solution (Stefanini *et al.,* 1967). Afterwards, the midguts of *T. absoluta* were dissected in insect saline solution (0.1 M NaCl + 0.1 M KH2PO4 + O.1M Na2HPO4), dehydrated in a graded ethanol series (70, 80, 90 and 95 %) and embedded in JB-4™ historesin for 24 h. The samples were sectioned at 5 μm slices, stained with hematoxyline and eosin, examined and photographed in light photomicroscope.

Scanning electron microscopy

To evaluate the silicon effect in the wear of the mandibles in all four larval stages, *T. absoluta* larvae, were collected each 12 h, two larvae per plant, and placed in flasks containing 2 mL of Zamboni fixative. Larvae were collected immediately before ecdysis, based on the duration of each larval instar (Giustolin *et al.*, 2002) to ensure greater exposure to treatments. The mandibles were removed, dehydrated in a graded ethanol series (70, 80, 90 and 95 %), transferred to HMDS (Hexamethyldisilazane) and after five min air drying. The mandibles were gold covered (20 nm) and analyzed under a scanning electron microscope LEO VP1430 at the Center for Microscopy and Microanalysis at the Federal University of Viçosa.

Results

The midgut of *T. absoluta* larvae showed a single layered epithelium with four cell types: digestive, goblet, regenerative and micetocyte cells (Figs 1a - e).

The digestive cells were tall, which apex showing a well-developed brush border and cytoplasm containing numerous vacuoles (Fig. 1c). In the middle portion of these cells was found an oval nucleus with a predominance of decondensed chromatin (Fig. 1b).

The goblet cells were characterized by a cavity formed by invagination of the apical surface of these cells that showed a basal nucleus (Figs 1a - c).

The regenerative cells were small, never reaching the midgut lumen, containing a relatively large nucleus in relation to the cytoplasm and were scattered between the base of the goblet and digestive cells isolated or forming nests till three cells (Fig 1a).

Throughout the midgut epithelium of *T. absoluta* were found some large and globular cells with spherical nucleus and cytoplasm almost filled with small basophilic bacteria-like particles (Fig. 1e), which allowed the characterization of this cell type as mycetocytes.

A well-developed peritrophic membrane line the midgut lumen (Fig. 1c).

The midgut epithelium was seated on a homogeneous basal membrane and a layer of circular muscles was found externally, followed by an outer layer of longitudinal muscles.

Larvae of *T. absoluta* from the control group and treatments where calcium silicate was applied to the soil showed no differences in the structural organization of the midgut that was described above.

Larvae of *T. absoluta* obtained from silicon-based treatments applied to leaves showed changes in the morphology of the midgut, a characterized by the detachment of the basal membrane of the digestive epithelium (Figs 1a, 1b), presence of cytoplasmic protrusions with strongly

