BIOLOGICAL CONTROL

Oviposition Preference of *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) and Rates of Parasitism by *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) on *Lycopersicon esculentum* in São José de Ubá, RJ, Brazil

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Preferência de Oviposição de *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) e Taxas de Parasitismo por *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) em *Lycopersicon esculentum* em São José de Ubá, RJ.

RESUMO – *Neoleucinodes elegantalis* (Guenée) é uma praga importante em diversas regiões tomaticultoras do Brasil. Logo após a eclosão, a larva penetra rapidamente no fruto, característica que limita a efetividade dos inseticidas e do controle biológico. O maior entendimento dos sinais que estimulam ou inibem a localização do hospedeiro e a oviposição por *N. elegantalis* permitiria o desenvolvimento de novas estratégias de controle. Observou-se que a mariposa depositou 89% de seus ovos em frutos pequenos (23,1±0,95 mm) e que 76% dos ovos foram colocados sobre os quatro primeiros frutos basais da penca de tomates. A média do número de ovos/massa de ovos foi de 2,9±0,17 (intervalo de 1 a 13) e aproximadamente 70% desses ovos foram depositados de uma só vez. A percentagem de plantas com uma ou mais massas de ovos aumentou de 11,8% para 100% durante o período de dez semanas de monitoramento em 1996, e de 8,7 para 80% durante o período de nove semanas de amostragem em 1997. No final da safra de 1996, quando o número de plantas contendo ovos de *N. elegantalis* estava aumentando e as aplicações de pesticidas estavam diminuindo, um parasitóide de ovos, *Trichogramma pretiosum* Riley, foi coletado. Durante as últimas três datas de amostragem de 1996, a taxa de parasitismo aumentou de 2,4 para 28,7%. Entretanto, na passagem do outono para o inverno da safra de 1997, parasitóides de ovos não foram encontrados.

PALAVRAS-CHAVE: Insecta, tomate, broca-pequena-do-tomate, interações tritróficas, tamanho de fruto, tricomas.

ABSTRACT – *Neoleucinodes elegantalis* (Guenée) is one of the most important pests in several tomato growing regions of Brazil. The neonate larva enters the fruit shortly after eclosion, a characteristic that limits the effectiveness of insecticides and biological control agents. A better understanding of the cues that elicit or inhibit host-plant location and oviposition by *N. elegantalis* could lead to novel control strategies. Our findings indicate that *N. elegantalis* deposited 89% of its eggs on small fruits (23.1±0.95 mm), and that 76% of the eggs were placed on the first four basal fruits within the fruit cluster. The average number of eggs/egg mass was 2.9±0.17 (range 1-13), and approximately 70% of these were deposited in a single oviposition bout. The percentage of plants with one or more egg mass increased from 11.8 to 100% during a 10-wk monitoring period in 1996, and from 8.7 to 80% during a 9-wk period in 1997. At the end of the growing season in 1996, when the number of plants on which *N. elegantalis* eggs were found was increasing and pesticide applications were declining, the egg parasitoid *Trichogramma pretiosum* Riley, was observed and collected. During the last three sampling dates of 1996, parasitism rates increased from 2.4 to 28.7%. However, no egg parasitoids were found during the following autumn to winter growing season of 1997.

KEY WORDS: Insecta, tomato, tomato fruit borer, tritrophic interactions, fruit size, trichomes.

Lycopersicon esculentum Mill., tomato, is the second most important vegetable crop in Brazil, with approximately 60,000 ha under production (Nehmi *et al*. 1999). In the state of Rio de Janeiro, 10 to 15% of the cost of production is due to insecticide applications against four major insect pests: *Tuta absoluta* (Meyrick), *Liriomyza sativa* Blanchard, *Bemisia argentifolii* Bellows & Perring, and *Neoleucinodes elegantalis* (Guenée) (Cruz *et al.* 1996). *N. elegantalis* was first observed in Brazil in 1922 (Leiderman & Sauer 1953) and has since become the principal pest in several tomato growing regions. Estimated losses range from 45 (Gallo *et al*. 1988) to 90% (Leiderman & Sauer 1953, Moreira *et al*. 1985). Female moths are capable of laying up to 160 eggs (Marcano 1991) on a variety of solanaceous hosts (Toledo 1948). Eggs are usually laid singly or in clusters, on the calyx or fruit, and after hatching, the larvae penetrate directly into the fruit where they feed until pupation (Toledo 1948, Muñoz *et al*. 1991). It takes only one larva to make the fruit unmarketable, but with moderate to severe infestations up to 14 larvae/fruit have been reported (Toledo 1948, Muñoz *et al*. 1991). Despite the current control strategies, which include two to three applications of insecticides per week, and cultural practices such as the destruction of infested fruits or wild solanaceous hosts (Gallo *et al*. 1988), the reduction in the number of infested fruits is less than satisfactory (Maranhão *et al*. 1996). Furthermore, insecticides are mostly ineffective once the larva enters the fruit, are costly, can lead to resistance problems, have a negative effect on the environment, on farm workers and consumers, can lead to a loss of natural enemies, and can affect non-target species.

