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**Possibilities and limits of mass trapping and mating disruption techniques
in the control of *Ephestia kuehniella* (Zell.)
(Lepidoptera Phycitidae) (*)**

Abstract - The possibility of mass trapping or mating disruption of *Ephestia kuehniella* (Zell.) in a department of an industrial bakery heavily infested has been evaluated. The trials lasted 20 months, using ever increasing quantities of TDA and laminar dispensers located on the walls and on machinery. It was noted that in these conditions even the use of high doses of TDA can reduce, but not block completely the reproduction of *Ephestia kuehniella*. As far as mass trapping is concerned more than 16.000 adults have been captured on the whole, but it was not possible to lower the population to acceptable levels. The use of high doses of TDA does not interfere completely with the reproduction of the insect, which is little active and often nests in pipes and machinery. Only using a dosage of 50 mg of TDA per erogator, with a concentration of 1.5 mg/m³ of environment, it was possible to inhibit mating. In order to be successful with these techniques, it is necessary to clean accurately, to remove the debris from the most neglected corners, reducing the possibilities of nesting of this insect.

Riassunto - Possibilità e limiti del mass trapping e della tecnica confusione nel controllo di *Ephestia kuehniella* (Zell.) (Lepidoptera Phycitidae).

È stata valutata la possibilità di attuare il mass trapping o l'inibizione dell'accoppiamento di *Ephestia kuehniella* (Zell.) in un reparto di un panificio industriale gravemente infestato. Le prove sono durate 20 mesi, impiegando quantitativi progressivamente crescenti di TDA e utilizzando erogatori laminari applicati a pareti ed impianti. Si è evidenziato che in queste condizioni l'utilizzo di pur elevate dosi di TDA riesce a ridurre, ma non a bloccare completamente la riproduzione di *Ephestia kuehniella*. Per quanto riguarda il mass trapping, sono stati catturati complessivamente oltre 16.000 adulti, ma non si è riusciti ad abbassare la popolazione a livelli accettabili. L'uso di pur elevate dosi di TDA non interferisce completamente nella riproduzione dell'in-

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setto, poco mobile, spesso annidato in tubazioni ed impianti. Solo alla dose di 50 mg di TDA per erogatore, con una concentrazione pari a 1,5 mg/m³ di ambiente è stato possibile inibire l'accoppiamento. Si conferma che, per avere successo con queste tecniche, è necessario provvedere ad accurate pulizie, per rimuovere i detriti dagli angoli più trascurati, riducendo così le possibilità di annidamento di *Ephestia kuehniella*.

Key words: mass trapping, mating disruption, *Ephestia kuehniella*.

INTRODUCTION

From the moment the components of female pheromones of *Ephestia kuehniella* (Zell.) (Brady et al., 1971; Kuwahara & Casida, 1973) are identified numerous studies have been carried out to perfect techniques of control of this Phycitid moth. First of all, the dosage of release of (Z,E)-9,12-tetradecadienylacetate (TDA) has been indicated, suitable for applications of monitoring (Bommer & Reichmuth, 1980). Süss & Trematerra (1985) in laboratory experiments and using wing traps, freed 30 couples of *E. kuehniella* in a 131 cubic metres room and they captured 76.66% of males in 3 days singling out in the natural rubber dispenser charged with 2 mg (the average daily release in the first 60 days was 13 µg) which was the best for practical uses.

Furthermore, different applications of attracticide techniques and of mass trapping have been carried out.

As far as mass trapping is concerned, positive results can be obtained using funnel traps, one for every 260-280 cubic metres, charged with 2 mg of TDA. As a result it was possible to defer fumigations with methyl bromide, apart from the localised insecticide treatments, stabilising the presence of the moth at very low levels, comparable to those that occur naturally in the least favourable months to the development of the insect (Trematerra & Battaini, 1987; Trematerra, 1988; 1990; 1994a; 1994b). It has been constantly underlined though, both in the application of mass trapping and in the use of attracticide methods (Trematerra & Capizzi, 1987; 1991; Trematerra, 1995; Süss & Locatelli, 1995), that it results indispensable to associate accurate cleaning, aimed at removing the debris from the most neglected corners of departments and machinery. Only in this way it is possible to reach an insectistasis as it is understood by Levinson (1974; 1977) and Levinson & Levinson (1977).