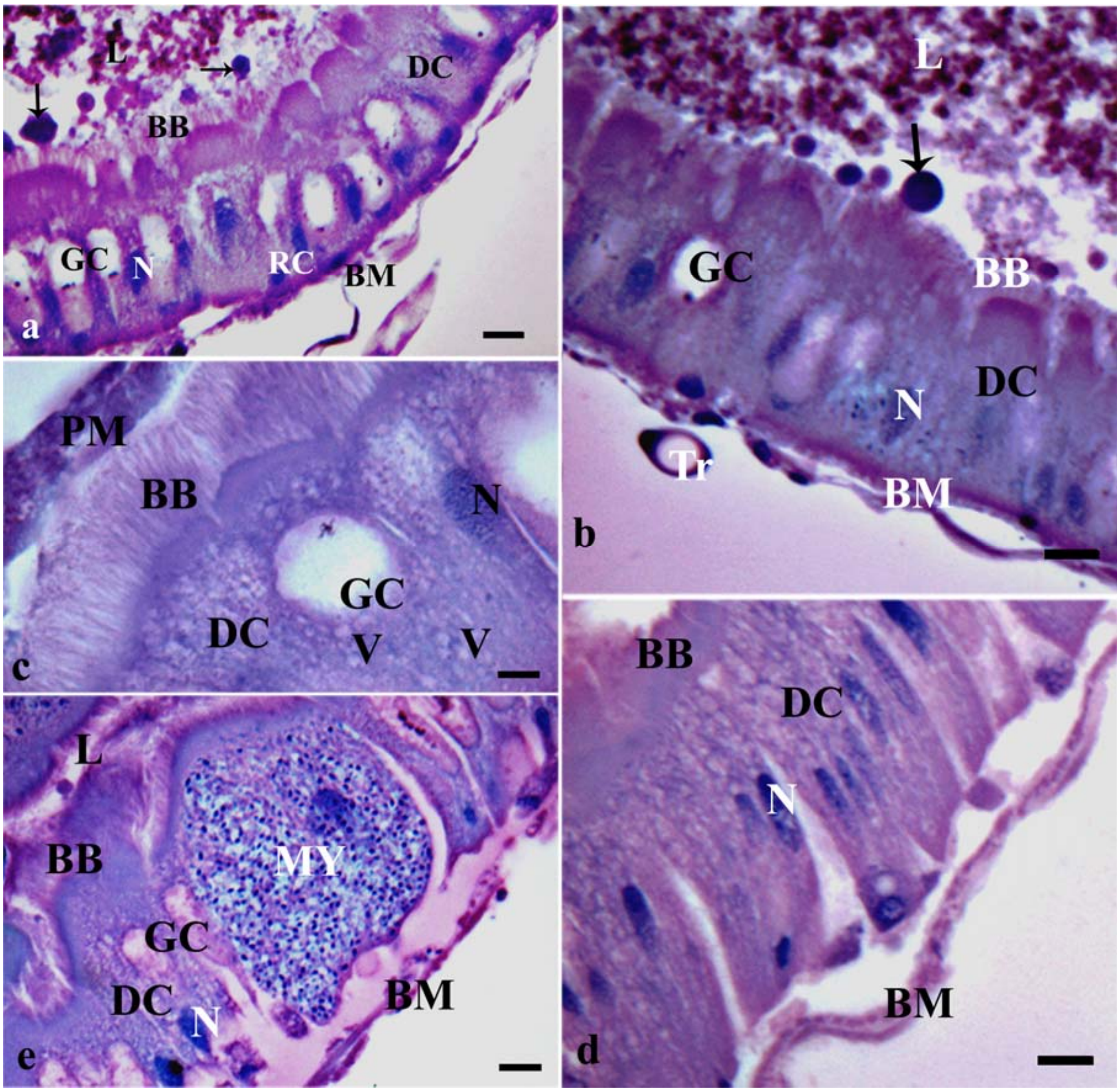

Fig. 1 Histological sections of the midgut of fourth instar larvae of *Tuta absoluta* (Lepidoptera, Gellechidae) fed on tomato plants treated with compounds containing silicon. **a)** Midgut epithelium of larvae fed on plants exposed to Agrosilício^{TM} leaves (40 t ha^{-1}) showing digestive cells (DC), globet cells (GC) and regenerative cell nidus (RC) Note basal membrane detached (BM), well developed brush border (BB) and apoptotic bodies (arrows) released to lumen (L) Bar = 20 μm; **b)** Midgut epithelium of larvae fed on plants exposed to Agrosilício^{TM} leaves (40 t ha^{-1}) showing basophilic apoptotic bodies (arrow) Bar = 10 μm; **c)** Midgut epithelium of larvae fed on plants exposed to Silicic Acid leaves (1,0 %) showing digestive (DC) and globet cells (GC) with vacuoles in the cytoplasm (V) Notice the peritrophic membrane (PM) Bar = 10 μm; **d)** Midgut epithelium of larvae fed on plants exposed to Sili-K^{TM} leaves (30 l ha^{-1}) showing detached basal membrane (BM) Bar = 10 μm; **e)** Midgut epithelium of larvae from plants the control group without any addition of compounds containing silicon and fed plants exposed to Sili-K^{TM} leaves (05 l ha^{-1}) showing mycetocyte (MY) characterized by basophilic granules bacteria-like N, nucleus with decondensed chromatin Bar = 10 μm.

basophilic content, some them containing the cell nucleus, which are released into the midgut lumen, similar to apoptotic bodies (Figs 1a, 1b).

