LABORATORY EVALUATION OF THE EFFICACY OF THE JUVENILE HORMONE ANALOG FENOXYCARB FOR CONTROLLING THE EASTERN SPRUCE BUDWORM *Choristoneura fumiferana* (CLEMENS) (LEPIDOPTERA: TORTRICIDAE)

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Laboratory Evaluation of the Efficacy of the Juvenile Hormone Analog Fenoxycarb for Controlling the Eastern Spruce Budworm, Choristoneura fumiferana (Clemens)(Lepidoptera: Tortricidae).

by

©Barry J. Hicks B.Sc. (Honours)

A thesis submitted to the School of Graduate Studies
in partial fulfilment of the requirements for
the degree of Master of Science.

Department of Biology
Memorial University of Newfoundland
October 1992

St. John's Newfoundland

ISBN 0-315-82640-1

Abstract

Topical application of the JHA, Fenoxycarb, to various developmental stages of Choristoneura fumiferana (Clms.) showed it to be an extremely promising compound for development as a control agent. Eggs 0 to 24 hr-old were most sensitive; eggs 48 to 72 hr-old and fifth instar larvae were less sensitive while third instar larvae were refractory.

Treatment of fifth instar larvae produced morphogenetic effects such as larval-pupal intermediates, supernumerary moulting and deformed pupae and adults.

Failure of hatching was the most striking effect when C. fumiferana eggs were treated with Fenoxycarb. Embryonic development was blocked by the compound at an early stage, or completed by embryos developing from the same egg mass. Observations of deformed unhatched embryos and some that completed embryogenesis, but died during hatching while partially emerged, suggests that Fenoxycarb disrupted the muscular/exoskeletal system that is needed to allow eclosion from the egg. Choristoneura fumiferana eggs treated with Fenoxycarb produced numerous abnormalities in the developing embryos such as miss-orientation and absence of walking appendages.

Treated eggs that, nevertheless, hatched developed increased mortality in post-embryonic development. Eggs that were treated with Fenoxycarb and reared to the adult stage exhibited increased mortality prior to or during the overwinter diapause stage

(hibernacula). The overall mortality to the adult stage was significantly higher in treated insects than in controls.

Temperature and relative humidity altered the efficacy of Fenoxycarb toward early eggs of C. fumiferana. Treated and control eggs were not affected by photoperiod. The optimum temperature to achieve greatest effects of the compound on eggs was 15°C. Relative humidity only became an influencing factor when the temperature was at 20°C. At that temperature a relative humidity of 60% was shown to be most effective.

Acknowledgements

I gratefully acknowledge the following people, without whose help this study would not have been possible.

- Catherine Power, for her caring support, patience and understanding throughout the whole ordeal.
- My parents, Gerald and Geraldine Hicks, for their guidance and financial assistance over the years.
- Dr Roger Gordon, my Supervisor, for his excellent academic and personal advice and for financial assistance.
- Dr. Hemendra S. Mulye for friendship and advice throughout.
- My Supervisory Committee, Drs Jean Finney-Crawley and Arthur Raske, for helpful suggestions and reviewing the manuscript.

I acknowledge and give thanks to:

- The Government of Newfoundland and Labrador for a "Special Scholarship in the Sciences Related to Resource Development" that provided me with the financial support to conduct this study.
- The Department of Biology, Memorial University of Newfoundland (financial support) and Mr. Roy Ficken (Photography).

Table of Contents

List of Tables

List of Figures

List of Plates

INTRODUCTION

The eastern spruce budworm , Choristoneura fumiferana (Clemens), is a serious pest of conifers of North America. It has a distribution that extends across the Boreal zone from the Atlantic coast to the Rocky Mountains. It is also found north to the arctic circle near the Mackenzie River and the Yukon, south to the Great Lakes - St. Lawrence region and Acadian forests (Harvey 1985). The spruce budworm is a major pest of balsam fir, Abies balsamea (L.) Mill., white spruce, Picea glauca (Moench) Voss., red spruce, Picea rubens Sarg., and black spruce Picea mariana (Mill.) B.S.P.(Otvos & Moody 1978). With the development of Canada's pulp and paper industry, the budworm has emerged as the most economically troublesome pest of the eastern forests due to its periodic outbreaks causing considerable tree mortality. The damage caused by severe infestations of the spruce budworm is shown by the characteristic reddish-brown scorched appearance of affected trees after the insect's feeding is completed. Tree mortality occurs after 4-5 years of repeated severe defoliation (Anonymous 1984).

The historical records show that extensive outbreaks of C. fumiferana occurred throughout New England in 1807 and again in 1890 (Freeman 1953). Studies by Blais (1965) of the radial-growth of basal discs from budworm-host trees from locations in eastern Canada showed suppressed growth dating back 300 years that strongly resembles the effect caused by budworm damage. These repeated

widespread infestations of C. fumiferana have occurred in eastern North America for centuries and perhaps millennia (Blais 1960).

In Newfoundland, the earliest recorded spruce budworm outbreak was on Bell Island, Conception Bay in 1942; this outbreak persisted until 1953 (Otvos & Moody 1978). Three further outbreaks occurred from 1953 to 1971. These latter outbreaks were rather small and scattered throughout the west and southwest part of insular Newfoundland, and collapsed without causing extensive tree mortality. The most recent outbreak (1971 - 1983) was the largest and most severe ever recorded in Newfoundland. It affected 484,000 ha of merchantable softwood stands and resulted in a estimated loss to the provincial economy totalling approximately $275,000,000 (1983 dollars); future softwood resources were jeopardized by the outbreak and recurring outbreaks are anticipated (Milne 1986). Factors that contributed to the severity of the outbreak included favourable weather, widespread moth dispersal from the mainland and an extensive supply of mature balsam fir as food. The outbreak population collapsed in most of the island in 1981 and by 1983 there was complete population collapse. The cause of the collapse in those 3 years is unknown (Raske 1986).

The life cycle, habits and ecology of the spruce budworm are well documented (McGugan 1954, Morris 1963). The eggs are laid on the host tree as pale green clusters (masses) in mid-summer. The oval eggs are about 750μm long, 500μm wide and 200μm thick (Stairs 1960). Clusters can occur on either side of the needles of balsam fir and the first eggs are laid nearest to the apex of the needles

(McGugan 1954). A laboratory study determined that development of eggs is temperature dependant (Regniere 1987). At 24±1°C the mean time of incubation is 8 days; under field conditions it was found to be about 10 days (McGugan 1954). The spruce budworm has six larval instars. First instar larvae are pale, yellowish-green with brown heads, and are approximately 2mm long. These larvae do not feed, but form hibernacula in which they moult and overwinter as second instar larvae (Atwood 1944; McGugan 1954). In Newfoundland, the second instar larvae emerge from the hibernacula in early June, and commence mining old needles, feed on staminate flowers, or enter the unopen vegetative buds (Crummey 1976). The larvae later change to yellowish-orange with a blackish-brown head and a pale-brown prothoracic shield, and have conspicuous whitish-yellow tubercles (McGugan 1954). Pupae are found at the larval sites or towards the centre of the branch wherever sixth instar larvae find a suitable place to pupate (Miller 1963). Newly formed budworm pupae are green or yellow, mature pupae are dark grey or dark brown, with no colour differences between the sexes (Campbell 1953). Adults are present from the last week of July to the middle of August (Crummey 1976). The development of the spruce budworm in Newfoundland is a few weeks behind the development of the budworm in eastern regions of mainland Canada (Crummey 1976).

The history of the aerial application of chemical insecticides for the control of the spruce budworm and many other forest pests has been documented by Prebble (1975). The first aerial applications of insecticides were made in several provinces between

1927 and 1929 using calcium arsenate in dust formulations. These control measures were deemed unsuccessful for the spruce budworm as only 50% larval mortality was achieved. Published reports of the early dusting projects contain no mention of adverse side effects on wildlife. However, in correspondence files of the day, there is reference to a small number of dead rabbits and birds on dusted plots (Otvos & Moody 1978). The use of calcium arsenate terminated in 1930 and no aerial applications of any kind for forest insect control were made until the mid-1940's when DDT was developed. DDT, an organochlorine insecticide, was used extensively in operational control programs in several provinces of Canada at various times from 1945 to 1968. The side effects of DDT on wildlife and other non-target organisms, and the long term build up of residual toxicity in the soil, were factors that culminated in the complete restriction of its use in 1969. Subsequently, several organophosphate and carbamate insecticides were used, since they were considered to be more acceptable than DDT from an ecological perspective.

The most widely used chemicals for the control of the spruce budworm on an operational scale in Canada have been fenitrothion (Sumithion®), phosphamidon (Dimecron®) and orthene of the organophosphate group and aminocarb (Matacil®), mexacarbate (Zectron®), and carbaryl (Sevin®) of the carbamate group. The mode of action of these insecticides on the spruce budworm is primarily as cholinesterase inhibitors. Because of continued public concern over the effect of these chemicals on non-target organisms, and the

possibility that reliance upon selected organophosphate and carbamate insecticides may increase the selection pressure for the development of resistance in these insects, there is a need to develop alternative insecticides.

The bacterial insecticide Bacillus thuringiensis Berliner is the only environmentally acceptable alternative to chemical control being used currently. Effective control of third and fourth instar larvae of C. fumiferana occurred when Bacillus thuringiensis was aerially applied in the Saguenay/ Lac St-Jean and Lower St. Lawrence regions of Quebec (Valero 1989). Other microbials such as viruses are being developed and methods are being investigated to develop insect specific compounds that act on novel systems within the bodies of the target pest (Morris et al. 1986). One category of compounds being tested are insect growth regulators (IGRs). There are five sub-categories of insect growth regulators: juvenile hormone analogs (JHAs), anti-juvenile compounds, chitin synthesis inhibitors, ecdysone analogs and metabolic inhibitors. All of these compounds exert their effects in various ways to disrupt the normal process of insect metamorphosis. The JHAs constitute the focus of my research on C. fumiferana.

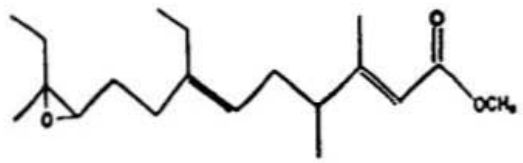
Insects control their development using hormones secreted by neurosecretory cells and glands of the endocrine system. The endocrine centres of insects involved in controlling metamorphosis are the medial neurosecretory cells of the protocerebrum of the brain, the corpora cardiaca, the corpora allata, and the prothoracic gland. The corpora allata produce a hormone known as

juvenile hormone (JH), while the neurosecretory cells produce the prothoracotropic hormone (PTTH) which is stored and released into the blood by the corpora cardiaca. The prothoracotropic hormone activates the prothoracic gland to produce ecdysone, the moulting hormone. Ecdysone or its derivatives act directly on the chromosomal mechanisms concerned with moulting (Retnakaran et al. 1985). During the insect's larval stages, the action of ecdysone is modified by the JH secreted by the corpora allata. The presence of JH ensures that the insect will retain larval characteristics during each moult; during the final instar, the corpora allata stops producing JH and the adult transformation occurs. Upsetting the titre of JH at certain periods during the life history will adversely affect metamorphosis (Retnakaran et al. 1985).

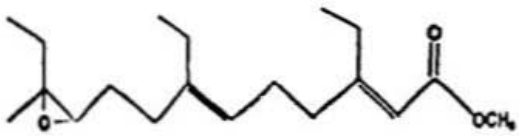
Fig. 1 shows the five naturally occurring JHs (Hoffmann & Lagueux 1985). All are terpenes with two sites of unsaturation and an epoxide ring; they differ only in the number and arrangement of carbon atoms.

The use of natural JHs as insecticides is not feasible, since they have low environmental stability and are difficult to synthesize. However, even before the chemical structures of JH was known, work showed that certain farnesyl compounds and a number of naturally occurring compounds exhibited JH type activity (Fig. 2). "Paper factor", later identified by Bowers et al. (1966) as Juvabione, was one such natural compound that was discovered from balsam fir trees. Most of the farnesyl derivatives and the natural compounds were relatively weak JHAs. Bowers (1969) opened up a new

Fig. 1. Chemical structures of the five known naturally occurring juvenile hormones of insects.



