CHAPTER : 2

OBSERVATIONS ON THE INFLUENCE OF EGG-WASH, PREPARED WITH DIFFERENT FLUID MEDIA, ON OVIPOSITION RESPONSE OF FEMALES AND ON THE BEHAVIOUR OF ADULTS AS WELL AS LARVAE OF *LASIODERMA SERRICORNE* (F.)

Selection of a proper site/host for oviposition by female insects is an important aspect of the life cycle. This fact is of greater relevance, as an adaptation, when considered in the light of the fact that the offsprings possess only a limited ability to disperse during their feeding stages (Tabashnik *et al.*, 1981). It is thus important that the mother insect selects a site/host that would provide a location more than marginal acceptability to her offsprings. Thus, when given a choice of sites/hosts, the mother, if able, would "select" that one which will provide optimum chances of survival for the offsprings such as adequate growth rate, low mortality, etc. Female insects make use of a variety of clues to locate suitable oviposition sites. Not surprisingly then, where the potential for competition among larvae is relatively high; the detection plus avoidance of already egg-laden sites/hosts seems of special adaptive value (Rausher, 1979). Females of certain insects mark oviposition sites with certain chemicals when they oviposit so that other females can recognize the site and refrain from ovipositing on the same site. Such oviposition deterring pheromone (ODP) would assure wider distribution of eggs over the available zone, and, thereby prevent overcrowding (Prokopy et al., 1978).

Perhaps, Salt was the first one to demonstrate in 1937 such chemical marking of oviposition site by the chalcid parasitoid, Trichogramma evanescens. ODP's are known to play important role in influencing the oviposition behaviour of a large number of phytophagous insects belonging to atleast 6 orders and 16 families (Prokopy, 1981 a and b; Prokopy et al., 1984). Several authors have reported about the marking of the oviposition site by some unknown chemicals, what they called as "Oviposition marker" Wasserman (1981) in C. maculatus; Szentesi (1981) in A. obtectus; Giga and Smith (1985) in C. maculatus and C. rhodesianus. Sakai et al., (1986) described it as " "Biological conditioning substances" (BCS) in the context of C. chinensis and C. maculatus. Messina et al., (1987) reported that the females of C. maculatus avoid ovipositing on host seeds already bearing conspecific eggs, and thus distribute eggs evenly among available seeds. They described that this behaviour was due to the presence of an ether soluble "Oviposition marker" that was deposited with the eggs. "Egg-load assessment" may be elicited by visual, olfactory or tactile stimuli (Rothschild and

Schoonhoven, 1977) but in most of the cases, it is mediated by contact chemoreception of pheromones deposited during or following egg laying (Prokopy et al., 1984; Stadler, 1984). It is thus obvious that deployment of such pheromones might prove to be an effective tool for prospective strategy of pest control. Behan and Schoonhoven (1978) demonstrated that in most cases, the ODP's are associated in some way with the eggs and suggested that females must be perceiving with antennal olfactory hairs as well as by tarsal contacts. In a number of species, this epideictic (Corbet, 1971) pheromone appeared to be a rather stable and water (sometimes also methanol) soluble, for example in various Rhagoletis species (Katsoyannos, 1975; Prokopy et al., 1976), Ceratitis capitata Wiedemann (Prokopy et al., 1978), Anastrepha suspensa Loew and A. fraterculus Wiedemann (Prokopy et al., 1977; Prokopy et al., 1982), Agromyza frontella (Mcneil and Quiring, 1983).

Oviposition deterrence due to feeding activity of larvae causing damage to the host plant and by extracts of frass applied to the host plant itself, in case of several species of Lepidoptera, has been described by Prokopy et al. (1984). Renwick and Radke (1980 and 1981) demonstrated an oviposition deterrent associated with frass from feeding larvae of the cabbage looper, Trichoplusia ni. The first instar larvae feeding on cabbage deterred oviposition by the adult moths for atleast upto

3 days. Oviposition deterrence by the feeding activity of larvae has also been described by Rothschild and Schoonhoven (1977) in _Pieris brassicae_. Dittrick _et al._, 1983 described a methanol-soluble oviposition deterrent from the frass of fifth instar larvae of _Ostrinia nubilalis_, the European corn borer. Extracts of nonhost plants also may act as oviposition deterrents for _Pieris_ sp. (Lundgren, 1975); and the tobacco bud worm, _Heliothis virescens_ F. (Tingle and Mitchell, 1984). Mitchell and Heath (1985) reported that extracts of pigweed and larval frass deter oviposition by the beet armyworm, _Spodoptera exigua_ (Hubner), and the southern armyworm, _S. eridania_ (Cramer).