The larvae of *T. absoluta* from all treatments including the control group, had six teeth in their mandibles without variation in the number or shape of them, and show no signs of damage from first to fourth instar (Figs 2a, 2b).

Discussion

The efficiency of silicon-containing products to control *T. absoluta* has been suggested to occur due its toxic and anti-feeding effect to the larval stage, playing a role as activator of tomato plants resistance (Rodrigues *et al.,* 2004; Côté-Beaulieu *et al.,* 2009). However, there were no studies proving this mechanism of silicon action, which is accumulated in higher concentrations as silica in the leaf tissues of plants (Basagli *et al.,* 2003; Correa *et al.,* 2005; Gomes *et al.,* 2005).

In this study, one of the effects caused by the application of silicon in the leaves was the detachment of the midgut epithelium from the basal membrane, which leads to the reduction of digestive capacity in insects (Barbeta *et al.,* 2008; Almeida *et al.,* 2009). However, there are no significant difference in the structural organization of the midgut epithelium of *T. absoluta* between the larvae in the control group and those of silicon treatments applied to the soil, as previously described for other Lepidoptera larvae (Levy *et al.,* 2004; Pinheiro *et al.,* 2008; Sousa *et al.,* 2009*,* 2010).

The mechanisms of silicon action in the gut cells of insects are not yet fully understood. Cellular detachment from basal membrane in the midgut of larvae of *T. absoluta* may indicate that the anti-feeding action of the compounds containing silicon may be related to physiological effects resulting from their ingestion. Compounds derived from neem seeds applied in leaves for use in integrated pest management also showed similar effects by altering the epithelial cells of the midgut of insects and, consequently, any effect on their development (Mordue and Nisbest 2000; Ndione *et al.,* 2007; Barbeta *et al.,* 2008; Almeida *et al.,* 2009, 2014). The midgut of *Alabama argillacea* (Lepidoptera: Noctuidae) caterpillar fed on *Bacillus thuringiensis* cotton leaves has morphological changes of goblet and digestive cells with increase in cytoplasm vacuoles amount, degeneration of muscle layer and absence of peritrophic membrane (Sousa *et al.,* 2010).

The presence of cell protrusions like apoptotic bodies being released in the midgut lumen of *T. absoluta* larvae treated with silicon applied to leaves, suggest a cytotoxic effect of silicon. Apoptosis is a morphological pattern of programmed cell death characterized by cell shrinkage (Ihara *et al.,* 1998). The intake of silicon may have a toxic effect caused by damaging the epithelial cells of the midgut and, therefore, a response would be the elimination of these cells by programmed cell death. Toxins such as Cry1Ac from Bt have similar effect, providing a toxic effect and causing apoptosis in cells of the insects midgut (Zhang *et al.,* 2005).

The occurrence of small basophilic granules found in globular cells of midgut of *T. absoluta* larvae in this study are similar to structures called mycetocytes found in some insect that depend on the obligatory mutualism with microorganisms, mainly bacteria (Moran and Baumann, 2000). However, the occurrence of such cells accumulating microorganisms has not been reported to occur in Lepidoptera and the identification of the microorganisms found inside mycetocytes of *T. absoluta* must be conducted in the future, in order to understand their function in the physiology of the insect or disease transmission to plants.

Our results showed no effect of silicon on the mandibles of *T. absoluta*, which have not wear signals in all larval stages. Silicon is beneficial to plants, acting as an inducing resistance agent against pest insects, and the silification of the

