Toxicity of Several Insecticides to the Southern Green Stink Bug, Nezara viridula L.[1]

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The southern green stink bug, *Nezara viridula* L., is known as a serious plant and fruit pest throughout the tropical, sub-tropical and temperate zones. It has become a serious pest since first observed on Oahu in 1961, and subsequently has spread to all the Hawaiian Islands.

Tests for the most effective insecticides included chlorinated hydrocarbons, organophosphates, and carbamates. DDT dusts or sprays generally were effective (Helson & Greaves, 1945; Sloan, 1945; Gellatley, 1949, 1951; Riherd, 1949; Everett, 1950; Martin, 1952; and May, 1958). Furthermore, organophosphorus insecticides such as parathion or malathion were effective also in the field control of this insect (Baranowski, 1959; Bowling, 1960; and Brogdon et al., 1960). Preliminary laboratory tests, however, showed that DDT was ineffective, although certain organophosphorus insecticides were toxic. The Hawaiian strain of the stink bug apparently was resistant to DDT, contrary to the results of field tests in other areas.

This study was undertaken to determine the toxicity of p, p[1]-DDT, heptachlor and Bidrin® to nymphs and adults of the stink bug.

Materials and Methods

The stink bug strain used in this study was collected in a field on Oahu in April, 1963. Since then, it has been reared without insecticidal pressure, but the insecticidal history of the stink bug before colonization is unknown. The insects were reared at temperatures of 24 and 27 °C and 50–70% RH. Sugar cubes, fresh string beans, corn and water were supplied to nymphs and adults for food.

Laboratory-reared stink bugs of 2 to 3–day-old second, third, fourth and fifth instar nymphs and 3–5-day-old adults of both sexes were used. Only the adults were sexed since the nymphs were unable to be separated.

The weights of the 2 to 3-day-old second, third, fourth and fifth instar nymphs and 3 to 5-day-old males and females were, respectively, 1.78 ± 0.33, 6.07 ± 0.73, 21.06 ± 3.99, 68.21 ± 6.94, 104.85 ± 9.08 and 125.04 ± 12.21 mg. Insects were weighed in groups of 10 or 20 with about 1,000 in each stage. Although the females apparently were heavier than the males, this difference was not significant.

The insecticides used were DDT (1, 1, 1-trichloro-2, 2-bis [*p*-chloro-

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phenyl] ethane), p, p¹-isomer, m.p. 108.5–109 °C; heptachlor (1, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanoindene), technical grade of 72% purity, m.p. 57–60 °C, and Bidrin® (3-[dimethoxyphosphinyloxy]-*N*, *N*-dimethyl-*cis*-crotonamide), technical, 75% purity. Stock solutions of these compounds dissolved in acetone were diluted to the required concentrations with acetone before testing. The stink bugs were lightly anesthetized with CO_2. One microliter of acetone solution of the insecticide was applied ventrally between the mesothoracic coxae and allowed to evaporate. The treated insects kept for observation were confined in 250-ml beakers with the bottoms covered by paper towel discs and containing either fresh string beans or a bouqet of tender foliage of *Asystasia coromandeliana* for food which was changed every 3 days. Each beaker was covered with Kimwipes® paper and kept at room temperatures between 21 and 27 °C. Mortality readings were taken daily for one week for the p, p¹-DDT and heptachlor treatments, and 4 times daily for Bidrin. Insects were recorded as unaffected if they moved around normally. All tests were replicated between 3 and 6 times.

The recorded mortality was corrected for natural mortality by Abbott's formula (Abbott, 1925) and the data analyzed by probit analysis as described by Finney (1952). All regression equations were calculated on the basis of mortality 7 days after treatment with p,p¹-DDT and heptachlor and 2 days after treatment with Bidrin. Ishii and Sherman (1965) pointed out the significance of holding periods necessary for maximum effect of the insecticide to appear. However, the natural mortality sometimes exceeded 20% after a 7-day holding period. Mortality of the insects treated with p,p¹-DDT or heptachlor were not considered beyond that period. The median-lethal dosages (LD-50's) and their fiducial limits were expressed in terms of micrograms of insecticide per gram of insect body weight.