An interesting possibility in the application of pheromones in the direct control of some Phycitid moth, which is still little studied for *E. kuehniella* it considers the use of TDA for the mating disruption.

Studies about this, by Barrer (1976), Haines & Read (1977), Sower & Whitmer (1977) and Prevett et al. (1989) have shown the validity of the method with

respect to *Ephestia cautella* (Walker). Hagstrum & Davis (1982) underline, instead how it is necessary to increase progressively the concentration of pheromone in the presence of an ever increasing density of population of this moth. Experiments with regards to *Plodia interpunctella* (Hubner) are described by Sower et al. (1975) and by Sower & Whitmer (1977). Finally, Trematerra & Capizzi (1987) report a reduction of a population of *Ephestia kuehniella* in a mill, applying a quantity of 0,2 mg of TDA per cubic metre.

During the study, one wanted to observe if it is possible to obtain the mass trapping or the mating suppression of *Ephestia kuehniella* in a department of an industrial bakery seriously infested by the insect.

MATERIALS AND METHODS

The experiment took place in the "dough" department of an industrial bakery. The environment is about 2300 cubic metres, constantly kept at 24°C and 55-60% r.h., with artificial light and a continuous production cycle. The laminar dispensers of pheromone, (Capizzi et al., 1986)⁽¹⁾ were put on the perimeter walls, at a height of about 2 metres, as well as on different structures of the machineries and on all the basins used for the doughs, which are built on wheels to allow for their movement. These basins, after mixing the raw materials, are left for the rising in the same room and later they are moved to a suited lifting apparatus to be emptied of their content in the dosage machines.

Applying the erogators on the support structures of the basins, constantly infested by *E. kuehniella*, on the one hand one wanted to localize the pheromone in the sure hotbed of multiplication for the moth, on the other to exploit the periodical movement of the basins themselves to favour the distribution of the TDA in the department.

From 30 to 60 erogators were used (the number varies according to the course of trials), and they were substituted periodically, approximately every 2 months.

For the control of the efficacy of the method 10 funnel traps⁽²⁾ were installed more or less uniformly distributed in the whole environment. The map of the department and the disposition of the traps are in fig. 1.

The traps were firstly used with the commercial rubber dispenser charged with

⁽¹⁾ The laminar dispensers, provided by Isagro Ricerca S.r.l. of Novara (Italy), are made by a layer of felted cellulose and polyethylene fibrils coated with a heat-sealed aluminium-polyethylene film (of square shape with 20 mm or 25 mm sides) with a variable thickness from 0.46 to 2 mm., in function of the quantity of TDA used for impregnation.

⁽²⁾ Funnel trap Mastrap - Isagro S.p.A. - Segrate (Milano) Italy.

2 mg of TDA, to verify the fortnightly captures before installing the laminar dispensers on the walls and machineries. Later, for over 4 months they were activated with the commercial dispenser but in the presence of laminar dispensers in the environment, to later go onto an activation with this last kind of dispensers, soaked with the same, progressively increasing doses, used for those distributed in the department.

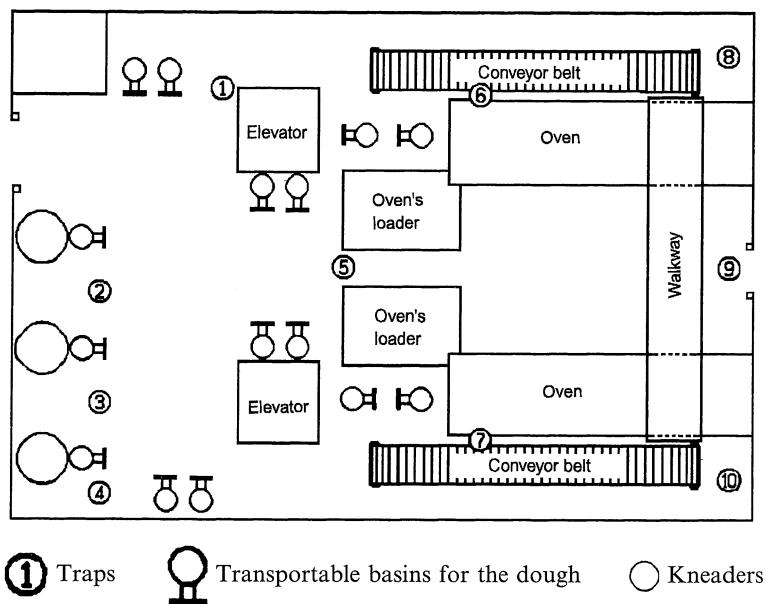


Fig. 1 - Map of the department with the traps disposition.