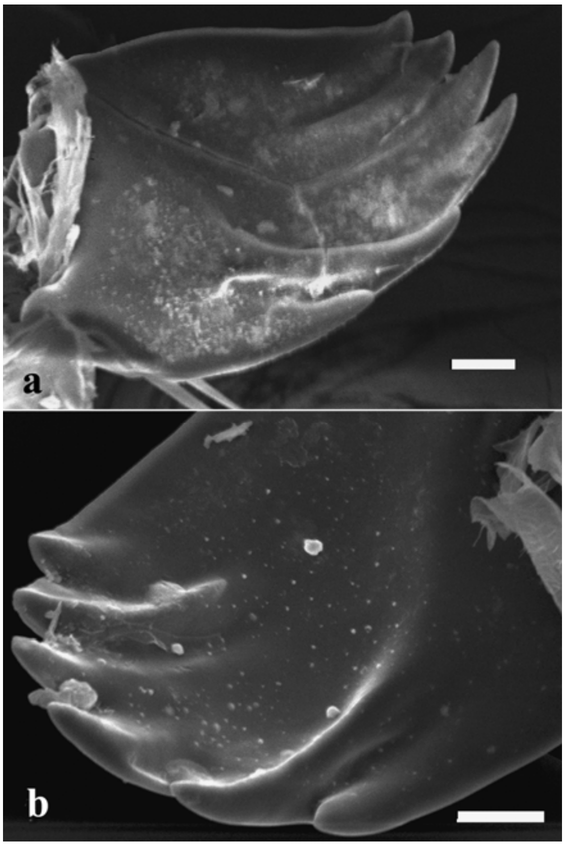

Fig. 2 Mandibles of *Tuta absoluta* (Lepidoptera, Gellechidae) larvae; a) Fed on tomato plants exposed to Silicic Acid leaves (0.5 %) Bar = 20 μm; b) Control group without any addition of compounds containing silicon Bar = 20 μm

epidermis prevents the penetration and chewing by insects due to the hardening of plant cell wall (Datnoff *et al.,* 2001). In larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed on corn plants treated with calcium silicate occurs a wearing in the mandibles teeth (Goussain *et al.,* 2002). However, in *Eldana saccharina* (Lepidoptera: Pyralidae) caterpillars fed on sugar cane treated with silicon, there was no significant effect in wearing in the mandibles (Kvedaras *et al.,* 2009).

Absence of wear in the mandibles of *T. absoluta* larvae fed on tomato plants treated with silicon, may be due to physiological characteristics of the plant and the feeding habits of these larvae. The tomato is a non-accumulating silicon plant because it absorbs little silicon through the roots (Ma and Yamaji 2006) and the increasing levels of this compound in tomato leaves is not proportional to their availability in the soil (Pereira *et al.,* 2003). The effect of mechanical protection of silicon in plants is attributed to its storage as amorphous silica (SiO_2nH_2O) in the cell wall (Datnoff *et al.,* 2001). The Agrosilicio™ (insoluble in water) applied to leaves forms only one layer of silica on the epidermis of the leaves while the Sili-K™ (silicon-soluble liquid) forms a silica layer evidenced by its polymerization with cuticular compounds of the plant (unpublished data). The larvae of *T. absoluta* feed and develop in the mesophyll of tomato leaves (Medeiros *et al.,* 2009) and, probably, due to the diet, to the compounds containing silicon does not penetrate the leaves of tomato plants and this plant not accumulate Si (Ma *et al.,* 2001), the mandibles of *T. absoluta* have been not stressed.

Conclusion

The foliar application of silicon-containing compounds in tomato plants was effective against the attack of *T. absoluta* caterpillars causing detachment of midgut cells from the basal membrane, which may result in digestion difficulties and larval mortality.

Acknowledgements

This research was supported by Brazilian research agencies CAPES, CNPq and FAPEMIG. Authors are grateful to Center for Microscopy and Microanalysis (UFV) for technical assistance, Prof. Picanço M (UFV) for make available *T. absoluta* samples, Prof. Sedyama T (UFV) for greenhouse assistance and to EMBRAPA.

References

Almeida GD, Zanuncio JC, Senthil-Nathan S, Pratissoli D, Polanczyk RA, Azevedo DO*, et al.* Citotoxicity in the midgut and fat body of *Anticarsia gemmatalis* (Lepidoptera: Geometridae) larvae exerted by neem seed extract. Inv. Surv. J. 11: 79-86, 2014.

Almeida GD, Pratissoli D, Zanuncio JC, Vicentini VB, Holtz AM, Serrão JE. Calcium silicate and organic mineral fetilizer applications rduce phytophagy by *Thrips palmi* Karny (Thysanoptera: Thripidae) on eggplants (*Solanum melongena* L.). Interciencia 33: 835-838, 2008.

