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22,25-Bisdeoxyecdysone: Pathological Effects on the Mexican Bean Beetle[1] and Synergism with Juvenile Hormone Compounds[2,3]

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ABSTRACT

All 4 instars of *Epilachna varivestis* Mulsant, when confined with bean plants dipped in solutions containing the ecdysteroid 22,25-bisdeoxyecdysone (BDE), exhibited lethal molting abnormalities. Treated 4th instars molted precociously into dwarf neotenic forms, often with abnormal wing pad extension. Newly emerged females confined for 10 days with beans that had been dipped into a solution containing 100 ppm of BDE were sterilized permanently. Ovipositing females confined for 7 days stopped oviposition by the 3rd day and gradually reduced their feeding to 17% that of the controls 8–10 days after treatment began.

Several juvenile hormone compounds and insecticide-synergists synergized BDE on 4th-instars and adult females. When ovipositing females were confined in field cages with beans sprayed once with a mixture of 50 ppm of BDE + 250 ppm each of sesamex and (*E*)-4-[(6,7-epoxy-3-ethyl-7-methyl-2-nonenyl)oxy]-1,2-(methylenedioxy)benzene, viable egg production did not exceed 12% that of the controls for any 5-day period during the following 30 days.

The ecdysone analogue 22,25-bisdeoxyecdysone (BDE) was reported to cause lethal molting abnormalities (Earle et al. 1970, Robbins et al. 1970), to suppress metamorphosis (Bowers 1968, Robbins et al. 1968), and to inhibit ovarian development (Robbins et al. 1968, Earle et al. 1970) in insects. Also, Kaplanis et al. (1971) reported that the insecticide synergists sesamex and piperonyl butoxide synergized the ovarian inhibition of BDE in the house fly, *Musca domestica* L., 5- to 10-fold. We, therefore, investigated the pathological effects of 22,25-bisdeoxyecdysone, alone and in combination with various juvenile hormone (JH) compounds or insecticide-synergists, on the Mexican bean beetle, *Epilachna varivestis* Mulsant.

METHODS AND RESULTS.—*Effects of BDE on Eggs.*—Ten egg masses on leaf disks were dipped for 5 sec into acetone:water (3:1) solutions containing various concentrations of BDE and incubated at 24°C in petri dishes containing a moist dental wick. Concentrations of BDE as high as 1000 ppm had no effect on egg hatch whether the eggs were 0–1 or 4–5 days old.

Effects of BDE on Larvae and Pupae.—Intact primary leaves of 'Henderson' bush beans were dipped into solutions of acetone: water (3:1) containing 0, 100, 500, or 1000 ppm BDE. Newly ecdysed larvae of each stage were confined with the treated bean plants in 1-ft^3 cages and held in a growth chamber at 24°C under constant light. Each treatment was replicated 3 times with 25 1st instars, 12 2nd instars, and 10 3rd or 10 4th instars. Those larvae that molted to the next stage were placed on untreated leaves and observed until adult emergence.

Growth and feeding of larvae of stages 1–3 did not appear to be inhibited when they were confined with plants dipped into solutions containing 1000 ppm of BDE (Table 1). Larvae attached themselves to the leaves in preparation for molting at the normal time interval or slightly precociously, but they were unable to molt, or they died shortly after ecdysis. The exuviae could be peeled readily from affected larvae, revealing a new cuticle beneath.

No delayed lethal effects were observed beyond the early following stage, except that one 2nd instar

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Table 1.—Mortality and suppression of feeding induced by confinement of Mexican bean beetle larvae with BDE-treated plants.

Concn of BDE (ppm)	% mortality when indicated instar was treated[a]				% feeding by 4th instar[b, c]
	1st	2nd	3rd	4th	
1000	100	84	90	100	
500	67	0	0	100	27 a
100	5	0	0	100	61 b
0	2	0	0	0	100 c

[a] 3 replications/treatment with 25 1st instars, 12 2nd instars, and 10 3rd or 10 4th instars/treatment.
[b] Means followed by the same letter are not significantly different at the 5% level by Duncan's multple range test.
[c] Based on percent of mm^2 of damaged leaf area in controls.

formed a supernumerary instar with extended wing pads, but was unable to form a normal pupa at the next ecdysis.