In the different phases of the experiment the quantity of pheromone was increased in the laminar dispensers, going from 2 mg (with a daily release of 20 μg) to 5 mg (release of 40 μg), to 10 mg (release of 70 $\mu\text{g}/\text{day}$) and finally to 50 mg (release of 200 μg)⁽³⁾, to verify what was the dosage to obtain a sure mating disruption.

To evaluate this, a test using as attracting sources vergin females of *E. kuehniella* was carried out. 5 funnel traps, with a female in each trap, were distributed in the department, each female emerged from the cocoon two days before and

⁽³⁾ Arsura E. and Capizzi A., Personal communication.

came from a laboratory of the Institute of Entomology of the University of Milan and was periodically changed every two days. The female was kept in a net cage, suspended over the trap, in the same position as synthetic pheromone.

During the research, which lasted 20 months, the control of the captures carried out in the traps were noted down fortnightly. During this period a calendar of treatments with pyrethrum synergised with PPB was kept, agreed upon directly between the company and a pest control operator, for a total of 20 treatments carried out with a fogging machine (Swingfog).

Considering the data that was being gradually obtained, further laboratory experiments were carried out to verify the possibility of mating of *E. kuehniella* in very restricted environments, in the presence of high concentrations of TDA.

Glass containers of 0.22 liters were used for these tests or crystal containers (cm 50x50x50, capacity of 125 liters), putting into them 1 or 2 couples of individuals emerged from the cocoon 2 days before, in the presence of laminar dispensers, charged with 2 or 10 mg of TDA. The dispensers were placed in the containers 2 hours before the freeing of the adults. On the bottom of the containers themselves a piece of black paper was placed to ease the collection of the eggs deposited. These were then incubated at 25°C and 60-70% r.h. to verify the egg hatch. 8 replicates were used for each test.

Results were evaluated by Anova.

RESULTS

The fig. 2 and table 1 underline the captures in the ten traps installed. First of all, the high number of captures (about 2000 individuals) in the first fifteen days must be noted with the lack of laminar dispensers, which is then followed by a clear reduction. This is due to the fact that in the initial months of the experiment the traps were not baited with a quantity of pheromone similar to that of the laminar dispensers applied in the department. Therefore the strong interference exerted by the TDA released by the dispensers installed on the walls and machineries can be seen, and this determines a high competition against to the traps, reducing their efficacy.

Things visibly changed later, that is when the dispensers applied on the walls and machineries were uniformed to those present in the traps. On the other hand, the total number of adults captured tends to increase together with the summer period, decreasing instead between autumn and spring. It can be deduced then, in the conditions in which the study was first carried out, the quantity of TDA distributed in the environment was not able to interfere in a substantial way with the multiplication of the insect, which continued to follow its traditional increase of flight, which can actually be registered in the summer

months in every environment infested by *E. kuehniella*. As the number of traps was kept constant, it can be noted that the coefficient of variability tends to evolve in a specular way, to the average, showing a more uniform behaviour of the traps in the moments of greatest infestation. In any case, the captures of moths of each trap underlines a different activity of these, based on their location and on the greater or lower quantity of food debris present in the immediate vicinity. In particular, the most colonised areas resulted to be those of the kneading machines and of the system of loading of the pastry itself, as well as the head of the ovens. These are areas difficult to clean characterized by the high number of cracks and crevices.