In case of stored product coleopteran pests; some of these pheromones have been reported - Tenebrionid beetles (Loconti and Roth, 1953) and Bruchid beetles (Yoshida, 1961; Umeya, 1966; Oshima _et al._, 1973; Wasserman, 1981; Szentesi, 1981; Giga and Smith, 1985, Sakai _et al._, 1986). Seed Beetles (Bruchidae) of the genus _Callosobruchus_ are well known to distribute their eggs evenly among seeds (Avidov _et al._, 1965). Gravid females of _C. maculatus_ F. not only distinguish between egg-laden and pristine seeds but also asses egg loads quantitatively, i.e. when all seeds bear eggs; females prefer to oviposit on seeds with below-average egg densities (Messina and Renwick, 1985 a). Giga and Smith, 1985; Wasserman, 1985;

Messina and Renwick, 1985 b demonstrated a chemical basis for the egg recognition in C. maculatus depending on the fact that the females lay more eggs on clean seeds than on seeds from which an egg has been removed. The possibility of physical (tactile) cues was demonstrated by Messina and Renwick, 1985 b as because attachment of eggs models to seeds deterred oviposition to some extent.

It has been known that the cigarette beetles deposit their eggs on non-food materials are very less, than on food stuffs (Powell, 1931; Dick, 1937 and Howe, 1957). Yamamota and Fraenkel (1960) demonstrated the suitability of tobacco leaves for oviposition by Lasioderma females and that this was dependent on (i) The nicotine content (ii) The adequacy of nutrients and (iii) materials which change the toxicity of nicotine.

It is apparent from the observations reported in the preceding chapter that females of L. serricorne were strongly deterred from the female-conditioned medium. Further, during the course of other preliminary experiments it was noticed that egg-laying by tobacco beetles was reduced significantly when presented with rice grains already partially utilized for oviposition by other individuals. This fact indicate that female beetles tend to avoid sites already oviposited upon by other females. However, there is no report about any oviposition deterring pheromone (ODP) in this species of pest. The present work was, therefore, undertaken to find out whether there is any ODP-like activity associated with the egg-washings in this particular pest species. It was also thought desirable to look into possible influence of egg-wash on behaviour (attraction/repulsion) of conspecific larvae as well as adults.

MATERIAL AND METHODS

Initially the adult <u>Lasioderma serricorne</u> were collected from a stock growing on stored rice. They were maintained in the laboratory on wheat flour mixed with 5% brewers yeast powder as food. The culture was maintained in an incubator set at 28 ± 2°C and 65 ± 5% relative humidity. Freshly emerged adults were allowed to lay eggs on rice. After 24 hours of egg laying; the rice grains were thoroughly shaken on sieves, the eggs were collected and transferred in glass vials, stored in a refrigerator at 4°C until after about 12,000 - 12,500 eggs were gathered for each wash preparation. The rice grains were changed between egg-collections for getting higher rates of egg laying. The eggs were then gently shaken for 5 minutes in following fluid media separately:- (i)distilled water, (ii) insect saline, (iii) methanol, (iv) acetone, (v) di-ethyl ether and (vi) hexane. The eggs were then removed by filtration using filter paper (Whatman no.1) and the washes thus collected were labelled after the names of respective fluid media.

According to Fletcher and Long (1971) tobacco leaves or leaf-extracts stimulate egg-laying by this species of beetle. It was, therefore, decided to provide uniformly punched 10 mm tobacco leaf discs (Virginia variety) for egg laying. In case of every type of egg-wash, 80 leaf discs were dipped into respective egg-wash as samples of a particular fluid medium to serve as the "treated" discs. Another lot of 80 discs were dipped into only the respective fluid medium and called as "control" discs. The third lot of 80 leaf discs were kept as "fresh". All the leaf discs were allowed to dry at room temperature. It has been shown that the female tobacco beetle prefers crevices for egg laying (Lefkovitch and Currie, 1967; Kohno and Ohnishi, 1986). The leaf discs were, therefore, pinned together closely making leaf disc-stacks, each having 5 discs, thus, in all 16 leaf disc-stacks of "treated", "control" and "fresh" in case every type of egg-wash were prepared for experiments. The ovipositional response of the beetle was examined using plastic petri-dishes as "Choice dish" (Fig.1.A.) On the bottom of the "Choice dishes" closely fitting circular corrugated papers were placed and covered with filter paper discs of the same size. Four of each type of disc-stacks - "treated", "control" and "fresh" - were arranged in serially alternate order at 12 equidistant sites in each "Choice dish" as shown in Fig.1.B. Adult males and females (5-6 days post-emergent age) were allowed to mate in separate containers and 24 hours thereafter, five pairs were released into each "Choice dish". The dishes were kept for 24 hours in dark at ambient temperature and humidity. The number of eggs laid in each type of disc-stacks were recorded after 24 hours and compared with each other. The possible deterrent or other-wise influence of different egg-washes were expressed in terms of percentage deterrence of egg laying behaviour in the following way:

