Donahaye, E.J., Navarro, S. and Leesch J.G. [Eds.] (2001) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Fresno, CA. 29 Oct. - 3 Nov. 2000, Executive Printing Services, Clovis, CA, U.S.A pp. 727-739

PHEROMONE TRAPS FOR MONITORING *LASIODERMA SERRICORNE* F. AND *EPHESTIA ELUTELLA* (HÜBNER) IN STORED TOBACCO (BEFORE FUMIGATION) IN PORTUGAL

MARIA OTÍLIA CARVALHO,* ANA PAULA PEREIRA AND A. MEXIA

*Centro de Estudos de Fitossanidade do Armazenamento/ Instituto de Investigação Científica Tropical 1300-142 Lisboa, Portugal [*e-mail: cefa@iict.pt]*

ABSTRACT

Studies were conducted, from May 1997 to December 1999, in 12 Virginia tobacco fields, in 9 on-farm and curing barns, in 1 green Virginia tobacco store, 1 processing factory (stemmery) 2 finished tobacco stores and a manufacturing room, located in Portugal. Two types of pheromone traps, Mini Delta and New Serrico, were used to detect and monitor *Lasioderma serricorne* F. Three types of pheromone trap, Delta, Funnel and Pherocon II, were used to detect and monitor *Ephestia elutella* (Hübner).

Our findings revealed the presence of both species from tobacco fields through to the manufactory room, and for both species the insect populations increased through these processes, although the abundance of both species was very low in the fields, on-farm stores and curing barns. In the factories and stores the relative density of cigarette beetle males was higher than tobacco moth males.

Iwao's patchiness regression was used to estimate the distribution pattern of both pests. *L. serricorne* can fit the linear regression, and β-slope suggested a definite tendency to aggregate but colony size may change with increase in population density: at low densities males did not tend to live together in the same quadrat but with higher densities the α-intercept suggested that small groups of individuals are formed ($1<\alpha+1<3$). *E. elutella* males, at low densities seemed to fit better a random distribution although with increasing density, more individuals tended to be distributed in a weak but definitive aggregation.

INTRODUCTION

Insect infestations by the cigarette beetle, *Lasioderma serricorne* F. (Coleoptera: Anobiidae) and the tobacco moth, *Ephestia elutella* (Hubner) (Lepidoptera: Pyralidae), in stored tobacco may begin even in on-farm stores and curing barns and continue until the tobacco is marketed (Anon. 1971).

L. serricorne is the most serious tobacco pest; it is particularly suited to tropical regions and is known to infest all stages of the product, resulting in a world-wide loss of at least US$ 300 million of stored tobacco stocks per annum (Anon. 1971). Because of its adaptability *L. serricorne* may become established in leaf storages, in processing facilities, in tobacco dust accumulations, in the finished product, and even in retail premises and vending machines (Massey, 1999). *E. elutella* can consume and damage stored tobacco but it is generally less widespread than *L. serricorne* in tobacco, as its distribution seems to be confined mainly to temperate regions of the world (Genève, 1996).

A comprehensive integrated pest management program is required to produce a quality product that is free from active insect infestation and/or punctation of tobacco leaves or cigarettes. This involves a team approach and must be practiced at all stages from curing on farms, through the processing factory (stemmery), to storage and manufacturing premises (Massey, 1999). The procedure of monitoring to learn of the presence and population densities of *L. serricorne* and *E. elutella* is a primary tool for determining both when and where action should be taken to ensure a good quality of finished product (Massey, 1999).

Nowadays, pheromone traps are the main tools available for detecting and monitoring both pests. Furthermore Carvalho (1998) found that the traps Mini Delta and New Serrico were the most efficient for catching *L. serricorne* among five commercially available pheromone traps with and with/without food lures.

In this work, data obtained from pheromone-trap captures provide information on the presence of both *L. serricorne* and *E. elutella* from the field, to the curing barns through manufacture, and to storage of the final product. The spatial distribution pattern for populations of each species, using the regression of mean crowding is analysed.

