

Effect of Orally Administered 5-Ethoxy-6-[4-Methoxyphenyl] Methyl-1,3-Benzodioxole on Reproduction of the Mediterranean Fruit Fly (Diptera: Tephritidae)

CHIOU-LING HSU, FRANKLIN CHANG, HOWARD F. MOWER,¹
LINDA J. GROVES,¹ AND LEONARD JURD²

Department of Entomology, University of Hawaii,
Honolulu, Hawaii 96822

J. Econ. Entomol. 82(4): 1046-1053 (1989)

ABSTRACT Ovarian growth was delayed at least 25 d in females of the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann), fed sugar cubes containing J2581 (5-ethoxy-6-[4-methoxyphenyl]methyl-1,3-benzodioxole) at a concentration of 0.57 mg/g. Ovarian development of females fed a sugar-protein hydrolysate diet containing the same concentration of J2581 was delayed by 9 d. Egg hatch (larval eclosion) was significantly reduced from 0 to 6%. Eggs that failed to hatch had fragmented yolk plasm and absence of embryonic development. A juvenile hormone analog, (7S)methoprene, concomitantly fed with J2581 to newly emerged flies, caused partially increased oviposition but no improvement in ovarian growth or percentage egg hatch. J2581 and seven other benzyl-1,3-benzodioxoles were not mutagenic in the Ames *Salmonella* microsome test with and without metabolic activators.

KEY WORDS Insecta, fruit flies, reproduction, benzodioxole

SEVERAL METHYLENEDIOXY DERIVATIVES of substituted benzylphenols (benzyl-1,3-benzodioxoles) (BBDs) modified from cinnamylphenols isolated from the Panamanian hardwood, *Dalbergia retusa* (Hemsley) (Jurd et al. 1972, 1979; Jurd & Manners 1980), are biologically active against several insect species (Jurd et al. 1979; Rawlins et al. 1979; Chang et al. 1980, 1984; Flint et al. 1980; Rawlins & Jurd 1981; Langley et al. 1982; Van Mellaert et al. 1983a,b,c; Matolcsy et al. 1986). Activities of BBDs and related benzylphenols include the induction of permanent or partial sterility in insects (Jurd et al. 1979; Rawlins et al. 1979; Chang et al. 1980; Langley et al. 1982; Van Mellaert et al. 1983a,b), interference with sex attractancy (Chang et al. 1984), and induction of precocious metamorphosis (Darvas et al. 1988).

Discovery that two BBDs blocked the effect of juvenile hormone in a modified *Galleria* cuticle wax bioassay (De Loof et al. 1982) and three BBDs induced precocious metamorphosis in *Hyphantria cunea* (Drury) larvae (Darvas et al. 1988) strongly suggested an anti-juvenile hormone function for the compounds. However, this mode of action has recently been questioned (Langley & Pimley 1986, Staal 1986, Beckage et al. 1987).

Although permanent sterility in insects can be achieved by oral administration of BBDs (Jurd et al. 1979, Langley et al. 1982, Van Mellaert et al. 1983b), eventual recovery of fertility after treat-

ment with BBDs or related benzylphenols (whether orally or topically administered) has been observed by Chang et al. (1980) and Flint et al. (1980). Chang et al. (1988) found that a single topical application of a sublethal dose of BBDs coded J2581 (5-ethoxy-6-[4-methoxyphenyl]methyl-1,3-benzodioxole) and J3263 (5-methoxy-6-(1-[4-ethoxyphenyl]ethyl)-1,3-benzodioxole) to 1-d-old *Ceratitidis capitata* (Wiedemann) adults temporarily prolonged oöcyte development by 9 and 13 d, respectively.

To test whether longer administration of BBDs (i.e., by continual feeding of BBD-treated diet to adults) would prolong or make its effect on reproduction permanent, we fed J2581 ad lib. to adult female *C. capitata* and assessed the effect of this mode of administration on reproduction. We also tested J2581 and seven additional BBDs shown previously to be biologically active (Chang et al. 1984) for potential mutagenicity in the Ames *Salmonella* microsome test with and without metabolic activators (Ames et al. 1975).

Materials and Methods

Insects. *C. capitata* puparia were obtained from the mass-rearing facilities of the USDA-ARS Tropical Fruit and Vegetable Research Laboratory in Honolulu. The sex of flies was determined upon emergence. Flies were held in separate cages at 23 ± 1°C and a photoperiod of 13:11 (L:D). They were allowed to feed ad lib. on either a sugar-yeast hydrolysate (3:1, wt/wt) mixture (Tanaka et al. 1969) or sugar cubes in which a BBD was incor-

¹ Department of Biochemistry and Biophysics, University of Hawaii, Honolulu, Hawaii 96822.

² Western Regional Research Center, Natural Products Chemistry Unit, USDA-ARS, Berkeley, Calif. 94710.

porated. In some experiments, oleic acid or olive oil was added as a supplement.

Chemicals. BBDs coded J2581, J2710, J2962, J3230, J3223, J3360, J3263, J2922, J2880, and J2315 (Table 1) were synthesized by L.J. Structures of the racemic compounds were confirmed by elemental (C,H), proton NMR (nuclear magnetic resonance), and mass-spectral analyses (Jurd et al. 1979). Thin-layer chromatography of all compounds on silica gel sheets (Eastman Kodak, Rochester, N.Y.), development in ethyl acetate/hexane (3:7, vol/vol), and subsequent charring showed a purity >99%. (7S)Methoprene (isopropyl-11-methoxy-3,7,11-trimethyldeca-dienoate) was a gift from G. B. Staal (Zoecon Research Institute, Palo Alto, Calif.).

