EFFECT OF AN INSECT GROWTH REGULATOR, EXHIBITING JUVENILE HORMONE ACTIVITY, ON LARVAE OF A PINE-DEFOLIATING SAWFLY, DIPRION SIMILIS (HARTIG)

W. H. FOGAL

PETAWAWA NATIONAL FORESTRY INSTITUTE

CANADIAN FORESTRY SERVICE

CHALK RIVER, ONTARIO

and

C. R. SULLIVAN and M.-J. KWAIN

GREAT LAKES FOREST RESEARCH CENTRE

CANADIAN FORESTRY SERVICE

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Information Office,
Great Lakes Forest Research Centre,
Canadian Forestry Service,
Department of the Environment,
Box 490,
Sault Ste. Marie, Ontario
P6A 5M7

ABSTRACT

Topical application of a chemical with active juvenile hormone (a mixture of the cecropia juvenile hormone with its isomers) to first instar larvae of *Diprion similis* (Hartig) causes complete mortality before the fourth instar at the highest concentration tested (10 µg/µl) and has less effect at lower concentrations. The effect of the same concentrations is less with applications to second instar larvae and no mortality is observed with applications to third instars. Applications to penultimate instar larvae appear to prolong larval life, depending on dose, particularly at the beginning or toward the end of the instar. The increase in duration of larval life results from a delay in ecdysis to last instar larvae or the occurrence of supernumerary feeding larvae. A pronounced reduction in the proportions of larvae spinning cocoons occurs with applications toward the end of the penultimate instar but there is little apparent effect on the larva-pupa or pupa-adult metamorphosis within cocoons. Small percentages of adults with missing or reduced legs were observed, but their frequency was not dependent on dose. Egg complements of treated females may be greater or less than those of untreated females, depending on the time of application of the hormone. While food quality may have some effect on egg production, it has little or no detectable effect on other responses to juvenile hormone.

Implications for the possible use of hormone-active chemicals in control of sawflies are discussed.

RÉSUMÉ

L'application locale d'un insecticide chimique contenant une hormone juvénile active (un mélange de l'hormone juvénile Cecropia avec ses isomères) sur des larves de premier stade du *Diprion similis* (Hartig) cause une mortalité complète avant le quatrième stade avec la plus forte teneur essayée (10 µg/µl) et a moins d'effet à des teneurs plus faibles. L'effet des mêmes teneurs est moindre par l'application aux larves du deuxième stade et on n'observe aucune mortalité chez les larves du troisième stade. Des applications sur des larves de l'avant-dernier stade semblent prolonger la vie larvaire, selon la dose, particulièrement au début ou vers la fin du stade. L'augmentation de la durée de vie larvaire résulte d'un délai dans l'ecdysis des larves du dernier stade, ou de la présence de larves se nourrissant en surnombre. On observe une réduction prononcée des proportions de larves filant des cocons avec les applications vers la fin de l'avant-dernier stade mais il y a peu d'effet apparent sur les métamorphoses de larve-pupe ou de pupe-adulte à l'intérieur des cocons. On a observé de faibles pourcentages d'adultes manquant des pattes ou ayant des pattes trop courtes mais leur fréquence ne dépendait aucunement de la dose administrée. Les oeufs des femelles traitées peuvent être plus ou moins nombreux que chez les femelles non traitées, selon le temps d'application de l'hormone. Alors que la qualité de l'alimentation peut agir sur la production d'oeufs, elle n'a que peu ou pas d'effet sur d'autres réactions aux hormones juvéniles.

Il est question des implications dues à l'utilisation possible d'insecticides chimiques contenant des hormones pour la répression des Diprions.

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INTRODUCTION

A number of diprionid sawflies are common defoliators of pine trees in Ontario (Wallace 1961) and insect growth regulators may have some potential in management strategies aimed at reducing insect numbers. Spraying egg clusters of *Neodiprion sertifer* (Geoff.) with chemicals exhibiting juvenile hormone activity reduces egg hatch under laboratory and field conditions (Fogal and Sullivan 1976) and application of an inhibitor of chitin synthesis to pine foliage blocks larval molting of *Diprion similis* (Hartig); if larvae are in early instars the latter chemical may provide foliage protection (Fogal 1977). Applications of insect growth regulators exhibiting juvenile hormone activity to larval stages of insects usually prolong larval life and will likely increase defoliation. However, population density and damage in the subsequent generation might be reduced by interference with metamorphosis and egg production.

This report describes laboratory studies to evaluate the effects of insect juvenile hormone on larval stages of *Diprion similis*. Chemicals with active juvenile hormone were applied to early instar larvae and were assessed for lethal effects up to the fourth instar, when damage from feeding becomes more evident. Penultimate (fifth) instar larvae were used to determine the effect of a chemical with active juvenile hormone on molting, metamorphosis and egg production. Larvae used in these tests were reared on foliage from four different hosts: jack pine (*Pinus banksiana* Lamb.), Scots pine (*P. sylvestris* L.), white pine (*P. strobus* L.), and red pine (*P. resinosa* Ait.), to determine possible interactions between host plant and hormone action. Differences in the nutritive value of foliage from these hosts have already been described (Fogal 1974).

MATERIALS AND METHODS

A. Comparison of Acetone and Octane as Solvents

Wigglesworth (1969) used octane in tests on *Rhodnius prolixus*. In the present experiments octane was compared with acetone as a solvent by treating 1- to 3-day-old male penultimate instar larvae with solvents only or with solvents plus 10 µg per µl of juvenile hormone (AY-22342-3: a mixture of the cecropia juvenile hormone with its isomers supplied by Ayerst Research Laboratories, Saint-Laurent, Quebec).[1]

B. Early Instar Treatments

First, second, and third instar larvae were obtained from a continuous culture of *Diprion similis* collected originally near Chatsworth,

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[1]The identification of commercial products is solely for the information and convenience of the reader and does not constitute endorsement by the Great Lakes Forest Research Centre.

Ontario and reared as described previously (Fogal and Webb 1976) under the following conditions: 21-23°C, 60-70% RH and a 16-hr photophase. Larvae were treated with 1 µl of a solution of AY-22342-3 or a juvenile hormone mimic (R-20458-B: a preparation containing 75% 4-ethyl phenyl-6, 7-epoxy geranyl ether supplied by Stauffer Chemical Co., Mountain View, California) in acetone to give the following doses: 0, .1, or 10 µg. Treated and untreated control larvae were reared in lots of 10 to 20 and assessed for survival to the fourth instar.