MATERIALS AND METHODS

Experimental sites

Studies were conducted in 12 Virginia tobacco fields (var: k 326), 9 on-farm stores and curing barns, 1 green tobacco Virginia store, 1 stemmery (processing factory), 2 finished tobacco stores and a manufacturing room (manufactory), in a number of locations in Portugal, from May 1997 to December 1999 (Table 1). Each tobacco field area ranged from 6 ha to 60 ha (Carvalho *et al.,* 2000), and the areas of on-farm stores, cured tobacco stores and factories are presented in Table 1.

All the cured tobacco was processed in the stemmery and was temporarily stored in green tobacco stores. After being processed, the finished tobacco was stored in part in the finished tobacco store No. 1, which is owned by the stemmery facility. The finished tobacco store No. 2 belongs to the manufacturing facility and is used to store several types of cured tobacco from different countries before being transformed into cigarettes in this factory.

Monitoring techniques

Lasioderma serricorne: Two types of pheromone traps were used: Mini Delta traps (from AgriSense-BCS, UK) and New Serrico (from Fuji Flavor Co., Ltd., Japan). The Mini Delta trap is an adhesive delta trap (~ 130 mm x 110 mm), which presents a pattern of black and white vertical stripes, and is supplied with the pheromone lure that is enclosed in a small plastic vial, positioned in the middle of the trap and lasts insert Table 1

4-6 weeks. The New Serrico trap is designed as a slim box (~180 mm x 80 mm x 7 mm) to protect and enclose the two adhesive surfaces. This type of trap is supplied with both food and pheromone lures. The pheromone lure is activated by removing a polythene film from fiber disks into which it is absorbed and has a duration of 6-8 weeks. The food lure is obtained by steam distillation of a few kinds of herbs in order to attract *L. serricorne* females and lasts 2 weeks.

The two traps were supplied with the pheromone lure (4S,6S,7S)4,6-Dimethyl-7-hydroxynonan-3-one (Serricornin). Mini Delta traps were used to detect *L. serricorne* in the tobacco fields, in the on-farm stores and in the curing barns, and were changed every 6 weeks. New Serrico traps were used in the warehouses and handling facilities and were changed every 6 weeks except in the manufacturing facility where they were changed every 4 weeks.

To detect insects in the fields, one pheromone trap for *L. serricorne* and for *E. elutella* were placed in each field, suspended in trees at a height of 1-2 m. To detect and monitor *L. serricorne* populations in on-farm stores curing barns, warehouses and the processing facility, the traps were fixed at a height of 1-1.5 m at distances from each other which ranged from 12 m x12 m to 25 m x 25 m. In the room located in the manufacturing facility the traps were set-up at a distance of 6 m x 6 m from each other, to target for mass trapping. The number of traps used in each site is presented in Table 1.

Ephestia elutella: Three types of pheromone traps were used: Delta and Funnel traps (from AgriSense-BCS, UK) and Pherocon II (from Trécé Inc., USA). The delta moth trap for monitoring *E. elutella* consists of corrugated plastic (280 mm high x 200 mm x 120 mm) and employs a replaceable sticky insert and polythene vials. The pheromone dispensers and sticky inserts are replaced every 6 weeks. The funnel trap, 230 mm high and 170 mm diameter, is made of plastic with a large base and removable cap. The pheromone dispensers are replaced every 6 weeks. Dead insects collect in the base of the trap. Both traps use polythene vials containing 2 mg of *(Z,E)*-9,12-tetradecadienyl acetate (ZETA). The Pherocon II trap consists of two glued surfaces and uses a rubber pheromone lure that lasts 6 months.

All the traps were placed at 2 to 3 m above floor level and the numbers of moths caught were recorded weekly.