Table 1. Contact toxicity of p,p¹-DDT to the southern green stink bug seven days after treatment

Stage of insect	Mean body weight (mg)	Median lethal dosage μg/g a/	Fiducial limits (P=0.05) μg/g a/	Dosage-mortality regression equation
Adult female	129.5	5.6	3.5— 8.8	Y=3.7189+1.7091X
Adult male	107.9	6.3	4.2— 9.6	Y=3.7564+1.5657X
5th instar	68.0	4.8	2.6— 9.0	Y=4.1722+1.1563X
4th instar	19.6	2.9	2.0— 4.4	Y=3.8414+2.3602X
3rd instar	5.7	3.3	3.5— 4.5	Y=3.1740+3.0617X
2nd instar	1.7	5.0	3.6— 7.0	Y=3.7420+1.7558X

a/ Calculated from mean body weight

RESULTS

Toxicity of p,p¹-DDT

No significant differences in susceptibility were found between the various stages.

Heterogeneity: The value of chi square (X^2), an index of dispersion, as a measure of heterogeneity is significant when the calculated value is greater than the theoretical one at the level of P=0.05. That is, the population tested is heterogeneous in its susceptibility to the insecticide used. The chi square values were clearly significant except for the third instar nymph. This value was especially large at the adult stage, i.e., the adult population was highly heterogeneous in its susceptibility to p,p¹-DDT. In fact, the mortality at the adult stage fluctuated greatly at each dosage level. The fiducial limits were broad and the slopes of the regression lines which also measure heterogeneity were gradual except for that of the third instar nymph.

Table 2. Contact toxicity of heptachlor to the southern green stink bug seven days after treatment

Stage of insect	Mean body weight (mg)	Median lethal dosage μg/g a/	Fiducial limits (P=0.05) μg/g a/	Dosage-mortality regression equation
Adult female	119.7	6.9	5.2— 9.2	Y =3.3256+2.0057X
Adult male	102.3	5.2	3.8— 7.1	Y =3.7058+1.8121X
5th instar	68.9	1.9	1.3— 2.7	Y =4.6105+1.3057X
4th instar	19.6	1.8	1.5— 2.1	Y =4.4247+2.2534X
3rd instar	5.6	1.9	1.7— 2.2	Y =3.7626+4.2550X
2nd instar	1.7	1.7	1.4— 2.2	Y =4.5351+1.8254X

a/ Calculated from mean body weight

Toxicity of Heptachlor

On comparing the LD-50's for heptachlor, significant differences in susceptibility between the adults and the nymphs were found. However, no significant difference in susceptibility was found due to the sex of the adult, or between the immature stages.

Heterogeneity: The chi square values at each stage were smaller than the theoretical values, i.e., apparently the stink bug population was not heterogeneous in its susceptibility to heptachlor. The slopes of the regression equations were small except for that of the third instar nymphs.

Toxicity of Bidrin

No significant differences in susceptibility to Bidrin were found between the adults and/or any of the immature stages.

As Bidrin is an organophosphorus insecticide, attempts were made to determine colorimetrically the brain cholinesterase activity of the adults *in vitro* by means of the method described by Cook (1954). However, there was insufficient enzyme activity to be measured.

Table 3. Contact toxicity of Bidrin to the southern green stink bug two days after treatment

Stage of insect	Mean body weight (mg)	Median lethal dosage μg/g a/	Fiducial limits (P=0.05) μg/g a/	Dosage-mortality regression equation
Adult female	122.4	0.35	0.31—0.40	Y=7.0702+4.6418X
Adult male	102.5	0.39	0.32—0.47	Y=6.4798+3.4153X
5th instar	68.8	0.36	0.31—0.41	Y=6.7507+3.9663X
4th instar	26.6	0.38	0.32—0.44	Y=6.4783+3.5105X
3rd instar	7.9	0.35	0.29—0.44	Y=6.4743+3.2909X
2nd instar	2.2	0.44	0.35—0.55	Y=6.0996+3.0201X

a/ Calculated from mean body weight

Heterogeneity: The southern green stink bug was a homogeneous population in its susceptibility toward Bidrin. The (X^2) values obtained at each stage were much less than the theoretical values and the slopes of the regression lines were relatively steep.

DISCUSSION

In many species of insects, the male is generally more susceptible to insecticides than the female (Shepard, 1951). However, in this study no significant difference in susceptibility between the sexes was found.