Almeida GD, Pratissoli D, Zanuncio JC, Vicentini VB, Holtz AM, Serrão JE. Calcium silicate and organic mineral fertilizer increase the resistance of tomato plants to *Frankliniella schultzei.* Phytoparasitica 37: 225-230, 2009.

Barbeta BL, Marshal AT, Gillon A, Craik DJ, Marlyn AA. Plant cyclotides disrupt epithelial cell in the midgut of Lepidoptera larvae. Proc. Natl. Acad. Sci. USA 105: 1221-1225, 2008.

Basagli MAB, Moraes JC, Carvalho GA, Ecole CC, Gonçalves-Gervásio RCR. Effect of soduim silicate on the resistance of wheat plants to green-aphids *Schizaphis graminun* (Rond) (Hemiptera: Aphididae). Neotrop. Ent. 32: 659-663, 2003.

Cherry R, Lu HJ, Wright A, Roberts P, Luo YG. Effects of silicon on resistance of St Augustinegrass to Southern chinch bugs (Hemiptera: Blissidae) and plant disease. J. Entomol. Sci. 47: 17-26, 2012.

Chérif M, Menzies JG, Benhamou N, Bélanger RR. Studies of silicon distribution in wounded and *Pythium ultimum* infected cucumber plants. Physiol. Molec. Plant Path. 41: 371-385, 1992.

Correa RSB, Moraes JC, Auad AM, Carvalho GA. Silicon and acibenzolar-s-methyl as resistance inducers in cucumber, against the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Biotype B. Neotrop. Ent. 34: 429-433, 2005.

Costa RR, Moraes JC. Efeitos do ácido silícico e do acibenzolar-s-methyl na resistência de plantas de trigo ao *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). Neotrop. Ent. 35: 834-839, 2006.

Côté-Beaulieu C, Chain F, Menzies JG, Kinrade SD, Bélanger, RR. Absorption of aqueous inorganic and organic silicon compounds by wheat and their effect on growth and powdery mildew control. Environ. Exp. Bot. 65:155-116, 2009.

Datnoff LE, Snyder GH, Korndörfer GH. Silicon in agriculture, Elsevier, Amsterdam, 2001.

De Priester W. Ultrastructure of the midgut epithelial cells in the fly *Calliphora erythrocephala.* J. Ultrastruct. Res. 36: 783-805, 1971.

Do Gramaci M, Arthurs SP, Chen JJ, Osborne L. Silicon applications have minimal effects on *Scirtothrips dorsalis* (Thysanoptera: Thripide) populations on pepper plant, *Capsicum annum* L. Fla. Ent. 96: 48-54, 2013.

Forkpah C, Dixon LR, Fahrbach SE, Rueppell O. Xenobiotic effects on the intestinal stem cell proliferation in adult honey bee (*Apis melliera* L) workers. PLOS One 9: e91180, 2014.

Ghanmi D, McNally DJ, Benhamou N, Menzies JG, Belanger RR. Powdery mildew of *Arabidopsis thaliana*, a pathosystem for exploring the role of silicon in plant-microbe interactions. Physiol. Molec. Plant Path. 64: 189-199, 2004.

Giustolin TA, Vendramim, JD, Parra, JRP. Número de ínstar larvais de *Tuta absoluta* (Meyrick) em genótipos de tomateiro. Sci. Agric. 59: 393-396. 2002.

Gomes FB, Moraes JC, Santos CD, Goussain MM. Resistance induction in wheat plants by silicon and aphids. Sci. Agricola 62: 547-551, 2005.

Goussain MM, Moraes JC, Carvalho J, Nogueira NL, Rossi NL. Efeito da aplicação de silício em plantas de milho no desenvolvimento biológico da lagarta-do-cartucho *Spodoptera frugiperda* (J. Smith) (Lepidoptera: Noctuidae). Neotrop. Ent. 3: 306-310, 2002.