REGRESSION TECHNIQUES FOR DESCRIBING DISPERSION PATTERNS

Iwao's Patchiness Regression

Lloyd (1967) created the term "mean crowding" ($\overset{*}{x}$) used to describe the mean number of other individuals per individual in an average sample unit, expressed as

$$\overset{*}{x} = \bar{x} + \left(\frac{s^2}{\bar{x}} - 1 \right) \qquad (\bar{x} > 0)$$

$\overset{*}{x}$ = mean crowding; $\bar{x}$ = sample mean; s^2 = sample variance
The regression equation is expressed as

$$\overset{*}{x} = \alpha + \beta\bar{x}$$

α = y-intercept; β = regression slope

The symbol α denotes the average number of other individuals living in the same sample unit or quadrat per individual and is termed the *index of basic contagion.* When α=0 the basic component is a single individual; if α<0 there is repulsion among individuals; for α>0 the basic component is a colony and α+1 gives a measure of clump size. The symbol β, also called *density-contagiousness coefficient* is used as a measure of the dispersion of the basic unit, it is the slope value and indicates the special distribution of the clumps: when β=1 the clumps are distributed in a random way, when β<1 the distribution behaves uniformly and when β>1 the data follow the aggregate distribution. SE_{α} and SE_{β} are the standard errors of the estimates α and β (Iwao, 1968; Southwood, 1978).

The r^2, represents the proportion of the $\overset{*}{x}$ variability explained by the linear relation with $\bar{x}$ (Bhattacharyya and Johnson, 1977).

RESULTS

Insect detection in Virginia tobacco fields

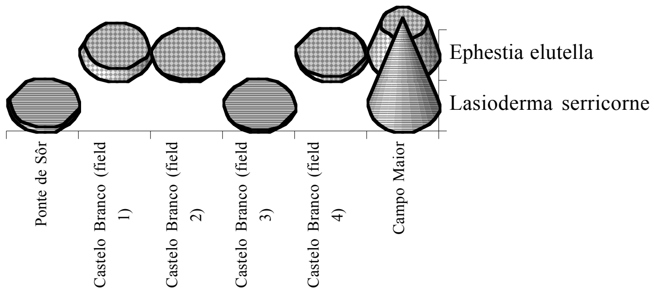
Of the 12 Virginia tobacco fields studied, one or both of the stored-tobacco pests were only detected in 6 tobacco fields (Fig. 1).

L. serricorne was detected in three tobacco fields, one field in each region, Ponte de Sôr, Castelo Branco and Campo Maior, with total catches of, 2, 1 and 44 cigarette beetles respectively. *E. elutella* was not detected in tobacco fields in Ponte Sôr but it was found in three tobacco fields in Castelo Branco, where the Delta traps caught a total of 5, 1 and 2 tobacco moths, respectively, and in one tobacco field in Campo Maior where Pherocon II traps collected a total of 16 adults.

Insect detection in on-farm stores and curing barns

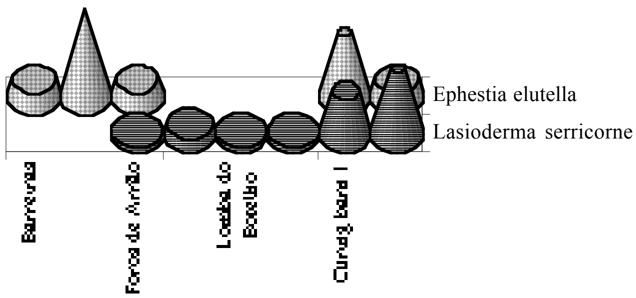
L. serricorne was present in six on-farm stores and curing barns and *E. elutella* was detected in five stores (Fig. 2).

The trap catches of *L. serricorne* in on-farm stores and curing barns in Ponte de Sôr (Foros de Arrão) and Castelo Branco (Ribeiro de Freixo, Lomba do Botelho and Cascalhal) were very low but in the two on-farm stores in the Campo Maior region (Curing barns 1 and 2) the number of male *L. serricorne* collected in the Mini Delta traps was higher although the temperatures registered during the trial period (-1ºC to 38ºC) were not favourable to *L. serricorne* development (Carvalho, *et al.*, 2000).



	Ponte de Sôr	Castelo Branco (field 1)	Castelo Branco (field 2)	Castelo Branco (field 3)	Castelo Branco (field 4)	Campo Maior
Lasioderma serricorne	2	0	0	1	0	44
Ephestia elutella	0	5	1	0	2	16

Fig. 1. Total of cigarette beetles and tobacco moths caught in the pheromone traps, placed in the tobacco fields.