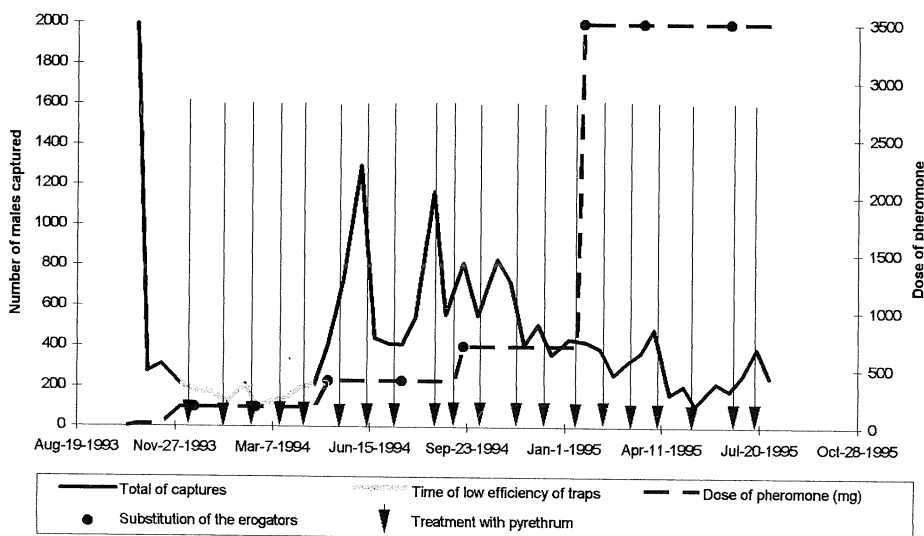


Fig. 2 - Captures of *Ephestia kuehniella* with Funnel traps.

From this point of view, the monthly treatments with fogs with pyrethrum were not efficacious for a global protection from infestation. In fact these treatments tend to clean out the department of moths in flight only for a few days, but cannot be correlated to the presence of larvae, nor with the total population of *E. kuehniella*, because the adults are frequently nested in the crevices which have not been reached by the insecticide, and where they can calmly mate, perpetuating the infestation.

Laboratory tests confirm how, in very restricted areas, even the use of a dispenser charged with 10 mg of TDA is unable to disrupt mating (table 2). The only thing that can be observed is a reduction of the cases of the laying of fertile eggs, which is conditioned by the concentration of TDA and by the number of couples present in a given space.

Table 1 - Captures of *Ephestia kuehniella* with Funnel traps in the department.

