



TOMATO PLANTS TRANSGENIC FOR AN *ARABIDOPSIS THALIANA* CYSTEIN PROTEINASE INHIBITOR (*ATCYS*) IMPAIR THE LIFE CYCLE OF *HELICOVERPA ARMIGERA* (HÜB.)



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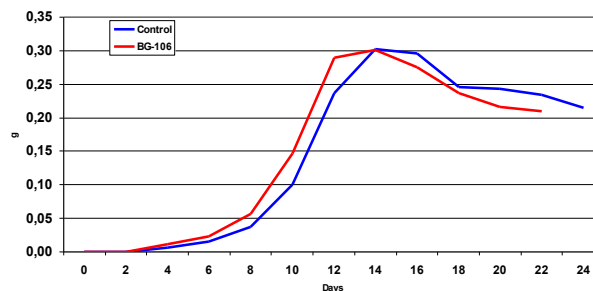
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Introduction:

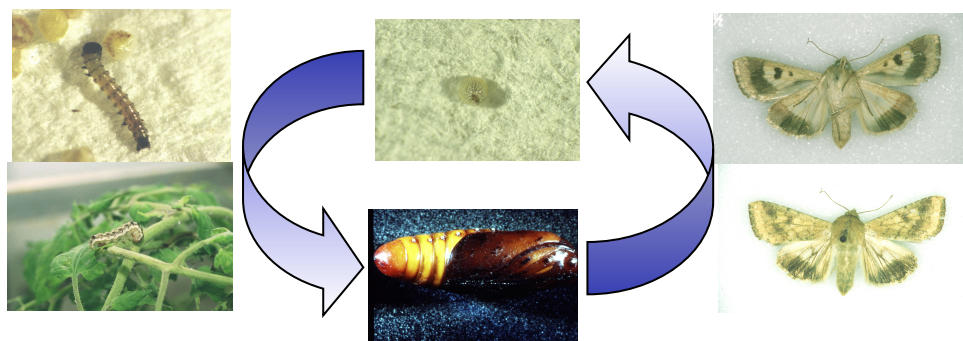
Helicoverpa armigera (Hübner) is a cosmopolite Lepidoptera Noctuidae able to injury several plant species (more than 180) among which tomato (*Lycopersicon esculentum*) (Pollini, 1998; Tremblay, 1990). The young larva is yellow with black head, then, at mature stage, it becomes green-yellowish with typical lateral light stripes. It accomplishes its life cycle in about a month, but in Central-Southern Italy, it has 2-4 generations per year, over wintering as pupa in the soil. In the last years, this noctuid has provoked infestations of so high degree to become a tomato key pest in Central Italy and in several other world regions (Speranza, 2001; Reed and Pawar, 1982). On the tomato plant, the larvae start eating away the leaves then feed themselves with flower buds and berries, by causing their dropping to the ground or rotting. At harvest (August in Italy) non marketable fruits exhibiting holes and tunnels without or with eating larvae, can be observed.

Proteinase inhibitors are able to interfere in several animal digesting processes and to harm on their growing (Broadway and Duffey, 1986; Liener *et al.*, 1988; Ryan, 1990).

Following introduction of the *CpTI* (cowpea trypsin inhibitor) gene into tobacco, Hilder *et al.* (1987) showed that its expression in the transgenic plants resulted in a significant reduction of damage by another lepidopterian Noctuidae *Heliothis virescens*.



Dynamic of average weight of *H. armigera* reared with BG-106 (line red) and with control (line blue).



Life cycle of *Helicoverpa armigera* (Hüb.)



Tomatoes damaged by *H. armigera*.

Materials and methods:

The tomato plant cv. Riogrande (RIG) and its *AtCys* transgenic version (T_2 homozygous plants, BG-106) were utilised for the insect bioassay. The transgenic BG-106 plants showed a cysteine inhibitory activity double than the control. *H. armigera* individuals from the Plant Protection Department of the University of Tuscia's rearing were used for the bioassays. In all the bioassays non-transgenic genotype RIG were used as control and the bioassays were carried out in a thermo-conditioned environment at 27°C (± 1), 18:6 photoperiod (light:dark) and 50% H.R.. Newborn larvae, taken from the above-mentioned rearing, were reared with artificial diet for 4 days to avoid the natural neonatal mortality. After this period 64 larvae per thesis (transgenic and RIG tomato), were taken by random and each of them put in a breeding box. Pieces of leaves of the testing plants were put in each box to let a continuous feeding. Every two days, from the beginning of the bioassay to the emerging from the cocoon, the larval dejections was take away; the boxes were sterilized by means of ethylic alcohol. Every two days also the larval weight and their mortality were recorded. At the pupating pupae sexing were recorded. The emerged insects were coupled, in particular boxes (9x9cm), in order to allow their coupling and the oviposition. Each couple were daily monitored and the newborn larvae from the laid eggs were noted and taken. At the death of the individuals of each couple, the number of the laid eggs were noted.

Results:

The mean weight was generally higher when larvae were fed with BG-106 leaves. No statistical difference in mortality was observed between larvae reared with control (RIG) and with BG-106 leaves. The percentage of adults emerged from the cocoon was 81% and 76% for the control and BG-106 respectively. The sex ratio (males/females) was in favour of the female sex both for the RIG (0.94) and BG-106 (0.79) cocoons. On average, the number of laid eggs of the BG-106 fed females was 33% lower than the control. By considering the percentage of hatched eggs (emerged larvae), the value obtained was 6.8% for BG-106 against 11% for RIG.

	N.	P	Pu	Pu	A	Ad	Ad	Co	n.	% of
Control	10	10	10	10	10	10	10	10	10	10
BG-106	10	10	10	10	10	10	10	10	10	10

Data results from bioassay

Conclusion:

According to these data, in *AtCys* transgenic tomato (BG-106), a level of cysteine proteinase inhibition double than the untransformed control, is sufficient to negatively influence the *H. armigera* biological cycle in particular on number of eggs laid and on fertility (percentage of emerged larvae per female), even if the weight of the larvae fed with the BG-106 leaves is on average higher than the control (RIG). The last datum is in agreement with similar experiments reported in literature where the effect of proteinase inhibitors is tested in different host-pest systems (Abhay *et al.*, 1999; Hilder *et al.*, 1987; Ryan, 1990).

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