% deterrence due to a particular egg-wash = $(F - T) \times 100/(F + T)$

% deterrence due to a particular fluid medium = $(F - C) \times 100/(F + C)$

where, F, T and C represent the number of eggs laid on the "fresh", "treated" and "control" leaf disc-stacks, respectively.

Responses of larvae and adults of the tobacco beetle to the leaf disc treated with different egg-washes were also tested employing plastic petri dishes (9 cm diameter) as "Choice chamber". The floor of the dish was fitted with a filter paper of the same size (Whatman no.1) to facilitate easy movements of the insect stages. For this purpose a choice between "treated" and "control" disc-stacks was offered separately to 2nd, 3rd and 4th larval instars and adults. Such two stacks, each of 10 discs, were placed at diametrically opposite points in the "choice chambers".

In the experiments with larval instars, 5 larvae of a particular instar were released at each time at the centre of the "choice chamber". The chamber was kept in dark and the attractiveness or otherwise was recorded after 15 minutes every time. The experiments for each larval instar were replicated 15 times with each set of egg-wash. The test larvae were collected an hour before testing. The filter paper and the leaf discs were changed between experiments with each larval instar to minimise contamination by the test larvae. For the convenience of handelling only 2nd, 3rd and 4th instar larvae were used. In the experiments with adults, 5 to 6 days old unmated males and females were tested. At each time only one adult insect was released at the centre of the "choice chamber". The males and females were tested using separate "choice chambers". The chamber was also kept in the dark and the attractiveness was recorded after 5 minutes every time. The tests with adult insects were replicated 50 times, using new adult individuals at each time. All the experiments were carried out within 48 hours of preparing an egg-wash.

The adults or larvae found near/over a particular egg-wash "treated" or washing fluid medium "control" leaf stacks were regarded as showing positive response either to the egg-wash treated "treated" or to the fluid vehicle treated "control", as the case may be. Those found elsewhere were assumed to have shown no response. Only those number of larvae or adults found near/over the "treated" and "control" leaf disc-stacks were taken into account. Data obtained in case of 3 larval stages as well as adult males and females are represented together in a table on the basis of types of egg-wash. Statistical tests were carried out using the methods of Bishop (1983).

RESULTS AND DISCUSSION

The egg laying response of the tobacco beetle, *Lasioderma serricorne* (F.) to tobacco leaf disc-stacks treated with egg washes (EW) prepared in 6 different fluid vehicles are given in Table 2.1. It was seen that the egg-laying in the tobacco leaf disc-stacks treated with egg-wash prepared in distilled water (DW) was slightly increased in comparison with those treated with only distilled water, (Table 2.1), whereas the number of eggs laid in the fresh tobacco leaf discs was very high. If one compares the respective percentage distribution of eggs laid in the "treated", "control" and "fresh" leaf disc-stacks (19.56, 14.94 and 65.50) it would be noticed that instead of deterring the females from egg-laying DW-EW attracted them. Similar behavioural response has been reported in *Culex* mosquitoes, where distilled water conditioned by egg rafts or that containing egg rafts attracted more mosquitoes for egg-laying than did just distilled water (Osgood, 1971; Hiroshi et al., 1978). On the other hand, Klijnstra (1986) found that, in the case of P. brassicae, the distilled water EW possessed a higher deterrent activity. However, according to him maximum ODP-like effect appeared to be associated with methanol-EW. Prokopy et al., (1982) demonstrated that the female Anastrepha fraterculus (Wiedeman) deposit a water soluble, durable ODP by dragging the ovipositor on the fruit after egg-laying. Water soluble ODP's were also reported in Rhagoletis cerasi (Katsoyannos, 1975) and in Agromyza frontella (Mcneil and Quiring, 1983).

The marked difference, in respect of the numbers of eggs laid, between the "control" and "fresh" tobacco leaf disc-stacks may be ascribed to higher humidity content of the former, which the insects apparently avoid.

