EFFECTS OF SEVERAL INSECT GROWTH REGULATORS ON THE CABBABE MAGGOT, *DELIA RADICUM* Linnaeus (DIPTERA: ANTHOMYIIDAE)

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EFFECTS OF SEVERAL INSECT GROWTH REGULATORS ON THE CABBAGE MAGGOT, DELIA RADICUM Linnaeus (DIPTERA: ANTHOMYIIDAE)

BY

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ABSTRACT

A procedure was developed for maintaining a stock colony of the cabbage maggot, Delia radicum (Linnaeus), in the greenhouse. A petri dish rearing method producing a high yield of adults was developed for laboratory screening of insect growth regulators against this pest.

Topical application of the chitin synthesis inhibitor, diflubenzuron (Dimilin®), at a rate of 1.0 µg per egg significantly reduced hatch of 24-28 hour old eggs and caused 100% mortality of first instar larvae before they could feed on rutabaga slices. Diflubenzuron had no effect on mature larvae or pupae of D. radicum. Methoprene (Altosid® 5E), a juvenile hormone analogue, applied at the same dose, did not adversely affect egg hatch but prevented the eclosion of adults from pupae that subsequently developed from treated eggs. Pupal-adult intermediates were observed in the treated puparia. Treatment of mature, post-feeding larvae and recently formed pupae with methoprene also produced non-eclosing pupal-adult mosaics. A dose of 0.05 µg per larva completely suppressed adult eclosion. However, diflubenzuron applied to third instar larvae or pupae did not affect adult eclosion. Juvenile hormone I and the anti-juvenile compound, precocene II, did not affect D. radicum when applied to the egg, larval or pupal stages.

Thin-layer chromatography and colorimetric analysis of hemolymph metabolites 36 hours after treatment of post-feeding larvae demonstrated that methoprene caused a significant reduction in trehalose, the predominant carbohydrate in the hemolymph. Methoprene did not disrupt hemolymph amino acid or protein concentrations.

This study suggests that methoprene and diflubenzuron are potential insecticides against D. radicum and that further studies on their effectiveness in the field and basis of disrupted development in D. radicum and other non-target insects is warranted.

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INTRODUCTION

The cabbage maggot (cabbage root fly) Delia radicum Linnaeus (Hylemya brassicae Bouché) (Diptera: Anthomyiidae) is a major pest of root and stem crucifers in North America and Europe. In North America, it is a particularly serious pest of Cruciferae in Canada and the northern regions of the United States, but is seldom injurious south of the 40 N latitude (Metcalf and Flint, 1962). In the Atlantic Provinces, the cabbage maggot is the most serious pest of rutabagas (swede turnips), cabbage, cauliflower, broccoli and Brussel sprouts (Read, 1972). Delia radicum is of particular concern to the agricultural industry in Newfoundland because it is the limiting factor in the commercial production of cabbages and rutabagas, two of the three principal crops grown in this region (Morris, 1959a). It is estimated that 75 per cent of the marketable crop would be forfeited yearly if this pest was not controlled (pers. comm. M. Stapleton, Provincial Agriculture, Research Station, St. John's West, Newfoundland).

Delia radicum adults, similar in appearance to the common house fly but only half as long, oviposit eggs on or near the soil surface, or underneath the foliage of planted crucifers. First instar larvae (L_1) hatch from eggs and feed on the roots of host plants below the soil surface. These small white maggots generally attack the secondary roots and then move up into the main root of their host as they feed and mature, developing into second (L_2) and third (L_3) instar larvae (Read, 1972). When maggots are abundant, the roots of cabbage, cauliflower and broccoli become honeycombed and rotten and young plants often die from the attack. Delia radicum larvae feeding on young rutabaga and radish cause root distortion and later in the season, tunnel into the fleshy roots, rendering them unmarketable (Morris, 1959a; Read, 1972). At the end of the feeding period, post-

feeding L_3 larvae leave the host in search of a suitable pupation site in the surrounding soil or, more rarely, pupate in their galleries, particularly if the surrounding soil is very dry (Read, 1972). Inside the puparium, the larva develops into a pupa and eventually into an adult. At eclosion, the adult breaks open the puparial case and pushes its way to the soil surface.