Iso-JH- 0



JH- 0

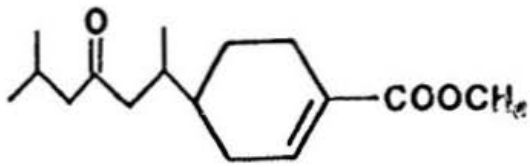
JH- 1

JH- 2

JH- 3

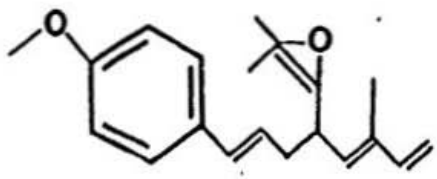
Fig. 2. Various naturally occurring and synthesized JHAs

Naturally occurring JHAs



JUVABIONE

BOWERS et al. (1966)



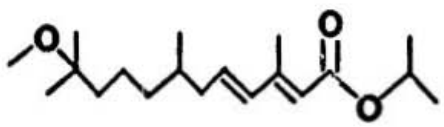
JUVOCIMENE-1

BOWERS & NISHIDA (1980)

FARNESOL

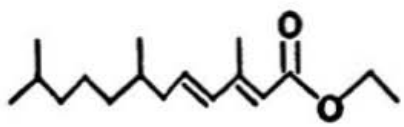
SCHIALEK (1961)

Synthesized JHAs



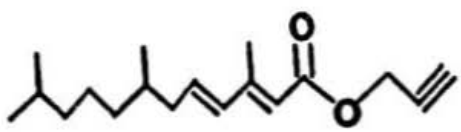
METHOPRENE

HENRICK et al. (1973)



HYDROPRENE

HENRICK et al. (1973)



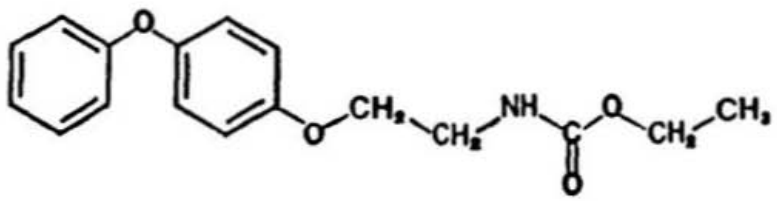
KINOPRENE

HENRICK et al. (1976)

area of research when he synthesized substituted aromatic terpenoid ethers that were more active than the natural hormones. The novel mode of action of JHAs, together with their specificity and environmental safety resulted in great interest in the development of these compounds by pesticide manufacturers (Retnakaran et al. 1985). The action of the JHAs resembles the insects endogenous JH; however they may or may not be similar to them in their chemical structures. Methoprene, hydroprene and kinoprene (Fig. 2) have juvenilizing effects on various insects and are structurally related to the natural hormones in that they are terpenoid compounds. A few compounds with chemical structures analogous to those of other categories of insecticides (eg. carbamates) exhibit juvenilizing effects, without displaying anti-cholinesterase activity. Such compounds are dissimilar in their chemical structure from the natural JH.

Preliminary studies done in the laboratory and field suggested that the terpenoid JHAs that were initially developed were relatively ineffective in suppressing the development of the eastern spruce budworm (Retnakaran 1970, 1973; Retnakaran & Grisdale 1970; Retnakaran et al. 1977, 1978; Outram 1975). The efficacy of using JHAs against a major defoliator such as the spruce budworm needs to be determined for non-terpenoid compounds that have been more recently synthesized. One such recently synthesized compound is Fenoxycarb, or RO-13-5223 (ethyl[2(4-phenoxyphenoxy)ethyl] carbamate)(Fig. 3) (Dorn et al. 1981; Kramer et al. 1981; Masner et al. 1981). Fenoxycarb belongs to a group of

Fig. 3. Fenoxycarb, ethyl[2(4-phenoxyphenoxy)ethyl] carbamate.


O
O
CH2
CH2
NH
C
O
O
CH2
CH3

chemical compounds known as phenoxy ethyl carbamates. Despite the carbamate moiety, it does not inhibit cholinesterase (Maag 1989). This compound has been shown in susceptibility tests to have the potential for controlling a variety of insect pests (Appendix 1).

In a recent laboratory study , Mulye & Gordon (1989) showed that Fenoxycarb was the most effective of seven JHAs that they screened against the sixth instar larva of C. fumiferana. Hicks & Gordon (1992) showed that the adult stage was more sensitive to the effects of Fenoxycarb than other stages. No information is available on stages other than adults and sixth instar larvae. Moreover, except for relatively few isolated studies on certain species of Lepidoptera, other than C. fumiferana (Benskin & Vinson 1973; Schooneveld & Wiebenga 1974; Smietanko et al. 1989; Varjas 1985), the possibility that the efficacy of JHAs may be modified by environmental factors has been largely ignored. To obtain a full appraisal of the potential of Fenoxycarb for controlling C. fumiferana, I determined the efficacy of the compound on selected developmental stages (eggs, third-instar larvae, and fifth-instar larvae) under laboratory conditions, as well as its efficacy against early embryos under selected environmental conditions.

LITERATURE REVIEW

2.1 Spruce Budworm Embryology

The embryology of the spruce budworm has been documented by Stairs (1960). A list of the critical events during the embryology of the spruce budworm is presented in Table 1.

The term blastokinesis comes from Wheeler (1893) who incorporated the embryonic movements of anatrepsis and katatrepsis under this term. However, since then many authors have inadvertently used blastokinesis as a synonym for ketatrepsis (Sander et al. 1985). The original terminology of Wheeler (1893), in which anatrepsis and ketatrepsis are sequential processes within the overall process of blastokinesis will be recognized in this study.

2.2 The role of JH during insect embryogenesis.

Little has been published on JH and its role during insect embryonic development (Hoffmann & Lagueux 1985). Bergot et al. (1980;1981) detected the presence of JH in the embryos several lepidopteran species. Preliminary investigations on the fluctuations of JH in eggs of Manduca sexta showed that neither JH I nor JH 0 were present in newly laid eggs, but both hormones

Table 1. Critical events that occur during the embryogenesis of Choristoneura fumiferana (Clem.), reared at 25°C as given by Stairs (1960).

Hour(hr)	Event
3	- union of sperm with oocyte to form zygote nucleus.
5	- cleavage of zygote to form solid sphere near centre of egg.
8	- superficial blastoderm is formed.
9	- differentiation of the blastoderm into the serosa and the embryonic rudiment, begins.
10	- differentiation is complete.
13	- rudiment sunken into the yolk, edges of rudiment folded inwards.
16	- embryo rotates either to the left or right around its longitudinal axis (anatrepsis).
19	- head lobes become evident.
23	- mesoderm is formed by invagination of cells along the mid-ventral side of the embryo.
40	- embryo divided into 17 segments each containing a pair of coelomic sacs. The CNS is not well differentiated. Gnathal and thoracic appendages appear as paired outgrowths from the side of the embryo.
72	- mouthparts are aligned. Paired appendages evident on abdominal segments. Lateral walls nearly closed along mid-dorsal line.
80	- dorsal closure complete. Embryo rotates around its longitudinal axis through 180° (ketatrepsis).
96	- mandibles are the only sclerotized structure.
100	- body muscles, stomodaeom and mesenteron appear functional.
120	- embryo breaks through amniotic sac and ingests the yolk which fills the anterior half of the egg.
144	- embryo has ingested all the yolk and embryonic envelopes and grows to fill the entire egg.
150	- emerges by chewing a hole in the anterior end of chorion.

exhibited a high titre after 2 or 3 days of development, with a drop prior to eclosion. In the locust, Locusta migratoria (Temin et al. 1986) and in the cockroach, Nauphoeta cinerea (Imboden et al. 1978), low levels of JH or no JH, respectively occurred through blastokinesis. The JH titre increased in both species after blastokinesis, then declined to lower levels by time of hatch. Roe et al. (1987) showed that in the house cricket, Acheta domesticus, JH esterase activity corresponded to the fluctuations in the JH titre. During early embryogenesis when the JH titres were low they recorded a period of maximum esterase activity. Subsequently, esterase activity declined, reaching its lowest level at the time of dorsal closure which corresponds to the peak in JH.

Speculation on the source of JH during early embryogenesis is that it originates from the female parent, and that the JH observed in later embryogenesis is due to the development and activity of the embryonic corpora allata (Dorn 1975). Since vitellogenesis in many insect species is controlled mainly by JH, the likelihood of maternal transfer of JH is possible. Direct evidence of this transfer is lacking. However it is well known that the ecdysteroids that are present in newly laid eggs of several species are maternal in origin and are synthesized in the ovaries of the female parent (Hoffmann & Lagueux 1985).

Insect post-embryonic development is controlled by three major categories of hormones: ecdysteroids, JHs and neuropeptides (Hagedorn 1985; Richards & Davies 1977). The presence of ecdysteroids and JHs in embryos (Hoffmann & Lagueux 1985) and the

occurrence of embryonic moulting (Jones 1956) suggest some similarity with post-embryonic development. However, the exact role of JH in embryonic development remains to be determined.

2.3 Effects of applications of JH and JHAs on embryonic development of insects.

Retnakaran & Grisdale (1970) showed that spruce budworm embryos that were topically treated with a dichloro analog of ethyl farnesoate developed to the point where black head capsules could be seen but they did not hatch. The embryonic development in the spruce budworm was blocked by five JHAs (Retnakaran 1970).

Eggs of many other insect species have been treated with JH or analogs of it and developmental disturbances recorded (see Hoffmann & Lagueux 1985, for review). The disorders observed depend on the time of application and the species examined (Slama & Williams 1966, Riddiford & Williams 1967, Novak 1969, Riddiford & Truman 1972, Hunt & Shappirio 1973, Wall 1974, Injeyan et al. 1979, Smith & Arking 1975, Charmillot et al. 1985, Kelly & Huebner 1986, Marchiondo et al. 1990, Gardner 1991). The general trend is that JHAs are effective in disrupting embryogenesis when applied during the early stages of embryonic development. Application of JH or JHAs before katatrepsis causes abnormal embryos, whereas application after katatrepsis has delayed effects that are not manifested until later in larval development (Riddiford 1970a, 1970b; Willis & Lawrence 1970; Riddiford & Truman 1972; Hunt &

Shappirio 1973). Treatment of the embryo early in development (i.e. prior to katatrepsis) results in a failure of the embryo to undergo the 180° rotational return that is required during embryogenesis (Wall 1974, Kelly & Huebner 1986). Therefore, such treated eggs fail to hatch. Dorsal closure was also affected when JHAs were applied to embryos of Oncopeltus apterus (Dorn 1982) and Rhodnius prolixus (Kelly & Huebner 1986). Hatching failure is the most common embryonic disturbance resulting from JHA treatment.

Failure of eggs treated with JH or JHAs to hatch is a common occurrence among many orders of insects. In the Hemiptera, Wall (1974) hypothesized that JHAs interfere with the process of hatching by upsetting the programming of the release of a hatching enzyme. Hatching failure is often the result of the anatomical defects associated with the interference of blastokinesis (Riddiford & Williams 1967; Enslee & Riddiford 1970; Kelly & Huebner 1986).

Delayed effects on post-embryonic development have been shown in several insects including hemipterans (Riddiford & Truman 1972, Hunt & Shappirio 1973, Willis & Lawrence 1970, Riddiford 1970a) and lepidopterans (Riddiford 1970b; 1972). Willis & Lawrence (1970) suggested that JHAs applied to eggs persist throughout growth until adult eclosion. However, it is equally possible that JHAs interfere with the programming of the embryonic corpus allatum in such a manner that the gland exhibited an abnormal continuation of hormone production at the time of metamorphosis. This hypothesis is supported by the studies of Riddiford & Truman (1972), who showed

that allatectomy of embryonically treated fifth instar larvae of Pyrrhocoris apterus resulted in normal metamorphosis, whereas the implantation of glands from fifth instar larvae, treated as embryos with JH1, into untreated fifth instar larvae interfered with metamorphosis. A third possible explanation for the delayed effects of JHAs on embryos is that the output of JH by the corpora allata throughout development may be quantitatively affected while the temporal pattern of the hormone's secretion may be unaffected (Hunt & Shappirio 1973).