The toxicity of the three insecticides to the various stages of this bug in order of decreasing effectiveness was as follows: *Adult*—Bidrin>p,p¹-DDT =heptachlor; *Fifth Instar Nymph*—Bidrin>heptachlor=p,p¹-DDT; *Fourth Instar Nymph*—Bidrin>heptachlor=p,p¹-DDT; *Third Instar Nymph*—Bidrin>heptachlor>p,p¹-DDT; and *Second Instar Nymph*—Bidrin>heptachlor>p,p¹-DDT.

In this study, there was no significant difference in the susceptibility to the insecticides between the stages except the nymphal stages which were more susceptible to heptachlor than the adult stage.

Hoskins and Gordon (1956) state that the log dosage-probit line is an expression of the reaction of the tested population to the chosen toxicant, and the slope measures the diversity of response or heterogeneity of the population to the toxicant used, and that a highly heterogeneous population has a wide range of susceptibility, i.e., a gradual slope of the log dosage-probit line. When the value of chi square (X^2) at the P=0.05 level exceeds expectation, such a high value proves that the procedure employed is subject to the wide variation of the treated population in susceptibility to the insecticide, i.e., the tested population is heterogeneous (Hoskins and Craig, 1962). Thus, all stages of the southern green stink bug population studied are heterogeneous in their response to p,p¹-DDT and the adults of this insect are probably also heterogeneous to heptachlor.

Although the results of specific laboratory tests on the toxicity of DDT to the southern green stink bug have not been reported in the

literature, many field test reports seem to suggest that DDT was an effective insecticide against this insect. In this study, however, the stink bug showed a high tolerance to DDT at all stages and to heptachlor at the adult stage. This local strain originated from one which obviously had been subjected to previous insecticidal pressure. Hoskins and Craig (1962) describe resistance from the genetic view point and state that a test performed upon a mixed population containing the susceptible and the resistant genotypes cannot give a straight log dosage-probit line but that the line has an inflection at the percentage mortality corresponding to the percentage of the susceptible genotype present. If too few experimental points were obtained, a statistical analysis would give no real biological significance to the regression line calculated. In the present study sufficient points were obtained and the straight lines best fitted to the points were drawn, although in general, they fluctuated greatly when p,p^1-DDT and heptachlor were applied. One possible explanation regarding the erratic data obtained from the p,p^1-DDT tests may be the mixture of susceptible and resistant individuals and that the percentage of the resistant individuals in the population varied in the various groups tested. This resulted in a fluctuating dosage-mortality response since the data employed were the means of several experiments conducted on different days using different sub-colonies of the insects. This could also explain the fluctuating data when the adults were treated with heptachlor.

During the observation periods, some of the nymphs treated with insecticide molted, and these insects usually survived treatments. Although all nymphs except those in the fifth instar should have molted by the end of the seventh day after treatment, ecdysis did not occur in most individuals. Handling of the insects appeared to interfere with ecdysis. Other factors also interfered such as: lower minimum temperatures during the observation period than those in the rearing room, carbon dioxide which was used for immobilizing the insects, and/or *Asystasia coromandeliana*, the food provided during holding periods in some experiments but not used during rearing of the cultures.

SUMMARY

This study was conducted to investigate the toxicity of DDT, heptachlor, and Bidrin® to both the adults and the nymphs of the southern green stink bug in the laboratory.

When the toxicities of the insecticides were compared on a basis of the LD-50's, no significant difference in susceptibility was found between the sex of the adults and/or between the immature stages except that the nymphs were significantly more susceptible to heptachlor than the adults.

The stink bug in all its stages was much more susceptible to Bidrin than to p,p^1-DDT or heptachlor. Significant difference in toxicity between p,p^1-DDT and heptachlor existed only in the second and third instar nymphs. Heptachlor and p,p^1-DDT took a longer time than Bidrin before

the appearance of their full effect on the insects, i.e., Bidrin was relatively quick-acting whereas p,p¹-DDT and heptachlor were slower acting.

Both the adults and the nymphs were heterogeneous in their susceptibility to p,p¹-DDT, but only the adults were heterogeneous in their response to heptachlor; however, all stages were homogeneous in their response to Bidrin.

Although the insecticidal history of the stink bug used is unknown, it seems that this local Hawaiian strain originated from one which apparently had been subjected to previous insecticidal pressure since they appeared to be resistant to p,p¹-DDT in all stages as well as to heptachlor in the adult stage.

The *in vitro* brain cholinesterase activity of the stink bug was too low to be measured.

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