CHAPTER : 3

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

It is usually known that two types of pheromones are secreted by insects. Firstly, the secretion of male insects, usually known as "Aggregation pheromone" which attracts both males and females of the same species. Secondly, the secretion by female insects, usually known as "Sex pheromone", attracts the conspecific males only.

Male produced aggregation pheromone has been reported from a large number of coleopteran pests, such as *Tribolium castaneum* (Faustini *et al*., 1981); *T. confusum* (Suzuki, 1981 and Ryan and O'Ceallachain, 1976); *Sitophilus oryzae* (Phillips and Burkholder, 1981); *Rhyzopertha dominica* (Khorramshahi and Burkholder, 1981); *Pissodes nemorensis* (Phillips *et al*., 1984); *Ips erosus* (Giesen *et al*., 1984) and *Cryptolestes pusillus* (Millar *et al*., 1985). A marcolide pheromone has been reported by Millar *et al*. (1985) in case of *C. turcicus* and by Cross *et al*. (1969) in case of *Anthonomus grandis*.

Except the aggregation pheromone, some other pheromones are known to be secreted by the male insects. Tanaka et al. (1981) reported the secretion of a different type of pheromone from azuki bean weevil, Callosobruchus chinensis, which they named as "erectin", that induces the males to extrude the genital organ at the time of copulation, which, is released by both sexes but affects only the males. Suzuki (1985) demonstrated the presence of a different aggregation substance in the frass of both males and females of T. castaneum which was attractive to both sexes. In case of a related species T. confusum also, Reville and Kannowski (1975) have found that both males and females produce pheromone that apparantly is effective on males.

Very little information is available on the oviposition deterring pheromone(s) of the stored product coleopteran pests, except a few reports on Tenebrionids and Bruchids. Deposition of chemically undefined substance(s) by the dry bean weevil, Acanthoscelides obtectus on the beans during various activities (most probably during defecation) were described by Szentesi (1981). Further, he has also mentioned that this kind of marking leads to avoidence of oviposition by other females on these beans. Szentesi (1981) obtained strong oviposition deterrents from seeds exposed to either sex of the weevils. Messina et al. (1987) also reported about secretion of a similar oviposition deterrent by C. maculatus, which they named as "oviposition marker". It was shown to be ether soluble. These authors suggested that general oviposition deterrents may be isolated from both sexes. The uniform egg deposition among available host azuki beans by C. chinensis and C. maculatus is expressed by their behaviour that the females prefer to oviposit on beans not conditioned by eggs and a biological conditioning substance (BCS) Sakai et al. (1986). They also reported that the BCS is excreted by both males and females, however, that from the females plays a major role. Oshima et al. (1973) and Messina and Renwick (1985b) extracted the chemical oviposition deterrents from males and females of the C. chinensis and C. maculatus respectively.

Strangely enough, no information is available about any pheromonal secretion by the male L. serricorne. However, during the course of present work, conditioning of the culture medium by the male L. serricorne was found to attract all the stages except the males. These clearly demonstrated the possibility of association of certain chemical(s)/pheromone(s) with the male insects. To test this assumption, male L. serricorne beetles were subjected to washings with different solvents like distilled water, insect saline, and various other organic solvents. These washings were then tested on conspecific larvae and adults for possible alteration of behaviour - attraction/repulsion and/or oviposition.

MATERIAL AND METHODS

The *L. serricorne* population were cultured in the laboratory as described in the previous chapter. The male insects were identified on the basis of morphological characters of genital papillae at pupal stage (Halstead, 1963). The isolated pupae were maintained in separate petri-dishes in the B.O.D. incubator for adult emergence. To prevent the fungal infection of the pupae 1% sodium benzoate solution soaked filter paper were used at the floor of the petri-dishes. Nacent post-emergence, the beetles were collected and allowed to age upto 5 days in the incubator. While the collection was going on, 5 - 6 days old males were stored in a refrigerator at 4°C until after about 1000 (2.5 gms) beetles were obtained. A batch of 1000 insects, in each case, was washed for 15 minutes with following solvents separately:- Distilled water, Insect saline, Methanol, Acetone and Hexane. The insect bodies were removed by filtration and the solutions collected were lebelled after the respective solvent. The tests for oviposition response, were carried out, employing "choice-dish" method as per the method already described. Possible influence of a particular male body-wash versus that of respective control were assessed by employing the same formula and the results are expressed as percent deterrence. In order to investigate the influence of various male body-washes on the behaviour of adults as well as larval instars similar "choice-chamber" method was followed as detailed in chapter 2. Statistical tests were performed as described in the previous chapter.