Depending on the locality and soil type, D. radicum may have several generations per year, the maximum recorded being five in some parts of the U.S.A. (Nair and McEwen, 1975). In Newfoundland, most F_1 adults do not emerge from puparia until the following spring however a small percentage of adults do and comprise a partial second generation (Morris, 1959a). In late May or early June, adults emerge from puparia that overwinter in fields in which cruciferous crops have been grown the previous year. Eggs are laid 3-6 days after the flies emerge and the larvae hatch within 3-7 days after oviposition and feed within the roots of susceptible plants for 3-4 weeks. Some adults will emerge from pupae 2-3 weeks after the larvae have left the plants to complete a second cycle. However, most pupae enter diapause to overwinter and emerge as adults the following spring (Morris, 1959a). The entire life cycle takes approximately six weeks.

The cabbage maggot was first described by Bouché in Germany in 1833 and is thought to be of European origin (Slingerland, 1894). Records show it was a serious pest in the early 1800's in Europe, particularly in Germany and England. In North America. it was first recorded in the U.S. in 1833 and fifty years later it was described as a serious pest of cauliflower in Canada (Slingerland, 1894). The most effective control methods recommended in the 1800's were the use of tarred paper cards fitted around the base of plants (as ovipositing deterrents) and inorganic insecticides such as carbolic acid and carbon disulphide against early larval instars (Slingerland, 1894). These methods were costly and inorganics were generally phytotoxic and not effective in all soil types. During the 1920s and 1930s, the chlorides of mercury were favored as seedbed drenches, furrow dusts or as seed coatings (Judge et al., 1968).

In the 1940s, the discovery of the insecticidal properties of DDT and subsequently, other organochlorides of unprecedented power and range of activity, led to their use against a wide array of insect pests. In Prince Edward Island, between 1952-1955, maggots were controlled with weekly foliage sprays of DDT, timed to coincide with the emergence of flies from overwintering pupae. However, the timing of the first spray was difficult to predict and control effectiveness was poor in wet weather (Read, Cannon, 1958). The cyclodienes, a class of organochloride insecticides, were generally more persistent in soil than DDT-type compounds and provided relatively long-term control. Several cyclodiene compounds, particularly aldrin, dieldrin and heptachlor, gave satisfactory control of cabbage maggots in Canada, the U.S. and Britain (Stitt, 1953; King and Forbes, 1954; Read and Cannon, 1958; Morris, 1959b; Metcalf and Flint, 1962; Coaker et al., 1963).

The success of the organochloride insecticides was short-lived. Reports of D. radicum populations that were no longer controlled with cyclodiene compounds first appeared in the literature in the early 1960's from Britain and the U.S. (Howitt and Cole, 1962; Coaker et al., 1963). At the same time, tests on soil insects from several regions across Canada demonstrated that four species of root maggots had developed cyclodiene resistance, including cabbage maggots from Newfoundland and P.E.I. (Harris et al., 1962; Morris, 1963; Read, 1964). Delia radicum and other soil insect pests had also developed cross-resistance to other cyclodiene insecticides not previously used against them (Harris, 1977). The development of resistance in soil insects was attributed to selection pressures of persistent organochloride residues in the soil (Coaker et al., 1963; McDonald and Swailes, 1975; Harris, 1977). Read and Brown (1966) demonstrated that dieldrin resistance was inherited in D. radicum.