Because of the risks associated with insecticides, alternative control strategies are needed for managing *N. elegantalis*. However, this can only be accomplished once we better understand the sensory cues that are involved in the acceptance and rejection of the host and/or oviposition site by the pest. Oviposition in Lepidoptera proceeds through a series of more or less distinct stages corresponding to host finding, host assessment, and oviposition (Renwick & Chew 1994). Olfactory responses to plant odors are known to be important in host location (Visser 1986, Ramaswamy 1988, Feeny *et al*. 1989), but upon contact with the plant, contact perception of both chemical and physical cues becomes paramount in determining the plant's suitability for oviposition (Kumar 1992). The choice made during oviposition is especially crucial for lepidopterans as recently eclosed larvae are often relatively immobile and suitable hosts may be widely spaced.

Here we present some of our findings regarding factors that influence the oviposition preference of *N. elegantalis*. Data on natural enemies are also presented and the potential for utilizing an egg parasitoid in a biological control program is discussed.

Materials and Methods

From late July until mid-October in 1996, and late April until the end of June in 1997, tomato fields (cv. 'Santa Clara') located near São José de Ubá, RJ were monitored for *N. elegantalis* and potential natural enemies. Tomato fields were selected based on accessibility and the presence of young fruits and *N. elegantalis* eggs. Fields were monitored on a weekly or bi-weekly basis, beginning about 45 days after transplanting, and continuing until harvest or until eggs were difficult to detect. Our objectives were to determine: (1) where eggs were laid on the plant, (2) whether there was a preferred fruit size for oviposition, (3) the number of eggs oviposited per plant, (4) the percentage of plants having one or more egg mass, and (5) whether there were any resident natural enemies that might be incorporated into a biological control program.

In 1996, 200 tomato plants were randomly selected and examined to determine the distribution of eggs. The exact location of the eggs was recorded (i.e., stem, foliage, petiole, upper or lower surface of the calyx, and fruit). The position of eggs within a fruit cluster was also noted. If eggs were located on the fruit or calyx, the maximum diameter (mm) of the fruit was measured by means of a Vernier caliper. All fruits were measured on 10 additional plants that had eggs. This provided us with a measure of all available fruits versus those that were selected by the female for oviposition. In 1997, counts were made of the number of eggs/egg mass and number of egg masses per fruit+calyx. An evenly dispersed, low number of eggs/egg mass or eggs/fruit could indicate that females were using an oviposition-deterring pheromone (ODP).

A preference for laying eggs on the underside of the calyx rather than on the upperside was noted. To determine if physical and/or chemical factors associated with trichomes were involved in this apparent preference, we used a dissecting microscope to count Type I and Type VI trichomes (Luckwill 1943) on the upper and lower surface of the calyx (N=25). These trichomes were the most common ones found on the calyx. Type I trichomes are chiefly responsible for villosity in the genus *Lycopersicon*, whereas Type VI trichomes contain a glandular head with two to four cells that contain methyl ketones. These compounds, especially 2-tridecanone, are acutely toxic to several insect pests of tomato (Luckwill 1943, Lin *et al*. 1987, Carter *et al*. 1989).

Throughout the monitoring periods in 1996 and 1997, *N. elegantalis* eggs were collected to determine rates of parasitism. Eggs were gently collected and placed on moistened filter paper in petri dishes. These eggs were maintained in an environmental chamber at 24 ± 1 °C, on a 12L:12D light regime, until the larvae hatched or until egg parasitoids emerged.

A t-test was used to determine if there was a significant difference in mean diameter between fruits containing eggs and fruits without eggs. All measurements of central tendency are expressed as means (± SEM).