Aside from the disputed effects of JHAs on the insect's endocrine system following treatment of egg stages, little is known about the biochemical consequences of treating eggs with these compounds. Retnakaran (1975) showed that the respiratory rate of spruce budworm embryos treated with JH and JHAs decreased progressively throughout the observation period indicating a slow down of metabolism until respiration ceased totally and there was no hatch. Protein electrophoretic analysis of Fenoxycarb treated embryos of Rhodnius prolixus showed that embryonic disturbances were due to alterations in the normal molecular events accompanying development (Kelly & Huebner 1987).

2.4 Effect of JHAs on the larvae of insects.

In general, the effects of exogenous application of a JHA to the last larval instar results in abnormal pupation (Retnakaran et al. 1985). In a holometabolous insect, such as C. fumiferana, the

titre of JH is normally high during the larval instars, except the last, in which it drops. The presence of a high JH titre during critical periods in the last larval stadium, results in larval-larval moults; when it is low it becomes a pupa, and when JH is absent the insect moults into the adult. Inhibition of metamorphosis is due to the JHAs ability to prevent imaginal differentiation of the insect's epidermal cells (Sehnal 1983).

In the Lepidoptera, JHAs readily induce formation of supernumerary larvae and various larval-pupal intermediates when applied to last instar larvae (Retnakaran 1973). Other morphogenetic effects observed among insect orders are: temporary or permanent arrest of moulting; adultoids; second pupae, and a number of anomalies that are due to allometric growth of body parts (Sehnal 1983).

Mulye & Gordon (1989) described the morphogenetic effects of Fenoxycarb when last instar larvae of C. fumiferana were topically treated. Larval-pupal intermediates, with precocious evagination of wing discs and the production of deformed pupae with hemolymph filled blisters in the thoracic region were recorded. In another tortricid species, the tufted apple moth, Platynota idaeusalis, Hull et al. (1991) found that the larva to pupa transformation was affected when late instar larvae were treated with Fenoxycarb.

Theoretically, treatment of early instar larvae with JHAs may be expected to have little or no effect on metamorphosis since at these stages the endogenous JH titre is high anyway (Retnakaran et al. 1985). Field application of JHAs to early instar larvae of C.

fumiferana showed them to be refractory (Retnakaran et al. 1978). However, Gadenne et al. (1990) found that Fenoxycarb, applied to early instars of the European Corn Borer, Ostrinia nubilalis, induced strong perturbation in development. No normal pupae were observed when early instar larvae were treated with 5µg of Fenoxycarb. They found that last instar larvae were also sensitive to Fenoxycarb, with disruptions in metamorphosis. Treatments produced supernumerary moults, larval/pupal intermediates and larvae that they classified as "permanent". These permanent larvae failed to moult to either supernumerary larvae, intermediates or pupae. King & Bennett (1988;1989;1990) compared the effect of Fenoxycarb on different life stages of Blattella germanica, and found that all stages were affected by the compound. A dose of 10 µg/µl applied topically to 1 day old oothecae caused 57.1% suppression of hatching (King & Bennett 1990), while mortality of nymphal stages treated with the same dose was high but decreased as older nymphs were treated (King & Bennett 1989). Last nymphal stages produced sterile adults when treated at much lower dosages than are required to sterilize adults or kill nymphal and oothecal stages (King & Bennett 1990).

There are numerous important biochemical mechanisms that exogenous application of JHAs may interfere with. Mechanisms such as secretion, transportation to target site, degradation, excretion and feedback control are all susceptible to the effects of JHAs (Retnakaran et al. 1985). In Diploptera punctata Tobe & Stay (1979) showed that the JHA, hydroprene, interfered with the feedback

control of the natural hormone titre; at low doses of JHA, JH synthesis was stimulated while at higher doses, a negative feedback mechanism depressed JH synthesis.

Juvenile hormone analogs inhibit DNA, RNA and protein synthesis in larvae (Himeno et al. 1979). Wyatt et al. (1987) showed that methoprene could replace the natural hormone in the locust (Locusta migratoria) fat body, and act directly on the fat body to induce expression of genes for the production of vitellogenin. Fenoxycarb was shown to regulate the level of translatable mRNA and thus the amount of hemolymph storage proteins in larvae of Trichoplusia ni (Jones et al. 1988).

2.5 JHA efficacy at different temperatures, photoperiods and relative humidities.

The influence of temperature upon JH and JHA efficacy has been studied in three species of Lepidoptera. In the tortricid moth, Adoxophyes orana, laboratory reared insects treated with either JH1 or the JHA, 6,7-epoxy-3,7-dimethyl-1-[3,4-(methylenedioxy)phenoxy]-2-noneme, at 13°C resulted in weak hormonal effects while, at 20°C maximal morphogenetic effects were obtained. At high temperatures (32°C) the response showed a sharp decrease (Schooneveld & Wiebenga 1974). There was a decrease in morphogenetic effects with decrease in post-treatment temperature when Heliothis virescens were treated with JH2 and the JHA, [E-4-(6,7-epoxy-3,7-dimethyl-2-octanyl)oxy-

1,2-(methenedioxy benezene)] (Benskin & Vinson 1973). In the larval wax moth Galleria mellonella, sensitivity to a JHA was dependent on the rearing temperature. Eighty percent of treated larvae reared at 30°C underwent supernumerary moulting while at 18°C, no extra-moults were observed (Smietanko et al. 1989). In Leptinotarsa decemlineata treated with the new non-terpenoid JHA, 2-(1-methyl-2-(20phenoxyphenoxy)ethoxy) pyridine, morphogenetic activity was modified by post treatment temperature (Santoso 1990).

Juvenile hormone analog sensitivities of larval instars reared at different photoperiods have not been explored intensively. Varjas (1985) concluded that the larvae of Pieris brassicae are JHA sensitive under different photoregimes. The rates of morphogenetic response increased when larvae were reared under long day conditions while JHA sensitivity was significantly decreased in the short day insects.

No information is available on the effects of relative humidity on JHA efficacy in any insects.

MATERIALS AND METHODS

3.1 Test Compound.

The chemical used was an emulsifiable concentration (125 g/L) of Fenoxycarb (ethyl [2(4-phenoxyphenoxy)ethyl] carbamate)(Fig. 3), supplied by Elanco, Eli Lilly Canada Inc., Scarborough, Ontario. A stock solution (100 µg/L) was made up by adding 7.92 mL of acetone to 80 µL of Fenoxycarb concentrate. The concentrations to be tested were obtained by diluting the stock solution.

3.2 Rearing of C. fumiferana.

Larvae were reared to the pupal stage from second-instar hibernacula supplied by the Forest Pest Management Institute (Forestry Canada), Sault Ste. Marie, Ontario. Larvae were reared under axenic conditions on an artificial diet at 24±1°C, 65±2% relative humidity, and 18L:6D photoperiod (Mulye & Gordon 1990). To obtain eggs for experimental purposes, pupae were sexed according to Jennings & Houseweart (1978) and 300 (10:1♂) were placed in a 0.5 m^3 fine mesh cage to undergo eclosion. Several branches of A. balsamea were included in the cage as sites of oviposition.

3.3 Treatment of C. fumiferana larvae.

A range (0.1 - 50.0 μg per μL) of concentrations of Fenoxycarb (liquid concentrate, technical grade) dissolved in acetone, were topically applied to the mid-dorsal region of 1-day-old third-instar or fifth-instar larvae with a Gilson micropipette. Ten replicates of 10-13 third-instar larvae and six replicates of six fifth-instar larvae were treated with each of the Fenoxycarb concentrations indicated. Controls consisted of acetone-treated and untreated 1-day-old third- or fifth-instar larvae. Larvae were monitored daily and developmental events such as moulting, pupation, and adult eclosion recorded. Treated larvae were maintained under the same environmental conditions as the larval colony.

3.4 Treatment of C. fumiferana eggs.

The balsam fir needles on which oviposition had occurred were placed singly under a stereomicroscope on filter paper circles lining the lids of Petri dishes (9 cm diam.). The egg masses were treated topically with a range of concentrations (5×10^{-5} to 25.0 μg per μL) of the JHA dissolved in acetone. In each case, the volume (1.5 - 2.5 μL) applied was adjusted in accordance with the number of eggs comprising the mass, to insure that the dose per egg (0.00025×10^{-2} to 1.25 μg) was constant among replicates (Appendix 2). Only egg masses of 30-50 eggs per mass were used, because eggs

laid singly or in small masses are usually indicative of unsuccessful mating (Retnakaran 1970). The filter paper circles were moistened with 2 or 3 drops of distilled water, and the Petri dishes assembled, inverted, then sealed completely around their edges with masking tape. A few cracks were placed in the inverted bottom to allow for exchange of humidity. Each Petri dish containing a single egg mass comprised one replicate; three to five replicates of 0 to 24 hr-old eggs and five to eight replicates of 48 to 72 hr-old eggs were treated with each of the Fenoxycarb concentrations. Controls consisted of acetone-treated and untreated eggs of similar stage of embryogenesis. Eggs were monitored daily for two weeks for hatching. All eggs were kept at 24 ± 1°C, relative humidity 55-60%, and 18L:6D photoperiod (Grisdale 1984).

In an attempt to determine the embryological events associated with age related sensitivity of treated embryos, both treated and control eggs were fixed at specific times during embryogenesis. Embryos were fixed in Kahle's solution and the chorion and yolk removed with fine forceps. The embryos were placed as whole mounts on slides in Ruben's fluid and observed under the compound and stereo-microscopes for abnormalities in embryonic development. Photographs were taken of the embryos using a microscope-mounted Nikon 35mm camera.

To determine if treated C. fumiferana eggs showed a delayed effect later on during post-embryonic development, eggs, 0 to 24 hr-old and 48 to 72 hr-old, were treated in the same manner and kept under the same environmental conditions as above; however,

larvae that hatched were allowed to form hibernaculae in cheesecloth (Grisdale 1984). Once moulting to the second instar larvae had occurred (2-3 weeks), the cheesecloth containing the hibernaculae were removed from the petri dishes and placed in small plastic bags (6 lb weight). Each bag contained the hibernaculae and the diapausing second instar larvae for each replicate. The bags were placed in a darkened incubator at 19 ± 1°C for three weeks; overwintering was established by maintaining the insects in a cold room at a temperature of 1°C in the dark for five months. After this time the cheesecloth containing the hibernaculae were placed in rearing cups containing artificial diet and the diapause was broken by exposing the insects to the normal larval rearing conditions. The survival of the insects was recorded through to the development of the adult.

The insects contained in each initial egg mass were considered one replicate. Twenty one replicates of 0 to 24 hr-old eggs (n=805) were treated with 0.0005 µg per egg of Fenoxycarb dissolved in acetone. Eleven replicates of 0 to 24 hr-old eggs (n=410) were treated with acetone as controls. Thirty-two replicates of 48 to 72 hr-old eggs (n=1144) were treated with 0.62 µg per egg of Fenoxycarb dissolved in acetone. Controls consisted of 14 replicates of acetone treated 48- to 72-h-old eggs (n=535). The above concentrations approximately correspond to the LD_{50} value for each stage.

3.5 Efficacy of Fenoxycarb against C. fumiferana eggs, reared under different environmental conditions.

The efficacy of Fenoxycarb was tested against the egg stage of the budworm under different environmental conditions. Eggs were obtained from the colony and were treated at 0 to 24 hours after oviposition. A range of concentrations (0.001 to 5.0 µg per µl) were topically applied to the eggs masses in the manner described previously. Three to six replicates of egg masses were treated with each of the Fenoxycarb concentrations, while 4-7 replicates were either acetone-treated or untreated as control groups. The environmental conditions tested were: temperature (15°C, 20°C, 25°C); relative humidity (60%, 100%), and photoperiod (6L:18D, 12L:12D, 18L:6D). Treated and untreated groups of insects were maintained under eighteen combinations of photoperiod, temperature and relative humidity; this arrangement insured that all possible combinations of the environmental parameters were tested (Appendix 3).

