Boll. Zool. agr. Bachic.
Ser. II, 30 (2): 197-206

30 december 1998

G. MINEO, M.C. RIZZO, B. MASSA, N. MINEO

On the control of *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) by selective insecticides and by manipulation of indigenous natural antagonists(*)

Abstract - A field trial looking at the efficacy of selective chemicals and biological procedures against the Citrus leaf miner (CLM) in a new plantation of citrus lemon, 14 Ha in extent, including about 3 years old plants, was carried out in Sicily. The citrus grove made of 3836 trees was divided into four blocks, one of which was treated with methomyl(1), one with paraffin-oil(2), one with imidacloprid(3) and another one with inoculative releases either of *Cirrospilus diallus* (Walker) or *C. pictus* (Nees), emerged from parasitized larvae of CLM collected in the field and bred in climatic room.
The final efficiency of the different procedures was measured by the percentage (in cm^2) of necrotized leaf surface due to the CLM larval trophic activity both on the sprayed and on the unsprayed plants. Results concerning various pest management procedures are discussed.

Riassunto - *Sul controllo di* Phyllocnistis citrella *Stainton (Lepidoptera Gracillariidae) mediante insetticidi selettivi e con la manipolazione di antagonisti indigeni naturali.*

Nel 1998 è stata effettuata in Sicilia una prova di controllo della Minatrice serpentina degli agrumi in un limoneto di 14 Ha costituito da 3836 piante di circa 3 anni. Esso è stato suddiviso in quattro parcelle; in tre di esse il controllo della Minatrice è stato effettuato eseguendo trattamenti rispettivamente con methomyl(1), olio di paraffina(2) e imidacloprid(3), mentre nella quarta sono stati fatti lanci inoculativi di adulti di *Cirrospilus diallus* (Walker) e *C. pictus* (Nees), ottenuti da larve parassitizzate raccolte in limoneti infestati dalla Minatrice.
L'efficacia finale dei diversi mezzi utilizzati è stata calcolata misurando la superficie fogliare necrotizzata conseguente all'attività trofica larvale della minatrice. Vengono infine discussi i risultati emersi.

Key words: *Phyllocnistis citrella; Citrus lemon* young plantation; cultural, chemical and biological control procedures.

(*) Research funded by Assessorato Regionale Agricoltura e Foreste Regione Sicilia (progetto di lotta integrata) and M.U.R.S.T. 40%.

Paper read at the Regional Symposium for Applied Biological Control in Mediterranean Countries, Cairo, 25-29/X/98.

(1) Lannate®. (2) Biolid®. (3) Confidor®.

INTRODUCTION

All the previous Authors (Garrido, 1995; Garrido Vivas & Gascon Lopez, 1995; Caleca *et al.*, 1996; Ortu & Acciaro, 1997), who studied the infestation phenology of *Phyllocnistis citrella* Stainton (CLM) on citrus groves of the Mediterranean Basin, agree that its control problem mostly looks at the young plantings and at nurseries. Argov & Rössler (1996) also agree with these conclusion. This is also supported from the evidences of Tirado's (1995) paper, in which the artificial elimination of all the CLM affected leaves on cvs *Sallustiana, Valencia late* and *Fortuna* were simulated, without any reported lost on the respective crops.

This work deals with an experiment in which the effect of cultural, chemical and biological techniques on the CLM control in Western Sicily (province of Palermo) were tested.

MATERIALS AND METHODS

The trial has been carried out in a lemon grove of 14 ha in extent including 3836 three years old trees. It was divided into 4 parcels, each one amounting the following number of plants: B_1 = 509, B_2 = 1055, B_3 = 1055, B_4 = 1213. In B_1 24 plants were marked, in B_2 42 plants, in B_3 42 and in B_4 46: the material for the observations was collected from each marked plant. Samplings of the tender shoots, during the water-stress period were made mostly on suckers. Four shoots per marked plant, belonging to the new flushing, were collected after the water-stress period. All the leaves of each shoot were examined for accounting the data concerning the effect of the applied techniques in each block for the CLM control. The practices performed in the four blocks are shown in Table 1.

Table 1 - Cultural, biological, and chemical techniques used in the lemon blocks.

