closely linked with a morphological trait. Thus, the usefulness of the linkage results is not so great as it might be. The placement of more morphological traits in the linkage map will undoubtedly rectify this situation in the future.

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Mexican Bean Beetle[1]: Compounds with Juvenile Hormone Activity (Juvegens)[2] as Potential Control Agents[3,4]

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ABSTRACT

Ovipositing *Epilachna varivestis* Mulsant were confined in cages in the laboratory with lima bean plants treated with compounds having juvenile hormone activity (juvegens). Egg masses deposited after the 1st day of confinement failed to hatch, although there was no reduction in egg deposition. Egg hatch returned to normal within a few days after the females were transferred to untreated plants. Last-stage larvae confined in cages with treated plants molted into larval-pupal or pupal-adult intermediates. In field-cage tests, 2 weekly foliar applications of 500 ppm of (*E*) -4-[6,7-epoxy-3,7-dimethyl-2-nonenyl) oxy]-1,2-(methylenedioxy) benzene reduced the hatch of egg masses from confined mated females by 98%. The same treatment completely prevented adult eclosion from last-stage larvae. In an open field test, weekly applications of 1 lb per 100 gal of 1-(*p*-chlorophenoxy) -6,7-epoxy-3,7-dimethyl-2-nonene reduced egg hatch by 90%, but, they also produced growth abnormalities of the lima bean plants closely resembling those produced by the phenoxy herbicides. The potential usefulness of juvegens as control agents for the Mexican bean beetle is discussed.

The potent ovicidal activity of certain synthetic compounds with juvenile hormone activity against young eggs of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Walker and Bowers 1970), stimulated further investigations of the possible usefulness of such compounds as control agents for this species. This report describes additional lethal morphogenetic effects produced by topical application or foliar residues of various compounds with juvenile hormone activity, and it discusses the possible usefulness of such compounds as control agents for this species. I have introduced the term "juvegens" and propose its adoption as a brief inclusive term for all compounds exhibiting juvenile hormone activity regardless of the source or chemical structure. The concern expressed by Berkoff (1970) and others over the too-liberal usage of the term "synthetic juvenile hormone" and lack of information concerning the possibility of chemically diverse juvenile hormones among insects suggests the need for such a term.

PROCEDURES AND RESULTS.—The synthetic juvegens (Bowers 1969, 1971) tested were (*E*) -4-[(6,7-epoxy-3,7-dimethyl-2-octenyl)oxy]-1,2-(methylenedioxy)benzene (Compound I); (*E*) -4-[(6,7-epoxy-3,7-dimethyl-2-nonenyl) oxy]-1,2-(methylenedioxy) benzene (Compound II); 1-(*p*-chlorophenoxy) -6,7-epoxy-3-ethyl-7-methyl-2-nonene (Compound III); 1-(*p*-chlorophenoxy) -6,7-epoxy-3,7-dimethyl-2-octene (Compound IV); and 1-(*p*-chlorophenoxy) -6,7-epoxy-3,7-dimethyl-2-nonene (Compound V).

[1] Coleoptera: Coccinellidae.

[2] The word "juvegens" is introduced as a brief inclusive term for all compounds exhibiting juvenile hormone activity regardless of their source or chemical structure. Received for publication Apr. 20, 1972.

[3] Mention of a proprietary product does not constitute endorsement by the USDA.

[4] The author is grateful to W. S. Bowers, Entomology Research Division, USDA, for supplying the juvegens and for helpful suggestions, and to Hoffman La Roche Inc., Nutley, N. J., for supplying additional quantities of Compounds II, III, and V for the field-cage and open-field tests.

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Table 1.—Percent eclosion of normal-appearing adult Mexican bean beetles after topical treatment of prepupae and young pupae with Compound I.[a]

Stage treated	% eclosion of normal-appearing adults after topical treatment with indicated amounts (μg) of Compound I				
	0.001	0.01	0.1	1.0	10
48–72 hr before pupation	95	90	95	100	95
0–24 hr before pupation	95	0	0	0	0
0–24 hr after pupation	100	0	0	0	0
24–48 hr after pupation	100	70	50	30	40
48–72 hr after pupation	100	95	100	100	100

[a] 20 insects/treatment. 90% or more of all control groups treated with the solvent emerged as normal-appearing adults.

All test insects were obtained from a laboratory colony maintained on 'Henderson' bush lima bean plants and incubated in an environmental chamber at 24°C under constant light.

Topical Treatments.—Doses of Compound I ranging from 0.001 to 10 μg/insect were applied in 1 μliter of acetone-water (3:1) to the abdominal middorsum of nearly mature larvae and young pupae. As shown in Table 1, topical treatments with dosages as low as 0.01 μg applied 1 day before to 1 day after pupation were 100% effective in preventing eclosion of normal-appearing adults. Three-day-old pupae were unaffected by doses as high as 10 μg. Affected pupae often completed development 2–3 days before the controls. If the pupal exuviae was peeled off the abdomen, retention of pupal epidermal characters was usually evident, often including the pupal urogomphi (Fig. 1).