RESULTS AND DISCUSSION

The egg laying response of the tobacco beetle, L. serricorne (F.) with respect to tobacco leaf discs "treated" with different male body-washes are given in Table 3.1. Reduction in egg laying was observed in case of the tobacco leaf discs-stacks treated with distilled water male body-wash. Considering such reduced egg-laying activity (20% inhibition), it could be surmised that some chemical(s)/pheromone(s) are associated with the male insects. A high concentration of this kind of male body-wash may be used to contain significantly the egg-laying of the beetle.

A slight reduction in egg-laying was observable due to the influence of insect saline male body-wash (Table 3.1).

Marked reduction in egg-laying was observed due to hexane male body-wash. The percentage of egg-laying on the "treated"and "control" leaf discs were 12.68, and 24.17 respectively. This demonstrate very clearly that the hexane male body-wash was carrying some chemical(s)/ pheromone(s) which deterred females from laying eggs. If one considers the percentage values of egg-laying on "treated" and "fresh" samples, it would be seen that about 50% reduction occured due to male body-wash. So, it can be concluded here that the chemical(s) pheromone(s) from the male beetles, extractable in hexane, can also be employed as the oviposition deterrent for this pest species.

Perusal of values given in Table 3.1 indicates a slight increase in egg-laying due to acetone male body-wash. It is pertinent to mention here the findings of Sridevi _et al_. (1987), who reported that the adult male accessory gland of _Spodoptera litura_ have an oviposition stimulatory factor. Presence of an oviposition stimulatory peptide in male accessory gland of _Drosophila melanogaster_ has been described by Chen _et al_. (1988). Possible involvement of a similar factor in the present experiment can not be ruled out.

There was no difference in the egg-laying when methanol male body-wash was tested. This demonstrated that whatever is extracted in methanol, does not contain factor(s) responsible for deterrent or stimulant action on oviposition behaviour of the beetle.

From the observation reported in table 3.1, it could be safely concluded that male body-wash does contain some factors that might influence behaviour of males or females.

Since, during the course of present investigation only the oviposition behaviour was studied; remarks can be made only on this aspect. On the basis of the results obtained, it could be said that the best possible solvent for extraction of oviposition deterring component was hexane.

Before concluding this part of discussion on adult L.serricorne behavioural patterns it would be pertinent to mention here that the acetone male body-wash did indicate presence of some kind of oviposition stimulant. However, this should need further investigation for confirmation.

Responses of the L.serricorne larvae as well as adults to the distilled water male body-wash are shown in Table 3.2. The wetness/humidity of the leaf samples might be responsible for the general reduction of attractiveness of the leaf discs for larval instars, as was evident from a large number (70% and above) of all the three instars wander away from either the "treated" or "control" leaf disc-stacks. Nevertheless, if the smaller numbers of larval forms are not be overlooked, then it can said that the 2nd and 3rd instar larvae did chose to prefer "treated" samples as compared the "control". As against this the last instar showed a negative response to distilled water male body-wash. Regarding the influence of male body-wash on adults; it could be seen that male beetles showed a negative response, whereas female ones did not show any preferential response as far as male body-wash was concerned.

Based on the above observations with male body-wash prepared in distilled water; it could said that this vehicle is not appropriate for the purpose under consideration.

Responses of larvae and adults to the acetone male body-wash are given in Table 3.3. The 2nd instar larvae exhibited a noticeable degree of attraction towards treated samples, but obviously were deterred by acetone as such in the "control" sample ($P < 0.05$). From the percentage distribution of 3rd and 4th instar larvae it is seen that a large majority of them showed positive response and that these did not distinguish between "treated" and "control" samples. The above results demonstrate that acetone extracted some chemical/pheromone from the male insects which could attract to an extent the early larval instars towards the "treated" samples. However, it is apparent that acetone of "control" sample was not agreeable to 2nd instar. About 72% of the male insects tested did show a negative response to either "treated" or "control" samples. The avoidence of acetone male body-wash by male insects may be explained by assuming that whatever chemical/pheromone acetone could extract from the male insects was seemingly not agreeable to other males. The females did not show any preferential response as far as "treated" and "control" samples were concerned, and were equally attracted to both the samples tested. From the above results, it may also be concluded that acetone is perhaps not a suitable extracting medium in the present context.

