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Influence of the juvenile hormone analogue, methoprene[1], on development of the southern pine beetle, Dendroctonus frontalis Zimm. (Col., Scolytidae)

By J. W. Van Sambeek and J. R. Bridges

Abstract

To determine the effects of juvenoids on the development and sensitivity of the southern pine beetle (SPB), *Dendroctonus frontalis* Zimm., we treated last-instar larvae, pupae, and callow adults with methoprene, a potent juvenile hormone analogue. From this study we identified a number of juvenoid effects on SPB. Methoprene has the greatest effect on last-instar larvae and on pupae when it has been applied within 24 hours after pupation. Methoprene either stopped developmental progress of C-shaped larvae or produced pupal-adult intermediates from post-feeding and prepupal larvae that pupated normally. The mean effective dose for 50 % adult juvenilization of day-old pupae was 0.16 ng. Methoprene treatment of older pupae and callow adults significantly reduced the percentage of fully pigmented adults produced. The pupal bioassay should be useful in determining the presence and effects of pine-produced juvenoids on SPB.

Introduction

Much research has been done recently on insect juvenile hormones (Slama et al. 1974; Staal 1975), especially concerning possible use of juvenoids as control agents. Relatively little attention has been given to bark beetles, though their sensitivity to synthetic juvenoids has been demonstrated in studies of flight muscle degeneration (Borden and Slater 1968), pheromone biosynthesis (Borden et al. 1969; Hughes and Renwick 1977; and Hughes and Renwick 1977), ovarian maturation (Sahota et al. 1970), egg hatchability (Ibaraki and Sahota 1976), fecundity, insect development and insect juvenilization (Novak et al. 1976).

Juvenoids have been found in wood and bark extractives from a number of conifers including several *Pinus* spp. (Mansingh et al. 1970; Jacobson et al. 1975; Van Sambeek 1978); however, the potential importance of host-produced juvenoids on the development and reproduction of bark beetles has not been recognized, nor has the possible role of host-produced juvenoids in host resistance been examined. Several studies have shown that disruption of the

[1] Mention of trade name is solely to identify material used and does not imply endorsement by the USDA

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normal microbial complex associated with bark beetles can reduce progeny production and cause atypical patterns of insect development (BARRAS 1973; FRANKLIN 1970; GOLDMAN and FRANKLIN 1977). Host-produced juvenoids might cause such a disruption.

This paper is part of a study aimed at determining whether *Pinus taeda* L. produces juvenoids and whether they affect the southern pine beetle (SPB), *Dendroctonus frontalis* Zimm. To discover the characteristics of a bioassay for the screening of juvenoids, we investigated the effects of methoprene, a potent synthetic juvenoid, on the development of SPB from larva through adult. We identified various developmental stages of the outerbark (fourth-instar) larvae and pupae and categorized several degrees of adult juvenilization in response to juvenoids.

2 Material and methods

We gathered last-instar larvae and pupae from the outer bark of field-collected billets of naturally infested *Pinus taeda* L. or *Pinus echinata* Mill. Larvae and pupae were then separated from the outer bark debris through a 10-mesh sieve and collected on a 20-mesh sieve before being transferred to petri dishes or to individual wells of glass-covered 9-well Pyrex spot test plates. We examined the larvae either daily or at 8-hour intervals and transferred freshly ecdysed pupae to individual wells of 9-well spot plates. Between observations all petri plates and spot plates were kept in the dark at 24 °C in 5-liter plastic boxes lined with moist tissue paper.

Insects were treated with 1 µl of a solution of methoprene in AR grade acetone containing 100 µg to 10 pg of methoprene (93 % active ingredient) per ml. Using a 25 µl Hamilton syringe with a 50-stop repeating dispenser, we applied the treatment as two 0.5 µl doses approximately 1 min apart to the venter of the abdomen. For checks, we either treated insects similarly with 1 µl of acetone or left them untreated.

Specimens were scored daily under a 12× binocular scope for developmental progress and morphological abnormalities until completion of eclosion, after which observations were made every other day until over 50 % of the morphologically normal adults had died. In the absence of normal adults, we discontinued observations when over 50 % of all specimens had died. Specimens were presumed dead when we could detect no movement of the mandibles, legs, or abdominal segments after the specimens were placed under an intense light beam from the microilluminator. At the time of death, we scored morphologically normal adults for the degree of adult pigmentation. Callow or immature adults were those having light or irregular pigmentation of the thorax and elytra; mature adults were those having pigmentation typical of adults emerging from naturally infested billets.