C. Hormone Application to Penultimate Instar Larvae on Four Different Hosts

Foliage was collected from plantations of red pine, jack pine and Scots pine at a field insectary near Sault Ste. Marie as described elsewhere (Fogal and Kwain 1972). White pine was collected from naturally regenerated trees of undetermined ages. First and second instar larvae were obtained from a continuous culture of *Diprion similis* collected originally near Thunder Bay, Ontario and reared as previously described (Fogal and Webb 1976) in separate batches on one of the four species of foliage. Penultimate instar larvae were treated with 1 µl of a solution of AY-22342-3 in acetone to give the following doses: 0, 1, 10 or 100 µg on the day of ecdyses or 3 or 6 days after for females; at ecdysis or 2 or 4 days after for males. Individual wet weights of up to 20 larvae were determined at time of treatment. Treated larvae and untreated control larvae were reared in screen-covered 16-oz (approx. 454 ml) jars on the same foods they had eaten in earlier instars. Up to 10 larvae were allotted to each jar. Average numbers of larvae per treatment per host per day were: males, 34 (minimum 26); females 23 (minimum 16), except for treatment day 6, where the value was 14 (minimum 6). Food in the jars was changed daily until all larvae had ecdysed. The following data were recorded: (1) number ecdysing to intermediate type larvae and supernumerary instars, spinning cocoons, and numbers of emerging adults (data presented in text as percentages of initial larvae); (2) number of diapause larvae and dead larvae, pupae or adults in cocoons remaining after 3 months of incubation in shell vials under rearing room conditions (data presented in text as percentages of cocoons examined); (3) number of adults (emerged and dead within cocoons) with appendicular abnormalities; (4) number of eggs in emerged females.

RESULTS

1. Comparison of Acetone and Octane as Solvents

Acetone alone, applied to penultimate instar larvae, had little effect on duration of the instar, percentage of last instar larvae

produced, or percentage of cocoons obtained. In contrast, octane appeared to have a deleterious effect, increasing the duration of the penultimate instar and reducing the numbers of last instar larvae. Therefore, acetone was used in all subsequent experiments herein reported.

2. Early Instar Treatments

Results shown in Table 1 reveal that no larvae treated in the first instar survived to the fourth at the highest concentration tested (10 µg/µl) with either insect growth regulator. A small reduction in survival appears to occur with treatment at 10 µg/µl in the second instar. No effect was noticeable in larvae treated at the third instar. At lower concentrations, effects were less pronounced. No consistent differences between the two growth regulators were observed. Since no differences were found between the two growth regulators and because AY-22342-3 contained natural cecropia hormone as a major component, it alone was selected for subsequent experiments.

Table 1. Effects of application of two insect growth regulators (AY-22342-3 and R-20458-B) to first, second, and third instar larvae of *Diprion similis* on percentage survival to the fourth instar.

Instar treated	Insect growth regulator	Untreated control	Acetone control	Regulator concentration µg/µl .1	1	10
1	AY-22342-3	70	65	39	60	0
	R-20458-B	--	--	45	45	0
2	AY-22342-3	90	53	50	80	20
	R-20458-B	--	--	75	40	45
3	AY-22342-3	80	85	95	80	80
	R-20458-B	--	--	85	90	80

3. Effect of Four Species of Foliage on Weights of Penultimate Instar Larvae

As a first step in determining the effect of AY-22342-3 on penultimate instar larvae, the wet weights of larvae reared on four host species were obtained to provide a basis for determining possible

relationships between larval weight and dose of applied hormone. Table 2 shows the wet weights of penultimate instar larvae feeding on the four species of foliage at days 0 (day of ecdysis), 2 or 4 for males and days 0, 3 or 6 for females. No differences were detectable at day 0 for either sex. For males at day 2, weights on jack pine and Scots pine were higher than those on white pine and red pine; at day 4, weights on Scots pine were higher than those on white pine and red pine, whereas an intermediate value was observed on jack pine. For females, at day 3, weights were highest on Scots pine and lowest on white pine, with intermediate values on jack pine and red pine: at day 6, results were essentially similar but levels of significance varied slightly.

Table 2. Wet weights (mg) (mean ±SE) at days 0, 2 or 4 for male and day 0, 3 or 6 for female penultimate instar larvae of *D. similis* reared on jack pine, Scots pine, white pine or red pine.

Sex	Day	Jack pine	Scots pine	White pine	Red pine
Males	0	53±2 (20)^{a}	55±2 (20)^{a}	49±2 (20)^{a}	51±1 (20)^{a}
	2	93±2 (20)^{a}	93±2 (20)^{a}	80±1 (20)^{b}	81±2 (20)^{b}
	4	112±3 (20)^{ac}	116±2 (20)^{a}	106±3 (20)^{bc}	105±2 (20)^{bc}
Females	0	74±2 (10)^{a}	78±4 (12)^{a}	73±3 (13)^{a}	74±3 (13)^{a}
	3	153±4 (13)^{ac}	162±7 (8)^{a}	133±4 (12)^{b}	141±2 (8)^{bc}
	6	184±3 (12)^{b}	201±5 (4)^{a}	170±5 (10)^{c}	184±6 (5)^{abc}

Note: Species of foliage compared with Duncan's multiple range test following analysis of variance. Means bearing the same letter are not significantly different. Numbers of larvae are given in parentheses.

4. *Effects of Dose and Time of Application of Hormone on Penultimate Instar Larvae of* Diprion similis *on Four Host Plants*

Ordinarily, penultimate instar larvae of *Diprion similis* molt to a non-feeding larval stage characterized most strikingly by reduced pigmentation in comparison with the feeding instar (Coppel et al. 1974). Intense black melanin pigmentation of the head capsule is reduced to brown or grey, black pigmentation of the body is less intense and the whole larva looks pale green (Fig. 1A). Treatments with hormone result in a molt to a supernumerary typically pigmented feeding instar (Fig. 1B)