To achieve 100% humidity, a beaker of water (200ml) was placed inside a 0.5m^3 humidity chamber constructed of clear plastic. After eggs were positioned inside the chamber , the entrance was sealed with a plastic bag and masking tape. The humidity chamber containing the eggs was then placed inside a Hotpack incubator (Waterloo, Ontario), where the temperature and photoperiod were controlled. To test eggs at 60% humidity, they were placed inside a Hotpack incubator that contained three large finger bowls (1L

each) of water in the bottom of the incubator. The humidity was monitored regularly in both set-ups using a standard wet/dry bulb hygrometer.

3.6 Statistical analysis

Variances of means were expressed as standard errors throughout. Probit analysis was used to calculate LD_{50} values (Finney 1971), after allowing for mortality in the controls (solvent-treated) using Abbott's formula (Abbott 1925). Significance between LD_{50} values were based on the criterion of non-overlap of 95% confidence limits. ANOVA was used to determine the significance in means among the environmental experiments; and between the treatments and controls of the overwinter diapause experiment. To preserve normality and homogeneity of variance in the data either $\log_e$(1+% survival) or arcsine(% survival) transformations were applied.

RESULTS

4.1 Efficacy of Fenoxycarb against C. fumiferana larvae.

The development of C. fumiferana was not affected by Fenoxycarb treatments of early third-instar larvae. At a dose of 50.0 µg Fenoxycarb per insect, survival of treated insects to the adult stage (74.1 ± 7.5%) was not significantly different from solvent-treated or untreated controls (60.5 ± 6.9% and 64.4 ± 7.4%, respectively). The LD_{50} value of this stage was therefore >50.0 µg/insect (Table 2).

Overall mortality (i.e. failure to survive to the adult stage) increased in a dose dependant fashion when early fifth-instar larvae were treated with Fenoxycarb (Fig. 4). Treatment of fifth-instar larvae resulted in supernumerary moulting (Plate 1B), and the formation of larval-pupal intermediates (Plate 1D) with mummification, precocious evagination of wing disks, and the production of deformed pupae (Plate 1E) with hemolymph-filled blisters in the thoracic region. Some adults that emerged were deformed (Plate 1G) with crumpled antennae and wings, attachment of pupal exuviae to heads and missing appendages (Plate 1H).

The LD_{50} value was obtained for insects that, when treated as early fifth-instar larvae, were successful: (a) in forming a chrysalis, (b) undergoing adult eclosion. These values are represented in Table 2 as larval mortality and overall mortality, respectively. Larval mortality takes into account mortality during

Table 2. Probit analysis of the lethal effects of Fenoxycarb on various developmental stages of Choristoneura fumiferana.

Developmental Stage	n[1]	Slope±SE	LD_{50} (µg/insect)	95% CL
0-24 h Eggs	1386	1.126 ± 0.039	0.0004	0.0002 - 0.0009
48-72 h Eggs	1537	0.760 ± 0.029	0.294	0.123 - 0.792
3rd instars:				
larval mortality[2]	187	-	> 50.0	-
overall mortality[3]	187	-	> 50.0	-
5th instars:				
larval mortality	180	0.735 ± 0.040	11.4	3.8 - 519.1
overall mortality	180	0.732 ± 0.035	2.4	0.8 - 16.9

[1] Total number of insects treated with compound or acetone.

[2] Mortality of treated insects prior to pupation.

[3] Combined mortality of larval and pupal stages.

Fig. 4. Dose-response curve, semi-logarithmic plot, for 1-day-old fifth instar larvae of Choristoneura fumiferana treated with Fenoxycarb. Each point represents mean combined mortality ± SE of larvae and pupae (six insects per replicate).

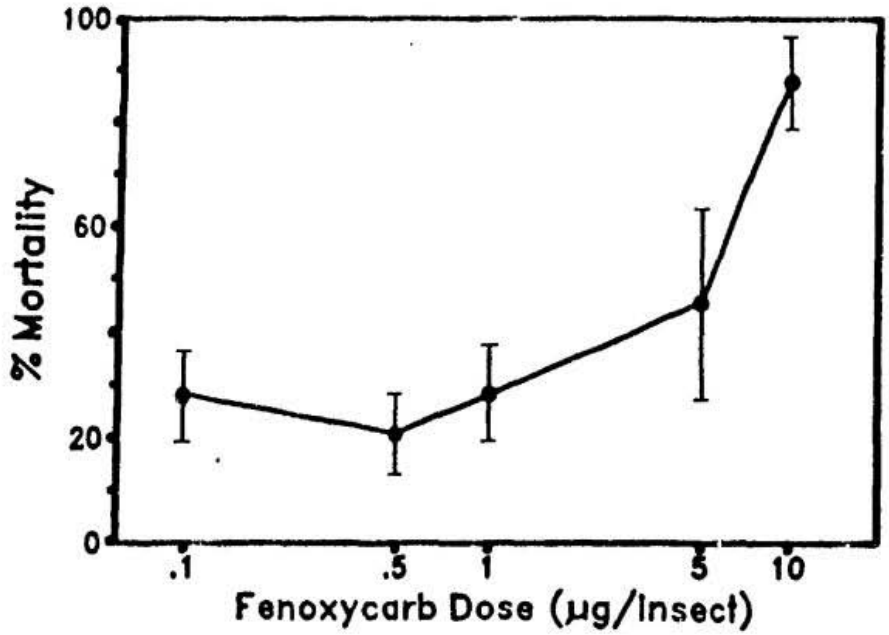
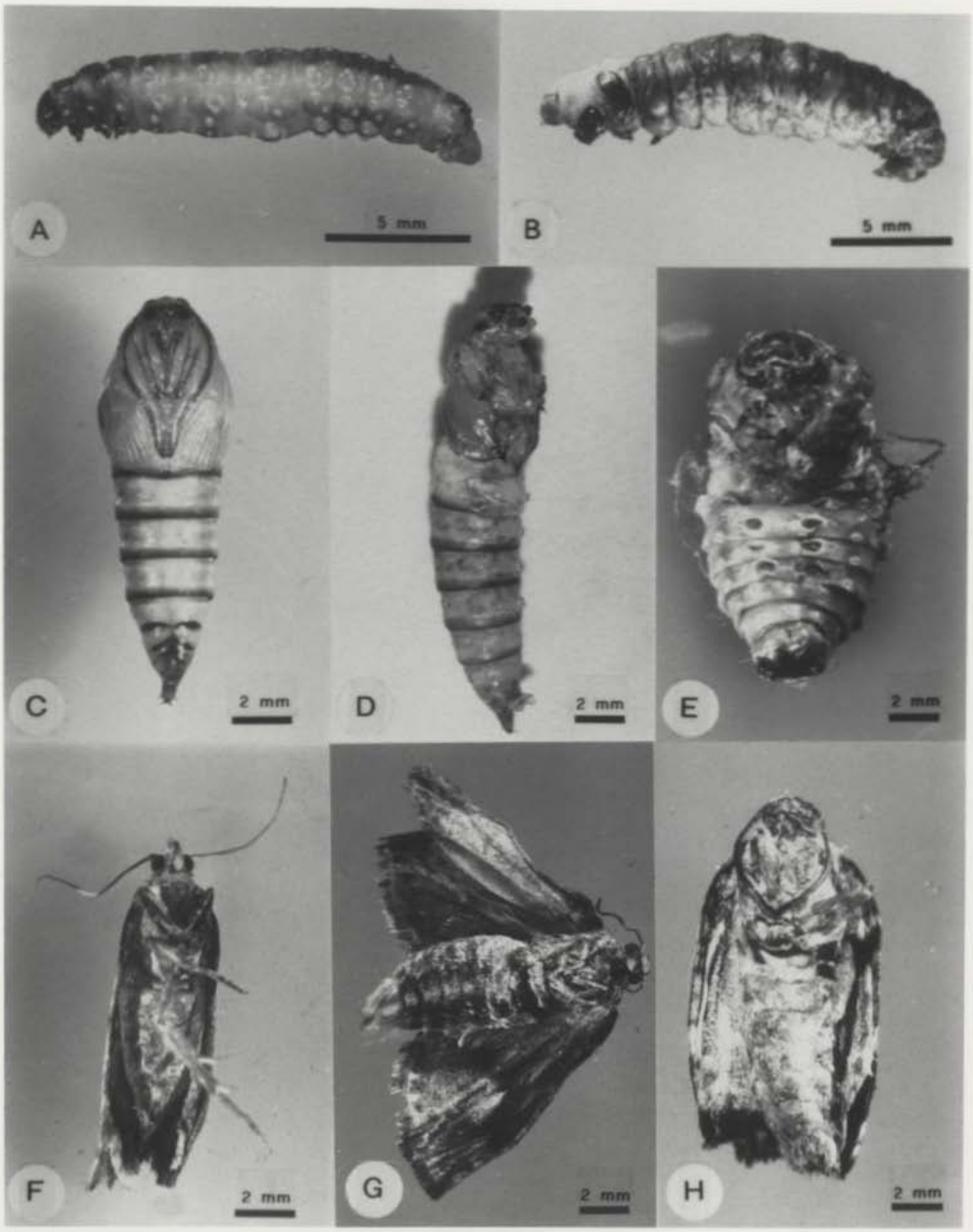

100
60
20
0
% Mortality
.1
.5
1
5
10
Fenoxycarb Dose (µg/Insect)

Plate 1. Morphogenetic perturbations resulting from Fenoxycarb treatment of 1-day old fifth instar larvae of Choristoneura fumiferana. A, Sixth instar larvae (Control). B, Supernumerary moulting. Note the head of instar seven larva breaking through the integument. C, Pupa (Control). D and E, larval-pupal intermediate and deformed pupae respectively. Note the remnants of larval characteristics. F, Adult (Control). G and H, deformed adults. Note the crumpled wings and antennae, missing walking appendages on right side of body of G, pupal exuvium attached to head in H.


A
5 mm
B
5 mm
C
2 mm
D
2 mm
E
2 mm
F
2 mm
G
2 mm
H
2 mm

the fifth and sixth stadia and during eclosion to the pupa. Overall mortality included the former as well as mortality during the pupal stadium and adult eclosion. The LD_{50} value for larval mortality was 11.4 µg/insect while overall mortality was 2.4µg/insect. However, since the 95% confidence limits overlap, I am unable to confidently conclude that the two values are different.

4.2 Efficacy of Fenoxycarb against eggs of C. fumiferana.

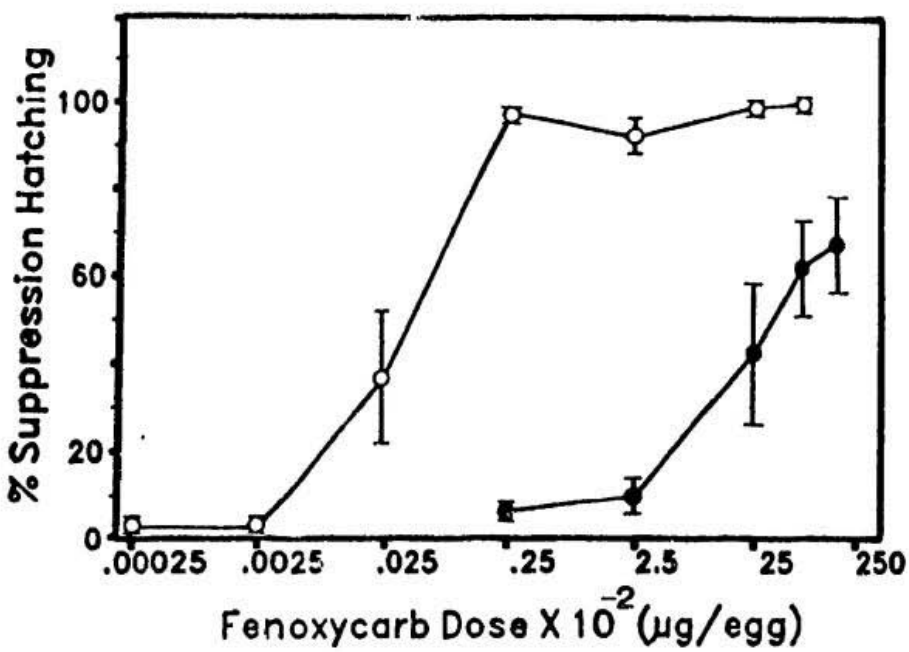
Hatching of eggs treated with Fenoxycarb was suppressed in a dose dependant fashion (Fig. 5). Eggs that were treated 0 to 24h-old were more sensitive than eggs treated 48 to 72 h-old. Close to 100% suppression of hatching occurred when eggs 0 to 24h-old were treated with 0.0025 µg/egg. At the same dosage, eggs treated later during embryogenesis had a significantly smaller suppression of hatching.