Fourth instars were more sensitive to BDE than the other instars (Table 1). Those treated with 1000 ppm BDE fed for only 3–4 days, compared with the normal 4–5 days before attaching themselves to the leaves in preparation for the pupal molt. The prepupal period also was abbreviated, and all treated larvae had attempted ecdysis within 4–5 days compared with the 7-day period for the controls. Many of the resulting neotenic forms (Fig. 1) partially or totally shed the exuviae, and some of them had short fully extended wing pads and walked around for several days in dishes provided with bean leaves before they died. The feeding of affected larvae was reduced significantly (Table 1). Although larvae confined with beans treated with 100 or 500 ppm of BDE attempted ecdysis after 4–5 days, larvae in the 500-ppm treatment fed only about half as much.

In other tests, nearly mature 4th instars were unaffected by foliar application of 100 ppm of BDE, and topical applications of as much as 10 μg of BDE applied to mature prepupae or to 0- to 1-day-old pupae had no effect on emergence of normal-appearing adults.

Several juvenile hormone compounds and insecticide synergists were tested for possible synergism with BDE on the larvae. A 2:1 ratio of sesamex or (*E*)-4-[(6,7-epoxy-3-ethyl-7-methyl-2-nonenyl) oxy]-1,2-(methylenedioxy) benzene (Compound 1) + BDE applied to bean leaves neither increased nor decreased effectiveness of BDE on 1st instars. However, with 4th instars (Table 2), BDE was synergized by addition to the dipping solution of 100 ppm of Compound 1, sesamex, piperonyl butoxide, (*E*)-1-(p-chlorophenoxy) 6,7-epoxy-3-ethyl-7-methyl-2-nonene (Compound 2), or (*E*)-6,7-epoxy-3-ethyl-7-methyl-1-phenoxy-2-nonene (Compound 3). Sesamex and piperonyl butoxide also are well established insecticide synergists; and homologues of Compound 1 are reported to synergize pyrethrums (Fales et al. 1970). All these compounds, except piperonyl butoxide, exhibit juvenile hormone activity on *Tenebrio* (Bowers 1968, 1969, 1971) and Mexican bean beetle pupae when 10 μg or less is applied topically (Walker, unpublished data). Only Compound 1 produced neotenic pupae when larvae were confined with leaves treated with the candidate synergists alone. This neoteny was distinguished from the neoteny produced by BDE by absence of mobile forms with extended wing pads and by a normal or slightly extended pre-ecdysial period in contrast to the precocious molting induced by BDE. However, distinction sometimes was difficult with combinations that exhibited marginal BDE activity.

Effects of BDE on Newly Emerged Adults.—Three replicates of 10 pairs of newly emerged adults were confined in 1-ft^3 cages for 10 days with bean plants dipped in acetone:water (3:1) solutions containing 0, 10, 100, or 1000 ppm BDE, with treated plants being replicated at 2-day intervals by freshly treated plants (females normally begin ovipositing on about the 10th day after emergence). During the subsequent 5 weeks, the adults were confined with untreated bean plants, and egg masses deposited on the leaves were collected daily. Adults treated with 1000 ppm of BDE produced no viable eggs; those treated with