Egg-laying on the insect saline egg-wash (IS-EW) "treated" leaf disc-stacks was reduced significantly as compared to "control" as well as "fresh" leaf discs (Table 2.1). This indicated the association of an oviposition deterring chemical with the IS-EW. However, the difference in the egg laying response between "control" and "fresh" leaf-stacks proved the unsuitability of the solvent. It can be suggested here that salinity of "control" leaf-stacks may have been responsible for such an inhibitory influence.

Methanol egg wash (M-EW) also reduced the egg-laying response of the beetles significantly (Table 2.1), but only about 17% additional reduction was observed when compared with the "control". This means that methanol itself had enough deterring effect. Alternatively, it may be said that the solvent might have either drained off some leaf disc chemicals or it might have chemically altered them so as deter the oviposition. Oviposition deterring activity of the M-EW and methanol itself were 64.70 and 32.54 respectively. The percentage egg distribution in the "treated", "control" and "fresh" leaf disc-stacks were 12.44, 29.53 and 58.03 respectively. So, it can be noted here that the M-EW was also carrying some ODP-like property, higher concentration of which may reduce the egg laying significantly. A methanol soluble oviposition deterrent was described by Dittrick et al., (1983) from the frass of 5th instar larvae of O. nubilalis, the European corn borer. Klijnstra (1986) reported high oviposition deterring activity of the methanol washings of the P. brassicae eggs.

Reduction in egg laying was also observed in the acetone egg wash (A-EW) treated leaf discs (Table 2.1). Though the inhibitory activity of the A-EW was relatively low; this solvent was considered as suitable for extraction of ODP, because the difference in egg-laying between the "control" and "fresh" leaf disc-stacks was marginal. The respective percentage egg distribution in the "treated", "control" and "fresh" were 22.28, 37.05 and 40.67. It may be concluded here that some chemical(s)/ pheromone(s) were associated with the A-EW, and higher concentration of which could possibly be used to deter the females from laying eggs. The present result is in contradiction with the findings of Bentley *et al*., (1976), who have reported that the acetone extracts of *A. triseriatus* eggs did not contain any oviposition deterrents.

The di-ethyl ether egg-wash (E-EW) also reduced the egg laying significantly and results were more or less similar to those observed with the A-EW (Table 2.1). The percentage egg distribution in the E-EW "treated", "control" and "fresh" disc-stacks were 23.27, 36.27 and 40.46, respectively. This demonstrated that some chemical(s)/ pheromone(s) are associated with the eggs, which can be used to deter the female insects from egg-laying. The little difference in the egg laying between the "control" and "fresh" samples indicates the suitability of the solvent. Giga and Smith (1985) reported about an ether soluble oviposition marker in *C. maculatus* and *C. rhodesianus* which was also associated with the eggs/ egg laying. Later, Messina et al., (1987) also described an ether soluble "oviposition marker" that is deposited with the eggs of *C. maculatus*.

The egg laying response of the tobacco beetle, *L. serricorne* in the hexane egg-wash (H-EW) treated tobacco leaf discs are given in Table 2.1. It was found that the H-EW reduced egg-laying in the "treated" samples. The percentage distribution of eggs in the "treated", "control" and "fresh" leaf discs were 22.22, 31.16 and 46.62, respectively. The above result also showed that the solvent itself had reduced egg-laying to a considerable extent. This may be ascribed to loss of some attractant properties of the tobacco leaves due to washing away and/or to chemical alterations brought about by the solvent itself in attractiveness of tobacco leaf for the female insects. From the results it may also be said that further concentration of the H-EW may deter the insect from egg laying. Some variation in the total number of eggs laid in the different solvent EW's may account for fluctuations in ambient temperature.