Percentage hatch in the controls was high (0-24h old: 97.9±1.0 untreated controls, 86.6±3.5 solvent-treated; 48-72h old; 91.5±1.8 untreated controls, 95.2±1.7 solvent-treated).

Of the unhatched eggs, it was observed that they frequently, though not always, completed embryogenesis to the black headed stage but the larvae did not initiate hatching or died during the process. Eggs treated with high doses of Fenoxycarb showed no embryonic development and remained pale green in colour.

Whole mounts of treated and solvent treated embryos of various ages were observed under compound and stereo-microscopes. Zero to

Fig. 5. Dose-response curves, semi-logarithmic plot, for 0-to 24-h-old eggs (O) and 48-to 72-h-old eggs (●) of Choristoneura fumiferana treated with Fenoxycarb. Each point represents mean ±SE suppression of hatching based on three to eight replicates; each replicate consists of an egg mass of 30-50 eggs.


% Suppression Hatching
100
60
20
0
.00025
.0025
.025
.25
2.5
25
250
Fenoxycarb Dose X 10^{-2} (µg/egg)

24 hr-old eggs were observed at 72, 96, 120 and 144 hours incubation, while eggs treated at 48 to 72 hr-old were examined at 120 and 144 hours incubation. In either case, the compound-treated and solvent-treated eggs showed no observable external difference in the development of the embryos prior to 120 hours incubation. At 120 hours, the acetone treated embryos appeared in a "C" orientation with their legs directed toward the inside. Fenoxycarb treated embryos were observed in a "S" orientation with their legs directed toward the outside. However, at 144 hours I observed other treated embryos that had the same apparent orientation as the solvent-treated embryos but with missing legs (Plate 2A). The solvent-treated embryos appeared to have normal appendages (Plate 2B). The time to complete normal embryogenesis, regardless of treatment, was approximately the same (Appendix 4). However, a number of abberations were observed in larvae that hatched from Fenoxycarb treated eggs. Plate 3A shows first and second instar larvae with malformations in their cuticles and Plate 3B shows the larvae of the same stages that hatched from acetone treated eggs. The instar I larva of Plate 3A has its head capsule distorted out of normal shape as compared to acetone-treated larvae of the same stage (Plate 3B). Unable to successfully shed it first instar exuvium, the instar II larva of Plate 3A will eventually die.

The LD_{50} values for eggs that were treated at 0 to 24 hours and those at 48 to 72 hours (Table 2) indicate that the early eggs are the most sensitive stage. The value (0.0004 µg/egg) is 735 times more sensitive than eggs treated at 48 to 72 hr-old, and 6000 times

Plate 2. Embryo of Choristoneura fumiferana (144 hour) treated at 0 to 24 hr-old with: A. Fenoxycarb (0.025µg/egg). Note missing thoracic walking appendages; B. Acetone (Control).

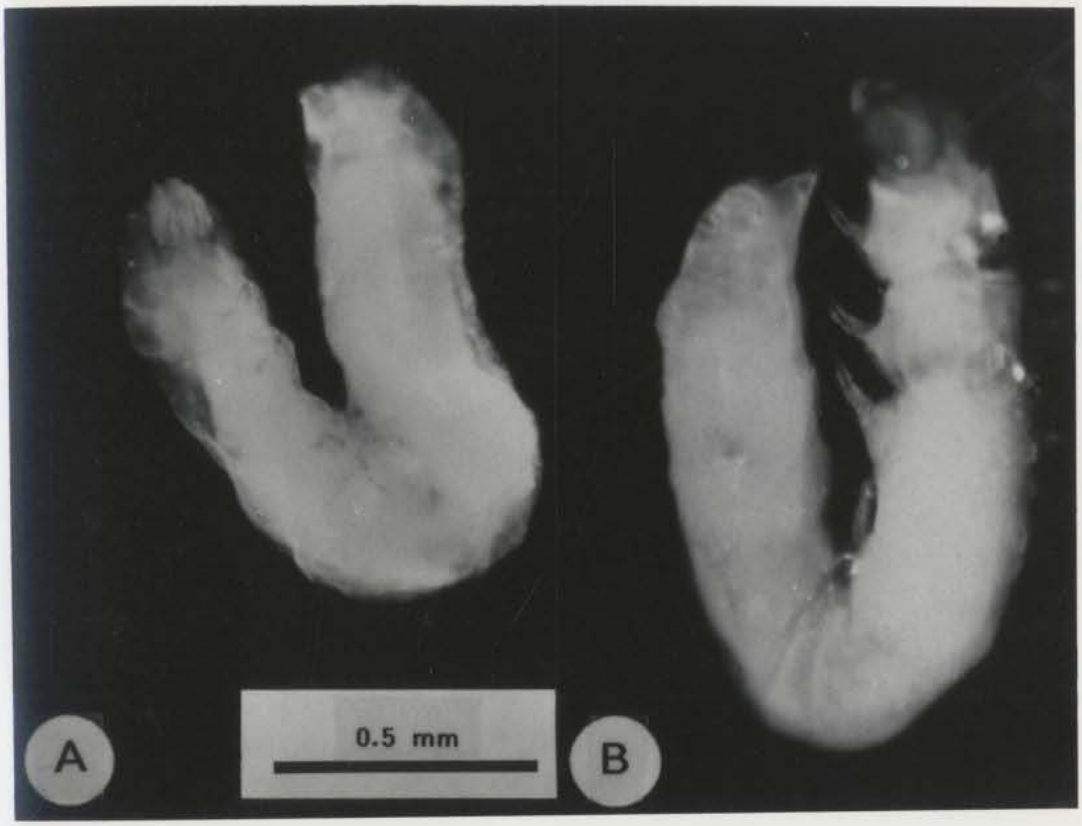
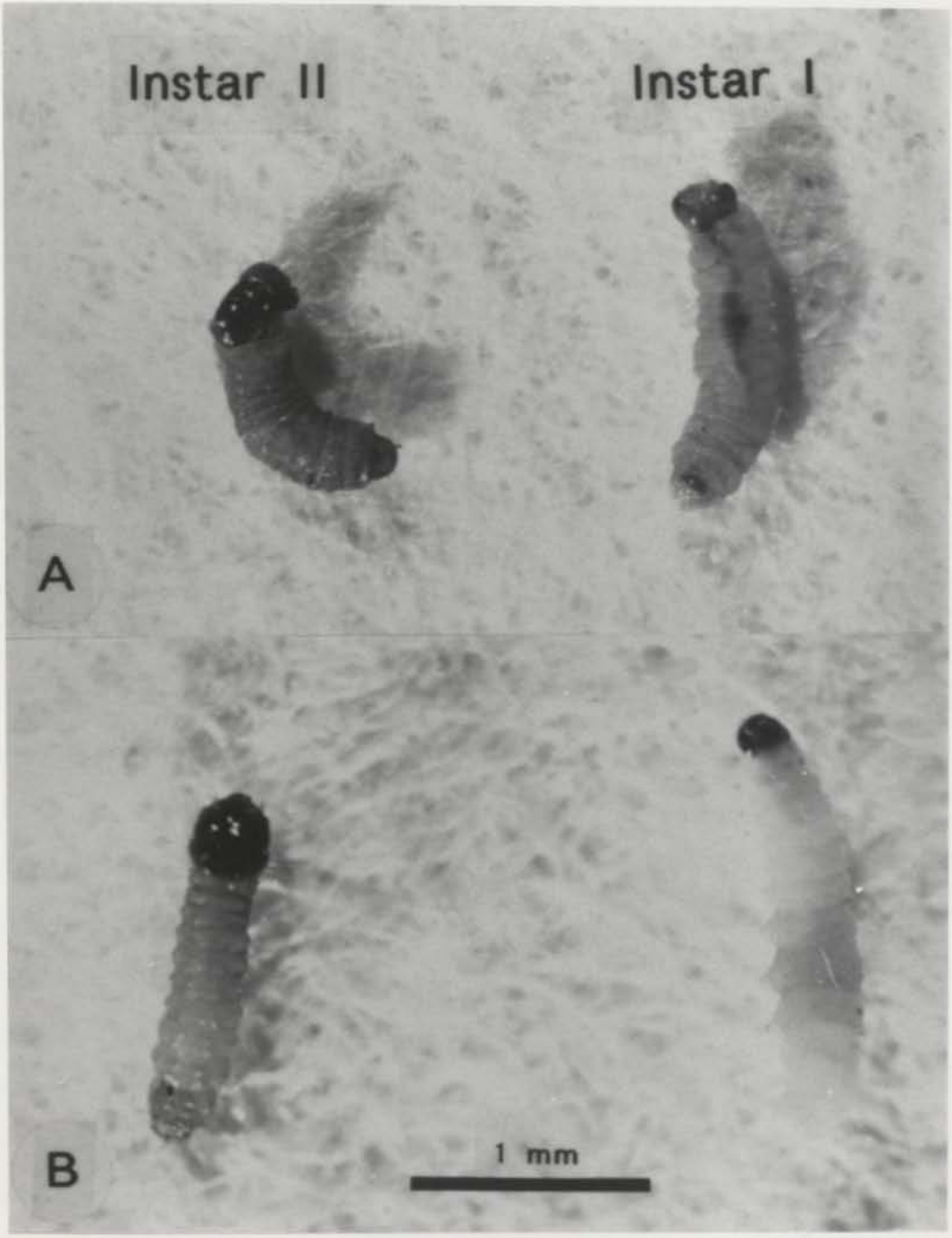

A
0.5 mm
B

Plate 3. Second and first instar larvae of Choristoneura fumiferana that hatched from (A) Fenoxycarb treated and (B) Acetone treated eggs. All treatments occurred 0 to 24 hours after oviposition. Note in A the first instar larva with deformed head and the second instar larva with first instar exuvium attached to head.


Instar II
Instar I
A
B
1 mm

more sensitive than larvae treated during the fifth instar.

As anticipated from my earlier experiments, the greatest effect of Fenoxycarb was manifested prior to egg hatching. Over 90% of the control eggs hatched, compared to only 22% of treated eggs (Table 3). In addition, the compound caused significant mortality ($p=0.024$) during the overwintering diapause stage. It was found that 37.4% of larvae that hatched from treated eggs and that successfully formed a hibernacula, survived the overwintering process and exited the hibernacula. In contrast, 67.0% of the acetone-treated controls survived to the same stage (Table 3). After the hibernaculae, the survival rate of controls declined more rapidly than did that of the treated insects (Fig. 6). Therefore, while the compound decreased overall survival to the adult stage by approximately 4 fold (Fenoxycarb-treated, 5.8±2.04%; Acetone-treated, 22.1±5.57%), it also appeared to displace the natural mortality of the insect to earlier stages of the life history.