	Barreiras	Tramaga	Foros de Arrão	Ribeiro de Freixo	Lomba do Botelho	Cascalhal	Curing barn 1	Curing barn 2
Lasioderma serricorne	0	0	1	3	1	1	12	18
Ephestia elutella	5	24	5	0	0	0	18	5

Fig. 2. Total of cigarette beetles and tobacco moths caught in the pheromone traps, placed in the on-farm stores and curing barns.

Although *E. elutella* was not recorded in the curing barns located in Castelo Branco, it was the main species found in the three on-farm stores and curing barns of Ponte de Sôr region (Barreiras, Tramaga and Foros de Arrão) and as with the cigarette beetle it was collected in both on-farm stores of the curing barns in Campo Maior region (curing barns 1 and 2) (Table 2). Tobacco moth populations increased as maximum temperatures decreased. However one adult was trapped in the on-farm store and curing barn in Tramaga when the highest temperature (40ºC) was registered (Carvalho, *et al.*, 2000).

TABLE 2
Iwao's patchiness regression estimates for *Lasioderma serricorne* caught by pheromone traps placed in the stores and facilities

Location	n[a]	$\alpha \pm SE_{\alpha}$	$\beta \pm SE_{\beta}$	r^2
Green tobacco store	23	-0.30034 ± 0.43594	1.963261 ± 0.09748	0.9508
Stemmery	28	0.00860 ± 0.41046	2.02783 ± 0.26873	0.6865
Finished tobacco store No.1	20	1.96975 ± 1.27825	1.29743 ± 0.13485	0.8372
Manufactory room	52	0.87477 ± 0.78367	2.03304 ± 0.08699	0.9161

[a] Number of $(\overset{*}{x} - \bar{x})$ pairs used in the regression

Insect Monitoring in warehouses and handling facilities
Lasioderma serricorne

Mean number of adults per trap: The mean trap catches registered in the stores of green tobacco and finished tobacco store No. 1, and the stemmery and manufacturing facilities are indicated in Fig. 3.

The seasonal fluctuations were more evident in the stores than in the factories. In the green and finished tobacco stores two distinct generations of cigarette beetle were present: the first generation between week 19 and week 28, and the second generation from week 34 to week 43. In the stemmery and in the manufacturing room, the number of generations was not so evident and several peaks in trap catches were registered during the period from the week 16 to week 45 in the stemmery and between the 8th and the 52nd week in the manufacturing room.

The thresholds upon which action should take place are set differently for each kind of store: 20-cigarette beetle/week/pheromone-trap for the green tobacco store and five-cigarette beetle/week/pheromone-trap for the finished tobacco store No. 1. For the factories, where it is not possible to apply any chemical control method, a preventive sanitation program was established and the machines, floor and walls were cleaned regularly. The manufacturer also used a five-cigarette beetle/week/pheromone-trap warning threshold to improve the sanitation programme.

In the green tobacco store the mean trap catches registered were relatively low; the highest average reached 11.8 insects/trap in week 22, and never reached the action threshold level. In the stemmery, the relative density of the *L. serricorne* population was low and the highest mean register was 4.8 insects/trap, in the 25th week, and 3.6 insects/trap in the 42nd week. In the finished tobacco store No. 1 and in the manufacturing room the mean trap catches were distinctly higher. In store No. 1, the action threshold level, for *L. serricorne* males, was exceeded twice and it