Haynes RJ. A contemporary overview of silicon availability in agricultural soils. J. Plant Nutrit. Soil Sci. 177: 831-844, 2014.

Ihara T, Tsukiko YMS, Ueno HOY. The process of ultrastructural changes from nuclei to apoptotic body. Virchows Arch. 433: 443-447, 1998.

Keeping MG, Miles N, Sewpersad C. Silicon reduces impact of plant nitrogen in promoting stalk borer (*Eldna saccharina*) but no sugarcane thrips (*Fulmekiola serrata*) infestations in sugarcane. Front. Plant Sci. 4: 289, 2014.

Kennedy GG. Tomato, pests, parasitoids, and predators, Tritrophic interactions involving the genus *Lycopersicon.* Annu. Rev. Ent. 48: 51-72, 2003.

Kvedaras OL, Byrne MJ, Coombes NE, Keeping MG. Influence of plant silicon and sugarcane cultivar on mandibular wear in the stalk borer

Eldana saccharina. Agric. For .Ent. 11: 301-306, 2009.
Lehane MJ, Billingsley PF. Biology of the insect midgut. Chapman & Hall, London, 1996.
Levy SM, Falleiros AMF, Gregório EA, Arrebola NR, Toledo LA. The larval midgut of *Anticarsia gemmatalis* (hübner) (Lepidoptera: Noctuidae), light and electron microscopy studies of the epithelial cells. Braz. J. Biol. 64: 633-638, 2004.
Ma JF, Miyake Y, Takahashi E. Silicon as a beneficial element for crop plant. In: Datnoff LE, Korndörfer GH, Snyder G (eds), Silicon in agriculture, Elsevier Science, Amsterdam, pp 17-39, 2001.
Ma JF, Yamaji N. Silicon uptake and accumulation in higher plants. Plant Sci. 11: 392-397, 2006.
Mahanil S, Attajarusit J, Stout MJ, Thipyapong P. Overexpression of tomato polyphenol oxidase increases resistance to common cutworm. Plant Sci. 74: 456-466, 2008.
Martins GF, Neves CA, Campos LAO, Serrão JE. The regenerative cells during the metamorphosis in the midgut of bees. Micron 37: 161-168, 2006.
Massey FP, Ennos AR, Hartley SE. Silica in grasses as a defence against insect herbivores, contrasting effects on folivores and a phloem feeder. J. Anim. Ecol. 75: 595-603, 2006.
Medeiros MA, Suji ER, Rasi GC, Liz RS, Morais HC. Padrão de oviposição e tabela de vida da traça-do-tomateiro *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Rev. Brasil. Ent. 53: 452-456, 2009.
Miranda MMM, Picanço MC, Zanuncio JC, Guedes RNC. Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Biocontrol Sci. Tech. 8: 597-606, 1998.
Moran NA, Baumann P. Bacteria endosymbionts in animals. Curr. Opin. Microbiol. 3: 270-275, 2000.
Mordue AJL, Nisbet AJ. Azadirachtin from the neem tree *Azadiracta indica* its actions against insects. An. Soc. Entomol. Brasil 29: 616-632, 2000.
Ndione RD, Faye O, Ndiaye M, Dieye A, Afoutou JM. Toxic effects of neem products (*Azadirachta indica* A Juss) on *Aedes aegypti* Linnaeus 1762 larvae. Afr. J. Biotech. 6: 2846-2854, 2007.
Neves, CA, Behring LL, Serrão JE. FMRFamide-like midgut endocrine cells during the metamorphosis in *Melipona quadrifasciata anthidioides* (Hymenoptera: Apidae). Micron 33: 453-460, 2002.
Pereira HS, Vitti GC, Korndorfer GH. Behavior of different silicon sources in the soil and in tomato crop. Rev. Brasil. Cienc. Solo 27: 101-108, 2003.
Pinheiro DO, Quagio-Grassiotto I, Gregório EA. Morphological regional differences of epithelial cells along the midgut in *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) larvae. Neotrop. Ent. 37: 413-419, 2008.