With the development of insecticide resistance and concern over persistent residues in the environment, use of organochloride insecticides has been restricted since the late 1950s. Other, less persistent compounds, such as organophosphates

and carbamates have been developed to take their place. Several of these chemicals have been shown to be effective against the cabbage maggot, although they generally do not give as consistent control as did the organochlorides prior to the development of resistance (Judge et al., 1968; Read, 1970; Goble et al., 1972). Resistance to non-organochloride insecticides has been slower to develop in insects. Low level organophosphate and carbamate resistance has been reported in the cabbage maggot and the onion maggot, Delia antiqua Meigen (Harris, 1977). The non-specific organophosphate resistance developed by D. antiqua in some regions of Canada and the U.S. has resulted in cross-resistance to carbofuran, a carbamate insecticide currently in use for cabbage maggot control (Harris and Svec, 1966).

The sole reliance on selected organophosphate and carbamate compounds to replace obsolete insecticides increases the selection pressure for resistance development in insect pests. Root maggots are particularly at risk in that they are continuously exposed to insecticide residues in the soil and may have several generation cycles per growing season (Harris, 1977). In the ongoing search for alternative insecticides, emphasis is being placed on the development of insect-specific compounds that act on novel systems within the target pest. One class of compounds being tested are insect growth regulators. This class encompasses a wide variety of compounds that act in various ways to disrupt the normal development of insects. Death by treatment is not immediate but results from a combination of secondary factors related to disrupted development. The compounds in this class generally fall into three major categories: juvenile hormone analogues (juvenoids), anti-juvenile compounds and chitin synthesis inhibitors. As a group, insect growth regulators are effective against a variety of insect pests, including those belonging to the cyclorrhaphid Diptera, are relatively non-toxic to mammals and other non-target organisms and do not persist for long periods in the environment (Retnakaran et al., 1985).

To date, several insect growth regulators have been shown to be effective

against the cabbage maggot. Juvenoids are ovicidal and prevent adult emergence in the laboratory (van de Veire and de Loof, 1973) and show promising control potential against D. radicum in the field (van de Veire and de Loof, 1974). Chitin synthesis inhibitors were effective in reducing crop damage due to cabbage maggots in field and greenhouse trials (Howard, 1977; Philip et al., 1977; Ritcey et al., 1977; Turnbull, 1982). Laboratory studies showed one such chitin synthesis inhibitor, diflubenzuron, to be ovicidal and larvicidal against D. radicum when the parental generation of adults or the eggs were treated (van de Veire and Delcour, 1976; Turnbull, 1982). Diflubenzuron was of low toxicity to Aleochara bilineata Gyllenhal. (Gordon and Cornect, 1986), a major predator/parasitoid of the cabbage maggot, found to parasitize up to 60% of field pupae in Newfoundland (Morris, 1959a). This is in contrast to the application of conventional insecticides which usually results in considerable mortality of parasites (Chapman and Eckenrode, 1973).

This study was done to evaluate the effectiveness of four compounds, representing the three major insect growth regulator groups, against eggs, larvae and pupae of the cabbage maggot in a laboratory setting. The study also provided an opportunity to obtain preliminary data on the physiological basis of disrupted development of a selected compound against the target insect.

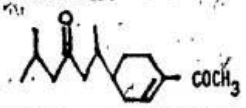
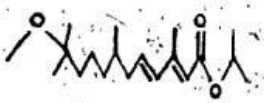
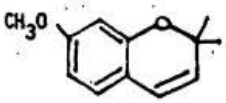
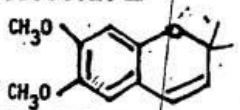
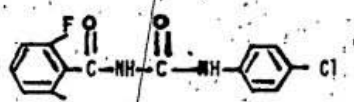
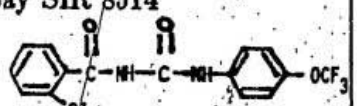
INSECT GROWTH REGULATORS: BACKGROUND INFORMATION