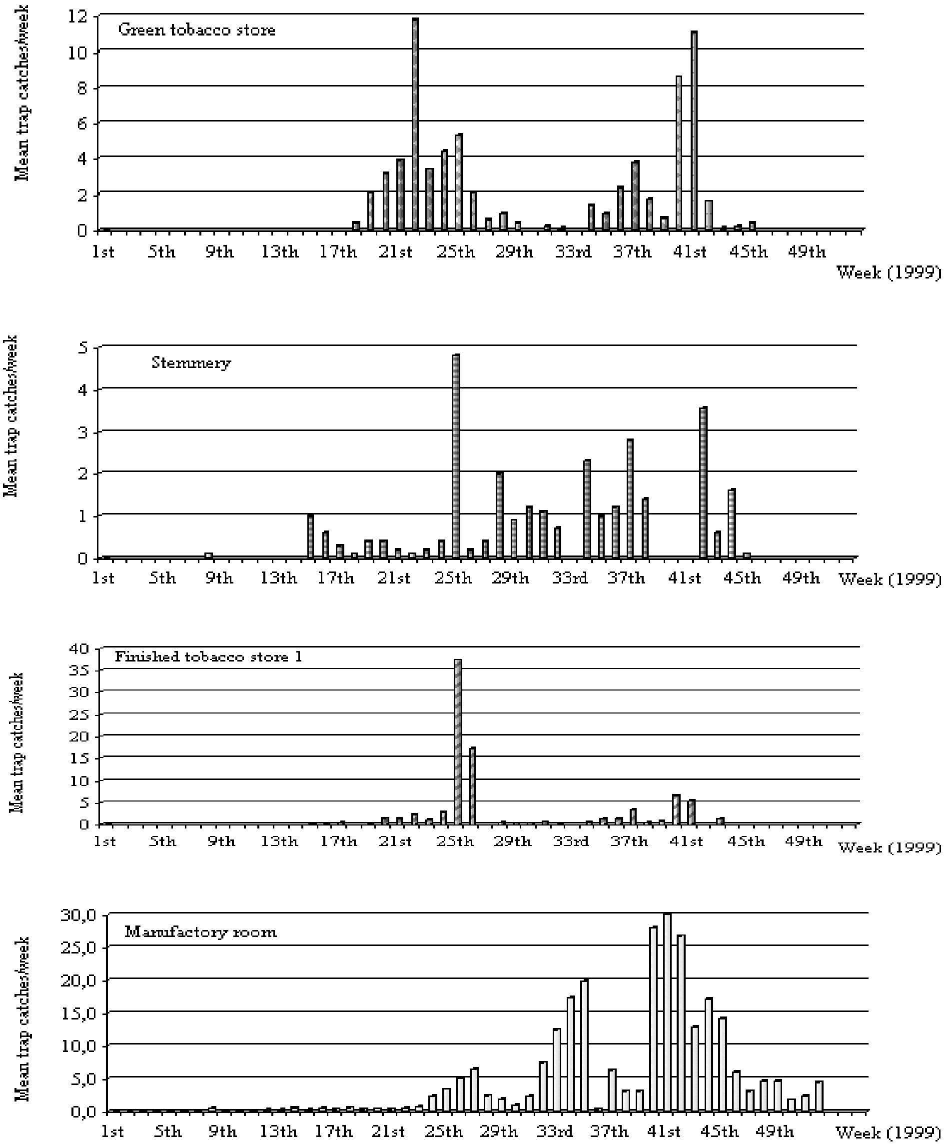


Fig. 3. Mean number of males of *Lasioderma serricorne,* caught per trap and per week, in the green tobacco store, stemmery, finished tobacco store and in a manufacturing room, during 1999.