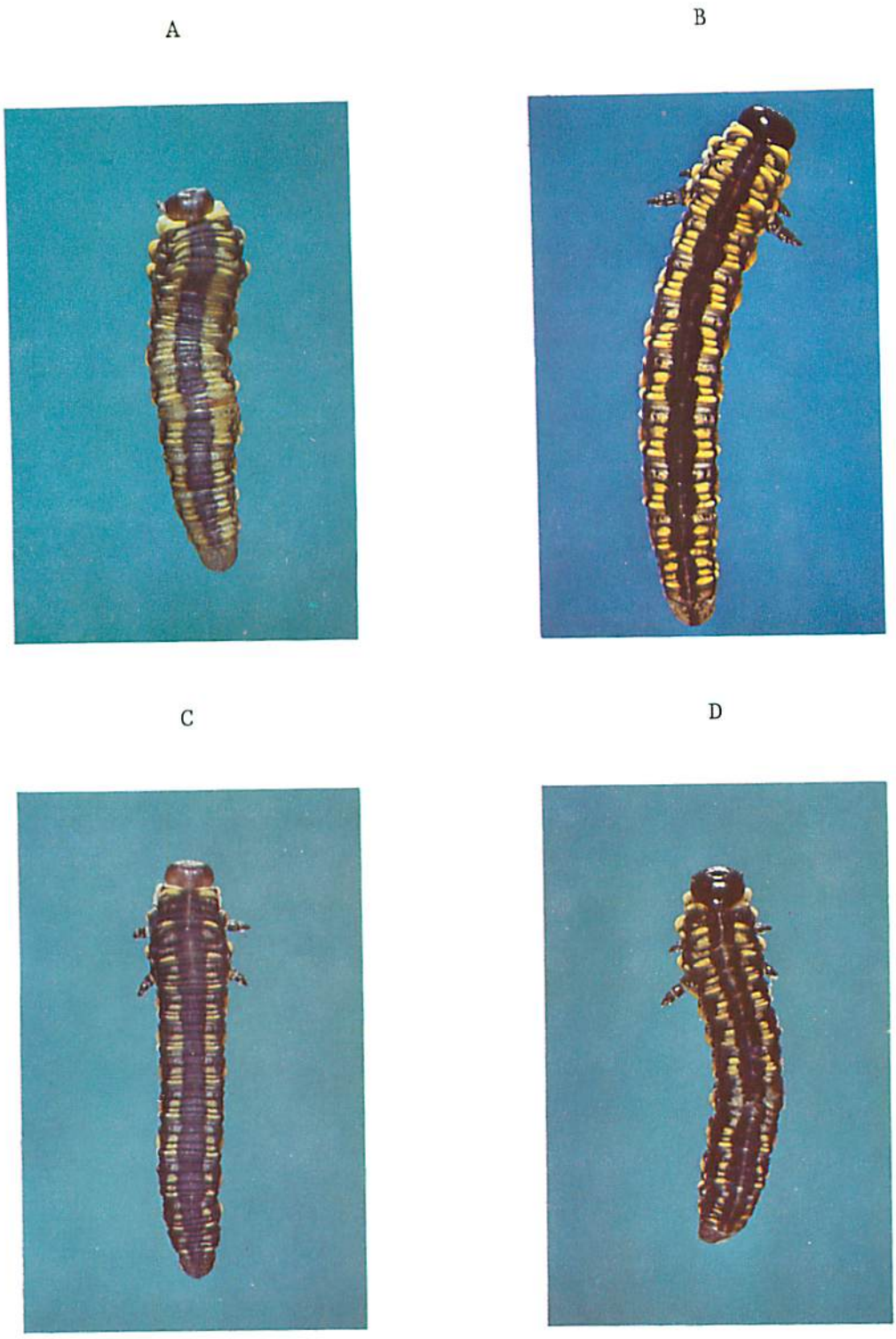

Figure 1. A, Last-instar larva of *Diprion similis*; B, perfect supernumerary larva; C, larva, intermediate between last instar and supernumerary instar with heavy melanin pigmentation of body wall but none on head capsule; D, larva, intermediate between last instar and supernumerary instar with melanin pigmentation of body wall and head capsule and retention of shape of last instar.

or prolongation of the instar without an additional feeding instar, or intermediate types with varying degrees of melanin pigmentation (Fig. 1C and D). Treatments also affect survival to cocoons or adults and egg production. Quantitative assessments of the results follow.

a. Incidence of intermediate larvae, supernumerary larvae, survival to cocoon spinning, and adult emergence: In males (Fig. 2) no intermediates occurred in untreated controls of day 0 or day 2 treatments; a small percentage occurred with acetone treatment. Slightly more occurred with hormone treatment; at day 0 no increase was apparent with increased dosage; at day 2 they were most abundant at the intermediate dose of 10 µg. At day 4 a small number of intermediate larvae resulted with the acetone treatment and with the two higher doses of hormone. At the lower dose (1 µg) of hormone, there was an apparent increase in the number of intermediate larvae produced, particularly among the sample fed on jack pine. Similar results were obtained with females (Fig. 3) except that acetone appeared to have a more pronounced effect on the last day of treatment, again, particularly for those feeding on jack pine.

The percentages of supernumerary larvae reached a high of 80% in males and about 70% in females at the highest dose with a clear relationship between inoreased dose and increase in percentage of supernumerary larvae (Fig. 2 and 3). No obvious relationships between percentages of supernumeraries and food type were evident. Day of treatment may influence responses; for both males and females relatively high percentages of supernumeraries occurred at the 10 µg concentrations in larvae treated at the beginning and near the end of the instar, but few or none occurred at the intervening treatment time. On the last day of treatment there appeared to be a trend to an inverse relationship between percentages of intermediates and supernumeraries as hormone dose increased.

Survival to cocoon spinning decreased as the hormone concentration increased. For males (Fig. 2) and females (Fig. 3) the effect of increasing concentration was most pronounced for larvae treated on day 4 and least pronounced for larvae treated on day 0. Food type had little noticeable effect on survival.

Percentages of adult emergence tended to follow the trends of survival to cocoon spinning at slightly lower levels. Few or no larvae treated near the end of the penultimate instar with doses of 10 and 100 µg survived to adults.