Gel Electrophoresis. Hemolymph protein profiles of both sexes of adult *C. capitata* (treated and controls) were obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of fresh hemolymph (a few crystals of phenylthiourea were added to the hemolymph to inhibit tyrosinase action before removal of hemocytes by centrifugation at $3,000 \times g$) on gel slabs (12%; 1.5 mm thick) in a Biorad Protean II electrophoresis unit (Biorad, Richmond, Calif.) and stained with Coomassie Blue G-250. Vitellin used as a standard in SDS-PAGE and in raising antiserum in New Zealand rabbits was obtained by homogenization of 2 g of prerinsed *C. capitata* eggs in phosphate-buffered saline (pH 7.0). The homogenate was subsequently processed by multiple ammonium sulfate fractionations and dialyses, followed by purification by gel chromatography on Sepharose CL-6B by a modification of the method of Mintzas & Kambysellis (1981). The presence of antivitelin serum was confirmed by double immunodiffusion precipitation patterns in agar (Ouchterlony 1967).

Treatment. Determination of time of BBD application to achieve maximum effect on reproduction in *C. capitata* was important, because earlier experiments (Chang et al. 1984) showed that BBDs were ineffective in interfering with normal oöcyte development if applied topically to females 4 d after eclosion. Based on light microscopic and SDS-PAGE analyses of developing oöcytes and hemolymph protein patterns from control *C. capitata*, females were considered previtellogenic within 24 h after eclosion. Therefore, the sex of adults emerging from approximately 0.5 g of pupae within 24 h after eclosion was determined. These adults were placed into separate cages and supplied with water and a diet of either a sugar-yeast hydrolysate mixture or sugar cubes containing a previously determined sublethal concentration of J2581 (0.57 mg/g diet). Diet treated with acetone served as controls.

Four groups of 20 control females or females fed with a BBD were allowed to mate with 20 males per group at various times after emergence in cages designed for mating and oviposition (Chang et al. 1988). Ovarian growth, number of eggs oviposited, and egg hatch (larval eclosion) were recorded. Diet containing J2581 continued to be fed to males and

Table 1. Formulas of benzyl-1,3-benzodioxole derivatives^a tested in this study

Code	Compound
J2581	5-ethoxy-6-(4-methoxyphenyl)methyl-1,3-benzodioxole
J2962	5-ethoxy-6-(1-phenylethyl)-1,3-benzodioxole
J3230	5-propyl-1-en-3-oxy-6-(1-[4-methoxyphenyl]ethyl)-1,3-benzodioxole
J3223	5-ethoxy-6-(1-[4-ethoxyphenyl]ethyl)-1,3-benzodioxole
J2710	5-methoxy-6-(1-[4-methoxyphenyl]ethyl)-1,3-benzodioxole
J3360	5-methoxy-6-(1-[4-fluorophenyl]ethyl)-1,3-benzodioxole
J3263	5-methoxy-6-(1-[4-ethoxyphenyl]ethyl)-1,3-benzodioxole
J2922	5-ethoxy-6-(1-[4-methoxyphenyl]ethyl)-1,3-benzodioxole

^a All compounds are racemic.

females in mating cages because earlier experiments showed that results were not significantly altered by cross matings between control males or males fed BBDs with females fed BBDs (data not shown).

Because increased growth by follicles also was reflected by increased external ovarian growth, measurement of ovarian length by a stage micrometer provided a rapid and reliable measure of the effect of various BBDs on oöcyte development. Ovaries were removed periodically from groups of 100 treated and control flies and placed in phosphate-buffered saline for lengthwise measurements. Spermathecae from treated females were also removed into phosphate-buffered saline for confirmation of successful insemination by males.

Data Analysis. Comparisons of measurements of ovarian length, numbers of eggs oviposited, and egg hatch between treated and control groups were made by analysis of variance and the Waller-Duncan *k* ratio test ($P < 0.01$; PROC GLM; SAS Institute 1982).

Ames Test for Mutagenicity. Because the major effect of sublethal doses of BBDs is on the reproductive system in insects, we examined J2581 as well as seven other closely related BBDs for potential mutagenicity in the Ames *Salmonella* microsome test (Ames et al. 1975). Three strains of *Salmonella typhimurium* his⁻ mutants (TA100 [point mutation], TA98 [frameshift mutation], and TA97A [frameshift mutation]) were used to test the reversion frequency induced by J2581, J2962, J3223, J3360, J3263, J2710, J3230, and J2922. All samples were tested at various concentrations up to a solubility limit of 250 µg BBD in dimethyl sulfoxide (DMSO) per plate in the presence or absence of a rat liver microsomal fraction (S9) and cofactors as described by Ames et al. (1975). A twofold increase of his⁺ colonies compared with negative control was considered indicative of mutagenicity. Known mutagens, 2,4-dinitrofluorenone, sodium azide, and IRC 191, were used as positive controls in the absence of 2-acetylaminofluorene in the presence of metabolic activator (S9 liver fraction). DMSO without BBDs was used as the negative (solvent) control.