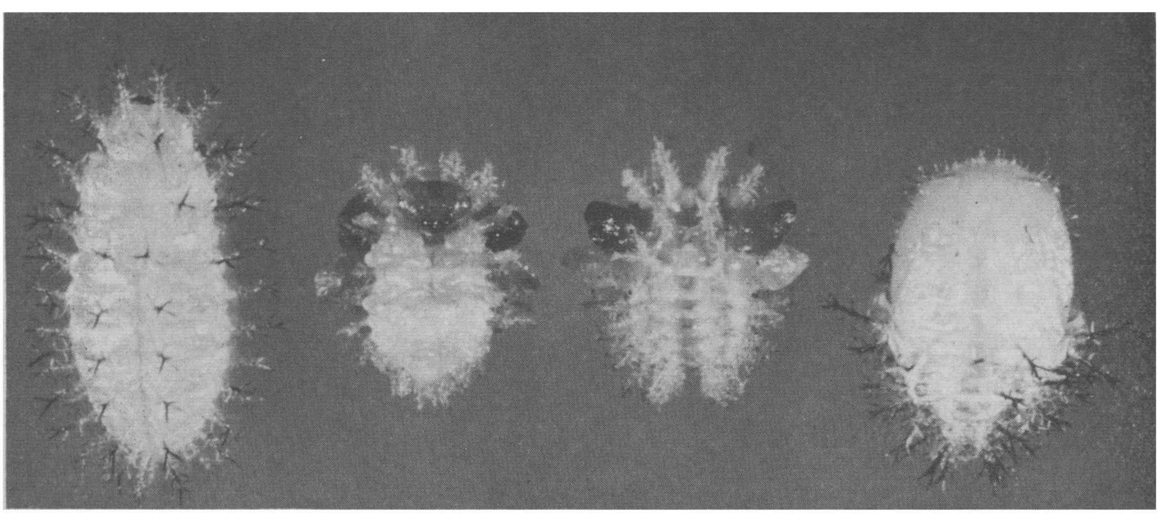

FIG. 1.—Mobile abnormal forms of the Mexican bean beetle resulting from confinement of last-stage larvae with bean plants treated with 22,25 bisdeoxyecdysone (center two) are compared with a normal mature larvae (left) and pupae (right).

Table 2.—Synergism of BDE on 4th-stage Mexican bean beetle larvae by insecticide-synergists and JH compounds.

Concn of BDE (ppm)	% BDE-type neoteny produced by candidate synergist (100 ppm)					
	None	Sesamex	Piperonyl butoxide	Compound 1	Compound 2	Compound 3
100	100	100	100	100	100	100
50	30	100	100	100	100	100
20	0	100	95	100	100	90
10	0	100	45	100	93	0
5	0	63	0	100	35	0
2	0	10	0	[a]	8	
1	0	0		0	0	
0	0	0	0	0	0	0

[a] Type of neoteny often not clearly distinguishable.

0, 10, and 100 ppm produced 100±43, 77±45, and 7±16 viable eggs, respectively. Also, during the latter part of the 10-day feeding period, feeding declined drastically compared with the controls, so by the 10th day there was virtually no feeding. A few adults of both sexes died on the 10th day, and by the 21st day, all females and most males were dead. Adults treated with 100 ppm of BDE produced only a few eggs, and feeding was depressed considerably; however, most lived as long as the controls. Adults treated with 10 ppm BDE had only slightly depressed egg production, and no noticeable effect on feeding or lifespan was apparent. Partially effective treatments resulted in the deposition of many undersized or deformed normal-sized nonviable eggs.

Effects of BDE on Ovipositing Females.—Newly emerged adult pairs were held in petri dishes until the females had oviposited 2–3 egg masses. Three groups of 10 such females were first confined for 7 days in 1-ft^3 cages and supplied daily with bean plants freshly dipped in BDE solutions and subsequently confined with untreated bean plants for 4 weeks. Egg masses were collected daily during the 5-week period, and the production of viable eggs was recorded (Table 3). BDE was as effective in inhibiting egg production in ovipositing females as it was in newly emerged females. Females treated with 100 ppm of BDE usually ceased oviposition of viable eggs during the 2nd day of treatment, and none recovered fertility during the remainder of the 5-week period. Feeding was not affected during the 1st 3 days of treatment (Table 2) but it gradually declined during the subsequent 4 days. During the 1st 3 days of confinement on untreated leaves (8–10 days after treatments began), females fed only 17% as much as the controls (Table 3), and there was no indication of the beginning of a recovery.

A 10:1 ratio of sesamex or Compound 1 plus BDE synergized sterilization and feeding depression of ovipositing females (Table 3). Although Compound 1 was a less effective synergist, its quick-acting ovicidal action is a desirable complementary quality when the possibility of using compounds with pathological effects similar to BDE as control agents for this insect is considered.

Field-Cage Tests on Ovipositing Females.—Henderson bush beans in 3×3×3½-ft wooden frame cages covered with cheesecloth were sprayed to runoff once with an acetone:water (3:1) solution that contained 250 ppm each of Compound 1 and sesamex with or without 50 ppm of BDE. Twelve mated females 2–3 weeks old were then introduced into each of 3 cages treatment. At 5-day intervals, egg masses were collected, and the number of live females found in each cage was recorded. These egg masses subsequently were held at room temperature in petri dishes to determine hatchability. Table 4 records the mean production of normal-appearing eggs and hatching eggs as a percentage of the controls and the number of live females per cage for each 5-day period. The combination of BDE-Compound 1-sesamex caused an immediate cessation of egg hatch, as did the Compound 1-sesamex combination. However, viable egg production returned to normal after ca. 7 days when BDE was omitted from the treatment, whereas when it was included, nearly complete sterilization resulted with only slight recovery during the 30-day period. Late in the test, the fewest live females were recovered consistently from cages containing bean plants which had been treated with the combination of BDE-Compound 1-sesamex but none of the differences were statistically significant at the 5% level of probability.