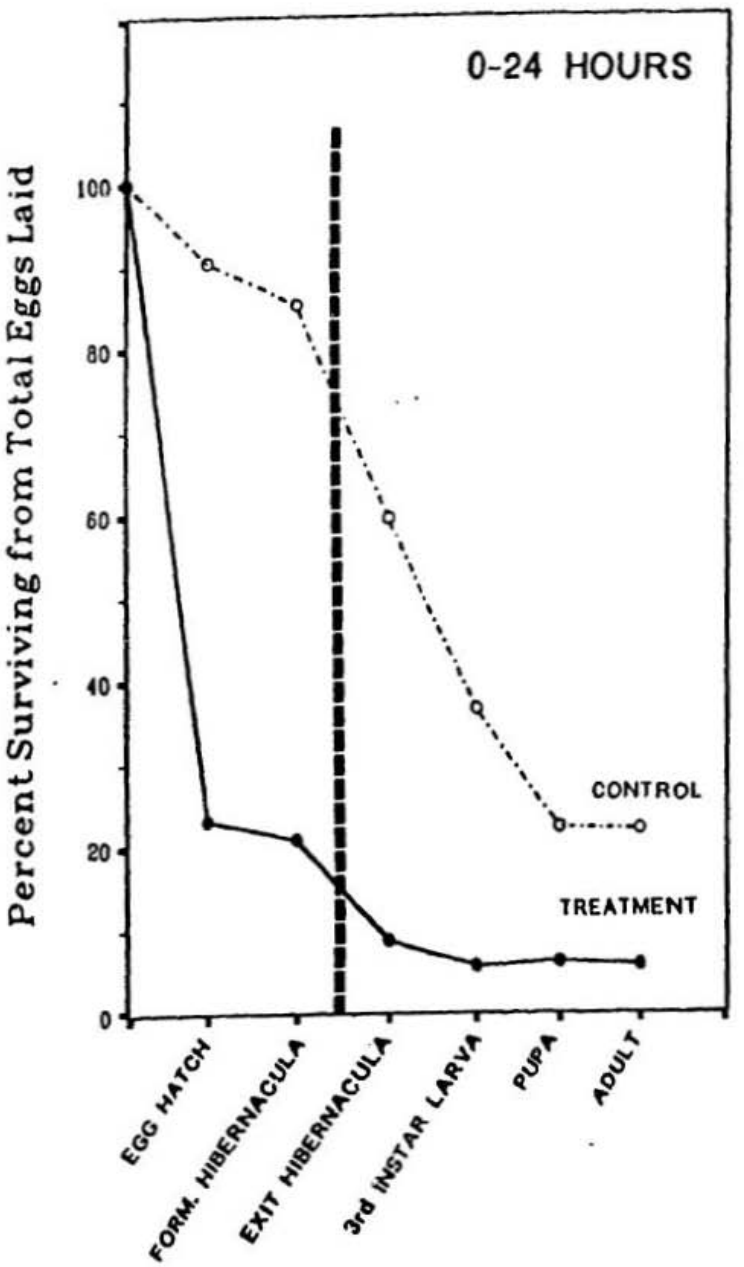
The effect of Fenoxycarb on various stages of the insect's life history was similar for 48 to 72 hr-old eggs as for 0 to 24 hr-old ones, treated with their respective LD_{50} doses. The predominant effect of the compound was on embryogenesis. Only 26.7% of treated eggs hatched, compared to 90.1% of control eggs (Table 3). In contrast to the situation observed for 0 to 24 hr-old eggs, however, significantly ($p=0.001$) fewer larvae that hatched from treated 48 to 72 hr-old eggs survived to form hibernaculae, compared to controls (Fenoxycarb-treated, 74.5±6.17%; Acetone-treated, 98.1±0.74%; Table 3). Survival of larvae during the

Table 3. Percent survival from preceding stages of Choristoneura fumiferana treated with Fenoxycarb at various times during embryogenesis and reared through diapause to the adult stage.

Age of eggs when treated (Conc.)	N	Egg Hatch (±SE)	Survival to Hibernacula (±SE)	Survival to exit of Hibernacula (±SE)	Survival to Third Instar (±SE)	Survival to Pupa (±SE)	Survival to Adult (±SE)
0-24 Hrs (0.01ug/ul)	C† 410	90.5* (3.35)	94.3 (4.20)	67.0* (7.34)	54.0 (6.62)	64.6 (11.0)	99.6 (0.43)
	T 805	23.3* (4.49)	85.4 (4.58)	37.4* (7.38)	72.6 (7.38)	77.7 (11.9)	88.9 (11.1)
48-72 Hrs (12.5ug/ul)	C 535	90.1* (3.98)	98.1* (0.74)	60.2 (4.59)	70.4 (3.37)	62.4 (5.6)	94.7 (2.22)
	T 1144	26.7* (4.90)	74.5* (6.17)	53.3 (8.46)	68.3 (9.83)	78.3 (8.67)	96.1 (3.93)

† C- Acetone Control; T- Treatment
* Significant at p=0.05.

Fig. 6. Survival of Choristoneura fumiferana, treated with Fenoxycarb (0.0005µg/egg) at 0 to 24 hours embryogenesis, over a winter diapause. The vertical line represents the position of the diapause in the life cycle. Total eggs treated: Acetone control, 410; Fenoxycarb treated, 805.


0-24 HOURS
Percent Surviving from Total Eggs Laid
100
80
60
40
20
0
CONTROL
TREATMENT
EGG HATCH
FORM. HIBERNACULA
EXIT HIBERNACULA
3rd INSTAR LARVA
PUPA
ADULT

treated, 98.1±0.74%; Table 3). Survival of larvae during the diapause stage was not affected by treatment. As was the case for larvae that developed from treated 0 to 24 hr-old eggs, the survival rate of control larvae that exited the hibernaculae declined more rapidly than treated ones (Fig. 7) and the compound significantly ($P < 0.001$) decreased overall survival to the adult stage (11.4 ± 4.1% treated; 23.6 ± 3.4% controls; Table 3). Thus, the JHA appeared to displace the natural mortality of the insect to earlier stages of the life history.

No morphogenetic effects such as larval-pupal intermediates or extra moulting were observed in either experiment. Moreover, treatment with the JHA did not delay development.

4.3 Efficacy of Fenoxycarb against eggs of C. fumiferana, reared under different temperatures, photoperiods and humidities.

Analysis of variance of the data demonstrated that photoperiod had no significant effect on the efficacy of the compound. By contrast, temperature and relative humidity, considered separately, each exhibited a significant effect on the mortality due to the compound (Table 4). Two-way interactions occurred between temperature and relative humidity; temperature and photoperiod; temperature and the concentration of the compound; and relative humidity and the concentration of the compound. Three-way interactions were shown to occur between all combinations of variables, except for relative humidity-photoperiod-concentration

Fig. 7. Survival of Choristoneura fumiferana, treated with Fenoxycarb (0.625 µg/egg) at 48 to 72 hours embryogenesis, over a winter diapause. The vertical dashed line represents the position of the diapause in the life cycle. Total eggs treated: Acetone control, 535; Fenoxycarb treated, 1144.

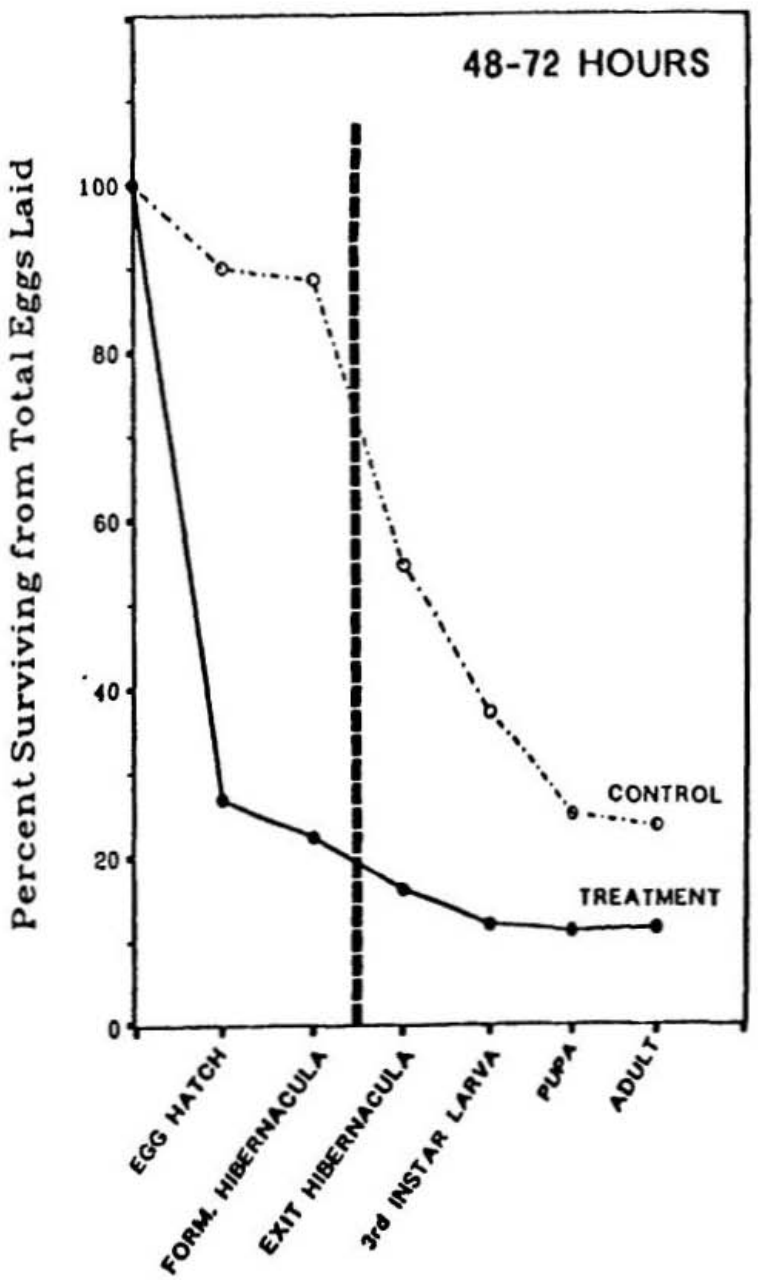

48-72 HOURS
Percent Surviving from Total Eggs Laid
100
80
60
40
20
0
CONTROL
TREATMENT
EGG HATCH
FORM. HIBERNACULA
EXIT HIBERNACULA
3rd INSTAR LARVA
PUPA
ADULT

Table 4. Analysis of variance of data obtained from Fenoxycarb treated eggs (0 to 24 hr-old) of Choristoneura fumiferana reared under different temperatures, relative humidities and photoperiods.

Source of variation	Df	Mean Square	F	Sig. of F*
Main effects				
Temperature	2	75.193	34.025	>.001
Relative Humidity	1	64.397	29.139	>.001
Photoperiod	2	3.240	1.466	.232
Concentration	5	335.101	151.631	>.001
2-Way Interactions				
Temp * RH	2	18.279	8.271	>.001
Temp * Photo	4	14.136	6.397	>.001
Temp * Conc	10	11.986	5.423	>.001
RH * Photo	2	.067	.030	.970
RH * Conc	5	16.609	7.516	>.001
Photo * Conc	10	1.650	.747	.680
3-Way Interactions				
Temp * RH * Photo	4	25.545	11.559	>.001
Temp * RH * Conc	10	6.650	3.009	.001
Temp * Photo * Conc	20	4.091	1.851	.014
RH * Photo * Conc	10	3.487	1.578	.111
4-Way Interactions				
Temp * RH * Photo * Conc	19	9.716	4.397	.000

* P > 0.05 indicates non-statistical significance.

used (Table 4). There was a significant four-way interaction between all variables that were studied.

From the analysis of variance it was shown that photoperiod had no effect on the efficacy of Fenoxycarb on spruce budworm eggs. Therefore, in calculating the LD_{50} values for the experiments, data pertaining to each temperature-relative humidity regime, that embodied three different photoperiods, were pooled, so that the three photoperiods were effectively eliminated as a consideration. Experimental groups were thereby reduced to six (Table 5). Probit analysis was performed on the data and LD_{50} values were obtained along with the slopes of the dose response curves and the 95% confidence limits.

Relative humidity was determined to be a factor that modified sensitivity of the eggs to the JHA, when temperature was maintained constant at 20°C. At 15°C and 25°C, there was no significant differences in the LD_{50} values at 60 or 100%. However, there was a difference at 20°C. At 20°C and 60%RH, eggs were more sensitive to the effects of Fenoxycarb than at 20°C and 100%RH.

Temperature was determined to be a factor that influenced sensitivity of the eggs to the JHA, when the relative humidity was maintained constant at 60%. Among the regimes where 60% RH was tested against the three temperatures, regime 1 (15°C,60% RH) was the most effective, in which the JHA had an LD_{50} of 0.00003 µg/egg. This value was statistically lower than the LD_{50} values obtained for the other two regimes (20°C, 25°C) at 60% and constituted the most effective regime of all that were studied.

Table 5. Probit analysis of the lethal effects of Fenoxycarb on eggs of Choristoneura fumiferana, reared under different temperatures and relative humidities.