Results and Discussion

Similar to reports by Salinas *et al*. (1993) in Columbia and by Salas *et al*. (1991) in Venezuela, eggs of *N. elegantalis* were found primarily on the tomato fruit and calyx. Salinas *et al*. (1993) found that most eggs (48%) were located at the interface between the tomato fruit and calyx. We found that the distribution of eggs varied with the degree of infestation, but that most eggs (85-99%) were located on the fruit or lower surface of the calyx (Table 1). No obvious preference for the interface between the calyx and fruit was observed. There was, however, a clear preference for the underside of the calyx in comparison to the upper surface of the calyx. We believe that this preference can at least partially be explained by the presence of Type I trichomes on the upper surface of the calyx, which had an average of 223.0 ± 14.76 trichomes (1.9 ± 0.14 mm long). The lower surface of the calyx had only 0.4 ± 0.25 Type I trichomes. The upper and lower surfaces of the calyx were not markedly different with regard to the number of glandular Type VI trichomes. Although this needs to be examined in more detail, it seems that the mechanism involved in the preference for the lower surface of the calyx is most likely due to mechanical rather than chemical interference. Consistent with these findings, Moreira *et al.* (1985) found that the number of glandular trichomes was not correlated with the degree of attack by *N. elegantalis* on several cultivars of tomatoes. A high density of long trichomes in maize, *Zea mays* L. has also been reported to act as an oviposition deterrent in a related pyralid moth, *Chilo partellus* (Swinehow) (Kumar 1992).

Table 1. Distribution of *N. elegantalis* eggs on *L. esculentum* cv. 'Santa Clara' with low to moderate and high infestations in 1996, in São José de Ubá, RJ.

	Low to moderate infestation*		High infestation**	
Location	Distribution (%)	n	Distribution (%)	n
Foliage & stem	0	0	0	0
Petiole	4.8	23	0	0
Upper surface of calyx	10.5	50	1.0	9
Lower surface of calyx	55.8	266	34.3	325
Fruit	28.9	138	64.7	612

* Low to moderate infestation was arbitrarily used when < 50% of the plants were infested;
** High infestation was used when > 50% of the plants were infested

Similar to previous reports from Columbia and Venezuela (Muñoz *et al.* 1991, Salas *et al.* 1991, Salinas *et al.* 1993), we found that the majority of eggs were laid on fruits with a mean diameter of 23.1 ± 0.95 mm. A comparison of the frequency distributions of available fruits versus fruits that received one or more oviposition bouts illustrates a distinct preference (Fig. 1; t= 8.18, P < 0.001, df =314). Seventy-three percent of all fruits between 11-30 mm in diameter

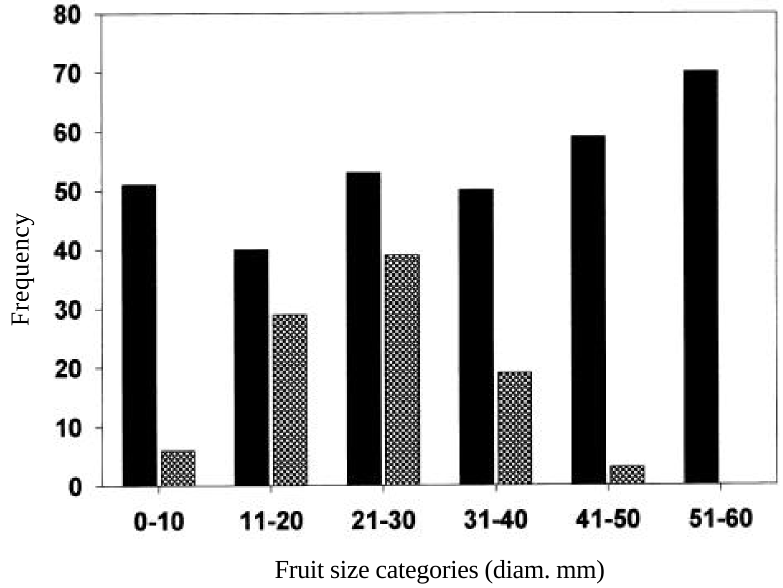

Figure 1. Frequency distribution for total number of *L. esculentum* fruits, cv. 'Santa Clara', available (solid bars), and fruits with *N. elegantalis* eggs (crosshatched bars), in São José de Ubá, RJ in 1996.

received one or more oviposition bouts, whereas only 27% of the fruits smaller or larger than this preferred size were selected for oviposition. Whether this preference is due to fruit size, color (fruits larger than 40 mm were beginning to turn red) and/or chemical changes needs to be examined.