Table 3.—Effects on viable egg production and feeding by ovipositing Mexican bean beetle females when confined with bean plants treated with BDE or BDE-candidate synergist combinations.

Treatment		Viable eggs produced[a]		Feeding (mm^2 damaged leaf area)[a] on days after treatment began	
Compounds	Concn (ppm)	During 7-day treatment period	During 4 weeks following treatment	0–3	8–10
None		100±41	100±33	100±14	100±18
BDE	10	75±46	65±18	99±16	95+18
BDE	100	19±3	0	91±22	17±25
Sesamex	100	84±27	101±55	92±30	93±25
Sesamex + BDE	100 1	65±37	45±39		
Sesamex + BDE	100 10	12± 2	1± 2	90±24	34± 6
Compound 1	100	0	89±34	102±10	118±31
Compound 1 + BDE	100 10	0	10±15	116±25	53±35

[a] % control means ± the 95% CL.

Table 4.—Effects of confining reproductively mature female Mexican bean beetles in field cages with bean plants treated with combinations of BDE, Compound 1, and sesamex.[a]

Time posttreatment (days)	Egg production[b, c]			Egg hatch[b, c]			Live females/cage[b, d]		
	Control	Compound 1 + sesamex	Compound 1 + sesamex + BDE	Control	Compound 1 + sesamex	Compound 1 + sesamex + BDE	Control	Compound 1 + sesamex	Compound 1 + sesamex + BDE
0–5	100 a	81 a	32 b	100 a	1 b	0 b	11.0 a	10.0 a	10.7 a
5–10	100 a	87 a	2 b	100 a	38 b	0 c	10.0 a	9.7 a	9.7 a
10–15	100 a	108 a	3 b	100 a	97 a	1 b	9.3 a	8.7 a	6.3 a
15–20	100 a	85 a	10 b	100 a	80 a	9 b	6.3 a	7.3 a	5.3 a
20–25	100 a	69 b	23 c	100 a	79 a	12 b	5.7 a	5.0 a	3.7 a
25–30	100 a	81 a	17 b	100 a	91 a	12 b	4.0 a	2.7 a	2.0 a

[a] The concentrations of BDE, Compound 1, and sesamex were 50, 250, and 250 ppm, respectively.
[b] Means in the same 5-day period followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.
[c] % of the control means for each 5-day period.
[d] 12 initially introduced into each cage.

DISCUSSION.—The pathological morphogenetic effects on larvae and adults of the Mexican bean beetle induced by confinement with BDE-treated bean plants provide hope that selective and relatively cheap compounds that produce similar effects may become a new type of control agent for this insect pest. It is suggested that a combination of a JH compound and a compound with effects similar to BDE would provide a more feasible control agent than either agent alone. In addition to the synergistic action of some JH compounds on BDE, they produce complementary ovicidal activity on young eggs (Walker and Bowers 1970) and a quick ovicidal effect on eggs deposited by females, before BDE-induced sterilization is completed. Also, contact treatment of prepupae or young pupae with JH compounds or confinement of nearly mature larvae with leaves treated with JH compounds suppresses adult development (unpublished data).

Extended abnormal wing pads associated with a neotenic pupal molt were observed previously in *Tribolium* (Nagel 1934) following exposure of nearly mature larvae to low temperatures, and in *Chilo suppressalis* (Walker) as a result of implantations of *Barathra* ecdysial glands (Fukaya and Mitsuhashi 1957) but they were not described previously as resulting from treatment with an ecdysteroid. Similar forms have been described previously (Landis and Davidson 1934) as occurring spontaneously in low frequency in Mexican bean beetle larvae.

When Mexican bean beetles are reared continuously under a 16D-8L photoperiod, ovarian maturation is inhibited, and feeding by both sexes declines gradually and stops completely after 2–4 weeks (Walker, unpublished data). Therefore, delayed gradual reduction in feeding by adults feeding on BDE-treated plants may be a response to the induction of the diapause syndrome. This hypothesis is suggested by the quantitative association between doses that sterilize females and those that suppress feeding. However, the delayed mortality associated with the higher doses of BDE suggests that the decline in adult feeding may be a response to a toxic effect. Further studies are necessary to clarify the nature of the effects of BDE on adult insects.

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