Data	Trap's number										Total	Mean	St. dev.	Var. coeff.
	A	B	C	1	2	3	4	5	6	7	8	9	10	
1993														
ott-9	0	0												
ott-19	0	2	20											
ott-30	0	2	20											
nov-13	0	2	20											
dic-2	30 × 5	2	170	2	20	18	7	50	52	4	24	31	7	215
dic-11	30 × 5	2	170											
dic-17	30 × 5	2	170	1	28	13	6	42	45	1	21	23	3	183
1994														
gen-3	30 × 5	2	170	2	16	11	12	25	59	2	21	14	7	168
gen-15	30 × 5	2	170											
gen-19	30 × 5	2	170	0	13	13	9	20	29	2	17	22	1	126
feb-7	30 × 5	2	170	0	17	12	16	34	70	3	33	12	13	210
feb-12	30 × 5	2	170											
feb-18	30 × 5	2	170	1	8	5	0	27	22	0	16	11	5	95
mar-4	30 × 5	2	170	1	8	10	9	27	36	2	20	8	2	123
mar-12	30 × 5	2	170											
mar-18	30 × 5	2	170	2	8	15	5	24	27	1	30	16	7	135
apr-5	30 × 5	2	170	6	18	10	14	36	36	1	38	23	15	197
apr-9	30 × 5	2	170											
apr-19	30 × 5	2	170	7	15	13	9	41	39	5	40	26	15	210
mag-3	30 × 10	10	400	28	39	21	13	106	48	49	58	27	26	415
mag-14	30 × 10	10	400											
mag-18	30 × 10	10	400	43	39	63	35	231	159	63	39	25	28	725
giu-6	30 × 10	10	400	146	155	94	154	312	216	85	41	70	23	1296
giu-11	30 × 10	10	400											
giu-20	30 × 10	10	400	40	95	36	74	73	20	41	15	17	29	440
lug-4	30 × 10	10	400	50	31	25	0	54	27	68	105	29	24	413
lug-9	30 × 10	10	400											
lug-18	60 × 5	10	400	32	54	15	53	96	48	34	16	42	19	409
ago-1	60 × 5	10	400	29	85	18	42	143	86	57	8	55	21	544
ago-20	60 × 5	10	400	136	139	72	128	272	221	79	33	64	22	1166
ago-23	60 × 5	10	400											
set-1	60 × 5	10	400	79	80	35	101	85	37	79	15	33	12	556
set-10	60 × 5	10	400											
set-19	60 × 10	10	700	41	92	43	98	145	107	70	95	56	66	813
ott-4	60 × 10	10	700	39	80	40	120	155	42	20	12	23	22	553
ott-8	60 × 10	10	700											
ott-24	60 × 10	10	700	15	39	51	162	170	49	40	55	131	119	831
nov-7	60 × 10	10	700	5	19	15	142	111	28	47	74	151	127	719
nov-12	60 × 10	10	700											
nov-21	60 × 10	10	700	10	7	5	51	23	52	59	50	100	50	407
dic-5	60 × 10	10	700	12	18	16	14	118	56	88	16	117	55	510
dic-10	60 × 10	10	700											
dic-19	60 × 10	10	700	5	32	85	13	12	99	39	20	15	40	360
1995														
gen-5	60 × 10	10	700	5	86	50	64	61	130	8	0	21	10	435
gen-14	60 × 10	10	700											
gen-23	60 × 50	50	3500	12	59	37	30	101	129	21	4	28	2	423
feb-6	60 × 50	50	3500	3	53	92	12	90	78	24	0	26	10	388
feb-11	60 × 50	50	3500											
feb-20	60 × 50	50	3500	4	61	27	8	85	33	11	0	18	13	260
mar-6	60 × 50	50	3500	10	60	41	15	81	48	24	0	21	20	320
mar-11	60 × 50	50	3500											
mar-20	60 × 50	50	3500	17	62	54	15	98	33	20	2	30	38	369
apr-3	60 × 50	50	3500	20	86	75	24	93	32	0	70	85	485	53,89
apr-8	60 × 50	50	3500											
apr-19	60 × 50	50	3500	7	38	22	6	20	7	11	0	43	10	164
mag-3	60 × 50	50	3500	12	45	27	6	30	6	15	0	50	15	206
mag-13	60 × 50	50	3500											
mag-15	60 × 50	50	3500	13	17	10	3	27	1	11	0	14	14	110
giu-6	60 × 50	50	3500	21	33	27	5	56	4	17	8	27	21	219
giu-19	60 × 50	50	3500	27	23	23	4	32	2	14	0	32	23	180
giu-24	60 × 50	50	3500											
lug-3	60 × 50	50	3500	30	40	25	5	29	24	38	2	21	45	259
lug-15	60 × 50	50	3500											
lug-17	60 × 50	50	3500	44	25	21	4	93	57	43	13	56	34	390
lug-30	60 × 50	50	3500	20	11	13	1	52	38	40	1	42	30	248
Total				977	1854	1298	1489	3380	2300	1268	942	1640	1128	16276
Mean				23,83	45,22	31,66	36,32	82,44	57,50	30,93	22,98	40,00	27,51	
St. dev.				31,99	35,29	24,21	47,01	68,41	51,60	26,71	25,65	32,93	28,23	
Var. coeff.				134,26	78,05	76,48	129,44	82,98	89,73	86,37	111,66	82,31	102,61	

A: Number of the laminar dispenser in the environment and milligram of TDA in each dispenser

B: Milligram of TDA in the dispenser used in each Funnel trap

C: Total dose of pheromone (mg) in the department (laminar dispensers + 10 Funnel traps); "©" change of dispensers

Table 2 - Cases of eggs laying of *E. kuehniella* in very restricted environment with high concentration of TDA.

Dose of pheromone mg	Number of couples		% of mating and laying of fertile eggs	
	0.22 liter	125 liter	0.22 liter	125 liter
2	2	2	100.00	87.50
10	1	1	62.50	25.00

On the other hand, around each laminar dispenser the presence of a number of males, even high has been noticed, absolutely still, for the whole duration of their life cycle. This testifies to the strong attracting effect of the dispensers applied (fig. 3).