Blocks	Water-stress period (in days)	Timing of irrigations	Pruning of suckers	Date of sprays		*a.i.* per 100 c.p., or wasp species	*cc* or *g/hl* of c. p.; total n° of wasps released
				1st flushing	2nd flushing		
B_1	37	2.VII; 17.VII; 21.VIII	9-6	24.VII	14/8; 22/8	methomyl 19%	250
B_2	37	2.VII; 17.VII 21.VIII	9-6	Biological control	Biological control	*Cirrospilus diallus;* C. *pictus*	644
B_3	37	2.VII; 17.VII 21.VIII	9-6	12.VII; 24.VII	14.VIII; 22.VIII	paraffin-oil	1500
B_4	37	2.VII; 17.VII 21.VIII	9-6	12.VII; 24.VII	14.VIII; 22.VIII	imidacloprid 20%	50

The leaf damage, due to the CLM larval trophic activity, was expressed according to the following procedure:

1) the total number of shoots produced in every marked plant per each flushing was counted;

2) two indices were used, i.e.:

a) *the average number of leaves per shoot*; it was performed on the basis of several groups of shoots (861 on the whole) collected in 11 lemon groves, and resulted to be 9 leaves per shoot;

b) *the total leaf surface per uninfested shoot*; this index was calculated by measuring the leaf surface of ten uninfested shoots (each constituted of 9 leaves) of each flushing, randomly selected in a group of 40 shoots collected from 10 plants per block. It corresponded to 341 and 263 cm^2, respectively in the first and second flushing. During the 1st flushing all the infested leaves were collected from the marked plants of each block, because of the very low level of CLM infestation observed. The percentage of necrotized leaf surface (*n.l.s.*), due to CLM larval activity, was measured on at least 3 leaves per shoot (that is 12 leaves per plant), or on all the available leaves with necrotized leaf areas when they were less than twelve. At the end of the 2nd flushing the *n.l.s.* was measured on 3 leaves per shoot. It should be stressed that in the 1st flush the number of affected leaves with silvery glare areas was higher than that of necrotized ones. Nevertheless, as above said, only leaves of both flushings presenting necrotized areas were considered to be an expression of leaf damage.

Adults of Eulophid wasps were released in the biological block (B_2); they were obtained from numerous samples of infested CLM leaves collected in other lemon orchards (11 on the whole). In laboratory they were examined for selecting the parasitized individuals, that were bred in climatic room until the emergence of the wasps. In total 796 adult wasps emerged, of which only the 445 *Cirrospilus pictus* (Nees) and the 199 *C. diallus* (Walker) were used for the releases. Table 2 shows the number and sequence of realeses of the cited antagonists in the biological block (B_2).

Further information on the released *Cirrospilus* spp. activity, as well as about the other antagonists, were also obtained as follows. The shoots of every sampling were put, separately per block, into proper cages in climatic room until the end of CLM antagonists' emergence. They were collected and identified almost every day; the living adults of *Cirrospilus* spp. were released, without counting their amount in Table 2.

All the data were statistically analysed and results checked by the Tukey's HSD test at $P = 0.05$ (Steel & Torrie, 1980).

