CHAPTER : 4

OBSERVATIONS ON THE INFLUENCE OF FEMALE BODY-WASH ON OVIPOSITION RESPONSE AND ON BEHAVIOUR OF ADULTS AS WELL AS LARVAE OF LASIODERMA SERRICORNE (F.)

Most of the reports available on the oviposition deterring pheromone (ODP) indicate it emanates from female body either during or after oviposition. Prokopy et al. (1982) have reported that female individuals of Anastrepha fraterculus (Wiedeman) leave behind a water-soluble, durable, ODP immediately after oviposition as the ovipositor is dragged along the fruit surface. Reports on such marking of oviposition sites by dragging of extended ovipositor after egg laying are available in case of many other dipterans such as that by Katsoyannos (1975) in Rhagoletis cerasi; Prokopy et al. (1978) in Ceratitis capitata; Averill and Prokopy (1981) in R. basiola; Averill and Prokopy (1982) in R. zephyria; Mcneil and Quiring (1983) in Agromyza frontella and Mumtaz and Aliniazee (1983) in R. indifferens. Employing behavioural and electrophysiological assay techniques, Prokopy et al. (1982) demonstrated that the posterior half of the midgut of female Rhagoletis pomonella is the principal site of production of a major component of the ODP. Wasserman (1981) described about an oviposition marker in cowpea weevil, Callosobruchus maculatus.

The female bruchids, chemically marks its oviposition site with each oviposition episode. Prokopy et al. (1982) isolated ODP from several body parts of the apple maggot fly, R. pomonella.

The previous experiments reported here with female-conditioned medium showed that female individuals of L. serricorne strongly avoided the conspecific female-conditioned medium, which demonstrated secretion of pheromonal chemicals that may profitably be used for deterring the female beetles from oviposition. The experiments with egg washes also demonstrated that some ODP like chemicals were associated with the eggs, logically the source of which would be the female body. Apart from the mention of sex pheromone secretion from the adult female insects (Burkholder, 1970; Coffelt and Burkholder, 1972), there are no reports of any other pheromonal secretions by the L. serricorne. Before going to the further detail of the egg wash or male wash associated secretions, in the light of above mentioned facts an investigation a was initiated to find out whether are any other pheromonal secretions from the adult female L. serricorne. It was also decided to investigate the possible role of female secretions in influencing the oviposition response as well as the behaviour of the larvae and adults regarding attractant and/or repellent activity.

MATERIAL AND METHODS

All the experimental details regarding culturing, sexing, age and numbers of female L. serricorne beetles utilized for each type of body-wash remain essentially similar to those described in the previous chapters. The five different solvents used for female body-washings were same as those described in case of male beetles (chapter 3). Oviposition response of the female beetles and behaviour of adult and larval instars were assessed employing testing techniques as outlined in previous chapters. Statistical tests were also carried out following the same procedure as described in the previous chapter.

RESULTS AND DISCUSSION

The egg laying responses of the tobacco beetle, L. serricorne to different female body washes are given in Table 4.1. The egg laying response tested with distilled water female body-wash is depicted in percentage-wise distribution of eggs laid on the "treated", "control" and "fresh" leaf discs-stacks as 11.66, 20.55 and 67.79 respectively. The reduction of egg laying in the control leaf discs may be explained that the humidity of the leaf may remain high or some chemicals from the leaf samples were washed away during dipping, which made the leaf discs different from the fresh samples. Considering the

percentage inhibitory activity, it was found that about 17% additional inhibition occured due to the female body-wash. This may logically mean that some pheromonal chemicals are associated with the female insects. A more concentrated dose of this may be used as an oviposition deterrent in the case of this pest. As previously cited Prokopy et al. (1982) had reported on the occurrence of a water soluble ODP in Anastrepha fraterculus (Wiedeman), secreted by female insects after egg laying by dragging the ovipositor on fruit surface.

Observations on female body-wash prepared in insect saline showed that the wash reduced the egg laying to a considerable extent. The percentage of eggs distribution on the "treated", "control" and "fresh" leaf disc-stacks were 17.33, 31.20 and 51.67 respectively. Taking percentage deterrence into account it may be seen that about 26% deterrence, over and above that observed with "control", occured due to female body-wash. It may, therefore, be inferred that some pheromonal chemicals can be extracted from the female insects that possess certain oviposition deterring function.