Considering the over-all egg-laying response of the tobacco beetle in respect of different solvent EW's and their respective controls, it may be concluded that an oviposition deterrent for the beetle may be obtained by washing the eggs in different solvents, such as insect saline, methanol, acetone, hexane and di-ethyl ether. Under these circumstances methanol was considered as the most suitable solvent for this purpose. It may, therefore be concluded that higher concentration of M-EW could profitably be tried out as a suitable oviposition deterrent in the case of this pest species. In the present experiment the process of application of the egg washes by dipping the leaf discs may have affected the ODP-like activity. This would easily be avoided by spraying the M-EW concentrate onto the stored food products. The results of the present experiment are contradictory to the findings of Mumtaz and Aliniazee (1983). These authors described that the oviposition deterring pheromone in R. indifferens (Diptera: Tephritidae) is an extremely stable compound both under acidic and basic conditions and the compound was unextractable into any of the organic solvents; including chloroform, hexane, ethyl acetate and ether. They, however, mentioned that the activity remained in the aqueous phase. That holds good probably in case of dipterans. For a coleopteran species, L. serricorne (F.) the egg-washes in organic solvents did show oviposition deterring activity, whereas the egg-wash in distilled water showed negligible oviposition attractant activity. The known involvement exclusively of tarsal chemoreceptors in case of dipterans and their preference for worm, moist/wet sites for oviposition stand out in stark contrast with the general antennal chemoreception and preference for comparatively dry sites for oviposition in coleopteran pests of stored-products. These sensory and behavioural differences might be the basis of adaptational mechanisms that, in all probability, find manifestation in the evolvement of different modes of pheromonal communications suited to respective habits and habitat.

In order to test the responses of adult beetles of both sexes and the larval instars to different egg-wash (EW) preparations, "choice chamber" method was adopted and the choice was only between EW "treated" and "control" leaf disc-stacks. The attractiveness of the egg-washes were gauged statistically by using Chi-square distribution methods (Bishop, 1983) based on an expected distribution of 50 : 50 in respect of "treated" versus "control" leaf disc-stacks. Due to very small size and extremely slow movements of the 1st instar larvae they were omitted from this set of observations. Actual recording are represented in Tables 2.2 to 2.7, each one for a particular type of EW, in respect of either positive or negative responses or otherwise by both adults and larval forms. In the discussion that follows first the larval behaviour and then that of adults will be delt with.

(i) Distilled water egg-wash (Table 2.2):-

It was found that in a general way the "treated" leaf discs were avoided by the larvae, but only in case of 2nd instar the results were statistically significant ($P < 0.01$). The percentage distribution of the 2nd, 3rd and 4th instar larvae between the "treated" and "control" samples were 21 and 60, 32 and 46 and 23 and 30, respectively. The above result may be explained on the assumption that some substances were associated with the eggs that were extractable with the distilled water and were capable of deterring the larvae from feeding on "treated" sample. The above result also indicate that the larvae may detect sites of high density of eggs, and may, therefore, avoid overcrowding over there.

The male insects showed strong attraction ($P < 0.01$) to the "treated" leaf discs, whereas the females showed no discernible preference for either the "treated" or "control" leaf discs. The above result also demonstrate the association of certain chemicals(s)/ pheromone(s) with the eggs; to which the male beetles responded positively.

(ii) Methanol Egg-wash (Table 2.3):-

Like distilled water egg-wash, the methanol egg-wash "treated" leaf discs were also avoided by the tobacco beetle larvae. Among the larvae showing positive response to "control" leaf discs, that of 2nd instar larvae only was significant ($P < 0.01$). The percentage distribution over "treated" and "control" samples were 14 and 42 for 2nd instar larvae, 15 and 27 for 3rd instar larvae and 21 and 29 for the 4th instar larvae.

Males showed avoidence ($P < 0.01$) of "treated" leaf discs. The percentage response of the *L. serricorne* males and females to the "treated" and "control" leaf discs respectively were 28 and 54 and 42 and 40. The above results indicate that the eggs of the beetle possess some methanol-extractable substances, which deter the larval instars as well as the male beetles.

(iii) Insect-saline egg-wash (Table 2.4):-

The larvae showed strong avoidence of the "treated" as well as "control" samples. The respective percentage distribution of the 2nd instar larvae to the "treated" and "control" samples were 10 and 15, 3rd instar larvae were 17 and 13 and 4th instar larvae were 11 and 20. Though statistically no significant difference was apparent in case of adult beetles of both sexes with regard to attractiveness or otherwise between the "treated" and "control" samples, the actual number of individuals on these leaf disc-stacks were comparatively more than elsewhere. Seemingly it appears that this EW/insect-saline itself obviously changed the surface properties of the leaf discs in such a way that adults of both sexes got confused and remained more with the "treated" and "control" stacks more than elsewhere. As opposed to this, all the larval forms remained away from both leaf stacks. It seems that the larval instars, being in their feeding stages, preferred to avoid insect saline affected leaf stacks. The adult male as well as female beetles, which are known not to be feeding stage, somehow found the thus affected leaf discs comparatively more agreeble than otherwise. Perhaps, little bit of salt present there had something to do with this confusion on the part of both sexes and selective avoidence by the larvae.