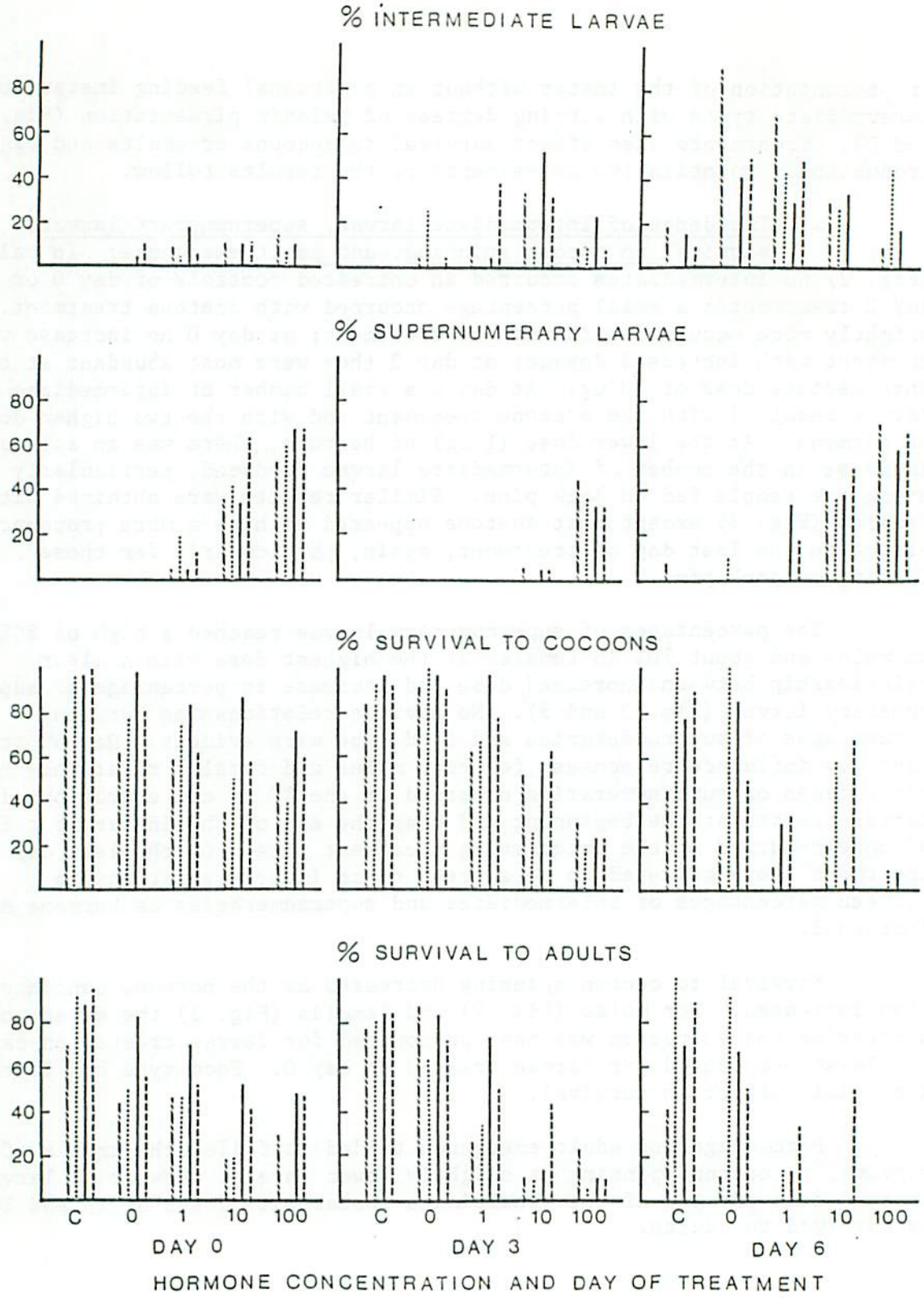

Fig. 2. Effect of hormone concentration (µg/µl) and time of treatment of male penultimate instar *Diprion similis* feeding on four different hosts, on percentages of intermediate larvae, supernumerary larvae, survival to cocoons and to adults. C = untreated larvae; 0 = acetone treated larvae (percentages based on numbers of initial larvae).

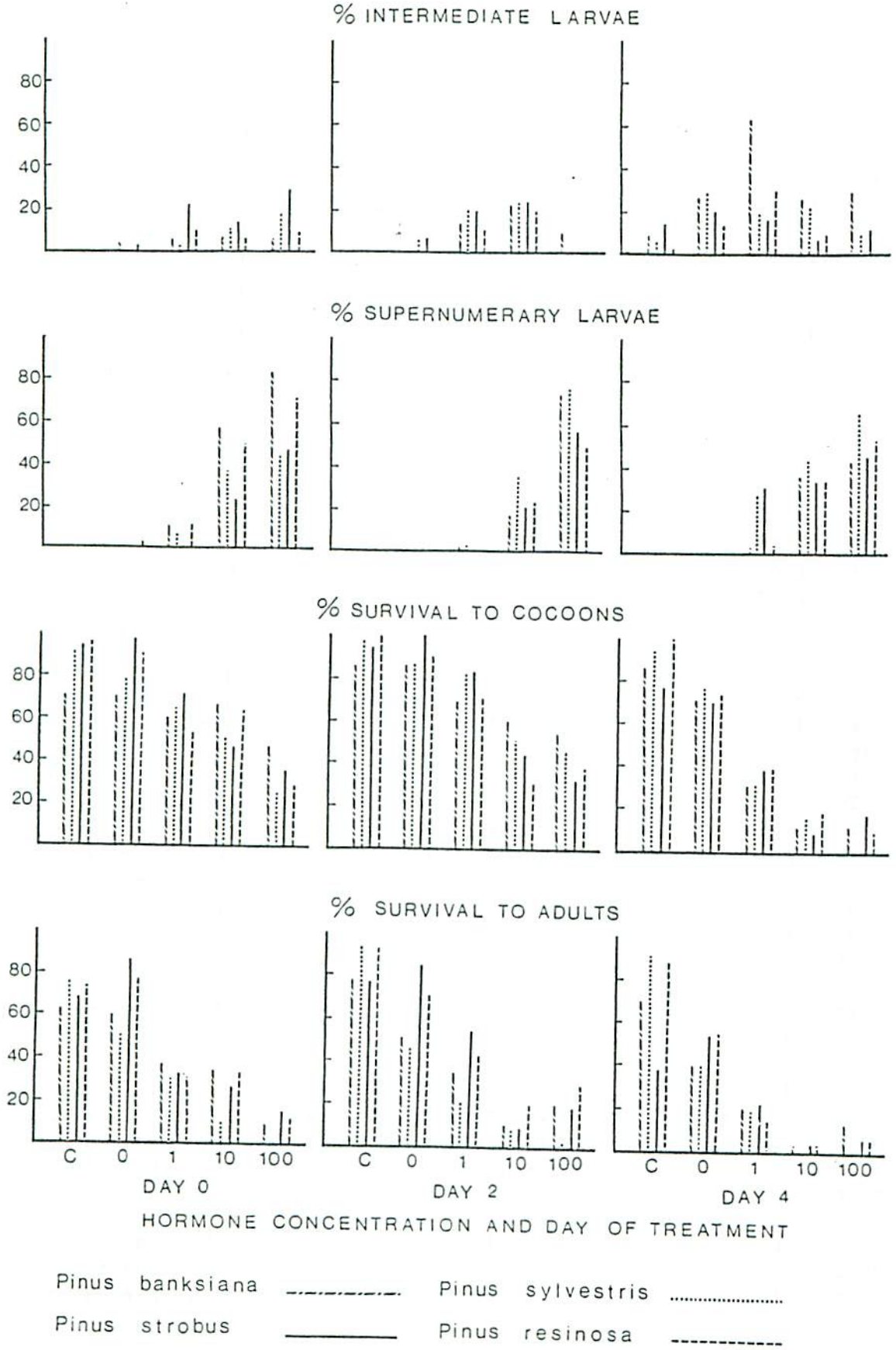

Fig. 3. Effect of hormone concentration (µg/µl) and time of treatment of female penultimate instar *Diprion similis* feeding on four different hosts, on percentages of intermediate larvae, supernumeraries, survival to cocoons and to adults (percentages based on numbers of initial larvae).

b. Duration of development: Application of juvenile hormone to penultimate instar larvae prolonged the duration of larval development either by prolonging the fifth instar or by inducing ecdysis to a sixth feeding instar. In penultimate instar larvae which ecdysed directly to last instar larvae, the duration of development increased progressively with increasing concentrations of hormone at treatment days 0 and 2 in males; at day 4 at the highest dose no increase over acetone controls was evident for jack pine and Scots pine whereas increases were evident on the other two foods (Fig. 4). For females (Fig. 5) the same was true with treatments on days 0 and 3; on day 6, there was little increase with dose.