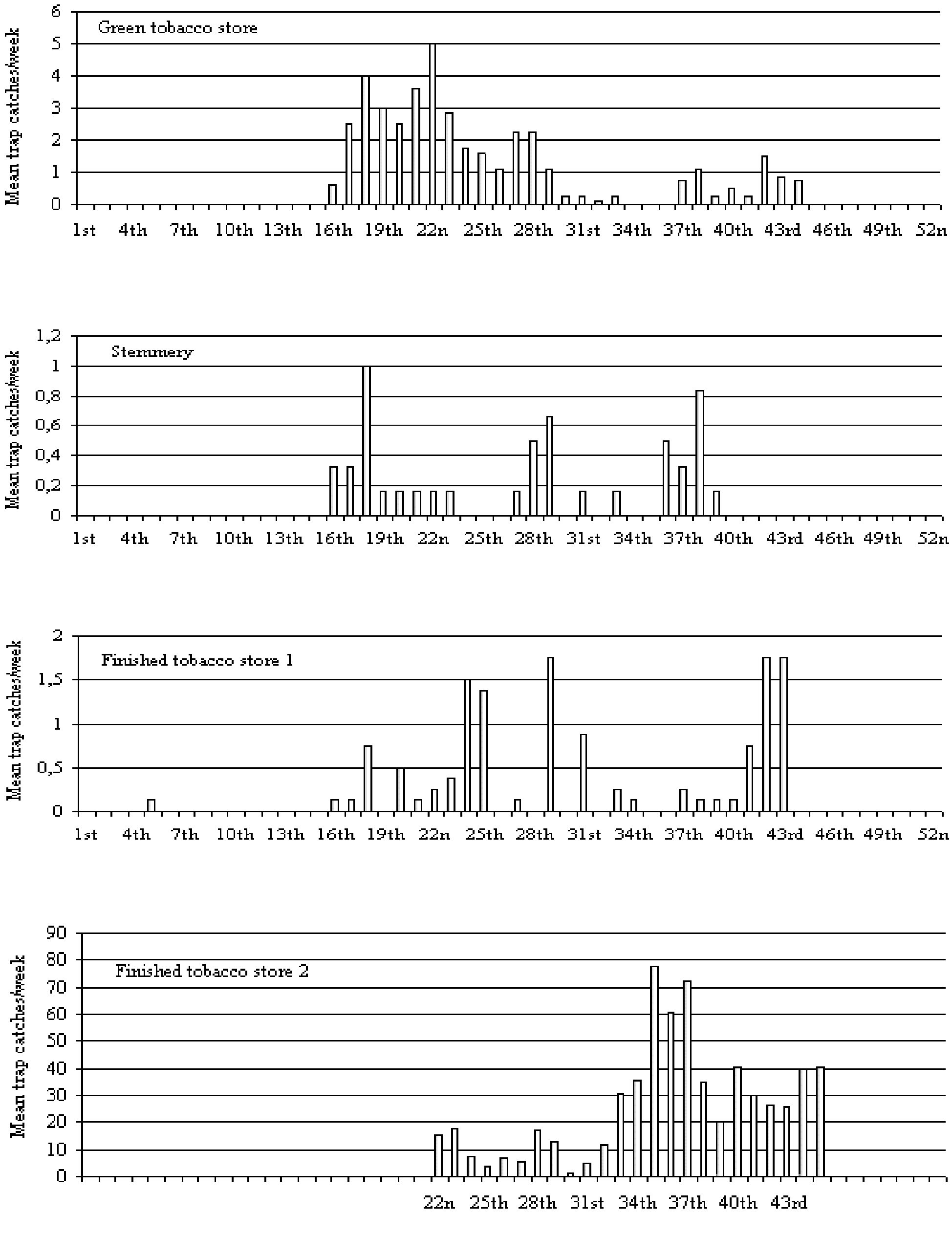


Fig. 4. Mean number of males of *Ephestia elutella,* caught per trap and per week, in the green tobacco store, stemmery, finished tobacco store No. 1, during 1999, and finished tobacco store No. 2, during 24 weeks in 1997.

was decided on each occasion to control the pest by fumigation (Fumicel): the first period was from the 25th to the 26th week when the mean trap-catches reached, 37.3 insects and 17.2 insects respectively; the second period, was in weeks 40 and 43 when the mean trap catches were 6.6 and 5.4 insects/trap respectively.

Because chemical control methods were not permitted in the manufacturing facility, as an addition to the preventive sanitation programme, supplementary pheromone traps were placed in this room to make mass trapping an alternative control method. In the manufacturing room, the relative density of cigarette beetle populations exceeded the five insects/trap/week threshold during three distinct periods: the first period, in weeks 26 and 27 with 5.1 and 6.4 insects/trap respectively; the second period, between weeks 32 and 37, when the mean trap catches ranged from 6.3 to 19.8 insects/trap; and the third period, from week 40 until week 46, when a mean of 6 to 30 cigarette beetles per pheromone trap were registered.

Regression techniques for dispersion patterns

Iwao's patchiness regression estimates calculated for *L. serricorne,* in the green tobacco and finished tobacco stores and in the stemmery and manufacturing room are shown in Table 2. The linear regression model fitted all the data obtained in the four different locals as it was indicated by the high r^2 values.

In the green tobacco store, the aggregative tendency (□= 1.963 ± 0.097) is masked by the tendency to repel between individuals (□= -0.3 ± 0.4359) at low densities (Fig. 4). It seems that at low densities the males of *L. serricorne* tend not to live together in the same quadrat and both repulsive and aggregative tendencies are inherent in the behavior of cigarette beetle males (Iwao, 1968).