Because last-instar larvae and pupae treated with methoprene produced an array of pupal-adult intermediates or juvenilized adults, we assigned activity ratings from 1 to 5 based on the degree of adult juvenilization as follows:

0 = morphologically normal adults with varying degrees of adult pigmentation
1 = adults that retain part of the pupal exuvium with little or no separation of the elytra
2 = adults with elytra slightly separated or ventrally pointed and with shrunken abdomens at the time of death
3 = pupal-adult intermediates with even cuticular tanning and with the first pair of femurs pointed slightly posteriorly and the tibiae extended
4 = pupal-adult intermediates with the first pair of femurs pointed perpendicular to the body axis, tibiae extended, and with lighter cuticular pigmentation of the abdomen than of the thorax
5 = pupal-like intermediates with little or no change in position of legs and with enlarged, unpigmented abdomens

The percentage of juvenilized adults is that portion of the tested specimens that had activity ratings greater than 0 (excluding those that failed to undergo eclosion), corrected for checks according to ABBOTT (1925). We calculated the activity ratings according to JACOBSON, REDFERN, and MILLS (1975) by summing the numerical activity ratings of all specimens in each experimental group and dividing by the total number of specimens (again excluding those that failed to undergo eclosion). We used log-probit analysis to calculate the median effective dose for juvenilization of 50 % of the adults (ED_{50}), slope function of dose-response curve, and confidence interval (LITCHFIELD and WILCOXON 1949).

The duration of the pupal instar was fixed as the time at which 50 % of the individuals had eclosed plus one-half the time interval over which the pupae were collected (normally 24 h intervals). The time at 50 % eclosion was determined by linear interpolation between the observations with less than and greater than 50 % change. Similarly, longevity of starved adults (time at 50 % dead) was calculated from the time of their collection as pupae minus the duration of the pupal instar. We used one-way analysis of variance to determine whether significant differences existed ($P < 0.05$) between treatment and check values. Because of the variability between check replicates of larvae and pupae collected from different trees, we calculated for each treatment the percentage reduction in the duration of the pupal instar, in the longevity of normal adults, and in the percentage of fully pigmented adults.

3 Results

3.1 Instar developmental sequence

The normal developmental sequence for outer bark larvae, pupae, and adults of the southern pine beetle at 24 °C is illustrated in table 1. Fourth-instar, C-shaped larvae near pupation initially displayed gradual thickening of the thorax. Prepupal larvae were unable to move their mandibles and underwent a transition from movement of each abdominal segment to movement only at the abdominal-thoracic junction. Quiescent or prepupal larvae just before pupation showed a general straightening along the body axis and a clearing of the head capsule. These larvae were extremely sensitive to handling and often failed to shed the larval exuvium at pupation.

Table 1. Normal developmental sequence for outer bark, fourth-instar larvae, pupae, and adults of the southern pine beetle at 24 °C