In cases where supernumerary larvae occurred, the duration of the fifth instar was similar to that of untreated larvae for both males (Fig. 4) and females (Fig. 5). The additional instar extended the duration of feeding of such larvae to approximately the same length as that of treated specimens which did not produce supernumeraries. In general, death of larvae tended to occur about the time they would normally molt to the last instar.

c. Fate of unemerged specimens: No larva-pupa or pupa-adult intermediates were detectable by examination of external morphology, but many specimens had died as larvae, pupae or adults. As expected for this sawfly (Coppel et al. 1974) some specimens were live diapause larvae. Percentages of diapause larvae, dead larvae and dead pupae or adults for each hormone treatment and treatment day are shown in Table 3. Because of the small numbers of cocoons available, results on all foods were lumped together. For all treatments except for those of females on day 3, the highest proportions of diapause larvae were found among untreated specimens. Smaller proportions occurred among specimens treated with acetone and the lowest hormone dose, whereas none were present in cocoons treated with the higher doses. Over half or nearly half of the insects had died as larvae except for day 0 and day 2 untreated males. The remaining specimens had died as pupae or adults with the largest proportions among specimens treated with acetone and hormone.

d. Abnormalities of unemerged and emerged adults: Examination of dead adults for abnormalities revealed that 13 (7 females, 6 males) out of 82 had abnormal appendages. Of these, 12 had abnormal legs (reduced or missing claws, tarsi, tibia or femurs) and on one an antenna was missing. From a total of 1,360 emerged adults, only 76 (29 females, 47 males) had abnormal development of appendages. There were 69 adults with leg abnormalities, four with missing antennae and three with reduced wings or sexual appendages. No relationships between abnormalities and dose, day of treatment or food type were evident.

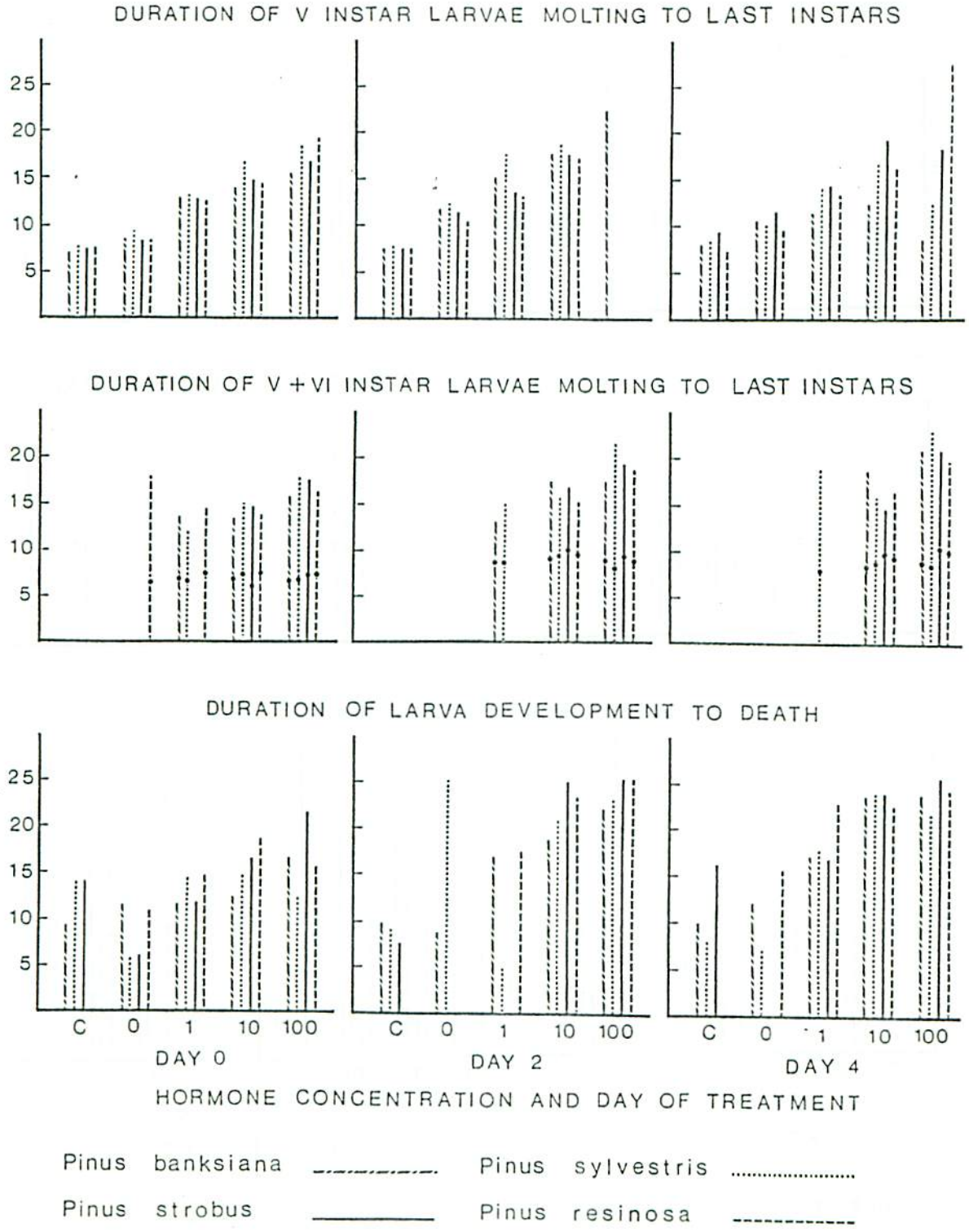

Fig. 4. Effect of hormone concentration (µg/µl) and time of treatment of male penultimate instar *Diprion similis* feeding on four different hosts, on duration of fifth instars molting to non-feeding last instars, fifth instars molting to non-feeding last instars, fifth plus sixth feeding instars molting to last instars, and larvae dying before the molt to the last instar.