In the stemmery, the Iwao's patchiness regression suggested a single male of *L. serricorne* being in the same sample unit, the average being around 1.0086 (= □ +1), which tends to be distributed in an aggregative pattern (□= 2.0278 ± 0.2687). In the finished tobacco store No. 1, the regression showed small groups of males, the average being around 2.9697 (= □ + 1). The slope □ of the regression is nearly equal to unity (□ = 1.2974 ± 0.1348), suggesting that small groups of males were distributed, although loosely but definitively with a tendency to aggregate. In the manufacturing room, the regression suggested the presence of small groups of individuals, with an average around 1.8748 (= □ + 1) distributed in an aggregative pattern (□ = 2.0330 ± 0.0870).

The relation "mean crowding" and "mean" sample of adult males of *L. serricorne* can be fitted to the linear Iwao's patchiness regression. The distribution of these males may change with increase in population density: since males tend to be attracted to females, at low densities these males do not tend to live together and may not need to compete with other males for the same female, although there is always an aggregative tendency. With higher population densities, the results observed in Table 2 suggested that small groups (1 □ □ +1 □ 3) produce an aggregative pattern.

Ephestia elutella

Mean number of adults per trap: The mean trap catches registered in the green tobacco store, stemmery and finished tobacco store No. 1 were relatively low (Fig. 4). In the green tobacco store two distinct generations of tobacco moth were present: the first generation between weeks 16 and 34, and a second generation from week 37 to weeks 43 and 44.

In the stemmery the highest mean trap-catch was one insect/trap in week 18, which indicates a very low density of tobacco moth population. In the finished tobacco store No. 1, density of tobacco moth was also very low and the highest mean trap catches registered were 1.8 moths/trap in weeks 29, 42 and 43. Only in the finished tobacco store No. 2, where the action threshold was set at 50 tobacco moths /week/pheromone-trap was the moth population density high. The number of generations was not clearly defined and several peaks of trap-catches were registered during the trials. From weeks 35 to 37 the mean number of insects caught per trap reached the threshold level, and based on these trap-catch data, it was possible to detect the sources of infestation, and the infested material was removed for fumigation in another store.

Regression techniques for dispersion patterns

Iwao's patchiness regression estimates for *E. cautella* in the green tobacco and finished tobacco warehouses and in the stemmery are shown in Table 3. At very low densities, as was observed in the stemmery and in the finished tobacco store No. 1 (Fig. 4), the linear regression model did not fit the majority of the data obtained in these locals, where r^2 was very low, at 0.2347 and 0.3495, respectively. In the green tobacco store, the regression suggested that a clump size of *E. elutella* males, with an average of 1.4596 (= α +1), tended to loosely aggregate (β= 1.1329 ± 0.1619).

In the finished tobacco store No. 2, the basic component was a colony, with an average around 19.8230 (= α +1), which tended slightly towards aggregation (β = 1.3513 ± 0.2133).

When the *E. elutella* population density was very low, the data obtained did not follow an aggregative pattern but approached a random distribution model and therefore did not fit the linear Iwao's patchiness regression. According to the increase in density, the individuals tended to become distributed in a very weak but definitive tendency towards aggregation, as the values of the β slope showed. From the intercept α, the mean size of clumps, (= α +1), might tend to be larger at higher densities.

DISCUSSION AND CONCLUSIONS

Infestations of some stored-product insect species that feed on the product prior to harvest, or on other alternative hosts, have already been studied. *L. serricorne* adults were found on flowers of six out of nine genera of thistles examined by Buchelos, (1989) in Greece. Our detection studies revealed the presence of *L. serricorne* and

E. elutella in tobacco fields and on-farm stores and curing barns although the relative abundance of both populations was extremely low.