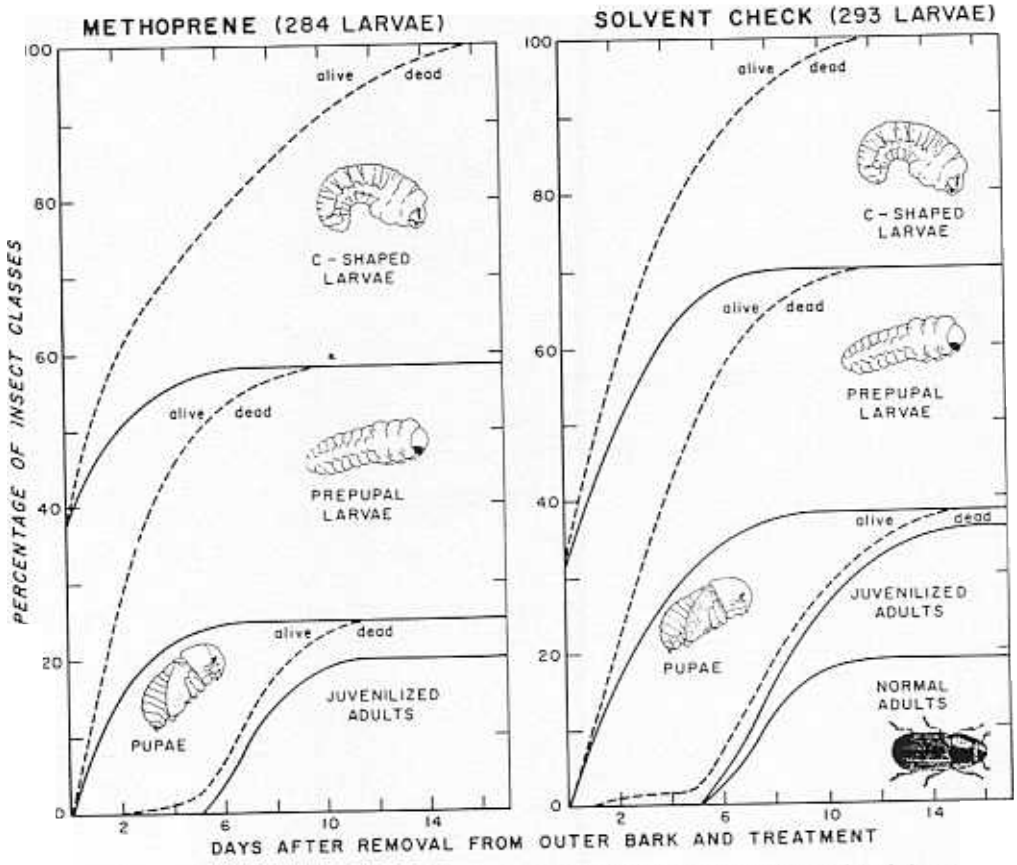
Time before or after pupation (d)	Development
−2.5	C-shaped larvae with gradual thickening of thoracic segments preventing flexing of abdomen against the head or thorax
−1.5	Prepupal larvae with immovable mandibles and abdominal movements primarily in the thoracic-abdominal area
−0.5	Quiescent prepupal larvae with general straightening along body axis and clearing of the head capsule
0.0	*Pupation*
0.1	Pupae without eye or cuticular pigmentation
2.2	Initiation of eye pigmentation
4.3	Initiation of mandibular pigmentation
4.6	Initiation of cuticular pigmentation in coxa, epistoma and epistomal process
4.8	Intiation of overall cuticular pigmentation
5.6	*Eclosion*
5.7	Callow adult with light to uneven cuticular pigmentation of the elytra and ventral side of thorax
10.0	Mature adult with cuticular pigmentation typical of beetles emerging from naturally infested billets
16.0	Median time for death of starved, mature adults

The pupal stage lasted 5.6 ± 0.3 d with no significant differences between

males and females. Pupation exhibited little if any diurnal fluctuations. The first 2 d after pupation, pupae showed no visible developmental progress. Up to 4 d after pupation, the only visible developmental progress was the initiation of eye pigmentation in the enclosed imago.

During the last day of the pupal instar, a rapid sequence of cuticular pigmentation occurred. Initiation of mandible and coxa pigmentation was the easiest pigmentation change to recognize and could be used to determine pupal age of the pupae collected directly from billets. Occasionally, pupae exhibited normal cuticular pigmentation but failed to develop normal eye pigmentation. In approximately 5 % of the pupae, the abdomen unexplainably shrank to less than one-half its normal thickness, and death occurred within a few days afterward. If pupae with either shrunken abdomens or abnormal eye pigmentation eclosed, they never became normal adults.

About 4 d after eclosion, newly eclosed callow adults developed dark brown cuticles characteristic of brood beetles emerging from naturally infested billets. After eclosion, starved adults usually lived about 8 to 10 d.



Developmental and mortality effects of 1 μg of methoprene or 1 μl of acetone on fourth-instar larvae removed from the outer bark of naturally infested billets

3.2 Sensitivity to methoprene

The figure illustrates the developmental and mortality effects of treating outer bark larvae of SPB with 1 μg of methoprene because greater concentrations significantly increased the number of pupae that failed to undergo eclosion. The effects of methoprene treatment on larvae were apparent in both the larval and adult stages. Of the methoprene-treated larvae, 43 % remained as undeveloped, C-shaped larvae, significantly more than the 31 % in the acetone-treated checks.

Methoprene also significantly increased the longevity of undeveloped larvae. Of the larvae that developed to the prepupal stage, methoprene decreased the percentage that completed pupation (48 % vs 61 %). Methoprene-treated larvae that did not pupate within 4 d after treatment never pupated, although some check larvae pupated as long as 8 d after being removed from the outer bark. In check larvae, however, the incidence of abnormal eclosion increased significantly if the larvae failed to pupate 3 or 4 d after removal from the outer bark. Of the larvae that pupated, all developed into morphologically normal pupae that became pupal-adult intermediates or juvenilized adults at the time of eclosion (table 2). Treatment of larvae within one day of pupation significantly reduced the duration of the pupal instar (table 3).