If one carefully observes the percentage distributions of eggs laid on the "treated" and "control" samples in case of female body-washes prepared with distilled water and insect saline, it could easily seen that dipping the tobacco leaf discs in distilled water led to a greater alteration

in the attractive nature of the tobacco leaves to female beetles, hence, comparatively less number of eggs were laid thereon. As apposed to this, probably treatment with insect saline did not make the leaf discs so unattractive to the female beetles. These remarks are of relevance, if one considers the differences between the percentage deterrence activity between "treated" and "control" samples of two egg washes.

The very high degree of reduction in egg laying was observed in case of hexane female body-wash. However, there was hardly any difference between number of eggs laid on the control and fresh samples. This indicated no adverse effect of the solvent on the tobacco leaves. It was found that more than 77% additional deterrence occured due to the hexane female body-wash, as compared to deterrence exerted by the control alone. The above result expressly demonstrated occurrence of pheromonal chemicals in the female tobacco beetle extractable with hexane have high ODP quality.

The acetone female body-wash also reduced the egg-laying to very highly significant degree (1/3), but, remarkably enough, acetone itself ("control") exhibited about 18% deterrence of oviposition response. Hence, the author suggests that, in comparison to hexane, acetone is not a very suitable extracting medium for ODP.

Lowest egg deposition was observed on the samples "treated" with methanol female body-wash and only 2.57% eggs were deposited on it. As against this more than 74% eggs were laid on the fresh leaf discs. Taking into consideration only these two readings the methanol body-wash appears to be a good solvent for ODP. Nevertheless, if one takes into account the percentage of eggs laid on the "control", then it can easily be surmised that methanol would not serve as a good solvent as far as the present experimental set up is concerned.

From the results presented in Table 4.1, it is very clear that a strong ODP is associated with the female beetles. Considering the egg laying inhibitory activity as the criterion, hexane was selected as the most suitable solvent. So it was concluded that the hexane female body-wash of L. serricorne may conveniently be used as a potent oviposition deterrent for the conspecific females. It is pertinent to mention here the findings of Hwang et al. (1982), who reported that lower aliphatic carboxylic acids induce negative oviposition response in various species of mosquitoes. The present result is somewhat similar to the findings of Messina et al. (1987), who have reported about an ether soluble oviposition marker is secreted by the females of C. maculatus. Secretion of ODP by the females of C. chinensis and C. maculatus reported by Oshima et al. (1973) and Messina

and Renwick (1985b) are also similar to the present findings.

The percentage distribution of larvae and adults with respect to tests conducted with distilled water female body-wash are given in Table 4.2. It was found that there was almost no influence on the 2nd and 3rd larval instars. Only the 4th instar larvae were found to get deterred ($P < 0.05$) by the treated samples. Majority of 2nd and 3rd instar larvae (78.67% and 72% respectively) were found to remain away from either "treated" or "control" samples. Higher number of larvae showing no response might have been due to higher humidity of the leaf samples tested or some chemicals may get washed off due to dipping making leaf discs inattractive. The male insects were found to exhibit noticeable attraction towards the "treated" leaf discs. The females did not show preference to any of the leaf discs and were more or less equally distributed. From these observations it is apparent that distilled water is not a good extracting medium for any substance(s) relevant to this work.

The percentage response of the larvae and adults with respect to tests carried out with insect saline female body-wash are shown in Table 4.3. Only in case of 2nd instar larvae significant attraction towards "treated" sample was observable, but the results with 3rd and 4th instars were not significant. With respect to the influence of female body-wash prepared with insect saline solution

on conspecific adults it can be seen from the tabulated records that a significantly high number of male beetles were attracted to "treated" samples. Regarding the distribution of female beetles apparently there was no preference shown between "treated" and "control" samples, nevertheless, it can be seen that 94% of them were found over these two samples. This latter fact indicates that though no choice on the part of female beetles was obvious, it can not be neglected that they get attracted these two stacks of tobacco leaf discs. It seems that the process of dipping the leaf discs in insect saline somehow made them attractive to females but the substance(s) coming in the saline body-wash apparently exerted a positive attraction on male beetles. It also indicates the presence of certain pheromonal chemicals (probably a sex pheromone) in the female body-wash prepared with saline solution.