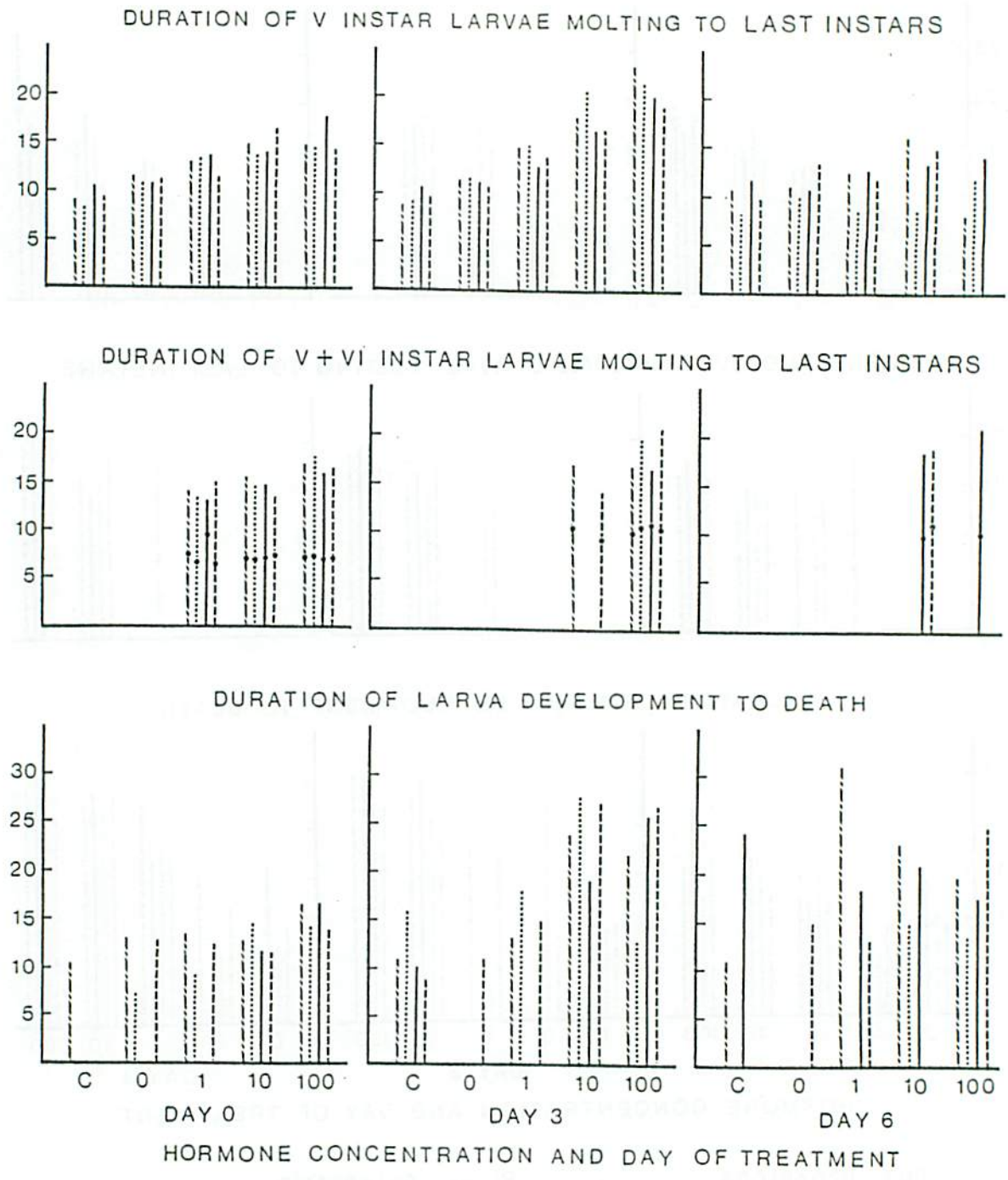

Fig. 5. Effect of hormone concentration (µg/µl) and time of treatment of female penultimate instar *Diprion similis* feeding on four different hosts, on duration of fifth plus sixth (supernumerary) feeding instars molting to non-feeding last instars, and larvae dying before the molt to the last instar.

Table 3. Effects of hormone concentration and time of treatment of penultimate instar *Diprion similis* on the percentage of diapause larvae, dead larvae, and dead pupae or adults within cocoons from which adults failed to emerge (results from four host foods combined).

Stage of development	Hormone concentration (µg/µl)	Sex and time of treatment (day)					
		Male			Female		
		0	2	4	0	3	6
Diapause larvae (%)	Untreated	68	66	28	25	18	50
	0	18	5	9	0	19	0
	1	13	0	0	0	5	0
	10	0	0	0	0	0	0
	100	0	0	0	0	0	0
Dead larvae (%)	Untreated	21	17	52	75	73	50
	0	46	65	63	78	50	60
	1	59	82	74	77	77	83
	10	79	77	93	48	72	75
	100	79	68	80	68	86	100
Dead pupae or adults (%)	Untreated	11	17	20	0	9	0
	0	36	30	28	22	31	40
	1	28	18	26	23	18	17
	10	21	23	7	52	28	25
	100	21	32	20	32	14	0

e. Egg production: The average number of eggs in females from larvae which were fed on jack pine and treated with hormone on day 0 increased with increasing doses (Fig. 6). The difference between the acetone control and the two highest hormone concentrations was found to be significant at the 5% level of probability with Duncan's multiple range test. On other foods increases with increasing doses were not evident. In females from larvae treated on days 3 and 6, egg counts for hormone treated specimens were not significantly higher than those for specimens fed on any diet and subject to acetone controls.

A notable feature in females receiving no treatment or acetone on days 0 and 3 was the larger number of eggs from larvae reared on jack pine and Scots pine in comparison with those from larvae reared on white pine and red pine: on day 6, egg complements were high on jack pine only. Duncan's multiple range test indicated that differences were significant at the 5% level of probability. In most instances on days 0 and 3, with hormone applications, females from larvae reared on jack

pine produced larger egg complements. Larger egg complements on jack pine and Scots pine may be a reflection of the higher nutritive value of these two species in comparison with red pine and white pine (Fogal 1974)..