Responses of the larvae and adults to "treated" and "control" samples of male body-wash prepared in insect saline are produced in Table 3.4. Around 50% of the larvae of each instar were showing no response. The avoidence of "treated" and "control" leaf discs by the test larvae may be due to the salinity or moisture itself. Among the larvae that showed response, comparatively higher numbers were attracted to the "treated" samples. No significant differences by way of preference to the"treated" or "control" samples were observed in case of both males and females. The above result lead to the conclusion that insect saline is not a solvent of choice as far as the present work is concerned.

Table 3.5 represents the responses of the *L. serricorne* larvae and adults to the tobacco leaf discs treated with hexane male body-wash. The above results demonstrated that whatever was extracted in hexane from the bodies of male insects has got very little influence on the larvae as well as females. As apposed to this the male insects showed a strong deterring tendency for the "treated" leaf discs, and 54% of the males rejected it. This high deterrent activity of male body-wash clearly indicated the presence of certain pheromonal components. This may possibly be used to deter the male insects significantly in order to prevent the population build up in already infested stores of preserved commodities.

Larval and adult responses to the methanol male body-wash are given in Table 3.6. The 2nd instar larvae were attracted significantly ($P < 0.05$) to the "treated" leaves, whereas the 4th instar larvae avoided it ($P < 0.05$). The 3rd instar larvae were more or less not influenced.

The male insects were strongly deterred ($P < 0.01$) by the male body-wash and majority of them (74%) moved away from both "treated" and "control" samples tested. The females did not show any preference for either the "treated" and "control" samples. This indicated that certain chemical/pheromone extractable with methanol from the male insects, if employed in concentrated dose may prove to be an effective agent for deterring the males and late instar larvae and at the same time attractant for the early instar larvae.

Taking into account the overall larval responses to the different male body-washes, it can be suggested that the chemical(s)/pheromone(s) present on bodies of male insects, which are extractable in different solvents employed here have some specific influence on particularly the early larval instars. Hence, it would be possible to utilize concentrated male body washes in appropriate doses as potent attractants for them. Similar larval attraction to the male-conditioned medium in the previous experiment (chapter - 1) also supports this suggestion. The observed congregation of 4th instar larvae on the control samples might have been the consequence of this instar being busy mainly with feeding just prior to pupation. Moreover the last instar larvae usually prefer to avoid overcrowding while pupating, hence, may go to less crowded "control" samples. The differences observed in numbers of 4th instar larvae in getting attracted to some "control" samples and not others might have been due to the variation in the age of their maturity. Larval responses to the male body-washes suggested that the male associated chemical(s)/pheromone(s) may be of aggregation pheromone in nature. It is pertinent here to mention the findings of Mondal and Port (1984) and Mondal (1985) wherein they reported that the larvae of T. castaneum getting attracted to the male conditioned medium was due to the aggregation pheromone. One of the characteristic properties of the male produced aggregation pheromone is to attract individuals of both sexes (Khorramshahi and Burkholder, 1981; Phillips and Burkholder, 1981; Ryan and O'Ceallachain, 1976). However, in the present case, since no preferential response by the female beetles could be noted with respect to almost all the male body-washes, it may be concluded that the chemical/pheromone of the male L. serricorne does not qualify as an aggregation pheromone. Additionally, as was observed in the early part of this chapter, some of the male body-washes did possess oviposition deterrent property. Hence, whatever was coming up in body-wash can not be considered as a mere aggregation pheromone. The present observations do not agree with the findings of Coffelt and Burkholder (1972) who reported that the male tobacco beetles secrete no pheromones.

Author himself feels that the findings obtained here could not be taken very seriously to represent desirably significant observations mainly due to one methodological draw-back. During the course of present work the tobacco leaf disc were dipped into the different male body-washes and the respective extracting media. This method of dipping might lead to loss of some components of the leaf discs themselves and thereby to alterations in their properties with respect to behavioural responses of the test insects. Better procedure would have been coating the leaf discs by spraying on them the washes or media so as to minimize the mentioned losses/ alterations. So, the author wishes to put on record that, in further work, this factor should be taken care of to obtain more meaningful data.