Foliar Residue Treatments.—Henderson bush lima bean plants in the primary leaf stage were dipped into acetone-water (3:1) juvegen formulations and placed in water bottles in 1 ft^3 screen cages. Each treatment consisted of 2 such cages each containing 10 larvae of a given stage incubated in an environmental chamber at 24°C under constant illumination until adult emergence.

Mature 3rd-stage and young (0–3 days old) 4th-stage larvae confined with bean plants dipped in 100 ppm of Compound I molted into terminal larval-pupal intermediates (Fig. 2), usually 1–2 days after the control larvae pupated. Feeding was slightly in excess of that of the controls. Mature larvae which were confined with treated plants and which prepupated within 1 day molted into normal-appearing pupae, but few adult eclosions resulted. Confinement of earlier stages with similarly treated beans plants was ineffective in inducing lethal morphogenetic effects.

Newly molted 4th-stage larvae confined with plants dipped in 10 or 100 ppm of Compound III produced normal or almost normal-appearing pupae, but no adults eclosed. Larval-pupal intermediates resulted when such larvae were confined with leaves dipped in 1000 ppm of the juvegen. First- and 2nd-stage

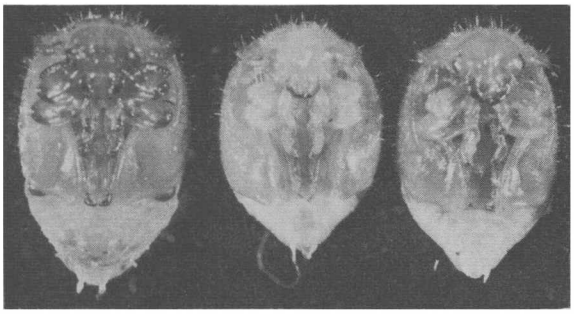

FIG. 1.—Left, a normal mature pupa. The 2 pupae at the right were treated as prepupae with 10 μg of Compound I. The molted pupal cuticle has been removed from the abdomen to reveal the retention of pupal characters.

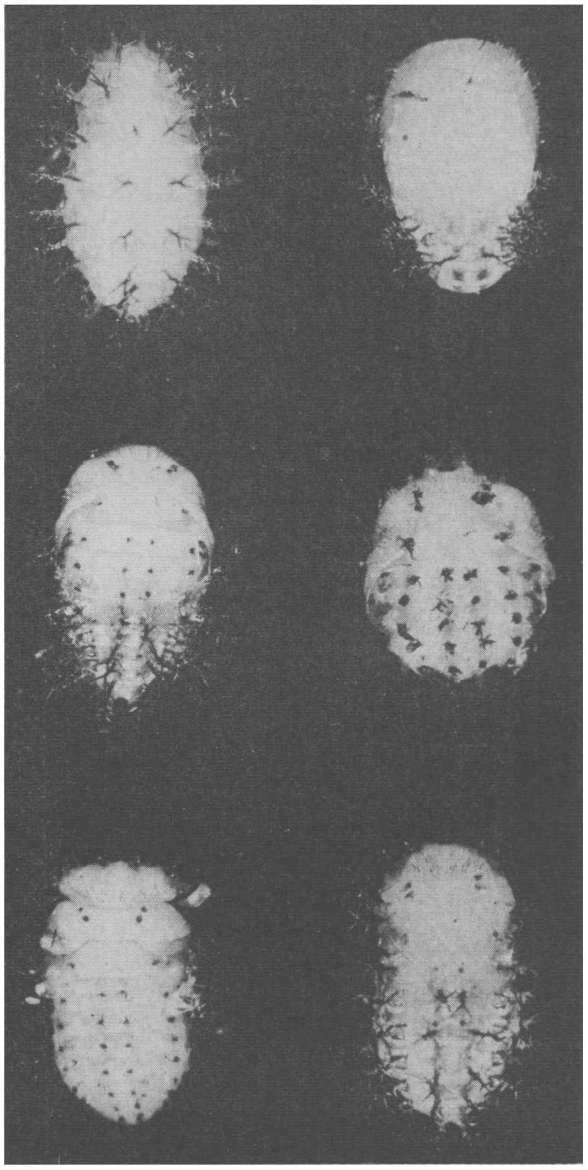

FIG. 2.—Upper left, a normal prepupa; upper right, a normal pupa. Others are various types of larval-pupal intermediates resulting from confinement of last-stage larvae with beans dipped in 100 ppm of Compound I.