During the egg distribution survey, tomato plants had an average of 5.2 ± 0.75 fruit clusters. We found that 89% of *N. elegantalis* eggs were laid on fruit clusters that approached the preferred fruit size (Table 2). Also, 76% of all eggs were laid on the first four basal fruits of the fruit cluster. This information on egg distribution could be used to develop a monitoring program for *N. elegantalis*. Searching efforts could be limited to the fruit and under the calyx of the first four basal fruits on fruit clusters with fruits 10-30 mm in diameter.

Table 2. Distribution of *N. elegantalis* eggs on *L. esculentum* cv. ‘Santa Clara’ in relation to fruit size and position in 1996, in São José de Ubá.

Cluster position[1]	Fruit size Diameter (mm ± SEM)	Number of eggs
1	8.5 ± 2.43	3
2	13.9 ± 7.65	56
3	22.0 ± 11.04	105
4	36.4 ± 11.21	17
5	45.8 ± 11.77	0
6	55.0 ± 4.92	0

[1] Position 1 is the upper cluster, position 6 the lower cluster

During a 9-wk sampling period in 1997, the number of eggs/egg mass ranged from one to 13. However, the average number of eggs/egg mass was relatively constant, ranging between 2.3 and 3.6 (Fig. 2). This is in spite of the fact that the number of egg masses found varied from a low of eight to a high of 408, which represented infestation levels of 8.7% (N=80) and 80% (N=105), respectively. Toledo (1948) and Salinas *et al*. (1993) also reported an average of 2-3 eggs/egg mass. These findings are highly suggestive of an egg-spacing or oviposition-deterring pheromone (ODP). Evidence that ODP’s are responsible for evenly spaced eggs has been demonstrated for a number of lepidopteran species (Gabel & Thiéry 1992, Renwick & Chew 1994). Total deterrency has never been observed, although reductions in oviposition by as much as 57% were reported for *Lobesia botrana* (Denis & Schiffermüller), the European grapevine moth (Gabel & Thiéry 1992). For *N. elegantalis*, the majority

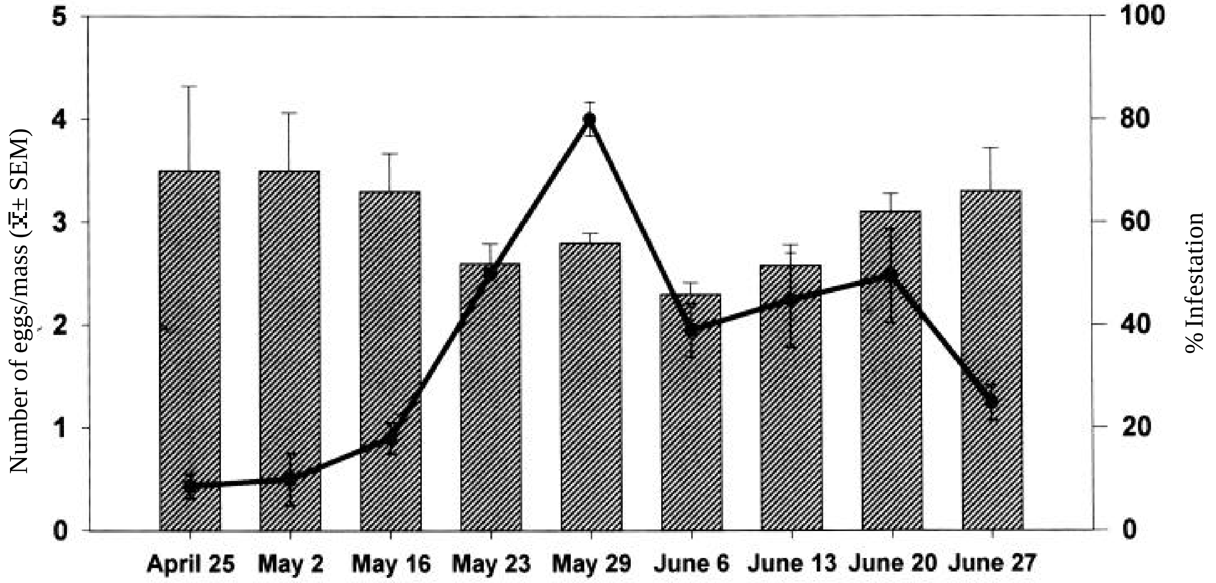

Figure 2. Frequency distribution for *N. elegantalis* egg mass size (diagonally-slashed bars) and percentage of plants infested (solid line) on *L. esculentum*, cv. ‘Santa Clara’, in São José de Ubá, RJ in 1997.