Regime No.	Temp. (°C)	RH (%)	n*	Slope±SE	LD_{50}†	95% C.L.
1	15	60	3776	1.34±0.04	0.00003	0.00002 - 0.00005
2	15	100	3856	1.03±0.03	0.00011	< 0.00034
3	20	60	4072	0.70±0.02	0.00014	0.00007 - 0.00023
4	20	100	4135	1.09±0.03	0.00053	0.00033 - 0.00081
5	25	60	3596	0.99±0.02	0.00016	0.00006 - 0.00029
6	25	100	3506	0.82±0.08	0.00011	0.00005 - 0.00020

* Total number treated with compound or acetone.

† in units of µg per egg.

DISCUSSION

This study has shown that spruce budworm eggs are more sensitive to the effects of Fenoxycarb than instar III, instar V or instar VI larvae (Mulye & Gordon 1989). However, they are probably less sensitive than the adult stage (Hicks & Gordon 1992). Hicks & Gordon (1992) showed that adult female moths had an LD_{50} value of 0.0009 µg per insect when topically treated with Fenoxycarb. This value represents the dose that when applied to adult females, resulted in a 50% reduction in the hatching of eggs that were subsequently oviposited. The LD_{50} for adult females resembles the LD_{50} for eggs that were treated soon after oviposition (0.0004 µg per egg). However, the values can be considered to be different because the ovaries are only one of several potential sites that the compound would reach after transversing the exoskeleton of the adult female. In addition, the dose of the compound affecting developing eggs would be lessened according to the number of eggs within the insect's ovaries. Hicks & Gordon (1992) concluded that eggs developing in the ovaries are more sensitive to Fenoxycarb than those that have been oviposited.

Little research has been done on the relative sensitivities of various stages of an insect's life history to JHAs. In general, the eggs of insect species that are affected by specific JHAs are sensitive, while early larvae are little affected. Late instar

larvae show morphogenetic effects when exposed to JHAs and JHAs affect adults by inducing sterilization of the females or possibly, by the transference of JHA to the female during mating by treated males (Retnakaran et al. 1985). The finding that all developmental stages of Blattella germanica were susceptible to Fenoxycarb and that mortality was better in younger larvae than in either adults or eggs may be viewed as an exceptional situation (King & Bennett 1988). The explanation may be that B. germanica is a hemimetabolous insect and reacts differently to Fenoxycarb during it's nymphal stages.

Based on combined mortality of larvae and pupae, the sensitivity of the penultimate larval (fifth) instar to Fenoxycarb was found to be 100-fold lower than that reported for the sixth instar (Mulye & Gordon 1989), and third instars were refractory. These findings may best be understood in terms of the insect's capacity to metabolize the compound before it develops to the sixth instar, when the endogenous JH titre drops and exogenous JHAs may adversely affect metamorphosis (Retnakaran et al. 1985). Thus, it seems reasonable to propose that the approximately two-week period required for third instar larvae to develop to sixth instars would be adequate to allow catabolism of the JHA to ineffective levels, but the five days needed to develop from fifth instars may permit only partial catabolism. In at least one tortricid, however, the susceptibility of the penultimate instar to Fenoxycarb does not appear to be linked to the persistence of the compound into the final instar, since it was shown to be degraded prior to the final

larval-larval moult; it was suggested that the JHA had a delayed effect by reprogramming the insect's endocrine system (Mauchamp et al. 1989). The physiological mechanisms underlying stage-related differential susceptibility and sensitivity of instars other than the final one to a JHA such as Fenoxycarb requires elucidation for C. fumiferana.

Juvenile hormone analogs have been shown to disrupt embryogenesis in many insects when applied to insect eggs prior to the blastokinesis phase of development (Staal 1975). Eggs of C. fumiferana that were treated 0 to 24 hr-old were more sensitive (735X) than eggs treated at 48 to 72 hr-old, indicating that the eggs are more sensitive to Fenoxycarb during the early stages of embryogenesis than during the later stages. This age-related differential susceptibility to Fenoxycarb has been further demonstrated in eggs of other lepidopteran species (Charmillott et al. 1985; Gardner 1991). Juvenile hormone analogs in general have shown this age-related sensitivity toward eggs of many different insects (Riddiford & Williams 1967; Riddiford 1970b,1972; Micciarelli 1977; Kelly & Huebner 1986; Miura & Takahashi 1987). During embryogenesis of Acheta domestica, cleavage and the formation of the blastoderm occur during the time when JH-esterase activity is the greatest (Roe et al. 1987). Juvenile hormone-esterase is the primary hormone deactivating enzyme and is inversely related to the JH titre (Retnakaran et al. 1985). During early embryogenesis, JH was shown to be virtually absent in Locusta migratoria embryos (Tenim et al. 1986) and totally absent in

Nauphoeta cinerea embryos (Imboden et al. 1987). Since early embryogenesis is the period of intensive organogenesis (Sanders et al. 1985), it seems plausible that exogenous application of Fenoxycarb to very early embryos may increase the endogenous JH titre at a time when levels are normally low and thus interfere with embryogenesis. The closer the application of JHA to the time of katatrepsis, the less effect it will have , since at this stage the endogenous JH titre has increased sharply. Research on JH and JH-esterase levels in embryos of C. fumiferana is required to understand the mode of action of Fenoxycarb on the endocrine events associated with embryogenesis.

Exogenous application of Fenoxycarb to one day old sixth instar larvae of the spruce budworm was shown to produce effects that included the formation of larval-pupal intermediates, with precocious evagination of wing disks, and production of deformed pupae with hemolymph-filled blisters in the thoracic region (Mulye & Gordon 1989). The application corresponded with the period when the normal JH titre was low and there was a peak in ecdysone. The presence of high levels of exogenous JH during the initial peak in ecdysone, negatively affects the expression of specific genes and interferes with molecular events associated with normal growth (Riddiford 1985). Like the last larval instar, embryogenesis has been shown in other insects to be characterized by levels of JH and ecdysone that change in a specific fashion as the embryo develops (Temin et al. 1986, Imboden et al. 1978, Roe et al. 1987). Studies should be done to investigate the effects of Fenoxycarb on

embryogenesis at the molecular level, to determine whether such molecular effects are analogous to those induced in the sixth instar larva.

Exogenous application of Fenoxycarb to eggs of C. fumiferana mostly prevented the completion of embryogenesis. However, eggs treated with the JHA sometimes completed embryogenesis to the terminal "black head capsule" stage but the larvae did not initiate hatching or died during the process. This pattern was observed in four tortricid species of orchards from Europe (Eupoecilia ambiguella, Lobesia botrana, Cydia pomonella and Adoxophyes orana). After treatment of eggs younger than two days, embryogenesis in all species proceeded to the "black spot" stage before the embryos died (Charmillott et al. 1985). In another lepidopteran, Adoxophyes orana, treatment of eggs with terpenoid JHAs resulted in embryos that developed to the size and shape of normal embryos prior to hatching but were unable to hatch and eventually desiccated (Schooneveld & Abdullah 1975). Pronounced morphogenic deformities were reported for Rhodnius prolixus embryos, following treatment of eggs with Fenoxycarb (Calli & Huebner 1986). Such embryos displayed body deformities such as misshapen appendages, contorted abdomens and thoraces and "free yolk" within the chorions. In the cat flea, Ctenocephalides felis, Marchiondo et al. (1990) showed that Fenoxycarb disrupted embryogenesis by causing extensive cellular and tissue damage including membrane lysis, burst cells and swollen and ruptured mitochondria. Similarly, C. fumiferana embryos treated with Fenoxycarb showed morphological abberations such as missing

appendages and orientation abnormalities, that would make hatching very difficult or impossible.

Application of high doses of JHAs are known to kill the embryos at any stage during embryogenesis (Wall 1974). Accordingly C. fumiferana eggs that were treated with doses of Fenoxycarb of greater than 2.5 µg/egg showed no signs of embryonic development and became dark in colour.

The underlying physiological causes of JHA-induced abnormal embryogenesis are not known. Thus, while the endocrine effects of Fenoxycarb treatment on eggs are speculative, it is reasonable to propose that the diverse morphological and orientational effects of the JHA result from a disruption in the pattern of protein synthesis, since protein synthesis is the major component of cellular differentiation (Chen 1978). Kelly & Huebner (1987) demonstrated that the normal pattern of protein synthesis in Rhodnius prolixus embryos is modified by Fenoxycarb. Although the physiological and associated embryological effects of the JHA warrant further study, the observation of deformed unhatched embryos within the same egg mass as embryos that completed embryogenesis, but died during hatching, suggests that Fenoxycarb, in addition to affecting morphogenesis, also disrupts the normal embryonic movements. Such disruptions result in deformities of the muscular and/or exoskeletal systems needed to effect eclosion from the egg.

Choristoneura fumiferana larvae that hatched from Fenoxycarb treated eggs and subsequently reared showed significant mortality

later on in post-embryonic development. When treated insects were compared to the controls, significantly fewer larvae hatched or successfully completed diapause. However, upon exiting the hibernaculae, these larvae survived better than those of the controls. In the control population, while a large percentage of the larvae hatched and successfully completed diapause, an increase in larval mortality up to pupation was observed. Overall percent survival of controls is greater than treated insects but the difference in survival is not as great as earlier in the life history, because mortality of the controls increased more rapidly then treated insects after the hibernacula stage. This data indicates that treatment of C. fumiferana eggs with Fenoxycarb displaces the natural mortality to an earlier time in the life history of the insect.

No delayed morphogenetic effects such as larval-pupal intermediates or extra moulting were observed when eggs of C. fumiferana were treated with the compound and larvae that hatched from the eggs reared through to the adult stage. Gardner (1991) similarly observed no delayed effects in the post-embryonic development of Spodoptera frugiperda when eggs were treated with Fenoxycarb. However, C. fumiferana differs from several other insect species, in which JHA treatment of the egg stage later during embryogenesis did not prevent hatch, but produced effects that were revealed later in post-embryonic development (Riddiford 1970a; Riddiford 1972; Marchiondo et al. 1990).

A reduction in the population of the feeding stages is the

main objective in controlling forest defoliators such as C. fumiferana. Larvae of C. fumiferana continuously feed during instars three to six with instars five and six causing the most damage. From the present study, it would appear that Fenoxycarb has the potential to be effective in field control situations in reducing the number of hatching and diapausing larvae, since larvae that hatch are prevented from completing diapause. This would result in fewer feeding larvae surviving later in development.

The present study showed that the efficacy of Fenoxycarb on eggs of C. fumiferana is altered by different environmental conditions. Fenoxycarb exerts its effects at a temperature optimum of 15 °C. Also, relative humidity affected JHA efficacy when the temperature was kept constant at 20°C. The underlying reasons why environmental factors such as temperature and relative humidity had an effect on the susceptibility of the eggs of C. fumiferana to Fenoxycarb, cannot be explained at this time. However, it is known that there are a variety of environmental factors that induce changes in the insect's physiology by action on the endocrine system, which serves as a link between the insect's physiology and it's environment (Highnam & Hill 1977). It has been suggested that JHAs function by disrupting the normal pattern of endocrine activity (Gordon & Burford 1984). This is supported by the studies of Elliott & McDonald (1976) who showed that the corpus allatum of adult Aphis craccivora displayed histological abnormalities following the application of hydroprene. Also, the development of the endocrine systems of adults of Sarcophaga crassipalpis were

inhibited by methoprene (Abou Halawa 1981). Given that the insect's neuroendocrine system is sensitive to JHAs and to environmental factors, it is not unreasonable to propose that JHAs and the environmental factors may interact, influencing the sensitivity of the insect's neuroendocrine system to each of these stimuli. Therefore, the changes observed in the insect's physiology and the consequent developmental effects resulting from JHA-induced neuroendocrinological effects would be affected by environmental factors to which the endocrine system is sensitive. Whether they act alone or together as synergists or antagonists requires determination. This theory presupposes that the Fenoxycarb administered to the eggs would not have been catabolized to ineffective levels by the time in embryogenesis when the endocrine system had been developed.