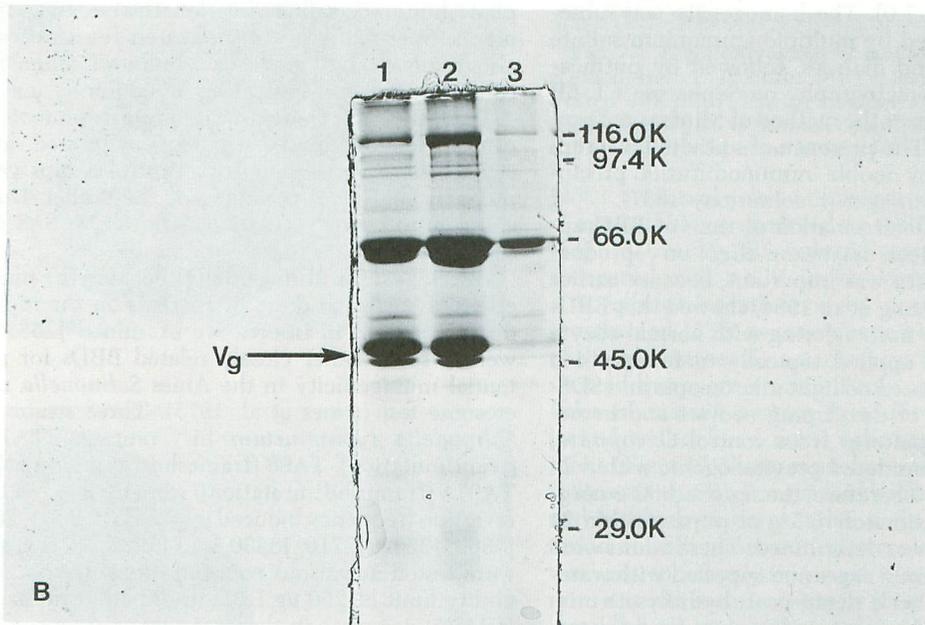
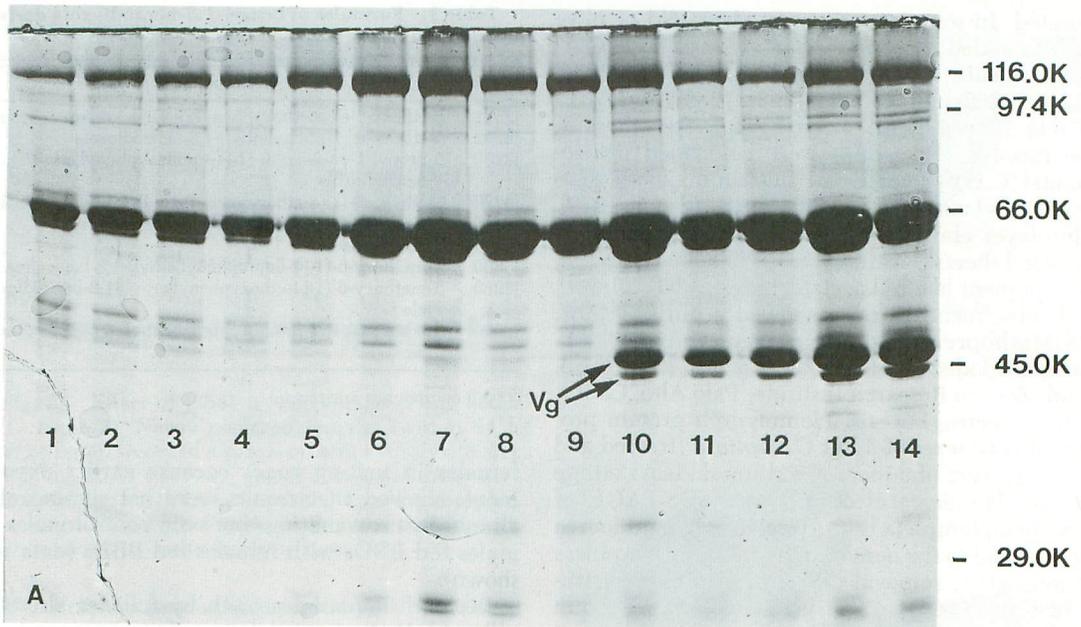


Fig. 1. Profile of proteins from whole hemolymph (without hemocytes) from adult *C. capitata* resolved by SDS-PAGE on 12% slab gel and stained with Coomassie Blue G-250. (A) Lanes 1-7 represent hemolymph proteins from 7- to 1-d-old males, respectively, lanes 8-14 from 1- to 7-d-old females, respectively. Significant amounts of hemolymph vitellogenin first appear on day 3, but is absent from males. Position of molecular weight markers at right (bands not shown). (B) Hemolymph vitellogenin appears in appreciable amounts from J2581-fed (lane 1) and control (lane 2) 6-d-old females compared with 1-d-old controls (lane 3). Position of molecular weight markers at right (bands not shown).

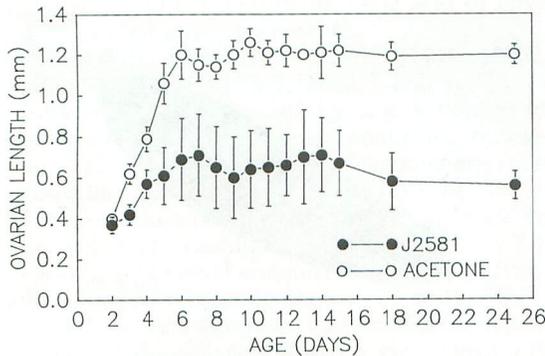


Fig. 2. Effect of J2581 on ovarian growth in *C. capitata*. Newly emerged females (within 24 h of eclosion) were fed sugar cubes containing J2581 (0.57 mg/g) and allowed to mate. Ovarian growth was followed from days 2 to 25 after eclosion by measuring ovarian length. Flies treated with acetone served as controls. Error bars, SEM.