(iv) Acetone egg-wash (Table 2.5):-

None of the larval instars showed significant preferential attraction or aversion towards "treated" or "control" leaf discs, whereas 80 percent of the males showed obvious aversion. Female beetles were, however, attracted towards "treated" stacks ($P < 0.05$) but the "control" stacks were apparently avoided. Probably acetone itself was not agreeble to females. The above results may be explained in two ways: firstly, the egg associated chemical(s) extractable with acetone have little or no influence on the behaviour of the larvae. Secondly, the solvent itself may have altered the general properties of the tobacco leaf during the process of dipping and thus the larvae did not find any difference between the "treated" and "control" samples. On the other hand, acetone egg-wash exhibited a positively deterrent influence on male beetles only, whereas, female beetles exhibited preference to "treated" stacks.

(v) Hexane egg-wash (Table 2.6):-

In general, a good number of larvae were found to be deterred by hexane egg-wash "treated" samples. If one considers the "treated" versus "control" readings then the result was significantly different only in case of 3rd instar. The respective percentage distribution of the 2nd, 3rd and 4th instar larvae to the "treated" and "control" samples were 19 and 17, 23 and 43 and 26 and 35.

However, on the basis of overall distribution of the larval instars it becomes obvious that the 2nd instar shunned off both the "treated" as well as "control" leaf stacks. This means not only the egg-wash sample but even the extracting medium that is hexane itself was not agreeble to the 2nd instar larvae.

As far as the adult beetle are concerned the male showed an aversion similar to 2nd instar larvae. Female beetles did not show much of an overt response, except that a comparatively high number of them could be observed over "control" stacks. The results obtained here prove that some chemical(s)/ pheromone(s) extractable with hexane was associated with the eggs, high dose of which may be used for deterring larvae and females in particular.

(vi) Di-ethyl ether egg-wash (Table 2.7):-

It was found that all the three larval forms exhibited a negative response to "treated" as well as "control" samples. This indicated that not merely the factors extractable in ether but the latter itself was equally effective in warding off the larval instars.

The males showed a definite avoidence ($P < 0.05$) of "treated" leaf discs. The female beetles were seen not to be influenced either by ether soluble matter from eggs or by ether itself; their distribution being more or less even. It may be seen that di-ethyl ether treatment might have resulted in alteration of general properties of the tobacco leaves in such a manner that all the larval forms avoided them. The chemicals associated with this egg-wash were certainly sufficient to deter the adult males. For practical application of this information it, therefore, appears possible that employment of ether extract of eggs would not only keep the larvae from feeding on the medium in which they are but this would also keep the males off the medium infested by this species. In short, it may provide a two-pronged weapon, by killing the larvae through starvation and by not allowing mating for further growth of population by keeping the males away.

By way of a caution it may be stated here that, in the present experiment only 10 tobacco leaf discs (10 mm diameter) were dipped for a single time in the egg-washes and stacked together by pinning, hence, concentration of the washes were not high. In all probability then, the leaf discs were containing a very little amount of the chemical(s)/pheromone(s). It may, therefore, be concluded here that using high concentrations of the egg-washes of suitable solvents may provide appropriate means for either deterring larval forms being in and feeding on culture media/stored products or driving the male beetles away.

In this light, the present findings point to the insect saline and di-ethyl ether as potent agents as deterrents for all the three larval forms. With respect to adult males, acetone and di-ethyl ether were found to be the agent of choice for keeping the males off the stored products. It is, therefore, likely that di-ethyl ether would be the best of the fluids for obtaining extractable egg-material(s) of significance. However, it remains to be studied as to how far the use of ether-extractable egg materials could be of practical value, nevertheless, the present work, at the least, opens up a new vista for trying a double-pronged management of L. serricorne (F.) infestation of stored products of economic importance. On one hand the larvae would not feed on treated food source and on the other hand the male beetles shunning away would reduce chances of population build-up.

An egg associated oviposition deterrent in the Hylema sp. (Diptera : Anthomyiidae) has been described as responsible for the regular distribution of its larvae (Zimmerman, 1979). In the present experiment, such noticeable larval repulsion was observed in case almost all the solvent egg-washes, especially the methanol, hexane and distilled water. All these egg-washes also showed a good degree of oviposition deterring activity. The present findings also agrees with Szentesi (1981) who reported that the 1st instar larvae of A. obtectus disperse more by some unknown chemical substance(s) deposited by the adult insects. Such substance(s) was also found to act as an oviposition deterrent.