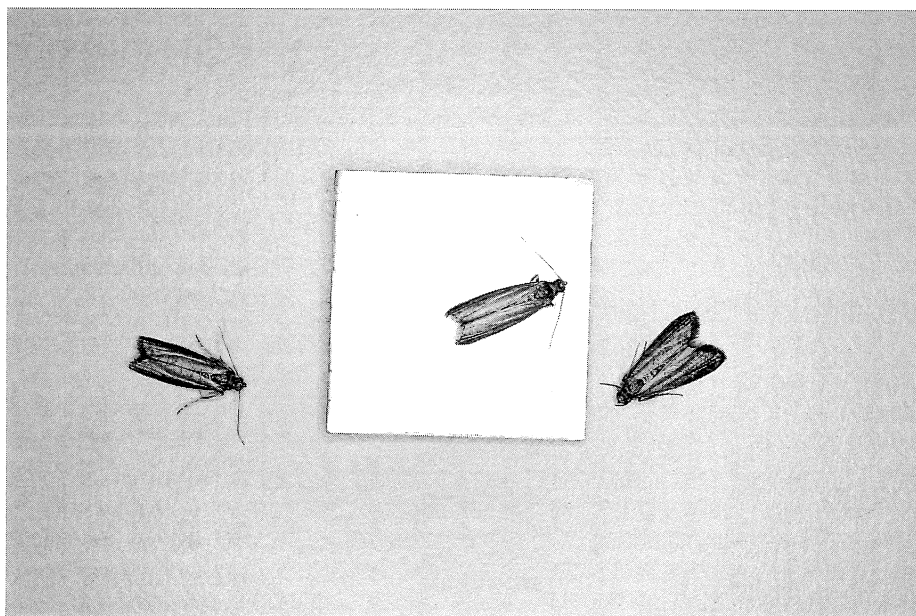


Fig. 3 - Males of *Ephestia kuehniella* attracted by a laminar dispenser.

On the basis of such results, at the same time taking advantage of both the natural decrease of the population of *E. kuehniella* in the winter period, and a very limited intervention of cleaning of the different areas previously neglected, laminar dispensers charged with 50 mg of TDA were used, activated with the same dosage the traps installed.

As can be seen, in the following months there was a sure decrease of the presence of adults in the environment. The efficacy of the attractiveness of the traps put into effect with such a high dosage of pheromone is confirmed by the comparison of the catches in the preceeding weeks of this phase of experimentation with the values obtained in the first period of use of the funnel traps, baited with 50 mg of TDA. The number of males captured does not differ much. Only after 3 months a halving of the captures can be determined, which stays at the same level in the following months, while a natural increase in the summer of the population was to be expected.

If the environmental and pabulum conditions are taken into consideration, on the basis of the data relative to the development of the insect supplied by various Authors (Jacob & Cox, 1977; Trematerra et al., 1982), *E kuehniella* presents a life cycle of about 80-90 days. Therefore the reduction of captures that can be seen from the beginning of mid April is mainly due to a decrease of egg laying following the use of high concentrations of TDA started in the previous January. Visually too, a much lower number of individuals laying on the walls and machineries could be seen.

To confirm that all this is thanks to the reaching of the efficacy of «mating disruption», a practical experiment of the captures was carried out, installing 5 traps with virgin females and comparing the data with that obtained at the same time with traps baited with 50 mg of TDA. The results (table 3) clearly show the

Table 3 - Difference in the captures with Funnel traps activated with 50 mg of TDA or with a natural source of pheromone during two periods of test.

Average of catches of 5 Funnel traps	June 24-July 03	July 03-July 17
Activated with 50 mg of TDA	25.9	39
Activated with 1 two days old virgin female	4.4	0.8

difference in the captures in the two cases. When there is a high concentration of TDA in the environment, *E. kuehniella* is not able to locate a natural source of pheromone, unless it is present in the immediate vicinity; in such a way mating of the moths present in the areas of the department reached by TDA is avoided.