Table 2. Effect on adult juvenilization of 1.0 μg methoprene treatment of fourth-instar larvae of the southern pine beetle at various times in the life cycle[a,b]

Treated stage	Number of Replicates (#)	Percentage Juvenilized Adults (%)	Activity Rating (Maximum = 5) Treated	Checks
C-shaped larvae 72 to 96 h before pupation	2	100*	3.50	1.00
C-shaped larvae 48 to 72 h before pupation	3	100*	3.96	0.72
Prepupal larvae 24 to 48 h before pupation	5	100*	3.98	0.68
Prepupal larvae 0 to 24 h before pupation	6	100*	3.94	0.54
Unpigmented Pupae 0 to 24 h after pupation	15	100*	3.29	0.86
Unpigmented Pupae 24 to 48 h after pupation	15	56*	1.87	0.86
Pupae with pigmented eyes 48 to 72 h after pupation	16	32*	1.54	0.91
Pupae with pigmented eyes 72 to 96 h after pupation	15	30*	1.18	0.90
Pupae with pigmented mandibles 96 to 120 h after pupation	12	18	0.94	0.66
Pupae with overall cuticular tanning 120 to 135 h after pupation	9	14	0.67	0.47

a Each replicate consisted of 9 to 14 specimens
b Percentages corrected for checks by Abbott's formula (1925); percentages followed by an* indicate values for methoprene-treated and checks were significantly different at the 5 % level. Average values for the checks were 27.1 ± 23.1 percent juvenilized adults with an activity rating of 0.80 for 141 replicates

Table 3. Effect of 1.0 μg of methoprene on percentage reduction in the duration of the pupal instar; longevity; and pigmentation of adults after treatment of fourth-instar larvae, pupae, and callow adults of the southern pine beetle at various times in the life cycle[a,b]

Treated Stage	Replicates (#)	Reduction in duration of pupal instar (%)	Reduction in longevity of starved adults (%)	Reduction in fully pigmented adults (%)
C-shaped larvae 72 to 96 h before pupation	2	11.3	–	–
C-shaped larvae 48 to 72 h before pupation	3	4.8	–	–
Prepupal larvae 24 to 48 h before pupation	5	3.1	–	–
Prepupal larvae 0 to 24 h before pupation	6	11.7*	–	–
Unpigmented Pupae 0 to 24 h after pupation	15	7.4*	–	–
Unpigmented Pupae 24 to 48 h after pupation	15	8.6*	22.8*	82.2*
Pupae with pigmented eyes 48 to 72 h after pupation	16	2.2	4.0	46.6*
Pupae with pigmented eyes 72 to 96 h after pupation	15	3.0	2.9	82.5*
Pupae with pigmented mandibles 96 to 120 h after pupation	12	0.0	10.2	73.6*
Pupae with overall cuticular tanning 120 to 135 h after pupation	9	3.0	18.6*	87.5*
Callow adults 0 to 35 h after eclosion	3	–	23.4*	96.0*

a Each replicate consisted of 9 to 14 specimens
b Percentages followed by an * were significantly different at the 5 % level between methoprene-treated and check values. The average values of 141 check replicates for duration of the pupal instar were 5.65 ± 0.43 d, for longevity of starved adults was 10.6 ± 2.6 d and for the percentage of fully pigmented adults at the time of death was 64.5 ± 30.6 %

Treatment of pupae during the last third of the instar produced morphologically normal callow adults, though many of these adults died earlier than checks. Most failed to develop normal adult pigmentation within 6 d after eclosion (table 3). Methoprene treatment during the first two-thirds of the pupal instar produced significant juvenilization (table 2). Pupae less than 24-h old were more sensitive to methoprene than were older pupae, and treatment with 1 μg caused juvenilization of all specimens at eclosion (table 2).

The activity rating for pupae treated within 24 h after pupation was 3.3 (table 2), while the rating was even higher (3.7) for pupae treated within 8 hours after pupation. Treatment of pupae of any age produced lower activity ratings than did the treatment of prepupal larvae (table 2). We found no significant difference in the response of male or female pupae to methoprene treatment.