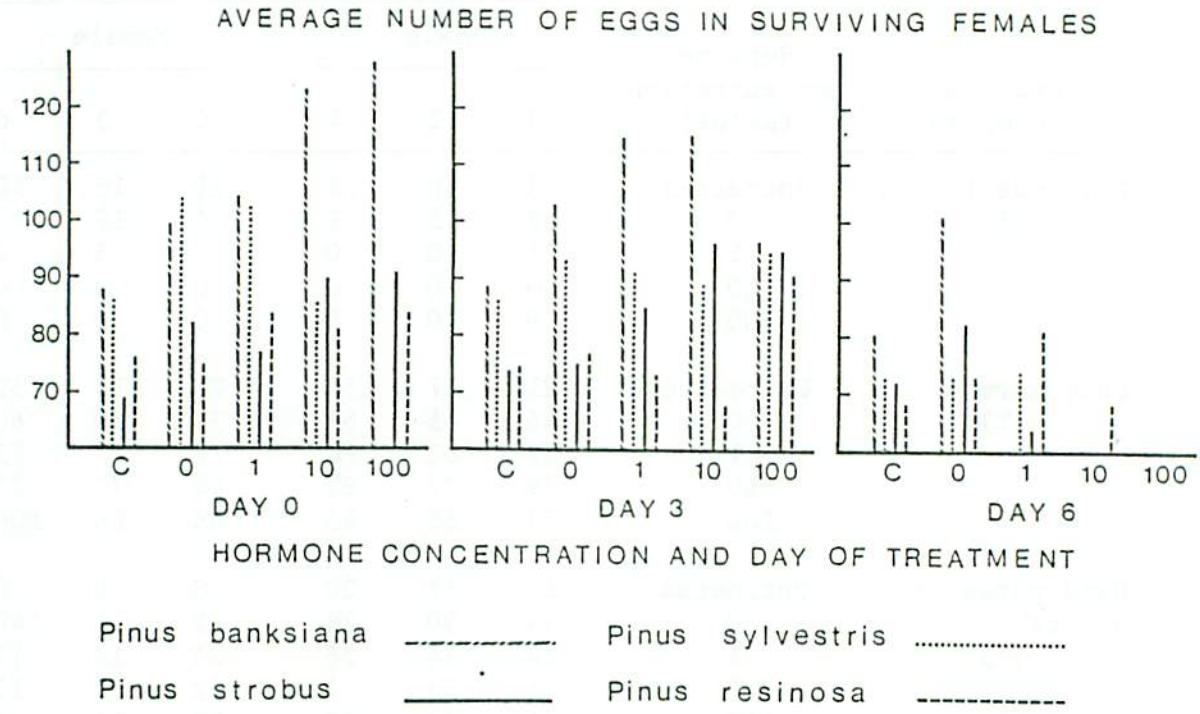

Fig. 6. Effect of hormone concentration (µg/µl) and time of treatment of female penultimate instar *Diprion similis* feeding on four different hosts, on number of eggs in surviving females.

DISCUSSION

Change in titre of juvenile hormone during development has been a central concept in explaining hormonal control of metamorphosis in endopterygote insects. According to this concept, excessive amounts of juvenile hormone result in larva-larva ecdysis; at intermediate concentrations or in the absence of hormone, pupae or adults are formed. Experience indicates that applications of chemicals with active juvenile hormone result in the formation of supernumerary larvae, increase in duration of larval life and interference in metamorphosis (Masner et al. 1976, Slama 1975). Time of application is crucial to the degree of expression of effects since cells and tissues are sensitive to hormone only at certain times (Slama 1975). Many cases of differential sensitivity at the organ, tissue and cell level have been reported (Willis 1974). Applied hormones may also be inactivated (Gilbert and Schneiderman 1960). Therefore, the compound may be lost before the

sensitive period if it is applied too early, and if it is applied too late, tissues are no longer sensitive. In addition, juvenile hormone treatments may lead to ecdysone deficiency (Masner et al. 1976, Srivastava and Gilbert 1969).

At the highest concentration tested (10 µg/µl) juvenile hormone prevents development to the fourth instar when applied to first instar larvae of *Diprion similis*. The effect is less pronounced in treatments of second instars and no effect is evident when third instars are treated. Perhaps this inhibition of development in the first instar is an antiecdysis effect resulting from ecdysone deficiency as suggested for mortality observed in the early instars of *Blatella germanica* (Hangartner and Masner 1973, Masner et al. 1976). The lower effect with second and third instars may result from lower doses per unit weight as the larvae gain weight in successive instars.

Hormone applications increase the duration of the penultimate instar of *Diprion similis*. The effect appears to be dependent on dose over the range tested except in females treated 6 days into the instar and males feeding on jack pine or Scots pine and treated on day 4. By then the sensitive period may have passed. Hormone induces supernumerary larvae in a dose-dependent fashion as well, but the response is most pronounced on the first and last days of treatment. Few or no supernumeraries occur in females treated during the intermediate period; the larger response in males may be due to larger doses per unit of body weight because of their smaller size. Within sexes, increase in body size appears to be overridden by other factors determining whether or not a supernumerary molt occurs. Tissues may be most sensitive at the beginning and toward the end of the instar. Perhaps applied hormone is readily inactivated in the intervening period. This is suggested by studies in other insects, e.g., *Galleria mellonella* (Hsiao and Hsiao 1977), in which endogenous level of hormone is low in the intermolt period of the larval instar. Alternatively, the relative indifference to hormone at the intermediate times of application may be due to a requirement of larvae to utilize metabolic capabilities fully for accumulation of reserve materials. This appears to be the time of maximum growth rate in a related sawfly, *Neodiprion sertifer* (Janda 1961). It is interesting that ecdysis to a supernumerary instar occurs close to the time of ecdysis to the last instar of untreated specimens, indicating that time of ecdysone release is not altered. In cases where the duration of the penultimate instar is prolonged without a supernumerary instar, juvenile hormone application may inhibit release or synthesis of ecdysone as it appears to do in *Blatella germanica* (Hangartner and Masner 1973) and *Sarcophaga bullata* (Srivastava and Gilbert 1969). If so, inhibition is less pronounced as dose increases.

Diprion similis, like all hymenoptera, has three metamorphic molts, whereas other holometabolous insects have two. The first, which we have already described, is the molt from a penultimate instar feeding

stage to a last instar which does not feed and spins a cocoon. Intermediates usually resemble the feeding stage in external pigmentation but are unable to feed, perhaps because the gut has undergone some degree of metamorphosis. If so, this would suggest that viscera are less sensitive than integumentary structures. This would be similar to the effect of juvenile hormone on the last instar of *Hyalophora cecropia* in which metamorphosis of the interal organs is unaffected when hormone is applied prior to the onset of spinning (Riddiford 1972). The largest numbers of intermediates were obtained at the lowest dose applied on the last day of treatment. There was an inverse relationship between the numbers of intermediate and supernumerary larvae obtained. Hence, while juvenile hormone produced the largest number of supernumeraries when applied at the beginning and toward the end of the instar, its effect on the first metamorphosis was most pronounced when applied toward the end of the instar. Survival to cocoons decreased with dose and the response became more pronounced with later times of treatment. Death prior to cocoon formation is likely due to interference in events associated with ecdysis since most larvae died about the time they would normally ecdyse to the last instar. For all treatments adult emergence was only marginally less than cocoon formation. Apparently hormone has little effect following cocoon formation.