Results

SDS-PAGE of hemolymph (without hemocytes) from control females showed the presence of female-specific proteins consisting of two major subunits ($M_r = 45K$ and $49K$), beginning on day 3 after eclosion (Fig. 1A). These protein bands also were detected in appreciable amounts from 6-d-old females fed J2581 and controls (Fig. 1B). We assumed that female-specific proteins were vitellogen based on comigration in SDS-PAGE with vitellin and immunological identity with vitellin when run in double diffusion Ouchterlony plates against a twofold male hemolymph-absorbed antiserum containing antivitelin activity.

Examination of oöcytes from control females revealed accelerated oöplasmic darkening and growth in size concomitant with increased lipid droplet formation within the oöplasm beginning on day 3 after adult eclosion. These events marked the beginning of vitellogenin uptake. To test the effect of J2581 on vitellogenin uptake, females were allowed to feed on diet containing J2581 at a time before vitellogenesis, i.e., within 24 h of adult eclosion.

Females fed a sugar-protein hydrolysate diet without J2581 had slightly longer ovaries ($\bar{x} \pm \text{SEM} = 1.6 \pm 0.1$ mm) than those fed sugar alone ($\bar{x} \pm \text{SEM} = 1.2 \pm 0.1$ mm). Maximum ovarian size was generally attained by day 6 after adult eclosion in females fed either diet. However, females fed sugar cubes containing J2581 (0.57 mg/g) had significantly shorter ovaries ($F = 39.16$; $df = 31$; $P = 0.0001$) over a 25-d recording period than ovaries from acetone controls, which attained full growth by day 6 after adult eclosion (Fig. 2). Ovarian growth of females fed a sugar-protein hydrolysate diet containing J2581 (0.57 mg/g) was significantly delayed ($F = 109.03$; $df = 7$; $P < 0.0001$): they attained full size by day 16 after eclosion compared with 6 d for the acetone controls (Fig. 3). Replacement of sugar-protein hydrolysate diet containing

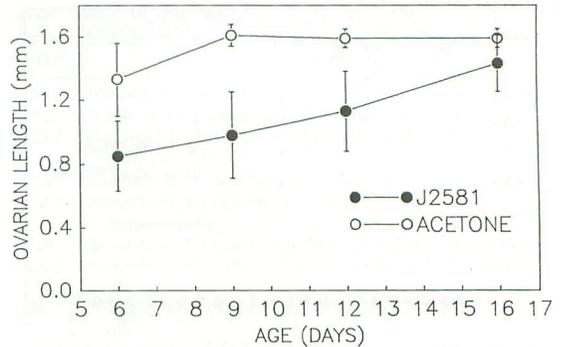


Fig. 3. Effect of J2581 on ovarian growth in *C. capitata*. Newly emerged females (within 24 h of eclosion) were fed a sugar-protein hydrolysate diet containing J2581 (0.57 mg/g) and allowed to mate. Ovarian growth was followed from days 6 to 16 after eclosion by measuring ovarian length. Flies treated with acetone served as controls. Error bars, SEM.

J2581 with freshly treated medium every 3 d prolonged the effect of J2581 on ovarian growth (data not shown). We suspect that J2581 may be degraded over time by microorganisms in the sugar-protein hydrolysate, resulting in recovery of ovarian growth. The absence of recovery in females fed sugar cubes containing J2581 may be attributable to low microbial levels maintained by its high sugar content.

Addition of oleic acid (Van Mellaert et al. 1983a) or olive oil (Matolcsy et al. 1986) to sugar diets increased the effectiveness of BBDs in reducing oöcyte growth in adult *Sarcophaga bullata* Parker and *Phormia regina* Meigen, respectively. Inclusion of oleic acid or olive oil to either of our diets did not appreciably enhance activity in *C. capitata* (data not shown).

Ceratitis capitata lay eggs as early as 6 d after eclosion; larval emergence occurs 3–4 d later at 23

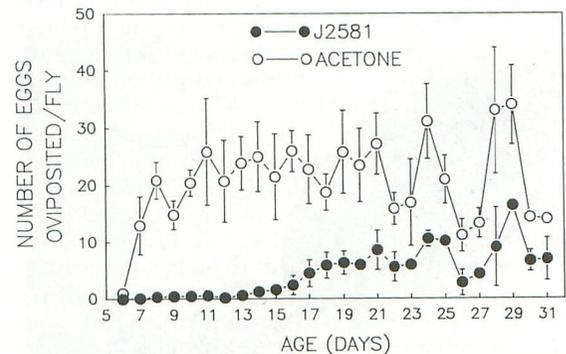


Fig. 4. Effect of J2581 on number of eggs oviposited per *C. capitata* female. Newly emerged females (within 24 h after eclosion) were fed a sugar-protein hydrolysate diet containing J2581 (0.57 mg/g). Treated females were mated and allowed to oviposit from days 6 to 31 after eclosion. Females treated with acetone served as controls. Error bars, SEM.