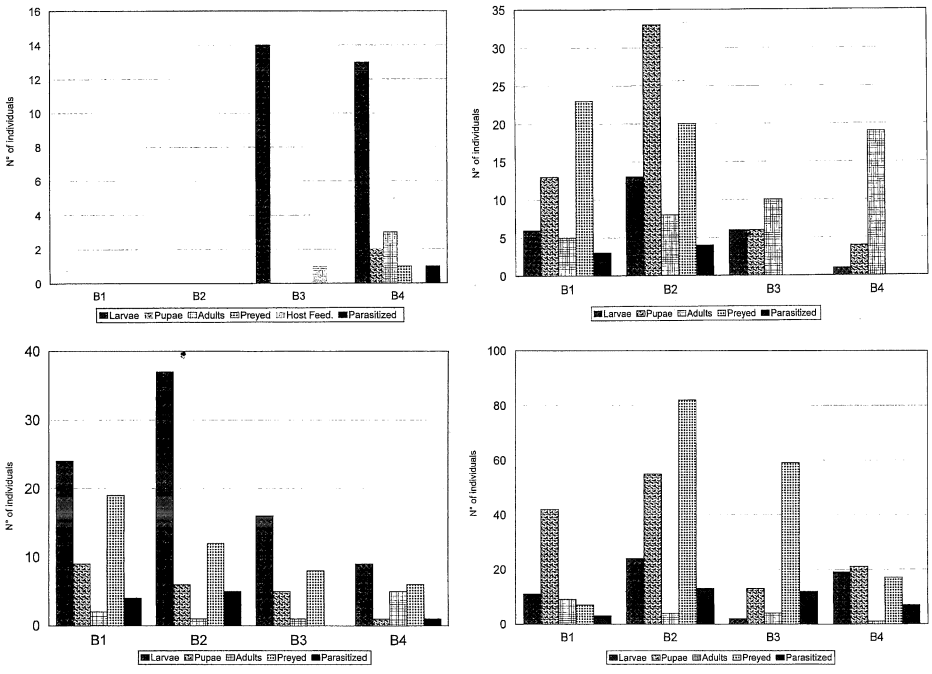
RESULTS AND DISCUSSION

Spring flushing

At the time the first sampling of shoots was made (27 May), the spring flushing was drawing to its close. After that date the plants were submitted to water-stress.

Table 2 - Inoculative releases of Cirrospilus diallus *and* C. pictus *carried out in the biological block B2.*

Date of releases	*Cirrospilus diallus* males/females	*Cirrospilus pictus* males/females
13-19/06	4/6	12/17
20-26/06	11/10	9/16
27/06-3/07	22/20	37/37
7-13/07	5/15	8/4
14-20/07	10/14	45/38
21-27/07	12/10	20/14
1-7/08	17/22	39/27
8-14/08	7/10	10/15
16-21/08	2/2	14/7
25-31/08	0/0	37/45
Total	90/109	228/217



Figs. 1-4 - Postembryonic CLM stages and antagonism levels observed in the blocks during the water-stress period: 27 May (Fig. 1), 10 June (Fig. 2), 18 June (Fig. 3), 2 July (Fig. 4).

Figs. 1, 2 and 3 show the composition of CLM population, as well as the activity of indigenous antagonists, respectively on 27 May, 10 June and 18 June(4).

1st flushing after water-stress

It lasted from about 8 July to the end of the month. Fig. 4 shows the phenology of CLM stages as well as the antagonist numbers resulted at the date of the water-stress breaking (2 July), that emerged from the samples of the tender leaves, mostly collected on suckers. These latters were cut, but not burnt, some day after the 1st irrigation. Table 3 shows the data of samplings performed on the new shoots one week after the 1st spray on B_3 and B_4. Between the treated blocks (B_1, B_3, B_4) and the unsprayed one (B_2) not significant differences were detected; the higher value of CLM antagonists emerged on B_2 are probably related to the releases of Eulophid wasps.

Table 3 - Total number of living postembryonic CLM stages, of parasitized and preyed individuals found on the 1st flushing after one week (19 July) from the 1st spray on B_3 and B_4. Numbers followed by different symbols are significant at $p = 0.05$.

	B_1	B_2	B_3	B_4
LARVAE	59 aa	165 aa	68 aa	74 aa
PUPAE	26 bb	106 bb	81 bb	12 bb
ADULTS	4 cc	3 cc	14 cc	4 cc
PREYED	58 dd	306 de	49 dd	36 dd
PARASITIZED	20 ee	195 ee	44 ee	3 ee

Table 4 shows the overall efficacy of the different treatments, expressed in terms of missing shoots per tree, and measured on the basis of *n.l.s.*. The minimum *n.l.s.* (211 cm^2) has been observed on B_4 (sprayed with imidacloprid), while the maximum (2734 cm^2) on B_2 (biological block); the *n.l.s.* of B_4 resulted lower and significantly different from those obtained on all the other blocks. In terms of missing shoots the *n.l.s.* ranged from 0.6 to 8.0 shoots per tree.

2nd flushing after water-stress

It lasted from 3 August to the first week of September, though a few number of shoots followed after. It should be pointed out that between 5 and 8 August a total

(4) In these and in other figs and tables through the paper, the term "parasitized" also includes mortality due to the host-feeding as well as to the injection into the body of CLM larvae and pupae of the secretion produced by the poisonous glands of the Eulophid females; predation in our experiment was almost entirely due to the ants.

Table 4 - Corresponding missing shoots per plant due to the necrotized leaf surface caused by CLM larval trophic activity, observed at the end of the 1st flushing. Numbers followed by different symbols are significant at p = 0.05.