Table 4.4 shows the percentage responses of L. serricorne larvae and adults to the hexane female body-wash. No significant influence could be observed in case of all the larval instars. However, one can not overlook the fact that the larval instars were keeping away from hexane itself. The males were highly attracted ($P < 0.01$) to the hexane wash (70% attracted to "treated" discs and only 18% to the "control"). No significant difference in the numbers of females attracted by "treated" and "control" leaf samples were recorded. The results

also indicate presence of a sex pheromone like influence on males due to hexane female body-wash.

The percentage response of the larvae and adults to the acetone female body-wash are shown in Table 4.5. The 2nd and 3rd instar larvae showed a slight attraction to the treated leaf samples but 73.75% and 70% respectively of them showed no response. The 4th instar larvae were attracted towards the "treated" samples. This result may be explained as acetone might have extracted some chemical(s)/pheromone(s) from the female insects, but the amount present were not enough to attract the larvae significantly. Only 30% of the males were found to show response, out of which 22% were deterring the treated samples ($P < 0.05$). The females were strongly attracted ($P < 0.01$) by the acetone female body-wash. This demonstrates that the chemical(s) extracted with acetone from the female insects were not like those extracted by other solvents.

The responsiveness of the larvae and adults to the methanol female body-wash are shown in Table 4.6. It was found that all the larval instars were significantly attracted. Among them, the attraction of 4th instar larvae was highly significant ($P < 0.01$). The above results clearly demonstrate that the chemical(s)/pheromone(s) extractable with methanol from the female insects possess highly attractive quality in respect of the larvae. Male

insects were also significantly ($P < 0.05$) attracted to the treated samples, though majority of them (64%) were found to remain away from the "treated" as well as "control" samples. The behaviour of the female tobacco beetles were not affected by the methanol female body-wash.

Kohno et al. (1983) reported that male tobacco beetles do not normally exhibit any attraction towards cured tobacco leaves. However, it was observed here that excepting acetone wash, all other washes when applied to leaf discs rendered the latter attractive to male beetles. This fact provides enough proof that the female bodies washed in the extracting media employed here, save acetone, give out a kind of sex pheromonal factor that attracts the male beetles but not the female ones. Additionally, the same exerted varying degrees of attractive influence on larval stages studied. Using hexane for extraction sex pheromone has been obtained from females of the tobacco beetles (Chuman et al. 1979a; Chuman et al. 1979b; Chuman et al. 1981; Chuman et al. 1982a and b; Chuman et al. 1985; Coffelt and Burkholder, 1972; Levinson et al. 1981; Levinson et al. 1983 and Levinson et al. 1986). But the oviposition deterring and larval attraction activity of the different solvent male and female body-washes indicates some similarity between the two washes. The above fact may be explained that the curicular chemical(s) from the adult males acted as oviposition deterrent for

the females and attractant/repellent for the larvae and the highly oviposition deterrent and more specific larval attraction activity of the female washes were due to combined effect of cuticular chemical(s) and the sex pheromone.

As was evident from the results presented here the two solvents viz. Hexane and Methanol, were found to be appropriate washing media. Further, it is also clear from various tables that what came in the female body-washings not only possessed the sex pheromonal activity, as was reported earlier by several authors, but also exhibited two other important pheromonal influences. One of them, that is that showing aggregation pheromonal activity with respect to larval forms and adult males has already been known to be associated with sex pheromone (Colwell et al. 1978). The second one was of greater interest to the present author namely the oviposition deterring influence. It was so because, as far as the present author is aware of, this has not been reported so far in the case of L. serricorne.

Choice between the two extracting media for further investigation was based on the percentage deterrence values of oviposition (Table 4.1). It is apparent from the table that methanol body-wash exerted maximum (93.31%) deterrence of oviposition, as the "treated" leaf-discs samples, whereas that with hexane registerer only 82.02% deterrence. Remarkably enough, the values for

"control" leaf disc-stacks for hexane and methanol were respectively 5.19% and 52.50. It was therefore, obvious that with the type of experimental set-up employed here, methanol "control" would always exhibit high deterrence, hence, hexane was chosen as the better of the two solvents for further studies to be reported in chapters that follow.