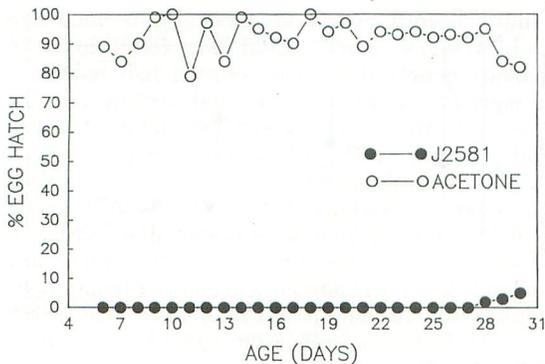


Fig. 5. Effect of J2581 on percentage of egg hatch in *C. capitata*. Newly emerged females (within 24 h after eclosion) were fed a sugar-protein hydrolysate diet containing J2581 (0.57 mg/g) and allowed to mate. Percentage of egg hatch from oviposited eggs was recorded from days 5 to 30 after eclosion. Females treated with acetone served as controls. Error bars, SEM.

$\pm 1^{\circ}\text{C}$. Females fed sugar-protein hydrolysate containing J2581 (0.57 mg/g) oviposited significantly fewer eggs per fly than controls ($F = 23.83$; $df = 51$; $P < 0.001$) during a 31-d recording period after eclosion (Fig. 4). Of the eggs laid by females fed

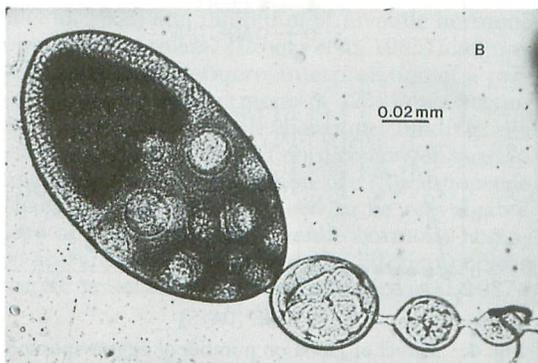
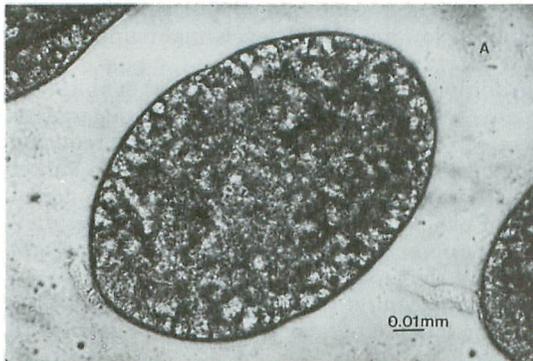


Fig. 6. Light microscopy of terminal follicles from 6-d-old females of *C. capitata*. (A) Typical follicle from female treated with J2581 and showing lack of well-defined nurse and follicular cells compared with (B) terminal follicle from control female.

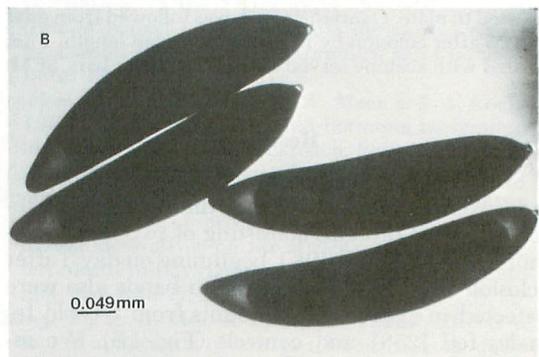
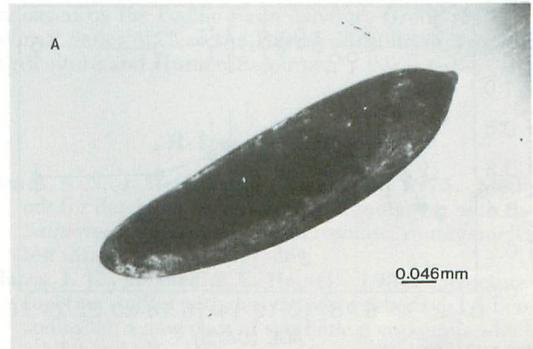


Fig. 7. Light microscopy of mature eggs (1 d before eclosion) from *C. capitata*. (A) Typical egg from female treated with J2581 showing fragmentation of yolk plasm and failure of yolk to contract compared with (B) normal embryonic development in eggs from control flies.

J2581, no larval eclosion (measured by percentage of egg hatch) was observed up to 27 d after eclosion (Fig. 5). By day 30, only 6% egg hatch resulted compared with $>80\%$ in the controls (Fig. 5).

Successful insemination by untreated males of females fed J2581 was confirmed by examinations of the spermathecal contents from 100 females that showed live spermatozoa. Terminal (primary) follicles in mature ovaries from females fed J2581 lacked well-defined nurse cells and follicular epithelia (Fig. 6A), features uncharacteristic of normal follicles (Fig. 6B). In eggs laid by females fed J2581 and in which embryos failed to develop, fragmentation of yolk plasm and absence of yolk contraction was commonly observed (Fig. 7A). This feature was not seen in controls (Fig. 7B). Effects on the yolk may explain the significantly low egg hatch from females fed J2581.