It should be pointed out here that, wherever decidedly positive results were obtained with respect to oviposition deterrence/stimulation and larval attraction/ repulsion, the responsible male body-washes could be put to practical applications after necessary concentration of respective body-washes or in high doses for controlling infestation of stored products by this pest species. Further, isolation of most effective component(s) of body-washes of concern would be of greater utility from this point of view.

SUMMARY

Assuming that the male *L. serricorne* beetles liberate some pheromonal substance(s), based on experimental observations (chapter : 1) on influence of male-conditioned culture medium, for the present experiment an attempt was to study the effect of washings of bodies of male beetles (1000 individuals/5 ml) in different extracting media on the behaviour of all stages of the conspecific individuals. Oviposition response of female beetles to the different male body-washes were tested. Among these hexane wash was found to lead to maximum reduction of oviposition on the treated samples. The conspecific male beetles were strongly deterred by this wash. However, hexane body-wash exerted no significant influence on the larval instars. In general, it was observed that the early larval instars exhibited varying degrees of positive response to most of the male body-washes tested, whereas the late instar larvae showed negative response, though such influence was of a mild nature. The above results demonstrate that cuticular or bodily substances of the male *L. serricorne* could be extracted

employing suitable media. The body-washes which were found to possess definite influences could profitably be used in concentrated doses for three different applications:- (i) oviposition deterrents (ii) as deterrent/repellent agents against the males (iii) and as attractant for the larvae to lure them away in suitable traps. The practical application of the above mentioned factors may prove to be of immense value in integrated management of this species.

Table 3.1. Oviposition response of the tobacco beetle, L. serricorne to the tobacco leaf discs treated with different male body washes

Fluid media employed for particular male 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	294	15.99	26.53	57.48	56.48	36.84
Insect saline	345	20.00	25.79	54.21	46.09	35.50
Hexane	331	12.68	24.17	63.15	66.53	44.64
Acetone	408	33.33	25.98	40.69	9.93	22.05
Methanol	495	25.05	29.29	45.66	29.14	21.83

Table 3.2. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with male body-wash prepared in distilled water

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	14.67	8.0	77.33	1.96 NS
3rd Instar	22.67	6.67	70.67	9.72 *
4th Instar	8.0	21.33	70.67	6.06 *
Male	34.0	56.0	10.0	5.38 *
Female	44.0	40.0	16.0	0.19 NS

* Significant at 5% level

NS Non significant

Table 3.3. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with male body-wash prepared in acetone

Insect stages	Percentage values of positive response to leaf stacks			
	Treated	Control	No response	χ^2
2nd Instar	30.0	12.5	57.5	7.20 *
3rd Instar	41.25	35.0	23.75	0.51 NS
4th Instar	37.0	45.0	17.5	0.68 NS
Male	10.0	18.0	72.0	2.28 NS
Female	40.0	34.0	26.0	0.48 NS

* Significant at 5% level

NS Non significant

Table 3.4. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with male body-wash prepared in Insect-saline

Insect stages	Percentage values of positive response to leaf stacks			
	Treated	Control	No response	χ^2
2nd Instar	26.67	17.33	56.0	1.98 NS
3rd Instar	30.67	22.67	46.67	1.20 NS
4th Instar	26.67	21.33	52.0	0.59 NS
Male	38.0	44.0	18.0	0.44 NS
Female	48.0	52.0	0.0	0.16 NS

NS Non significant

Table 3.5. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with male body-wash prepared in hexane

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	34.67	22.66	42.67	2.51 NS
3rd Instar	30.67	37.33	32.0	0.65 NS
4th Instar	25.33	34.67	40.0	1.45 NS
Male	10.0	54.0	36.0	30.25 **
Female	40.0	38.0	22.0	0.05 NS

** Significant at 1% level

NS Non significant

Table 3.6. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with male body-wash prepared in methanol

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	37.50	21.25	41.25	4.50 *
3rd Instar	33.75	31.25	35.0	0.97 NS
4th Instar	27.5	47.5	25.0	5.33 *
Male	4.0	22.0	74.0	12.46 **
Female	42.0	38.0	20.0	0.20 NS

* Significant at 5% level

** Significant at 1% level

NS Non significant