SUMMARY

Oviposition responses of the tobacco beetle, L. serricorne (F.) were tested against different body-washes of conspecific females. It was noted very interestingly that all the solvent female body washes reduced egg deposition by the beetles on the "treated" leaf discs in comparison to respective controls. Highest deterrence in oviposition (93.31%) was observed with the methanol body-wash but the solvent itself was found to affect the egg deposition to a considerable extent. The second highest deterrence (82.02%) was observed with the hexane body-wash, however, in this case the solvent had very negligible effect on the oviposition of the beetle. The above result clearly demonstrated that female L. serricorne body-wash prepared with suitable media can be used as potent oviposition deterrents in the case of this pest. Responses of the different larval instars and adults showed varying degrees of aggregation behaviour,

maximum being shown to methanol body-wash. It may be stated here that the methanol female body-wash was the most effective sample on all counts, except for the notable fact that the solvent itself was deterrent in many ways, hence, hexane was a better choice for further studies, as the latter was not a deterrent in itself.

Table 4.1. Oviposition response of the tobacco beetle, L. serricorne to the tobacco leaf discs treated with different female body washes

Fluid media employed for particular female 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	326	11.66	20.55	67.79	70.66	53.47
Insect saline	391	17.13	31.20	51.67	50.18	24.69
Hexane	324	4.93	45.07	50.0	82.02	5.19
Acetone	521	11.52	36.28	52.21	63.85	18.0
Methanol	389	2.57	23.14	74.29	93.31	52.50

Table 4.2. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with female body-wash prepared in distilled water

Insect stages	Percentage values of positive response to leaf stacks			χ^2
	Treated	Control	No response	
2nd Instar	10.67	10.67	78.67	0.0 NS
3rd Instar	16.0	12.0	72.0	0.57 NS
4th Instar	13.33	28.0	58.67	5.20 *
Male	46.0	30.0	24.0	3.36 NS
Female	30.0	36.0	34.0	0.54 NS

* Significant at 5% level

NS Non significant

Table 4.3. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with female body-wash prepared in Insect saline

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	36.0	16.0	48.0	7.69 *
3rd Instar	22.67	18.67	58.67	0.39 NS
4th Instar	26.67	21.33	52.0	0.59 NS
Male	62.0	24.0	14.0	16.79 **
Female	44.0	50.0	6.0	0.38 NS

* Significant at 5% level

** Significant at 1% level

NS Non significant

Table 4.4. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with female body-wash prepared in hexane

Insect stages	Percentage values of positive response to leaf stacks			χ^2
	Treated	Control	No response	
2nd Instar	16.0	18.67	65.33	0.21 NS
3rd Instar	26.67	26.67	46.67	0.0 NS
4th Instar	32.0	26.67	41.33	0.48 NS
Male	70.0	18.0	12.0	30.72 **
Female	38.0	46.0	16.0	0.76 NS

** Significant at 1% level

NS Non significant

Table 4.5. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with female body-wash prepared in acetone

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	16.25	10.0	73.75	1.93 NS
3rd Instar	18.75	11.25	70.0	1.87 NS
4th Instar	36.25	22.5	41.25	3.22 NS
Male	8.0	22.0	70.0	6.53 *
Female	50.0	22.0	28.0	10.88 **

* Significant at 5% level

** Significant at 1% level

NS Non significant

Table 4.6. Responsiveness of the L. serricorne (F.) larvae and adults to the tobacco leaf discs treated with female body-wash prepared in methanol

Insect stages	Percentage values of positive response to leaf stacks: Treated	Control	No response	χ^2
2nd Instar	38.75	22.50	38.75	4.31 *
3rd Instar	45.0	26.25	28.75	4.93 *
4th Instar	41.25	15.0	43.75	12.25 **
Male	24.0	12.0	64.0	4.0 *
Female	34.0	40.0	26.0	0.49 NS

* Significant at 5% level

** Significant at 1% level

NS Non significant