(7S)Methoprene added in equimolar amounts to J2581 in a sugar-protein hydrolysate diet and fed to newly emerged females resulted in an increase in numbers of eggs laid (Fig. 8) but no significant increase ($F = 5.94$; $df = 41$; $P > 0.0001$) in ovarian growth or percentage of egg hatch compared with females fed only J2581 in their diet (data not shown).

All eight BBDs failed to revert his^{-} to his^{+} appreciably in the absence or presence of a rat liver

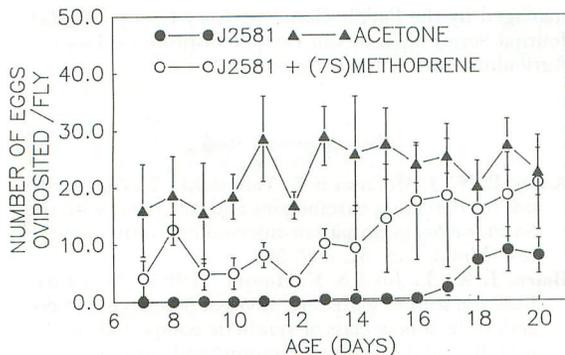


Fig. 8. Effect of methoprene and J2581 on number of eggs oviposited per female in *C. capitata*. Newly emerged females (within 24 h after eclosion) were fed a sugar-protein hydrolysate diet containing equimolar amounts of J2581 and (7S)methoprene. Treated females were mated and allowed to oviposit from days 7 to 20 after eclosion. Females treated with acetone or J2581 served as controls. Error bars, SEM.

microsomal fraction (S9) when dissolved in DMSO up to a solubility limit of 250 μg per plate (Table 2). Although not a definitive test for carcinogenicity for a number of reasons, the Ames test is commonly used as a primary screen to detect genotoxic substances and shows excellent correlation with the potential to induce mammalian cancers (Ames et al. 1975).

Discussion

At sublethal doses, topical application of biologically active BBDs appears to affect only the female reproductive system in *C. capitata*, whereas higher doses immediately kill adult flies (Chang et al. 1984). Likewise, long-term observation of both sexes of *C. capitata* fed sublethal concentrations of BBD revealed no apparent abnormal behavior compared with untreated flies in terms of the ability to feed, fly, display courtship behavior, and mate. Longevity likewise was not affected.

Reduction in egg hatch after topical or oral BBD treatment has been reported for a number of insect species (Rawlins et al. 1979, Rawlins & Jurd 1981, Van Mellaert et al. 1983b, Nelson et al. 1985). Chang et al. (1980) found that a BBD-related substituted benzylphenol, 2,4-bis(1,1-dimethylethyl)-6-[(4-methoxyphenyl)methyl]phenol, reduced egg hatch when included in the diet of newly emerged females of the house fly (*Musca domestica* L.) for 5 d; normal egg hatch occurred by day 14 after eclosion. A BBD, J2922 (5-ethoxy-6-[1-(4-methoxyphenyl)-ethyl]-1,3-benzodioxole), also was tested and likewise reduced egg hatch in house flies (Chang et al. 1980).

Eventual recovery of fertility has been observed in *C. capitata* after topical application of a single sublethal dose of BBDs to newly emerged flies (Chang et al. 1988). Partial recovery of fertility also was noted in the pink bollworm, *Pectinophora*

Table 2. Reversion of *S. typhimurium* strains exposed to eight benzyl-1,3-benzodioxole derivatives with and without metabolic activator in the Ames *Salmonella* microsome test

Test compound code	No. revertants per plate					
	Without S9			With S9		
	TA98	TA100	TA97A	TA98	TA100	TA97A
J2581	33	118	114	35	110	114
J2962	30	129	94	42	107	119
J3230	26	130	110	33	100	113
J3223	27	116	62	40	102	95
J2710	31	113	109	44	121	98
J3360	39	137	82	43	103	98
J3263	29	127	86	47	128	94
J2922	23	106	101	49	102	92
Controls						
Negative	31	122	95	47	105	138
Positive	1,443 ^a	6,134 ^b	1,223 ^c	1,589 ^d	—	—

Benzodioxole compounds were dissolved in DMSO up to a solubility limit of 250 μg /plate. S9 refers to an Aroclor-induced rat liver microsomal fraction enhanced with cofactors as described by Ames et al. (1975). DMSO served as the solvent control.

^a 2,4-dinitrofluorenone (20 μg /plate).

^b Sodium azide (5 μg /plate).

^c IRC 191 (1 μg /plate).

^d 2-acetylaminofluorene (20 μg /plate).

gossypiella (Saunders), the week after moths were exposed to surfaces treated with J2581 (0.1 mg/cm²). In both cases, females were not continually exposed to BBDs. Results obtained in this study suggest that prolonged exposure to orally administered BBDs is more effective than topical application in interfering with reproduction in insects.

Several BBDs appear to have direct anti-juvenile hormone effects when assayed in the *Galleria* cuticle wax bioassay (Van Mellaert et al. 1983a,c). For example, Van Mellaert et al. (1983a) demonstrated that protein (especially vitellogenin) accumulated in the hemolymph of female *S. bullata* fed J2581; the accumulation suggested that juvenile hormone-mediated yolk protein uptake by oöcytes was prevented. Darvas et al. (1988) reported that 50 and 100 μg J2710 induced precocious metamorphosis in 27 and 44% of *H. cunea*, respectively.