Flushing	Blocks	Average number of shoots per tree	Average leaf surface per shoot (in cm^2)	Total amount of n.l.s. (in cm^2)	Equivalent of missing shoots per tree
1st	B_1	117	341	514aa	1.51
	B_2	96	341	2734ab	8.0
	B_3	90	341	1323ab	3.9
	B_4	99	341	211ac	0.6

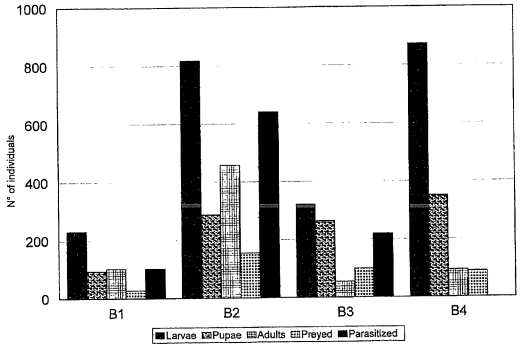


Fig. 5 - Postembryonic CLM stages and antagonism levels observed on 2nd flushing's shoots in the blocks six days before the first spray (8 August).

amount of 90 mm of rainfall occurred in the citrus planting examined. Fig. 5 shows the composition of CLM population as well as the incidence of the antagonism observed in the four blocks on 8 August. Because of technical reasons, the first spray on B_1, B_3 and B_4 was performed far from this date (14 August). In Table 5 the results

Table 5 - Total number of living postembryonic CLM stages, of parasitized and preyed individuals found on the 2nd flushing after one week (21 August) from the 1st spray on B_1, B_3 and B_4. Numbers followed by different symbols are significant at p = 0.05.

	B_1	B_2	B_3	B_4
LARVAE	390 aa	571 ab	124 aa	192 aa
PUPAE	124 bb	769 bc	265 bb	344 bb
ADULTS	13 cd	355 cc	267 cc	259 cc
PREYED	288 dd	290 dd	80 de	58 de
PARASITIZED	342 ee	1071 ef	99 eg	62 eg

Table 6 - Corresponding missing shoots per plant due to the necrotized leaf surface caused by CLM larval trophic activity, observed at the end of the 2nd flushing. Numbers followed by different symbols are significant at p = 0.05.

Flushing	Blocks	Average number of shoots per tree	Average leaf surface per shoot (in cm^2)	Total amount of n.l.s. (in cm^2)	Equivalent of missing shoots per tree
2nd	B^1	37	263	5372bb	20.4
	B^2	38	263	3984bc	15.1
	B^3	34	263	4925bd	18.7
	B^4	91	263	15279be	58.1

recorded a week after this spray (21 August), on the samples collected from the new flushing, are reported. Table 6 reports the overall efficacy of the different CLM control techniques applied in the blocks, again presented in terms of missing shoots per tree. Statistical analysis indicates that the *n.l.s.* value was significantly higher in respect to the others in the plants treated with imidacloprid (B_4). In fact such *n.l.s.* values in B_1, B_2, B_3 and B_4 amounts to an equivalent leaf surface of 20.4, 15.1, 18.7 and 58.1 missing shoots, respectively.

Antagonists' activity

The number of Eulophid wasps and of predators emerged from the samples of each block put into cages in climatic room is reported in Table 7. The higher number emerged from the biological parcel, followed from that in which the plants were sprayed with Parafin-oil. Among the wasp species some adults of *Semielacher petiolatus* (Girault) were also reported, emerged between the first and the second week of September. About the finding of this exotic Eulophid in Sicily cf. Mineo *et al.* (1998). See Plates 1 and 2.

Table 7 - Total number of Eulophids and predators emerged from the samples collected during the experiment.

Blocks	*A. p.* m/f	*C. d.* m/f	*C. p.* m/f	*N. f.* m/f	*P. a.* m/f	*S. p.* m/f	un. m/f	Total Eulophids	*Orius* sp.	*Chrysopa* sp.	Total predators
B_1	2/0	10/10	23/29	0/3	7/5	0/0	6/1	96	14	2	16
B_2	1/0	5/15	131/120	0/1	17/7	0/2	0/3	302	7	3	10
B_3	1/2	13/14	66/93	0/1	22/13	0/0	0/0	225	8	1	9
B_4	3/1	10/10	27/31	0/2	15/7	0/1	0/0	97	6	2	8

Legenda: *A.p.*= *Apotetrastichus postmarginalis* (Boucek); *C. d.* = *Cirrospilus diallus* (Walker); *C. p.* = *Cirrospilus pictus* (Nees); *N. f.*= *Neochrysocharis formosa* (Westwood); *P. a.* = *Pnigalio agraules* (Walker); *S. p.* = *Semielacher petiolatus* (Girault); un. = unidentified