(70%) of suitable oviposition sites received one oviposition bout (Fig. 3). However, as infestation levels increased, the number of oviposition bouts/fruit increased (Figs. 2 and 3), indicating that if *N. elegantalis* has an ODP then adaptation to the pheromone may be occurring as population pressure increases.

In 1996, during a 10-week sampling period, the percentage of plants with eggs increased from 11.8 to 100% (Fig. 4). When approximately 90% of the sampled plants were infested with an average of 16.08 eggs/plant, we observed an increased incidence of the egg parasitoid, *Trichogramma pretiosum* (Riley). Parasitism rates increased from 2.4 to 28.7% within a two-week period. In the following autumn to winter growing season of 1997, no egg parasitoids were found. Seasonal variations in temperature and rainfall, between the two growing seasons, may account for the lack of parasitoids in 1997. Alternatively, since tomatoes are generally not grown in this region during the summer months (December – February), there may have been a shortage of suitable hosts for *T. pretiosum*, leading to a decline in their numbers, emigration or perhaps aestivation.

During our search for biological control agents, only one other potential natural enemy was encountered. This wasp was a polyembrionic, egg-larval parasitoid belonging to the family Encyrtidae; however, it was found in only one out of several hundred larvae that were examined. Leiderman & Sauer (1953) encountered *T. minutum* Riley on eggs of *N. elegantalis* in the state of Rio de Janeiro, and Salas *et al.* (1991) have encountered *Trichogramma* spp. in Venezuela. In Columbia, Muñoz *et al.* (1991) found that on *Solanum quitoense* Lam. 1.6% of the larvae of *N. elegantalis* were parasitized by encyrtid wasps, 0.08% by tachinid flies, 0.4% by ichneumonid wasps, and 55% of the pupae were infected by *Beauveria* spp. In tomato, they found that 82% of the eggs of *N. elegantalis* were parasitized by *Trichogramma* spp. The abundance and types of natural enemies may be so much higher in Columbia because all eight wild species of tomato, as well as the wild form of *L. esculentum* originated in this region of South America (Warnock 1991). Exploration in this region for natural enemies of *N. elegantalis* could be worthwhile. As for *T. pretiosum*, it offers potential for the biological control of *N. elegantalis*. This species readily parasitizes the eggs of *Diatraea saccharalis* (Fab.), a lepidopteran species that can be mass reared in the laboratory, thus making it possible to rear *T. pretiosum* for releases. Augmentative or inundative releases of this parasitoid throughout the growing season could be an important component of an IPM program. Currently, the frequent use of "hard" pesticides makes such a program untenable, but with the advent of many of the new biorational pesticides this approach may become feasible.

We are currently investigating the effects of tomato genotypes and fruit characteristics on oviposition behavior of *N. elegantalis*, as well as the effects of plant and host (*N. elegantalis*) chemistry on host location by *T. pretiosum.* Additional work is needed before a comprehensive IPM program can be developed for *N. elegantalis*. Perhaps by using the sex pheromone of *N. elegantalis* as a monitoring