Table 2.—Results of weekly foliar applications of emulsions containing 500 ppm of juvegens in field cages (20 larvae + 6 mated females of the Mexican bean beetle/cage).[a, b, c]

Juvegen	Larvae		Adult females	
	% survival to the pupal molt	% adult eclosion	Mean egg production	% egg hatch
Residual treatment				
Compound II	63 a	00 a	908 a	1.5 a
Control	67 a	67 b	967 a	71.3 b
Compound III	58 a	0 a	869 a	33.1 a
Control	53 a	53 b	814 a	77.6 b
Compound IV	62 a	7 a	850 a	53.0 a
Control	72 a	72 b	947 a	75.2 b
Topical and residual treatment				
Compound IV	68 a	0 a	840 a	7.7 a
Control	78 a	78 b	704 a	66.1 b

[a] 10 young and 10 old 4th-stage larvae/cage in tests with Compounds II and III. Ten 3rd-stage + 5 young and 5 older 4th-stage larvae/cage for Compound IV.
[b] 3 replicates (cage)/treatment.
[c] Pairs followed by the same letters are not significantly different at the 5% level by the student "t" test.

larvae confined on bean plants dipped in 100 ppm of Compound III produced normal-appearing adults.

When 10 pairs of newly emerged adults were transferred daily to plants freshly dipped in 100 ppm of Compound I, no egg hatch resulted during the 7 days after oviposition commenced. However, when these adults were transferred to a cage containing untreated plants, egg hatch became normal after 3 days. Similar results were obtained when adults were alternated daily during the oviposition period between treated and untreated plants. Also, except that an occasional egg mass hatched during the 1st day, the same result was obtained when these treatments commenced 2 weeks after adult emergence. An adult treatment producing permanent nonviability of eggs has not yet been found.

A group of ten 14-day-old males was transferred daily for 5 consecutive days to bean plants freshly dipped in 100 ppm of Compound II. During the next 5 days these males were transferred daily for an 8-hr period from the treated foliage to a cage containing 10 virgin females of the same age, feeding on untreated foliage. Egg hatch remained normal during the 5-day period of male female pairings (74% compared with 67% hatch in the controls), when the experiment was terminated. In another test, ten 2- to 3-week-old virgin beetles of each sex were transferred daily to bean plants freshly dipped in only 10 ppm of Compound II. After the 1st day, no eggs from this treatment hatched. Therefore, it was concluded that the transfer of this compound from males to females was not a significant factor in determining the threshold foliar dosage for producing ovicidal effects.

Field-Cage Experiments.—In the summer of 1970, Compounds II, III, and IV were tested for lethal morphogenetic effects on the Mexican bean beetle as foliar treatments in field cages. In each experiment, 6 cheesecloth-covered 3×3×3½-ft wooden cages were secured over young Henderson bush lima bean plants. Three cages were chosen at random for the treatment and 3 cages for the controls.

The juvegens were formulated as emulsifiable concentrates with Velsicol AR-60-Triton® X-100-juvegen (2:1:1). Emulsions containing 500 ppm AI were applied twice, 1 week apart, to the bean plants with a small hand sprayer. Soon after the 1st foliar treatment, 6 mated females, 10 young 4th- and 10 older 4th-stage larvae were introduced into each cage containing bean plants treated with Compound II or III. Six mated females, ten 3rd-stage + 5 young, and 5 older 4th-stage larvae were introduced into each cage containing bean plants treated with Compound IV. All stages of the insect were removed just before the 2nd treatment, and the adults and feeding larvae were reintroduced after the treatment. At the end of the 2nd week, all stages were removed, and leaves with attached egg masses, prepupae, and pupae were held in water bottles at 24°C under constant illumination.

Table 2 shows percent survival to the pupal molt and percent eclosion from the introduced larvae, egg production by the introduced females, and percent egg hatch resulting from the 2-week treatment period. All 3 compounds reduced the number of adults recovered from the introduced larvae by 90% or more. Compounds III and IV did not demonstrate effective residual ovicidal activity, but Compound II reduced egg hatch by 98%. None of the treatments had a significant effect on egg production.

In addition, the effect of a combined topical plus residual application of Compound IV was determined by the same procedure used in the residual tests, except that 3 foliar treatments were made 1 week apart, and only adults were removed before the 2nd and 3rd treatments. No adults eclosed (Table 2), and egg hatch was reduced by 88%.