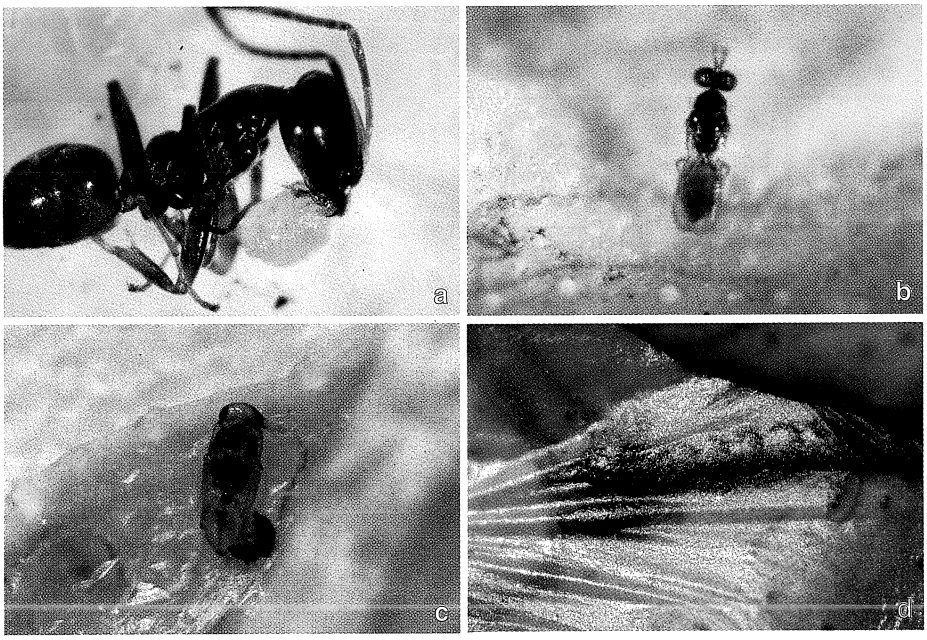


Plate 1 - a) Ant showing between the mandibles a larva of *Phyllocnistis citrella*; b) *Semielacher petiolatus* (Girault): adult; c) *Semielacher petiolatus*: young pupa; d) *Phyllocnistis citrella* Stainton. After the stinging made by the parasitoid, the body colouration of the CLM larva becomes brownish or dark-brown.

Remarks

Results above reported refer only to the first year of our experiments, and it seems hazardous to make some conclusion, particularly due to the lack of other similar experiments in citriculture against *Phyllocnistis citrella*. Nevertheless, some considerations could be made. Apart from the effects of different techniques and relative killing agents used, results shown in Table 4 (5^{th} column) sound in a way that, during the time of the first flushing after the water-stress ended, the CLM population in the field was yet at such a level that cannot determine a high infestation. Following this hypothesis it could be suggested, where it is possible, a management of such a cultural practice in order to obtain an earlier summer flushing, to avoid a massive CLM population in the field. Whenever it is not possible, the procedures for an augmentation of CLM natural antagonists, that also include the use of high selective sprays like oils, should be strengthened.