The juvenilization effects due to varying the concentration of methoprene on day-old pupae are summarized in table 4. The effective dose for juvenilization of 50 % of the adults (ED_{50}) was 0.16 ng per pupa (95 % confidence

Table 4. Effect of methoprene on adult juvenilization of 24 h old pupae of the southern pine beetle[a,b]

Concentration of methoprene (ng)	Juvenilized adults (%)[c]	Activity rating (Maximum = 5)	Frequency of Activity rating 5	4	3	2	1	0
1.0×10^5	100*	3.61	3	16	5	3	1	0
1.0×10^4	98.9*	3.27	4	33	27	17	0	1
1.0×10^3	96.5*	3.18	3	35	23	14	2	3
1.0×10^2	94.0*	3.19	0	38	14	13	0	4
1.0×10^1	92.1*	2.65	1	17	31	18	5	6
1.0×10^0	74.2*	2.14	2	17	17	48	2	20
1.0×10^{-1}	46.6*	1.32	0	4	25	24	14	49
1.0×10^{-2}	15.6	0.93	0	3	10	5	4	38
Acetone only	20.0	0.48	3	18	36	62	44	590
Untreated	11.0	0.26	2	9	26	24	36	710

a Each concentration replicated 8 or more times with 9–12 pupae/replicate
b Values followed by an * indicate treatment values were significantly different from checks at the 5 % level
c Treatment percentages corrected for checks with Abbott's formula (1925)

Table 5. Effect of methoprene on 0–24 h old pupae for % eclosion failure and for % reduction in the duration of pupal instar, longevity and pigmentation of starved adults[a,b,c]

Concentration of methoprene (ng)	Eclosion failure (%)	Reduction in duration of pupal instar (%)	Reduction in longevity of starved adults (%)	Reduction in fully pigmented adults (%)
1.0×10^5	79.4*	0.0	–	–
1.0×10^4	34.1*	1.3	–	–
1.0×10^3	10.8	7.8*	–	–
1.0×10^2	14.8	5.2*	–	–
1.0×10^1	11.8	4.6	–	–
1.0×10^0	12.3	5.0	2.4	38.0*
1.0×10^{-1}	8.5	0.7	9.6	8.4
1.0×10^{-2}	16.5	0.0	0.0	0.0

a Each concentration replicated 8 or more times with 9–12 pupae/replicate
b Values followed by an * indicate treatment values were significantly different from checks at the 5 % level
c The checks had 12.0 ± 12.5 % eclosion failures, and an average duration for the pupal instar of 5.65 ± 0.50 d, an average longevity of starved adults of 9.75 ± 2.6 d and an average percentage of fully pigmented adults at death of 67.0 ± 37.0 %

interval was 0.1–0.25) or about 50 to 70 µg/kg live weight. The slope function of the dose-response curve was 16.6 with a confidence interval of 9 to 32. Frequency of activity ratings from 5 to 1 for over 750 specimens treated with 10 pg to 100 µg of methoprene were 3, 32, 30, 29 and 6 %.

In the checks, activity ratings of 2 and 1 made up more than 65 % of juvenilized adults, because they had retained the pupal exuvium or had partially separated elytra. Juvenilized adults with activity ratings of 5 to 2 never escaped the pupal exuvium. Acetone-treated checks produced significantly more juvenilized adults than untreated checks (20 % vs 11 %). We attributed this high percentage in acetone-treated checks to the damage caused

by handling them and completely covering them with two applications of 0.5 µl of acetone.

Concentrations of methoprene greater than 10 µg/pupa significantly increased the percentage of eclosion failures (table 5). When they were not acutely toxic, concentrations above 100 ng significantly decreased the duration of the pupal instar. Treatment with methoprene, however, occasionally prolonged the pupal instar. For instance, 7 of 250 pupae treated with 1.0 µg of methoprene, but only 3 of over 300 check pupae, remained white, unpigmented pupae that failed to show developmental progress for more than 10 d. The remainder of both groups eclosed within 6 d of treatment.

Callow adults treated within 24 h after eclosion died significantly earlier than checks, and nearly all treated adults failed to develop normal adult pigmentation (table 3). Methoprene treatment of pupae slightly reduced longevity of the resulting starved adults, while higher methoprene concentrations significantly reduced the percentage of these adults that attained normal adult pigmentation.