In the stores and factories, the relative density of cigarette beetle populations (as detected by male trapping) was higher than tobacco moth populations. In the stores, where the ambient temperature range is greater than in the factories, *L. serricorne* presented two distinct generations, whereas in the factories this was not so evident. The highest mean trap-catch was 11.8 insects in the green tobacco store, 4.8 insects in the stemmery, 37.3 insects in the finished tobacco store No. 1, and 30 insects in the manufacturing room. As Iwao's patchiness regression showed, with the increase in density of the *L. serricorne* population, so the relationship "mean crowding" to "mean" became more significant. This regression line also suggested that at higher densities, male cigarette beetles tended to aggregate in small clumps.

The population density of *E. elutella* males was very low in the stemmery and stores, of the processing factory, and very high in the finished tobacco store No. 2 of the manufacturing facility, which contained imported tobacco, originating from North Europe, where climatic conditions were more favourable to tobacco moth development. From Iwao's patchiness regression, it seemed that the majority of the *E. elutella* data did not fit this linear regression and followed a random distribution, but at higher densities males of tobacco moth tended to distribute in an aggregative pattern with bigger clump sizes than those registered for cigarette beetles. However, the few adults, of both species, trapped by the pheromone traps in tobacco fields and on-farm stores and curing barns, even when the climatic conditions for development were not favorable, may provide an important source for later infestations of tobacco leaves in the cured tobacco warehouses and handling facilities. Low populations may persist in the green tobacco stores and stemmery where the process of removing the stem takes place. The finished tobacco is then stored and aged for several months to enhance the smoke characteristics, before being shipped for manufacturing (Ward, 1999). Consequently the survival and abundance of these populations can provide serious sources of infestation in the processing factories.

ACKNOWLEDGEMENTS

The authors thank all the tobacco farmers, and store owners, who kindly permitted access to their premises, and also the members of tobacco factories in particular, Eng. Quintero, Eng. Amílcar David, Eng. Paulo Abrantes, Mr. José António, Antónia Fernandes, Ana Rita, Eng. José Paulo Sequeira, Mr. Arnaldo Gomes and Mr. Bragano for their sympathetic collaboration.

We are also grateful to Prof. Pedro Amaro and Prof. Passos de Carvalho for helpful discussion, to Prof. Manuel Correia who provided information on tobacco field and storage practice and, especially, to Prof. Laura Torres for her important and valuable help in the course of the data analysis.

REFERENCES

Anon. (1971). Stored tobacco insects – biology and control. *USDA Handbook*, 233 pp.

Bhattacharyya, G.K. and Johnson, R.A. (1977) *Statistical concepts and methods*. John Wiley & Sons, Inc., New York, 334-367.

Buchelos, C.T. (1989) A contribution to *Lasioderma* spp. and other Coleoptera collected from thistles in southern Greece. *Entomol. Hellenica,* **7,** 7-12.

Carvalho, M.O. (1998) Studies on efficacy of five types of pheromone traps for monitoring *Lasioderma serricorne* F. (Coleoptera, Anobiidae) in stored tobacco. *Proc. IOBC/WPRS study group "Integrated protection of stored products", Aug. 31- Sep 2, 1997, Zurich, Switzerland*, **21,** 119-127.

Carvalho, M.O., Pereira, A.P. and Mexia, A. (2000) Occurrence of *Lasioderma serricorne* F. and *Ephestia elutella* (Hb.) in tobacco Virginia fields and curing barns. *Proc. IOBC Bull.*, **23,** 91-101.

Genève, R. (1996) International survey of pests and mould in stored tobacco 1994. *Congrès du Coresta,* Nov. 1996, Yokohama, Japan, 9 pp.

Iwao, S.A. (1968). A new regression method for analysing the aggregation pattern of animal populations. *Res. Popul. Ecol.,* **10** 1-20.

Massey, E.D. (1999) Stored tobacco: insects and their control. In: *Tobacco. Production, chemistry and technology*. (Edited by Davies, D.L. and Nielsen, M.T.) (1999) World Agric. Ser., CORESTA - Blackwell Science Ltd, USA, 241-249.

Southwood, T.R.E. (1978) Ecological methods with particular reference to the study of insect populations. Chapman and Hall, John Wiley & Sons, Inc., New York: 524 pp.

Ward, B. (1999) Green leaf threshing and redrying tobacco. In: *Tobacco Production Chemistry and Technology*. CORESTA, Blackwell sciences Ltd., USA, 330-337.