If, in fact, BBDs functioned as anti-juvenile hormone agents, addition of a juvenile hormone mimic should negate their effects. Chang et al. (1988) showed that (7S)methoprene, when added in equimolar amounts with J2581 and topically applied to newly emerged females of *C. capitata*, neutralized the effect of J2581 in delaying ovarian growth. However, when (7S)methoprene was added to the diet and fed to adult female *C. capitata*, significantly greater ovarian growth or percentage of egg hatch did not result compared with females fed only J2581 in their diet. Van Mellaert et al. (1983a) were unable to restore normal ovarian growth by either ecdysterone or methoprene supplementation in the diet in *S. bullata* fed J2581. Langley & Pimley (1986) also could not restore fertility in the tsetse fly, *Glossina morsitans morsitans* Westwood, with methoprene application be-

fore, concurrently with, and after topical application of J2581 and J2922. These workers also concluded that because juvenile hormone apparently has no function in the regulation of reproduction in adult tsetse flies, ovarian atrophy and sterility in this fly induced by BBD was not the result of an anti-juvenile hormone effect (Langley & Pimley 1986). Sparks et al. (1987) found that compared with fluoromevalonate used as a standard, a number of BBDs did not delay or inhibit larval-pupal ecdysis in prepupae of *Trichoplusia ni* (Hübner) when topically applied in either high or low doses. Furthermore, Beckage et al. (1987) observed no anti-juvenile hormone action when larvae of *Manduca sexta* Johannsson were topically treated with J2710 immediately after ecdysis to the fourth instar; abnormalities of the mouthparts and cervix were, however, observed upon ecdysis to the fifth instar. Moreover, J2710 apparently had no effect on methoprene action in the *black* mutant bioassay for activity resembling that of juvenile hormone. Our results showed that J2581 affected oöcyte and embryonic development in *C. capitata* by means that are still unclear.

J2581 did not affect vitellogenin biosynthesis in *C. capitata* because appreciable amounts in hemolymph of females fed J2581 in the diet were detected by SDS-PAGE 3 d after eclosion. Van Mellaert et al. (1983a) also demonstrated the presence of hemolymph vitellogenin in *S. bullata* fed J2581 in sugar. Because the juvenile hormones and ecdysterone are clearly involved in regulation of vitellogen biosynthesis in Diptera (Raabe 1986, Kelly et al. 1987), an anti-juvenile hormone effect by BBDs on vitellogen biosynthesis in *C. capitata* appears unlikely.

Although the major effect of BBDs in insects is on reproduction, mechanisms other than, but not excluding, juvenile hormone antagonism apparently may be involved. BBDs can damage tissues directly by enzymatic conversion to quinone methides (Jurd et al. 1979), although this has not yet been demonstrated in insects. Cytotoxic effects (Batra et al. 1984) and inhibition of juvenile hormone biosynthesis or release (Brooks et al. 1985) also may be involved. Furthermore, interpretation of a proposed anti-juvenile hormone of J2581 in *C. capitata* because of juvenile hormone binding site blockage or damage based on recovery of ovarian growth by topical application of (7S)methoprene (Chang et al. 1988) may need to be reevaluated based on the report that juvenile hormone homologs and analogs apparently share different nuclear receptor proteins in *M. sexta* (Riddiford et al. 1987).

Acknowledgment

We thank Harris Chang and the staff of the mass-rearing facility of the USDA Fruit and Vegetable Research Laboratory in Honolulu for assistance in obtaining insects for this study. This research was supported by the USDA under CSRS Special Grant No. 85-CSRS-2-2652

managed by the Pacific Basin Advisory Group (PBAG). Journal Series 3276 of the Hawaii Institute of Tropical Agriculture and Human Resources.