In many insect species, photoperiodism is a known modifier of the neuroendocrine system (Williams 1969) and is one of the principal physical factors regulating the induction and termination of insect diapause in all stages of development (Yamashita & Hasegawa 1985). However, since C. fumiferana undergoes an obligatory diapause (independent of environmental cues) during the second instar larvae, the newly developing neuroendocrine system of embryos may not be sensitive to photoperiod. This may explain why sensitivity of C. fumiferana embryos to Fenoxycarb was not altered by photoperiod.

The finding that early developing eggs, adults (Hicks & Gordon 1992) and sixth instar larvae (Mulye & Gordon 1989) of C.

fumiferana are highly sensitive to Fenoxycarb suggests a control potential for this JHA. Because the life stages of C. fumiferana are reasonably synchronous in field populations, application of the compound should be against the adult and egg stages, i.e. the most sensitive stages. The window in the field when these stages occur simultaneously in Newfoundland is from mid-July to early-August (Crummey 1976). It is interesting to note that the environmental temperature and relative humidity conditions experienced in Newfoundland during the time the adults and eggs are present in the field are conducive to high efficacy of Fenoxycarb (Appendix 5). The relatively high field persistence of this compound, shown to be four-weeks in a study from the Netherlands (de Reede et al. 1984) and three-four weeks in a study from Pennsylvania, U.S.A (Hull et al. 1991), should help advance applications against targeted stages.

While no studies of the effects of Fenoxycarb on C. fumiferana parasites or predators are available, some effects were observed on the endoparasite, Apanteles ater, when it's tortricid host, Adoxophyes orana was sprayed with Fenoxycarb. However, several other endoparasites, ectoparasites and predators exhibited considerable tolerance to the effects of the compound (de Reed et al. 1984). No detectable damage was recorded to predatory anthocorids when orchards were sprayed with Fenoxycarb to control the pear sucker, Cacopsylla pyricola (Solomon & Fitzgerald 1990). Moreover, Fenoxycarb did not disrupt the biological control of the European red mite, Pananychus ulmi, by coccinellid predators,

although it was suggested that there may be a potential for undesirable effects to immature coccinellids (Hull et al. 1991). Studies directed at determining the susceptibility to Fenoxycarb of non-target insects species, and other fauna within the biosphere of targeted spruce budworm populations, will be required prior to instituting field applications of the JHA.

The rationale behind integrated pest management (IPM) systems is to reduce and maintain the pest population at levels below those causing economic injury by using all available environmentally acceptable strategies, chemical or biological. An IPM system targeted against C. fumiferana would involve a strategy to reduce the loss from the current feeding stage, probably with Bacillus thuringiensis. Since current years' foliage cannot be protected with Fenoxycarb but subsequent years' can, it could be included as a strategy for the long term control of C. fumiferana. Further greenhouse and field studies should be undertaken to examine the efficacy of Fenoxycarb against populations of C. fumiferana and to ascertain the degree to which the JHA may negativively impact upon natural predators, parasitoids and other fauna within the ecosystem of the spruce budworm.

SUMMARY

Topical application of the JHA, Fenoxycarb, to various developmental stages of C. fumiferana showed it to be an extremely promising compound for development as a control agent. Eggs 0 to 24 hr-old were most sensitive; eggs 48 to 72 hr-old and fifth instar larvae were less sensitive while third instar larvae were refractory. Ranking the stages according to the LD_{50} value, the order of sensitivity of the stages was: adult females (Hicks & Gordon 1992) > 0 to 24 hr-old eggs > 48 to 72 hr-old eggs > sixth instar larva (Mulye & Gordon 1989) > fifth instar larva > third instar larva.

Treatment of fifth instar larvae produced morphogenetic effects such as larval-pupal intermediates, supernumerary moulting and deformed pupae and adults.

Failure of hatching was the most striking effect when C. fumiferana eggs were treated with Fenoxycarb. Embryonic development was blocked by the compound at an early stage, or completed by embryos developing from the same egg mass. Observations of deformed unhatched embryos and some that completed embryogenesis, but died during hatching while partially emerged, suggests that Fenoxycarb disrupted the muscular/exoskeletal system that is needed to allow eclosion from the egg. Choristoneura fumiferana eggs treated with Fenoxycarb produced numerous abnormalities in the developing

embryos such as miss-orientation and absence of walking appendages.

Treated eggs that, nevertheless, hatched developed increased mortality in post-embryonic development. Eggs that were treated with Fenoxycarb and reared to the adult stage exhibited increased mortality prior to or during the overwinter diapause stage (hibernacula). Insects that developed from treated eggs and that subsequently completed diapause had a lower morality throughout the remainder of the development than control insects. It thus appears that the natural mortality of C. fumiferana treated as eggs was shifted toward earlier stages in the life history (egg hatch and overwintering stages). Moreover, the overall mortality to the adult stage was significantly higher in treated insects than in controls.

Temperature and relative humidity altered the efficacy of Fenoxycarb toward early eggs of C. fumiferana. Treated and control eggs were not affected by photoperiod. The optimum temperature to achieve greatest effects of the compound on eggs was 15°C. Relative humidity only became an influencing factor when the temperature was at 20°C. At that temperature a relative humidity of 60% was shown to be most effective.

This compound is an excellent candidate for inclusion in integrated pest management programs directed against C. fumiferana.

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Appendix 1. Control of various insect pests with the Insect Growth Regulator, Fenoxycarb.

Order	Family	Species	Reference
Coleoptera	Cucujidae	Oryzaephilus surinamensis (L.)	Dorn et al. 1981 Edwards et al. 1991 Kramer et al. 1981 Thind & Edwards 1986
		Rhyzopertha dominica (F.)	Dorn et al. 1981 Edwards et al. 1991 Kramer et al. 1981 Thind & Edwards 1986
	Curculionidae	Pantomorus cervinus (Bohemen)	Coats 1990
		Sitophilus granarius (L.)	Dorn et al. 1981 Edwards et al. 1991 Edwards & Short 1984
		Sitophilus oryzae (L.)	Edwards & Short 1984 Kramer et al. 1981
		Sitophilus zeamais (Schonherr)	Edwards & Short 1984 Kramer et al. 1981
		Cryptolestes ferrugineus (Stephens)	Thind & Edwards 1986
		Cryptolestes pusillus (Schonherr)	Kramer et al. 1981
	Dermestidae	Trogoderma variabile Ballum	Dorn et al. 1981
	Tenebrionidae	Tribolium castaneum (Herbst.)	Dorn et al. 1981 Edwards et al. 1991 Kramer et al. 1981 Thind & Edwards 1986
		Tribolium confusum J du V	Dorn et al. 1981 Kramer et al. 1981
Diptera	Culicidae	Culex pipiens L.	Dorn et al. 1981
		Psorophora columbiae (Dyar & Knab)	Bassi et al. 1987 Weathersbee et al. 1988 Ogg & Gold 1988

Appendix 1. Control of various insect pests with the Insect Growth Regulator, Fenoxycarb. Continued.

Order	Family	Species	Reference
Hemiptera	Lygaeidae	Oncopeltus fasciatus (Dallas)	L-Kaelin & Masner 1981 Masner et al. 1981
	Reduvidae	Rhodnius prolixus Stal.	Kelly & Huebner 1986
		Triatoma infestans	Picollo et al. 1987
Homoptera	Diaspididae	Aonidiella aurantii Mask.	Dorn et al. 1981
Hymenoptera	Formicidae	Solenopsis invicta Buran.	Banks et al. 1988 Glancey & Banks 1988 Phillips & Thorvilson 1989
Lepidoptera	Bombycidae	Bombyx mori (L.)	Plantevin et al. 1991
	Noctuidae	Spodoptera frugiperda (smith)	Gardner 1991
		Spodoptera littoralis	Masner et al. 1981
	Phycitinae	Plodia interpunctella (Hubn.)	Kramer et al. 1981
		Sitotroga cerealella (Olivier)	Kramer et al. 1981
		Ephestra cautella (Walker)	Kramer et al. 1981
		Cacopsylla pyricola (Forester)	Solomon & Fitgerald 1990
	Pyralidae	Ostrinia nubilalis Hbn.	Gadenne et al. 1990
		Galleria mellonella (L.)	Masner et al. 1981
	Tortricidae	Adoxophyes orana F.v.R.	Charmillot et al. 1985 de Reede et al. 1984
		Adoxophyes reticulana Huebn.	Masner et al. 1981 Dorn et al. 1981
		Archips podana (Scop.)	de Reede et al. 1984
		Choristoneura fumiferana (Clem.)	Mulye & Gordon 1989 Hicks & Gordon 1992
		Cydia pomonella L.	Charmillot et al. 1985
		Eupoecilia ambiguella Hb.	Charmillot et al. 1985

Appendix 1. Control of various insect pests withthe Insect Growth Regulator, Fenoxycarb. Continued.

Order	Family	Species	Reference
Lepidoptera	Tortricidae	Heliothis virescens (F.)	Masner et al. 1987
		Lobesia botrana Den & Schiff.	Charmillot et al. 1985
		Pandemis heparana (Denn & Schiff.)	de Reede et al. 1984
		Platynota idaeusalis	Hull et al. 1991
Siphonaptera	Pulicidae	Ctenocephalides felis (Bouche)	Marchiondo et al. 1990
Orthoptera	Blattellidae	Blattella germanica (L.)	King & Bennett 1990
			Masner et al. 1981

Appendix 2: Volumes of Fenoxycarb added to the egg masses of Choristoneura fumiferana.

# of eggs in egg mass	Volume added to each egg mass (µl)	Amount of compound (per egg) at each concentration							
		25.0 µg/µl	12.5 µg/µl	5.0 µg/µl	0.5 µg/µl	5×10^{-2} µg/µl	5×10^{-3} µg/µl	5×10^{-4} µg/µl	5×10^{-5} µg/µl
30	1.5	1.25	0.625	0.25	0.025	2.5×10^{-3}	2.5×10^{-4}	2.5×10^{-5}	2.5×10^{-6}
31/32	1.6								
33/34	1.7								
35/36	1.8								
37/38	1.9								
39/40	2.0								
41/42	2.1								
43/44	2.2								
45/46	2.3								
47/48	2.4								
49/50	2.5								

Appendix 3. Outline of the experimental protocol for experiments involving eggs of the spruce budworm reared under different environmental conditions.

Temperature (°C)	Relative Humidity (%)	Photoperiod (L:D)	n*
15	60	6:18	1465
		12:12	1029
		18:6	1035
	100	6:18	1021
		12:12	1258
		18:6	1078
20	60	6:18	1089
		12:12	1122
		18:6	1184
	100	6:18	1320
		12:12	998
		18:6	1148
25	60	6:18	985
		12:12	1037
		18:6	881
	100	6:18	952
		12:12	1016
		18:6	858

* Total number of eggs untreated or treated with Fenoxycarb and Acetone.

Appendix 4. Mean number of incubation days for eggs of Choristoneura fumiferana that successfully hatched after treatment with acetone and various concentrations of Fenoxycarb. Eggs were reared at 24°C, 60% RH and 18L:6D photoperiod.

0- to 24-h old

	Days	SD	n
No treatment	6.9	1.26	331
Acetone	6.9	1.91	305
2.5×10^{-6}	6.8	1.74	128
2.5×10^{-5}	6.2	0.58	133
2.5×10^{-4}	6.3	1.69	97
2.5×10^{-3}	9.3	1.80	4
0.025	8.1	1.82	11
0.25	11.0	–	1
0.625	–	–	–

48- to 72-h old

	Days	SD	n
No treatment	6.3	0.55	314
Acetone	6.1	0.62	283
2.5×10^{-3}	5.9	1.27	193
0.025	6.0	0.69	181
0.25	6.1	0.62	98
0.625	5.9	1.79	87
1.25	6.3	1.13	93

Appendix 5. Temperature (°C) and relative humidity (%) information for July and August at St. John's, Newfoundland. (Environment Canada).

Temperature

Month	mean Max.	mean Min.	mean Daily
July	20.2	10.7	15.5
August	19.4	11.1	15.3

Record Temperatures

July	31.5(1983)	-1.2(1976)
August	30.0(1944)	0.2(1976)

Relative Humidity

Month	mean Low	mean High
July	60.0	97.0
August	63.0	96.0