Pinto DG, Aguilar MAG, Souza CAS, Silva DM, Siqueira PR, Cao JR. Photosynthesis, growth and incidence of insect pest in cacao genotypes sprayed with silicon. Biosci. J. 30: 715-724, 2014.
Rodrigues FA, McNally DJ, Datnoff LE, Jones JB, Labbé C, Benhamou N, *et al*. Silicon enhances the accumulation of diterpenoid phytoalexin in Rice, a potential mechanism for blast resistance. Phytopathol. 94: 177-183, 2004.
Santos MC, Junqueira AMR, Sá VGM, Zauncio JC, Serrão JE. Efeito do silício em aspectos comportamentais e na história de vida de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Rev. Brasil. Agropec. Sust. 2: 76-88, 2012.
Savant NK, Korndorfer GH, Datnoff LE, Snyder GH. Silicon nutrition and sugarcane production, a review. J. Plant Nutrit. 22: 1853-1903, 1999.
Serrão JE, Cruz-Landim C. Ultrastructure of midgut endocrine cells in workers of stingless bee (Hymenoptera: Apidae: Meliponinae). Iheringia 81: 151-156, 1996a.
Serrão JE, Cruz-Landim C. Ultrastructure of digestive cells in stingless bees of various ages (Hymenoptera: Apidae: Meliponinae). Cytobios 88: 161-171, 1996b.
Sidhu JK, Stout MJ, Blouin DC, Datnoff LE. Effect of silicon soil amendment on performance of sugarcane borer, *Diatraea saccharalis* (Lepidoptera: Crambidae) on rice. Bull. Ent. Res. 103: 656-664, 2013.
Sommer M, Kaczorek D, Kuzyakov Y, Breuer J. Silicon pools and fluxes in soils and landscapes, a review. J. Plant Nutrit. Soil Sci. 169: 310-329, 2006.
Sousa MEC, Wanderley-Teixeira V, Teixeira AAC, Siqueira HAA, Santos FAB, Alves LC. Ultrastructure of the *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae) midgut. Micron 40: 743-749, 2009.
Sousa MEC, Santos FAB, Wanderley-Teixeira V, Teixeira AAC, Siqueira HAA, Alves LC, *et al*. Histopathology and ultrastructure of midgut of *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae) fed Bt-cotton. J. Ins. Physiol. 56: 1913-1919, 2010.
Stefanini M, Demartino C, Zamboni L. Fixation of ejaculated spermatozoa for electron microscopy. Nature 216: 173-174, 1967.
Terra WR. The origin and functions of the insect peritrophic membrane and peritrophic gel. Arch. Ins. Biochem. Physiol. 47: 47-61, 2001.
Terra WR. Physiology and biochemistry of insect digestion, an evolutionary perspective. Braz. J. Med. Biol. Res. 21: 675-734, 1988.
Terra WR, Costa RH, Ferreira C. Plasma membranes from insect midgut cells. An. Acad. Brasil. Cienc. 78: 255-269, 2006.
Vianna UR, Pratissoli D, Zanuncio JC, Lima ER, Brunner J, Pereira FF, *et al.* Insecticide toxicity to *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) females and effects on descendent generation. Ecotoxicol. 18: 180-186, 2009.
Vilela NJ. Situação da produção de tomate no Brasil. EMBRAPA-Hortaliças. http://pt.slideshare.net/nirlene/situao-da-produo-de-tomate-no-brasil-5166847, 2009.

Vivan LM, Torres JB, Veiga FSL, Zanuncio JC. Comportamento de predação e conversão alimentar de *Podisus nigrispinus* sobre a traça-do-tomateiro. Pesq. Agropec. Brasil 36: 581-587, 2002.

Zanuncio TV, Serrão JE, Zanuncio JC, Guedes RNC. Permethrin-induced hormesis on the predator *Supputius cincticeps* (Stal, 1860) (Heteroptera: Pentatomidae). Crop Protec. 22: 941-947, 2003.

Zhang X, Candas M, Griko NB, Rose-Young L, Bulla LA. Cytotoxicity of *Bacillus thuringiensis* Cry1Ab toxin depends on specific binding of the toxin to the cadherin receptor BT-R(1) expressed in insect cells. Cell Death Differ. 12: 1407-1416, 2005.