Failure of adults to emerge from cocoons is due to the occurrence of diapause (Coppel et al. 1974) or to the death of larvae, pupae or adults. In untreated specimens a high proportion of cocoons failing to produce adults contained diapause larvae, whereas specimens treated with acetone and the lowest hormone dose contained a small proportion of diapause larvae, and none were found in cocoons receiving the two higher doses of hormone. The largest proportions of dead pupae and adults were found among specimens treated with acetone or hormone. The data suggest that acetone or hormone treatments reduce the number of larvae entering diapause, allowing some to develop to pupae or adults before dying. Juvenile hormone inhibits diapause in *Hyalophora cecropia* (Riddiford 1972) and induces diapause when applied to last instar larvae of the southwestern cornborer, *Diatraea grandiosella* (Yin and Chippendale 1973) and the rice stem borer, *Chilo suppressalis* (Yagi and Fukaya 1974). In our experiment acetone applications also affected diapause.

No larval-pupal or pupal-adult intermediates were obtained in the sawfly. Leg abnormalities were observed in emerged and unemerged adults but these were thought to be artifacts resulting from injury in the larval stages since they occurred in untreated and acetone controls and their occurrence did not increase with increased doses of juvenile hormone. The absence of effect on the larval-pupal and pupal-adult transformations suggests that hormone was applied before the critical time for affecting development of adult cuticular structures; perhaps the hormone is inactivated or excreted well before the time that precursor cells of adult structures are competent to respond to hormone.

Hence, juvenile hormone applied to penultimate instar larvae has little influence on larval-pupal and pupal-adult transformations.

Response of *Diprion similis* differed little with food type, but in other respects, molting and metamorphosis are modifiable by nutrition. It has been suggested that this is the result of changes in ecdysone production or ability of tissues to react to ecdysone supply (Fourche 1967). It has also been suggested that factors associated with somatic size play a decisive role in controlling the number of larval instars and hence the onset of metamorphosis (Nijhout 1975). In *Diprion similis*, size differences of larvae feeding on different foods at the beginning of what was presumed to be the penultimate instar were significant but they may not have been large enough to influence production of supernumerary larvae.

Practical suggestions concerning assays of juvenile hormone or mimics emerge from these investigations. First, in assessing effects of applications of juvenile hormone to larval stages it might be prudent to separate effects on metamorphosis from effects on larval development. In the case of the sawfly, lumping scores for supernumerary larvae with scores for low survival to adults would have masked some effects of time of administration on responses. Second, possible effects of acetone or any other solvent should be considered when one is assessing effects of topical applications of juvenile hormone. Comparisons of octane and acetone as solvents indicated that the latter had less deleterious effects, yet acetone alone does have effects which could be misinterpreted as effects of juvenile hormone. Effects were noted on the formation of intermediates and the induction of diapause. Acetone is used widely as a solvent for laboratory insecticide tests and has been shown to have adverse effects on other insects (Critchley and Almeida 1973).

The results also have implications for the possible use of chemicals with active juvenile hormones in control of diprionid sawflies. Treatments may afford little protection from damage by a current generation of larvae unless applied very early in larval life. Applications to first instar larvae prevent development to the fourth instar. Up to this stage larvae cause little feeding damage. This knowledge coupled with the knowledge that sprays applied to eggs cause mortality in the first instar or prior to hatch (Fogal and Sullivan 1976) permits an extension of the period of time over which sprays might be applied. When sprays are applied to later instar larvae, there is a likelihood of increased feeding and damage because of the increased length of larval life. However, when they are applied late in the penultimate instar interference in ecdysis and metamorphosis of feeding to non-feeding larvae is highly likely and will result in decreases in the number of adults.

Reductions in adult numbers are not likely to be counterbalanced by increases in female egg complements, which may occur as a result of application to larvae near the beginning of the penultimate instar.

This conclusion is reinforced by the results of a simulation study of the total number of eggs produced from samples of 20 larvae from each treatment. Egg complements of the appropriate number (based on actual percentage survival figures) of surviving adults were selected at random from experimental data except when actual larval samples were fewer than 20. In these cases mean egg complements for the treatment were used to fill in missing data. Sums of egg complements for each treatment are shown in Table 4. A three-way factorial analysis of variance (Sokal and Rohlf 1969) of the simulated data indicated significant difference at the 5% level of probability among treatment days and hormone concentration, but not among foods. However, differences among days depend on the type of food eaten. Reductions in egg population with increased hormone concentration are evident at all treatment days but are most pronounced on the last day. Hence, the application of hormone to late penultimate instar larvae will likely reduce a population of larvae in the subsequent generation.

Table 4. Total number of eggs produced by simulated samples of 20 female larvae treated with different concentrations of juvenile hormone on 3 successive days (days 0, 3, and 6) in penultimate instar larvae reared on four different pine species. (Total eggs = the sum of egg complements in actual specimens selected at random from survivors. Differences among treatments tested by three-way ANOV.)

		Treatment				
				Hormone concentration (µg/µl)		
Day of treatment	Species	Untreated control	Acetone treatment	1	10	100
0	jack pine	1165 (13)	802 (8)	952 (9)	371 (3)	768 (6)
	Scots pine	1347 (16)	1005 (10)	929 (9)	343 (4)	no survivors
	white pine	1314 (18)	1370 (17)	1010 (13)	896 (10)	821 (9)
	red pine	1365 (18)	679 (9)	836 (10)	566 (7)	672 (9)
3	jack pine	1180 (14)	1635 (16)	575 (5)	230 (2)	393 (4)
	Scots pine	1183 (14)	1120 (12)	634 (7)	178 (2)	95 (1)
	white pine	1235 (17)	1259 (17)	1259 (15)	289 (3)	190 (2)
	red pine	1030 (14)	1086 (14)	737 (10)	638 (9)	182 (2)
6	jack pine	565 (7)	202 (2)	no survivors	no survivors	no survivors
	Scots pine	1348 (17)	1311 (18)	222 (3)	no survivors	no survivors
	white pine	996 (14)	1061 (13)	189 (3)	no survivors	no survivors
	red pine	1017 (15)	731 (10)	568 (7)	476 (7)	no survivors

NOTE: The number of female survivors is given in parentheses.

Three-Way Factorial ANOV

Source of variation	df	SS	MS	F
day	2	1975231.3	987615.7	22.27*
dose	4	7572312.7	1893078.2	42.69*
food	3	338468.2	112822.7	2.54
day x dose	8	725761.0	90720.1	2.05
day x food	6	710424.2	118404.0	2.67*
dose x food	12	852887.1	71073.9	1.60
day x dose x food	24	1064188.8	44341.2	
Total	59	13239273.3		

*Difference significant at the 5% level of probability.

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