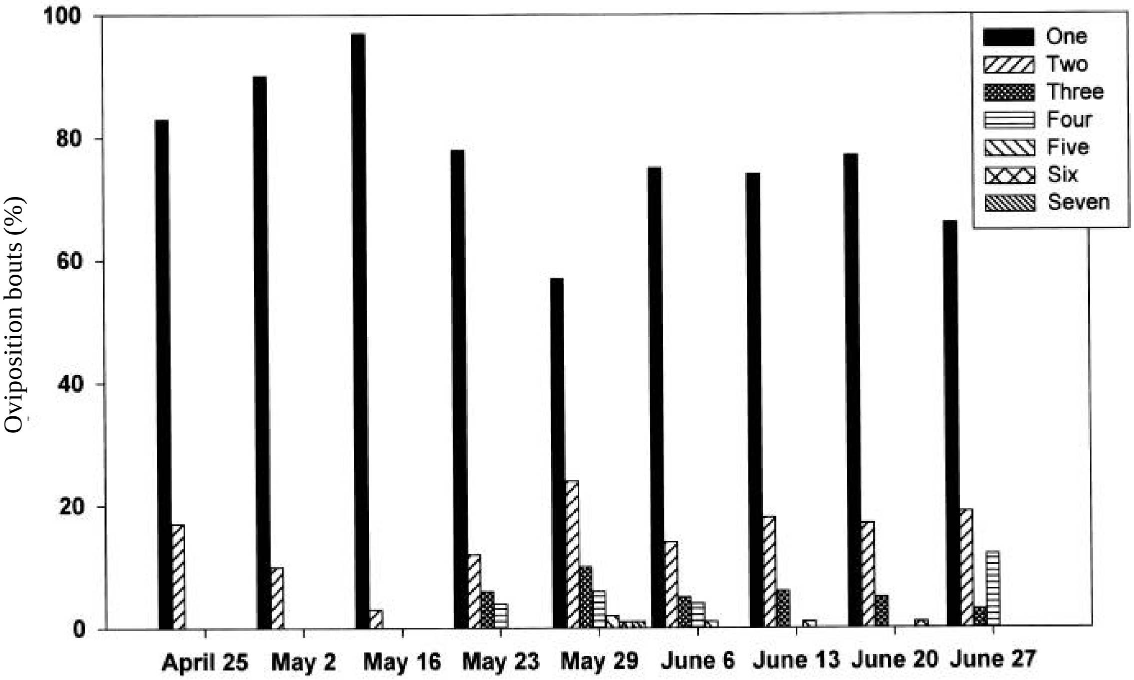

Figure 3. Percentage of plants receiving 1-7 separate oviposition bouts from *N. elegantalis* on *L. esculentum*, cv. 'Santa Clara', in São José de Ubá, RJ in 1997.

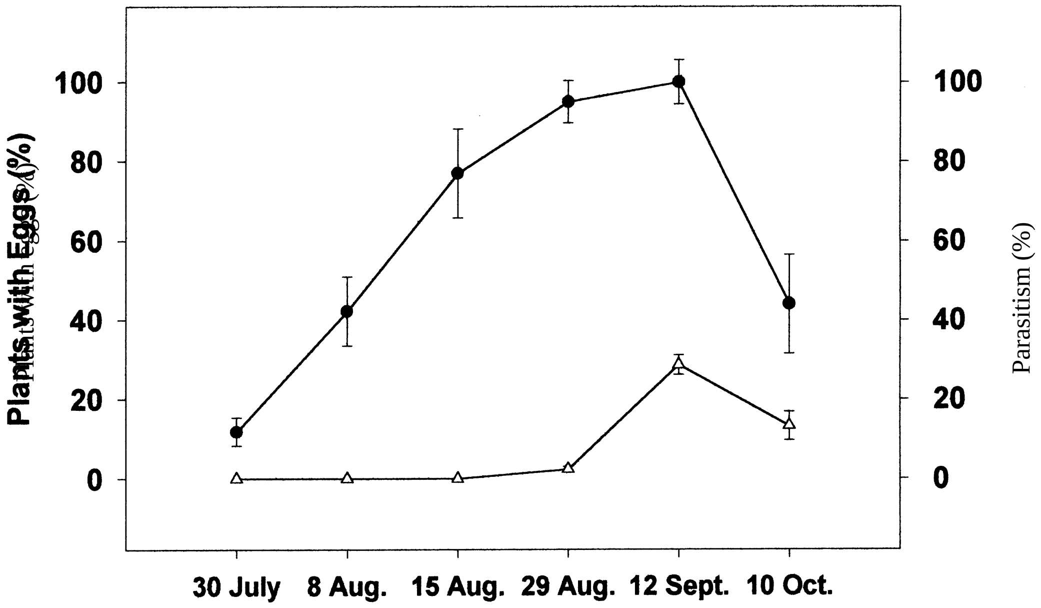

Figure 4. Percentage of *L. esculentum,* c.v. 'Santa Clara', with *N. elegantalis* eggs (circles) and percentage of eggs parasitized by *T. pretiosum* (triangles) in 1996, in São José de Ubá, RJ.

tool, along with a better understanding of the cues that the moth uses in selecting oviposition sites, and the factors that affect host location and selection by *T. pretiosum,* it may be possible to reduce the impact of this pest.

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