Bean-Plant Growth Abnormalities.—No phytotoxic symptoms were observed during the field-cage tests. However, in a subsequent open-field test, lima bean plants ('Thorogreen' variety) were treated with 1 lb/100 gal of Compound V at 3 weekly intervals followed by 2 sprays at 10-day intervals. Laboratory assays had indicated this compound possessed high juvenile hormone activity, and Mexican bean beetle egg hatch during the field test was reduced by 90%. However, by the 3rd week, stunting and growth malformations of the bean plants, suggestive of the effects produced by the phenoxy herbicides, had become quite noticeable. Leaf malformations included cupping, twisting, and failure to expand laterally. These effects may have actually been produced by the plausable (although unsubstantiated) oxidation product, *p*-chlorophenoxyacetic acid, well known for its potent auxin activity and pathological growth effects on plants (Brown and Weintraub 1962). It should be of interest to investigate this possibility.

DISCUSSION.—The Mexican bean beetle appears to possess favorable characteristics for use of synthetic juvegens as control agents. Lethal abnormalities during embryonic, pupal, and adult morphogenesis resulted from the residual activity from small amounts of some of these compounds on bean leaves. Undesirable effects (such as an extended larval feeding period or increased egg production) appeared to be minimal. Although juvegens would not appear to

offer the flexibility of conventional insecticides in controlling this species, when used to fullest advantage they might have a role in an integrated control program for this insect.

If the initial adult population is not great enough to require insecticidal control, but if it is judged to be capable of producing enough progeny to require later insecticidal applications, juvegen treatments soon after oviposition begins could prevent significant damage by the 1st larval generation. If the initial adult population is great enough to require insecticidal control, juvegens might be used to suppress the development of progeny from the reduced adult population in an effort to reduce the probability of the development of insecticide resistance.

Another possibility is to combine juvegens with other agents which complement their effectiveness as control agents by such effects as feeding suppression, repellency, or lethal developmental abnormalities of young larvae. For example, certain ecdysone analogues greatly suppress the feeding of last-stage larvae and adults and produce additional morphogenetic derangements when combined with juvegens (unpublished data). Treatment of beans with relatively low concentrations of certain vitamin antimetabolites (Gothilf and Waites 1968) or hypocholesteremic agents (unpublished data) causes high mortality among early larval instars. Combining such agents with juvegens might provide a more flexible control agent than either agent alone.

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Electrophysiological Screening of Queen Substance and Analogues for Attraction to Drone, Queen, and Worker Honey Bees[1,2]

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ABSTRACT

Electrophysiological responses were determined on the antennae of the 3 castes of *Apis mellifera* L. to *trans*-9-oxo-2-decenoic acid ("queen substance") and its *cis* isomer. In addition, electroantennograms (EAG's) were obtained with 3 structural analogues of queen substance on the drones. The EAG's correlated with the results of previous field tests and queen substance elicited much stronger responses than the *cis* isomer or the other test compounds. Marked differences were noted between the response of drone, worker, and queen honey bee antennae to the *trans* isomer.

The versatile pheromone *trans*-9-oxo-2-decenoic acid produced in the mandibular glands in the queen honey bee, *Apis mellifera* L., was studied first by Butler (1954) who named it "queen substance." The compound was isolated and identified independently by Barbier et al. (1960) and Butler et al. (1961). The pheromone was determined to function as a sex attractant for drones (Gary 1962) and as an inhibitor of ovarian development and queen-rearing in workers (Butler et al. 1961). Also, Kaissling and Renner (1968) showed by electrophysiological analysis that the response to queen substance was triggered by specialized cells situated in the antennal pore plates of all 3 castes of the honey bee.

Since the effect of both gross and minor changes in the structure of queen substance on its biological activity should contribute toward understanding the mechanism of olfaction in insects, several studies were made with a variety of compounds. *cis*-9-Oxo-2-decenoic acid inhibited queen-rearing by honey bee workers (Pain et al. 1962) but did not attract drones in the field (Doolittle et al. 1970). Doolittle et al. (1970) found also that *cis* acid had some activity as an inhibitor of queen cell construction but that it was significantly less active in this regard than the *trans* isomer. Therefore, the activity demonstrated may have occurred because of conversion of the *cis* to the *trans* isomer in the hive. In addition, the effect of structural changes on the biological activity of *trans*-9-oxo-2-decenoic acid was investigated by Blum et al. (1971) who evaluated several alkanoic acids structurally related to queen substance. Some of these were prepared by Tribble (1966[3]) and by Blum et al. (1971), who evaluated them as sex attractants. Since none showed any attractiveness to drones, *trans*-9-oxo-2-decenoic acid appears to be both a highly potent (as little as 6 μg of the acid attracts drones in the field) and a highly specific pheromone.

The purpose of this investigation was to find cor-

[1] Hymenoptera: Apidae.

[2] Received for publication Apr. 13, 1972.

[3] M. T. Tribble. 1966. Insect sex attractants. I. Synthesis of the sex attractant of the queen honey bee (*Apis mellifera*) and related substances. M.S. thesis, Louisiana State University, Baton Rouge, 69 p.