2.1. Juvenile hormone analogues

In 1956, Carroll Williams prepared the first active juvenile hormone extract from insect corpora allata and found it easily penetrated insect cuticle to block metamorphosis (Williams, 1956). Subsequent physiological studies led Williams to suggest juvenile hormone extracts be used as insect-specific insecticides to which pest insects would be unable to develop resistance (Williams, 1967). However, pure juvenile hormone compounds are seldom used in field experiments because of their cost of synthesis and instability in the field. Two major advances have been made in the practical application of juvenile hormone compounds. The first was the discovery of juvenile hormone activity in balsam fir (Sláma and Williams, 1966). The compound responsible for the juvenile hormone activity was isolated and named juvabione; being structurally similar to insect juvenile hormone, it represented the first of the naturally occurring juvenile hormone analogues (JHAs, juvenoids) (Bowers et al., 1966) , or compounds that functionally resemble juvenile hormone and may or may not be similar to it in structure (Table 2-1) (Retnakaran et al., 1985). The second major advance in the practical application of juvenoids was the laboratory synthesis of substances that were several hundred-fold more active than natural juvenile hormone (Bowers, 1969).

To date, four homologues of natural insect juvenile hormone (JH0-JHIII), all epoxy methyl dodecadienoates with distinct carbon chain lengths, have been identified (Röller and Bjerke, 1965; Meyer et al., 1968; Judy et al., 1973; Bergot et al., 1980). Efforts to optimize the chemistry of juvenoids for use as insecticides

Table 2-1: Summary table of the insecticidal properties of three major insect growth regulator groups with representative compounds

Compound Class	Insecticidal Properties	Representative Compounds
Juvenile Hormone and Analogs	. Penetrate insect cuticle to prevent metamorphic change in the epidermis . Function as juvenile hormone agonists and/or antagonists . Interfere with gene activation preventing imaginal development . Cause incomplete metamorphosis in immatures producing extra larval, nymphal or pupal stages . Inhibit egg hatch due to disrupted blastokinesis when applied to adults and/or eggs	Juvenile Hormone I (Cecropia JH)  Juvabione  Methoprene (ZR-515) 
Anti-Juvenile Hormone Agents	. Act as selective cytotoxins of insect corpora allata . Cause precocious metamorphosis of nymphs into miniature sterile adults . Sterilize treated adults of hemi- and holometabolous insects . Disrupt embryogenesis preventing egg eclosion	Precocene I  Precocene II 
Chitin Synthesis Inhibitors	. Inhibit the final stage of chitin synthesis by epidermal cells . Interfere with the deposition of chitin into the cuticular matrix of new developing cuticle . Cause interstadial mortality: larvae unable to shed old cuticle . Ovicidal: fully developed larvae unable to break out of egg case	Diflubenzuron  Bay SIR 8514 

have resulted in the synthesis of thousands of compounds that collectively are disruptive to members of all the major insect orders (Staal, 1975; Retnakaran et al., 1985). While insects may be unable to develop resistance to the natural hormone, they can develop resistance to juvenile hormone analogues (Staal, 1982; Retnakaran et al., 1985). To date, only two compounds, both products of Zoecon/Sandoz Corporation, have been registered for use in North America. In the U.S.A., methoprene has been approved by the Environmental Protection Agency for the control of flood water mosquitoes and fleas and kinoprene for the control of several homopteran pests (Table 2-1) (Staal, 1982; Retnakaran et al., 1985) . In Canada, methoprene is registered (Pesticide Division, Agriculture Canada) for use against mosquitoes.

Juvenile hormone is a natural component of the neurohormonal milieu of insects whose presence suppresses metamorphic change during a molt. It is evident that presenting exogenous juvenile hormone activity during periods when it is normally low or absent, ie. in preparation for the imaginal molt in Hemimetabola or for the larval-pupal or pupal-adult molt in Holometabola will adversely affect metamorphosis. The treatment of hemimetabolous nymphs with juvenoids causes the production of supernumerary larvae or various degrees of nymphal-adult forms (adultoids). The treatment of larvae or young pupae of susceptible Holometabola results in the production of larval-pupal or pupal-adult intermediates (Williams, 1956; Sehnal and Meyer, 1968; Srivastava and Gilbert, 1968; Jakob and Schoof, 1971; van de Veire and de Loof, 1973; Garris and Adkins, Jr., 1974; Mckague and Wood, 1974; Staal, 1982; Sparks, 1984; Retnakaran et al., 1985) . The period of greatest sensitivity for metamorphic inhibition by juvenoids is the ultimate or penultimate instar (or young pupa in the Holometabola) (Sláma and Williams, 1966; Wright, 1970; Staal, 1975) .