References Cited

- Ames, B. N., J. McCann & E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella/mammalian-microsome* mutagenicity test. *Mutat. Res.* 31: 347-364.
- Batra, J. K., L. Jurd & E. Hamel. 1984. Structure-function studies with derivatives of 6-benzyl-1,3-benzodioxole, a new class of synthetic compounds which inhibit tubulin polymerization and mitosis. *Mol. Pharmacol.* 27: 94-102.
- Beckage, N. E., B. A. Stirling, T. J. Templeton & B. D. Nielson. 1987. Disruptive developmental effects of benzyl-1,3-benzodioxole derivatives on unparasitized and parasitized *Manduca sexta* larvae. *J. Insect Physiol.* 33: 603-611.
- Brookes, G. T., G. E. Pratt, D. W. Mace & J. A. Cocks. 1985. Inhibition of juvenile hormone biosynthesis in corpora allata of the cockroach, *Periplaneta americana* (L.) *in vitro*. *Pestic. Sci.* 16: 132-142.
- Chang, F., C. L. Hsu, L. Jurd & D. L. Williamson. 1984. Effect of precocene and benzyl-1,3-benzodioxole derivatives on sex attractancy in the Mediterranean fruit fly (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 77: 147-151.
- Chang, F., C. L. Hsu & L. Jurd. 1988. Effects of topical application of benzyl-1,3-benzodioxole derivatives on reproduction in the Mediterranean fruit fly. *Insect Sci. Applic.* 9: 381-388.
- Chang, S. C., A. B. Borkovec & A. B. Demilo. 1980. Effects of substituted benzylphenols on reproduction of house flies. *J. Econ. Entomol.* 73: 745-747.
- Darvas, B., L. Varjas, A. I. Farag & H. Van Mellaert. 1988. Effects of benzyl-1,3-benzodioxoles on development of sensilla trichodea of *Hyphantria cunea* (Lepidoptera, Arctiidae) larvae, pp. 581-585. *In* F. Sehnal, A. Zabza & D. L. Denlinger [eds.], *Endocrinological frontiers in physiological insect ecology*, vol. 1. Wroclaw Technical University Press, Wroclaw, Poland.
- De Loof, A., H. Van Mellaert & L. Jurd. 1982. Two new compounds with anti-juvenile hormone activity as shown by the *Galleria* bioassay. *Gen. Comp. Endocrinol.* 46: 377.
- Flint, H. M., L. Jurd & J. Merkle. 1980. Pink bollworm: sterilizing effects of benzylphenols and benzyl-1,3-benzodioxoles. *J. Econ. Entomol.* 73: 710-714.
- Jurd, L. & G. D. Manners. 1980. Wood extractives as models for the development of new types of pest control agents. *J. Agric. Food Chem.* 28: 183-188.
- Jurd, L., K. Stevens & G. D. Manners. 1972. Quinonoid constituents of *Dalbergia retusa* heartwood. *Phytochemistry* 11: 3287-3292.
- Jurd, L., R. L. Fye & J. Morgan, Jr. 1979. New types of insect chemosterilants. Benzylphenols and benzyl-1,3-benzodioxole derivatives as additives to housefly diet. *J. Agric. Food Chem.* 27: 1007-1016.
- Kelly, T. J., T. S. Adams, M. B. Schwartz, M. J. Birnbaum, E. C. Rubenstein & R. B. Imberski. 1987. Juvenile hormone and ovarian maturation in the Diptera: a review of recent results. *Insect Biochem.* 17: 1089-1093.
- Langley, P. A. & R. W. Pimley. 1986. A role for juvenile hormone and the effects of so-called anti-

- juvenile hormones in *Glossina morsitans*. J. Insect Physiol. 8: 727-734.
- Langley, P. A., M. A. Trewern & L. Jurd. 1982.** Sterilizing effects of benzyl-1,3-benzodioxoles on the tsetse fly *Glossina morsitans morsitans*. Bull. Entomol. Res. 72: 473-481.
- Matolcsy, G., R. Feyereisen, H. Van Mellaert, A. Pal, L. Varjas, I. Belai & P. Kulesar. 1986.** Molecular modifications of benzylphenol and benzyl-1,3-benzodioxole types of insect chemosterilants. Pestic. Sci. 17: 13-24.
- Mintzas, A. C. & M. P. Kambysellis. 1981.** The yolk proteins of *Drosophila melanogaster*: isolation and characterization. Insect Biochem. 12: 25-33.
- Nelson, R. S., A. K. Mohamed & P. Vattikutti. 1985.** Efficacy of three insect growth regulators on the development of *Aedes aegypti*. J. Am. Mosq. Control Assoc. 1: 240-242.
- Ouchterlony, O. 1967.** Immunodiffusion and immunoelectrophoresis, pp. 665-706. In D. M. Weir [ed.], Handbook of experimental immunology. Blackwell, Oxford, England.
- Raabe, M. 1986.** Insect reproduction: regulation of successive steps, pp. 30-154. In P. D. Evans & V. B. Wigglesworth [eds.], Advances in insect physiology, vol. 19. Academic, New York.
- Rawlins, S. C. & L. Jurd. 1981.** Influence of the mode of administration of benzylphenols and benzyl-1,3-benzodioxoles on screwworm fertility. J. Econ. Entomol. 74: 215-217.
- Rawlins, S. C., L. Jurd & J. W. Snow. 1979.** Anti-fertility effects of benzylphenols and benzyl-1,3-benzodioxoles on screwworm flies. J. Econ. Entomol. 72: 674-677.
- Riddiford, L. M., E. Osir, C. Fittinghoff & J. M. Green. 1987.** Juvenile hormone analog binding in *Manduca* epidermis. Insect Biochem. 17: 1039-1043.
- SAS Institute. 1982.** SAS user's guide: statistics. SAS Institute, Cary, N.C.
- Sparks, T. C., R. M. Roe, A. Buehler & B. Hammock. 1987.** The evaluation of anti-juvenile hormones using last stadium larvae of the cabbage looper, *Trichoplusia ni* (Hübner). Insect Biochem. 17: 1011-1016.
- Staal, G. B. 1986.** Anti-juvenile hormone agents. Annu. Rev. Entomol. 31: 391-429.
- Tanaka, N., L. F. Steiner, K. Ohinata & R. Okamoto. 1969.** Low-cost larval rearing medium for mass production of oriental and Mediterranean fruit flies. J. Econ. Entomol. 62: 967-968.
- Van Mellaert, H., A. De Loof & L. Jurd. 1983a.** Physiological effects of a benzyl-1,3-benzodioxole chemosterilant on *Sarcophaga bullata*. Pestic. Biochem. Physiol. 20: 124-130.
- 1983b.** Insecticidal activity of 5-methoxy-6-(1-(4-methoxyphenyl)ethyl)-1,3-benzodioxole against the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 76: 990-992.
- 1983c.** Anti-juvenile hormone effects on newly described chemosterilants: benzyl-1,3-benzodioxoles and benzylphenols. Entomol. Exp. Appl. 33: 83-88.

Received for publication 25 August 1988; accepted 15 November 1988.