During the present work the best extracting medium for extraction of ODP-like substance(s) from the eggs was found to be methanol (64.7%) and the least (26.97%) suitable was di-ethyl ether. On this basis, it is logical to suggest that the suspected ODP-like activity of egg wash in methanol is different from the observed deterrent activity of the ether egg-wash effective against 2nd, 3rd and 4th instar larvae as well as adult male beetles. It is, therefore, desirable to analyse chemical nature of these two egg-washes which exhibited divergent influence on the behaviour of L. serricorne (F.).

SUMMARY

Oviposition response of the female tobacco beetle, L. serricorne (F.) were tested against different solvent egg-washes. Among the different solvents used, insect saline, methanol, acetone, hexane and di-ethyl ether were found to reduce the egg-laying by the beetle. Considering the percentage egg laying inhibitory activity, methanol was found to be most effective. When the responsiveness (attraction or repulsion) of the different larval instars and adults were tested against the food media treated with different solvent egg-washes, it was also found that all the larval instars showed deterrence from the egg-wash treated samples. In this case also methanol egg-wash was found to be most effective. The males also showed

deterrence to the treated samples but the females showed no preference for it. So the present result demonstrate that methanol would be the best of the fluids for obtaining extractable egg-materials of significance, high density of which can be used for three different functions:- (i) as an oviposition deterrent (ii) feeding deterrent for the larvae and (iii) keeping the male beetles away from the treated source and thus would reduce the chances of population build-up. The present findings also point to the insect-saline and di-ethyl ether as potent deterrent for all the larval forms. With respect to the responsiveness of the adult beetles, di-ethyl ether was found to be the agent of choice for keeping the males off the treated source. It is, therefore, likely that di-ethyl ether may also provide a solvent for obtaining extractable egg-materials of significance which may provide appropriate means for deterring larval forms from in and feeding on treated medium on the one hand and on the other driving the male beetles away and thus will reduce the chances of population build-up. The large scale isolation and subsequent chemical synthesis of the above mentioned factors may prove to be of immense practical value in the integrated management of this particular pest species.

Table 2.1. Oviposition response of the tobacco beetle, L. serricorne to the tobacco leaf discs treated with different egg-washes

Fluid media employed for particular egg-wash	Total number eggs recorded	Percentage of egg distribution on leaf-stacks of types -			Percentage deterrence in respect of	
		Treated	Control	Fresh	Treated stacks	Control stacks
Distilled water	542	19.56	14.94	65.50	54.01	62.84
Methanol	579	12.44	29.53	58.03	64.70	32.54
Acetone	413	22.28	37.05	40.67	29.23	4.67
Insect saline	399	19.05	29.32	51.63	40.10	27.55
Hexane	459	22.22	31.16	46.62	35.44	19.89
Di-ethyl ether	477	23.27	36.27	40.46	26.97	5.46

Table 2.2 Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with egg-wash prepared in distilled water

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	21	60	19	18.77 **
3rd Instar	32	46	22	2.51 NS
4th Instar	23	30	47	0.92 NS
Male	54	22	24	13.47 **
Female	44	44	12	0.0 NS

** Significant at 1% level

NS Non significant

Table 2.3. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with egg-wash prepared in methanol

	Percentage values of positive response to leaf stacks			
Insect stages	Treated	Control	No response	χ^2
2nd Instar	14	42	44	14.0 **
3rd Instar	15	27	58	3.42 NS
4th Instar	21	29	50	1.28 NS
Male	28	54	20	8.24 *
Female	42	40	18	0.05 NS

* Significant at 5% level

** Significant at 1% level

NS Non significant

Table 2.4. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with egg-wash prepared in insect saline

	Percentage values of positive response to leaf stacks			
Insect stages	Treated	Control	No response	χ^2
2nd Instar	10	15	75	1.0 NS
3rd Instar	17	13	70	0.53 NS
4th Instar	11	20	69	2.61 NS
Male	40	34	26	0.49 NS
Female	40	40	20	0.0 NS

NS Non significant

Table 2.5. Responsiveness of the L. *serricorne* (F.) larvae and adults to the tobacco leaf discs treated with egg-wash prepared in acetone

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	29	29	42	0.0 NS
3rd Instar	29	41	30	2.06 NS
4th Instar	31	24	45	0.89 NS
Male	6	14	80	3.2 NS
Female	44	22	34	7.34 *

* Significant at 5% level

NS Non significant

Table 2.6. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with egg-wash prepared in hexane