CONCLUSIONS

The experiment has underlined that in an environment seriously infested, the use of even high doses of TDA manages to reduce, but not to completely inhibit, the reproduction of *E. kuehniella*. This is because the males of the Phycitid moths,

not very mobile themselves, are able to locate anyway a female present in the immediate vicinity. In the 20 months of the experiment, more than 16000 adults were captured with 10 traps, but it was not possible to reach low levels of presence that can be neglected, despite the confirmation (thanks to the activation of some traps with virgin females) that in the last months the permeation of the environment with synthetic pheromone was obtained.

That real mass trapping was therefore not put into effect. This manages to lead the infestation to insectistasis again, leaving out from other preventive infestations or from a direct control. In these operative conditions, using a trap every 230 cubic metres approximately, the presence of *E. kuehniella* resulted high in any case.

This research cannot confirm the positive results obtained, with mass trapping of the above-mentioned Authors, but it underlines the need to locate and destroy the hotbeds of nesting, to be successful in the control of infestations.

Using 50 mg of TDA for each dispenser, with a total use of 3.5 g in 2300 cubic metres (equal to 1.5 mg/m^3) an effect of inhibition to mating of the males of *E. kuehniella* was obtained. The individuals nesting in the crevices, on the contrary, only a few centimeters from one another are anyhow able to mate and perpetuate the attacks.

It is interesting to note that Prevett et al. (1989) obtained in *Ephestia cautella* a reduction of emergence by over 97% when a density of population was of 0.25 moths/m² surface area, with an airborne pheromone concentration of 1-3 $\mu\text{g/m}^3$.

On the basis of these conclusions, it is necessary to make other considerations.

The results, both with mass trapping and with the mating disruption are above all conditioned by the density of the populations present.

In the cases of success of the application of these techniques referred to by other Authors, probably the infestations were lower than those met in this experiment. The problem of the evaluation of the density of a population of Phycitid moths in an industrial department is complex and has not been resolved yet. In laboratory tests, Süss & Trematerra (1985) free couples of *E. kuehniella* in such a way to have 0.2-0.3 adults per square metre. Hodges et al. (1984) operate with a density of *E. cautella* equal to 0.1-0.3 adults per square metre.

In reality, though, the nesting of insects in the remotest corners, within the tubes and the machineries, makes it impossible at present a sufficiently precise evaluation of populations that can be met in real cases. Even in the works of Trematerra (1988; 1990); Trematerra & Battaini (1987); Trematerra & Capizzi (1987) the entity of the infestation in act is not indicated precisely. In practice, therefore, the reference to the number of individuals present on the walls and visible areas leads to an underevaluation of the colonization.

To find a remedy to this, the number of captures carried out with pheromone traps are usually referred to, starting from the presupposition that they are very efficient.

About this, Knipling & McGuire (1966) have proposed an equation able to represent the competition between pheromone traps and the presence of males and females of moths in a given environment. This formula has subsequently been criticized by Nakamura & Oyama (1978), who developed a new equation where it is assumed that the number of possible coupling per female is limited. Anyhow, they neglect eventual superimpositions between the areas of influence of the traps and the females present. This is what in reality happens in confined environments as those in question, and consequently, this formula is inapplicable.

To be able to evaluate the real entity of a population in a given environment, the studies by different Authors on the life cycle of *E. kuehniella* in environmental and pabulum controlled conditions are of help (Brindley, 1930; Bell, 1975; Jacob & Cox, 1977; Trematerra et al., 1982; Cox et al., 1984; Cox, 1987).

Siddiqui & Barlow (1973) and Subramanyan & Hagstrum (1993) have furthermore perfected mathematical models which are able to allow a forecast of the development of the insect, in the presence of constant temperatures or alternated and of values of relative humidity well singled out, as well as in function of the pabulum.

The knowledge on the efficacy of traps used must be deepened, the possible interferences caused by constant artificial lighting in the department, meanwhile other parts of the same are still in the dark. Furthermore the fact that trapping of males can take place after one or more successful mating has to be taken into consideration.

For this reason, to reach a rationalization of the mating disruption techniques it will be necessary to develop a mathematical model which integrates the previous knowledge on the influence of various parameters on the development of the populations of the insect with the elements still not defined and which allows to have at one's disposal surer data on the density of the populations in a given environment. In such a way the quantity of TDA to be used case by case can be correlated in function of the presence of the insect.

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