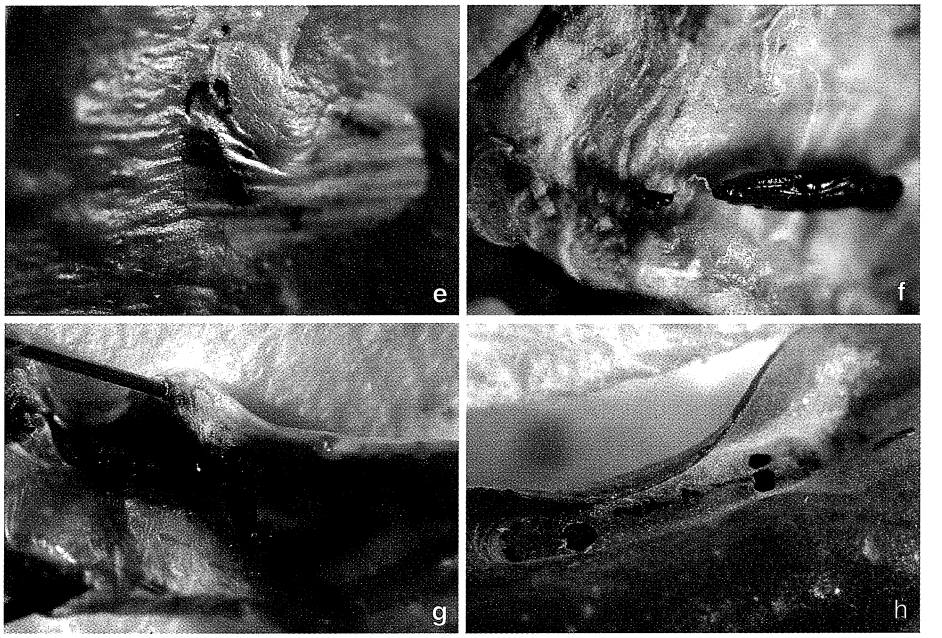


Plate 2 - e) *Cirrospilus pictus* (Nees). The full grown larva usually puts small pellets of its meconium close to each others in a horse-shoe shape at the end of the last segment of its gaster. Whenever the leaf tissues harden, either in the nature or in the laboratory rearing, the larva distributes the pellets along the sides of its body to build a sort of "pillars". Pillars are made between the roof and floor of the mine; by this expedient the larva makes easier the emergence of the adult parasitoid, avoiding leaf tissues folding. This strategy, well known for many Eulophid wasps, has been observed by us, other than in *C. pictus*, also in *C. diallus* (Walker), *Ratzeburgiola incompleta* Boucek and others; f) *Cirrospilus pictus*: pupa exhibiting the "pupal cord". The young pupa, in order to make easier the adult emergence from the pupal case, forms a cord that may be sticked either on the floor of the pupal cell or on the meconium. This kind of "pupal cord" has also been observed by us in other CLM parasitoids, like *Cirrospilus diallus*, *Ratzeburgiola incompleta* and others; g) *Cirrospilus pictus*: a very rare case observed in the nature, in which three larvae developing onto a single host have reached the pupal stage from which two adult females and one male emerged; h) *Cirrospilus pictus*: although three emergence holes may be seen, the number of meconia indicates that only two adults emerged. This occurrence, in which two larvae developed onto a single host, is also a rare case observed in the nature.

AKNOWLEDGMENTS

We thank Mario Lo Valvo and Antonino Mineo for their collaboration.

REFERENCES

ARGOV Y., RÖSSLER Y., 1996 - Introduction, release and recovery of several exotic natural enemies for biological control of the citrus leafminer *Phyllocnistis citrella* Stainton in Israel. - Phytoparasitica 24 (1): 33-38.

CALECA V., LO VERDE G., MASSA B., 1996 - Indagine su *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) in un limoneto della Sicilia occidentale. - Boll. Zool. agr. Bachic. 28 (2): 165-183.

GARRIDO A., 1995 - El minador de las hojas de las citricos (*Phyllocnistis citrella* Stainton): morfologia, biologia, comportamiento, daños, interaccion con factores foraneos. - Phytoma 72: 84-92.

GARRIDO VIVAS A., GASCON LOPEZ I., 1995 - Distribucion des fases immaduras de *Phyllocnistis citrella* Stainton, segùn el tamaño de la hoja. - Bol. San. Veg. Plagas 21: 559-571.

MINEO G., CALECA V., MASSA B., 1998 - *Semielacher petiolatus* (Girault) (Hymenoptera Eulophidae), natural antagonist of *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae), new for the italian entomofauna. - Naturalista sicil. 22: 3-6.

ORTU S., ACCIARO M., 1997 - Indagini sui danni prodotti dalla minatrice serpentina degli agrumi (*Phyllocnistis citrella* Stainton) in Sardegna. - Phytophaga 7: 13-21.

STEEL R.D.G., TORRIE J.H., 1980 - Principles and procedures of statistics. - 2nd ed. Mc Graw-Hill, New York.

TIRADO GONZALES L., 1995 - Daños causados par las ataques de *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae), y su repercusión sobre la produccion de arboles adultes de citricas en el suroeste español. - Bol. San. Veg. Plagas 23 (1997): 73-91.

PROF. GIOVANNI MINEO, DR. MARIA CONCETTA RIZZO, PROF. BRUNO MASSA - Istituto di Entomologia agraria, Università, Viale delle Scienze 13, I-90128 Palermo.

DR. NICOLA MINEO - Viale Michelangelo 200, I-90145 Palermo.

Accepted 30 November 1998