4 Discussion

A bioassay for the routine screening of effects of juvenoids on SPB could use either outer bark larvae or pupae collected from naturally infested trees. Larvae collected too early in the fourth instar will not pupate and, without a suitable artificial diet, could not be used in a bioassay. Methoprene treatment of older fourth-instar larvae either prevented further development or produced pupal-adult intermediates but apparently did not affect the ensuing pupae. A good bioassay, however, should produce intermediates at the first molt after treatment (Staal 1972). Similar responses have been observed for fourth-instar of *Ips typographus* (Novak et al. 1976), *Tribolium castaneum* (Edwards 1976), and most other coleopteran larvae (Staal 1972).

A frequent response of other coleopteran larvae to methoprene has been the delay of metamorphosis. In SPB the failure of larvae to pupate if treated more than 4 d before pupation indicated delayed metamorphosis as did the increased longevity of C-shaped larvae after the 1 µg methoprene treatment. Non-toxic concentrations of methoprene slightly reduced the duration of the pupal instar rather than prolonging it as in some other coleopteran insects (Novak et al. 1976). As in *I. typographus* (Novak et al. 1976), treatment of last-instar larvae of SPB often caused death at larval-pupal ecdysis. Some of the treated SPB larvae, however, produced morphologically normal pupae that later developed into pupal-like, juvenilized adults unable to leave the pupal exuvium.

Although pupae were slightly less sensitive to methoprene than were larvae, they more nearly met the requirements for a juvenilization bioassay (Staal 1972). They were relatively insensitive to handling, and solvent-treated checks produced fewer than 20 % juvenilized adults. Pupae treated during the first part of the instar produced pupal-adult intermediates at eclosion. In other coleopteran bioassays, the degree of pigmentation was used to synchronize the pupae of *Tribolium castaneum* (Edwards 1976), *Hylobius abietus*, and *Ips typographus* (Novak et al. 1976). For SPB this technique was ineffective because pupal sensitivity to methoprene declined significantly before initiation of eye pigmentation. For bioassays, then, SPB should be collected as larvae and

observed until pupation. The sensitive period of SPB pupae for juvenoids, like other coleopterans (EDWARDS 1976, SLAMA et al. 1974), is the first part of the instar. Based on activity index values for SPB pupae, the routine screening of juvenoids should be done on pupae less than 8 h old.

The slope function of the dose-response curve of SPB to methoprene is characteristic of that of other coloepteran pupae (SLAMA et al. 1974) and reflects the relative insensitivity of SPB to juvenoids. Reduced longevity of adults after juvenoid treatment of SPB pupae has also been observed in *Tribolium* (EDWARDS 1976) and may be a consequence of an increased metabolic rate as seen in adult cockroaches (THATTE and TONAPI 1978).

This study has established that certain immature stages of the southern pine beetle are extremely sensitive to synthetic juvenoids and could, therefore, be affected by host-produced juvenoids in the inner bark microenvironment. Because of the inaccessibility of these immature stages under the bark, the potential of synthetic juvenoids as control agents is very limited. We hope future investigations of the potential role of host-produced juvenoids in host tree resistance may lead to development of additional approaches to control of the southern pine beetle.

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Zusammenfassung

Über den Einfluß des Juvenilhormon-Analogons Methopren auf die Entwicklung von Dendroctonus frontalis Zimm. (Col., Scolytidae)

Zur Bestimmung der Wirkungen von Juvenoiden auf die Entwicklung und Empfindlichkeit des Südkiefern-Borkenkäfers *Dendroctonus frontalis* wurden Altlarven, Puppen und unreife Käfer mit Methopren, einem Juvenilhormon-Analogon, behandelt. Aus diesen Untersuchungen ergab sich eine Anzahl von Juvenoid-Wirkungen auf *D. frontalis*. Methopren zeigte die stärkste Wirkung auf erwachsene Larven sowie auf Puppen innerhalb von 25 h nach der Verpuppung. Es stoppte entweder den Entwicklungsprozeß der Larven oder produzierte Puppen/Adulte-Zwischenformen bei den spätfressenden und Vorpuppen-Larven, welche normal zur Verpuppung kamen. Die mittlere wirksame Dosis für 50%ige Adulte-Juvenilisation von 1 d alten Puppen betrug 0,16 ng. Die Methopren-Behandlung von älteren Puppen und unreifen Adulten verringerte signifikant den Prozentsatz vollpigmentierter Adulter. Die Behandlung der Puppen könnte nützlich zur Bestimmung der Anwesenheit und Wirkung kiefernbürtiger Juvenoide von *D. frontalis* sein.

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