Insect stages	Percentage values of positive response to leaf stacks			
	Treated	Control	No response	χ^2
2nd Instar	19	17	64	0.11 NS
3rd Instar	23	43	34	6.06 *
4th Instar	26	35	39	1.33 NS
Male	22	22	56	0.0 NS
Female	28	46	26	4.38 *

* Significant at 5% level

NS Non significant

Table 2.7. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with egg-wash prepared in di-ethyl ether

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	10	11	79	0.05 NS
3rd Instar	10	11	79	0.05 NS
4th Instar	14	17	69	0.29 NS
Male	8	20	72	5.14 *
Female	24	38	38	3.16 NS

* Significant at 5% level

NS Non significant

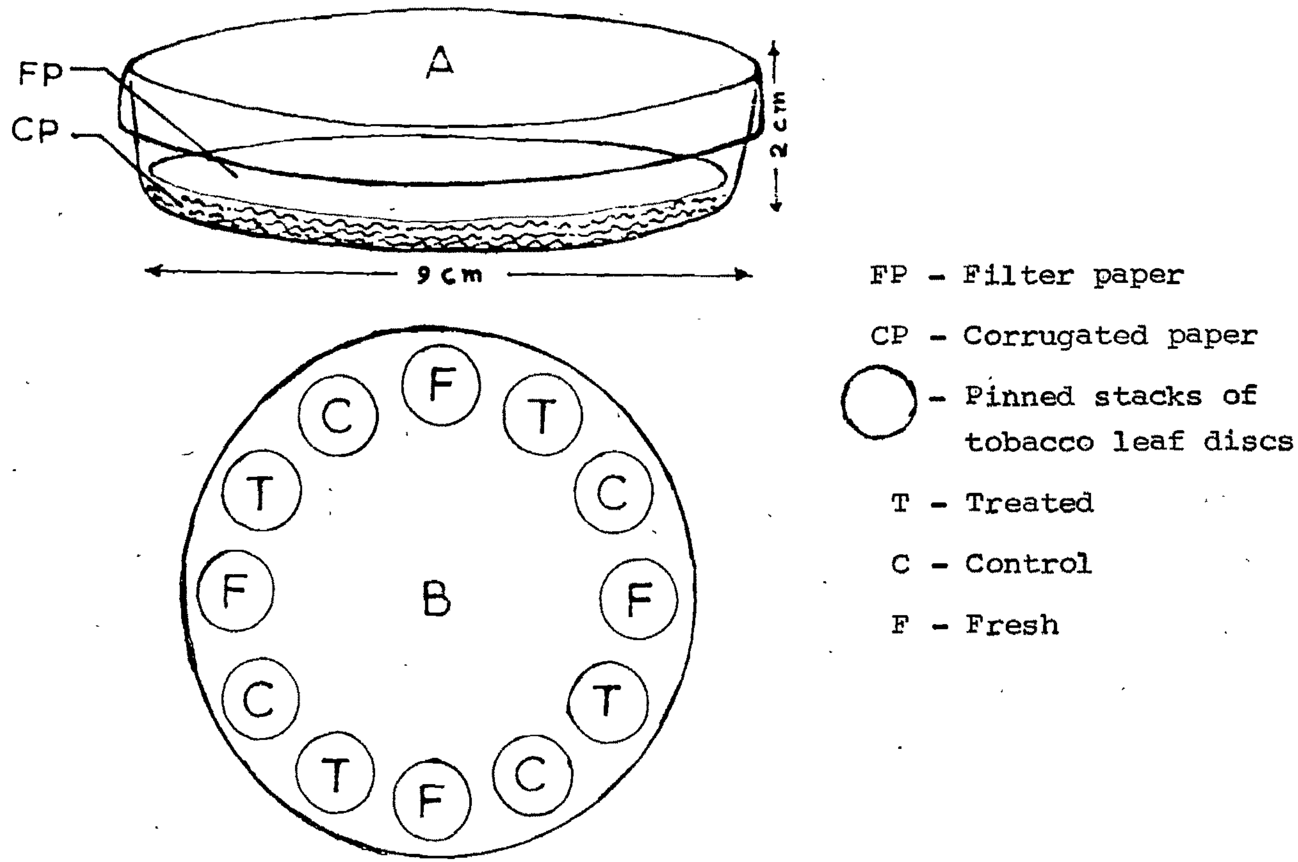

Fig.1. Schematic diagrams of the arrangements of disc-stacks in "Choice dishes". (A) Choice dish (B) Experimental design