The corpora allata of insects are inactive during the ultimate stages of metamorphosis, but secrete juvenile hormone again in the adult stage to bring about sexual maturation. Generally, the administration of juvenoids has little

effect on the adult insect or on reproduction (Staal, 1982; Retnakaran et al., 1985). Cases where adult sterility is induced due to juvenile hormone analogue treatment are usually a result of the transferral of the compounds to developing eggs within the maternal body where they block embryonic development. Juvenoids inhibit embryogensis when applied to the eggs of all insect species examined, which include representatives from all major orders (reviewed by Staal, 1975; Sehnal, 1983). Insect eggs are most sensitive prior to or just after being oviposited (Sláma and Williams, 1966; Riddiford and Williams, 1967). Juvenojds block embryogenesis during blastokinesis (Riddiford and Williams, 1967; Staal, 1982) or result in delayed effects on metamorphosis further in oncogonesis similar to those obtained by treating immature stages (Willis and Lawrence, 1970; Riddiford and Truman, 1972).

The biochemical effects of juvenoids are complex and vary from one compound to another. They can function as juvenile hormone agonists or antagonists or both (Henrick, 1982; Retnakaran et al., 1985). Juvenile hormone is thought to act at the genetic level. A widely-accepted model of action proposes that juvenile hormone enters the epidermal cell and after complexing with receptors activates or inactivates certain genes preventing imaginal development (Laufer and Borst, 1983).

The lethal morphogenetic disturbances of juvenoids late in larval development have resulted in their registration for use against insects of economic importance during the adult stages, such as public health and veterinary pests (Staal, 1982; Retnakaran et al., 1985). They also show promise for control of stored products pests (Nickle, 1979; Mian and Mulla, 1983).

2.2. Anti-juvenile hormone agents: the precocenes

Research with juvenoids as insecticides demonstrated that a more practical approach to the control of crop feeding larvae would be to disrupt the action of endogenous juvenile hormone. Application of an anti-juvenile hormone agent would induce those results historically produced by surgical allactectomy, i.e. precocious metamorphosis of larvae into miniature sterile adults and sterilization of treated adults. Bowers and his colleagues, led by the previous discovery of insect hormone activity in plants, randomly searched for anti-juvenile hormone activity in plant extracts (reviewed by Bowers, 1982). After exposing milkweed bugs (Oncopeltus fasciatus) to approximately 300 plants, young nymphs skipped instars to form miniature adults and exposed adults were sterilized by topical treatment or exposure to vapors of an extract from Ageratum houstonianum, an ornamental bedding plant (Bowers et al., 1976). In support of the apparent anti-juvenile hormone action of the extract, both effects could be reversed by concomitant application of exogenous juvenile hormone. Two distinct anti-juvenile hormone compounds, both dichromene compounds (Table 2-1), were isolated and named precocene (I-II) (Bowers et al., 1976). Though the precocenes have significant anti-juvenile hormone activity against several hemimetabolous taxa, precocious development has been induced in only two species among insects with complete metamorphosis (Bowers 1982, 1985). A number of other anti-juvenile hormone agents have been reported but the precocenes remain the most well researched (reviewed by Staal, 1986).

Despite the absence of a developmental effect in holometabolous insects, the precocenes disrupt reproduction in both hemi- and holometabolous species, including Dipterans. Sterilization results from the inhibition of ovarian development (Bowers et al., 1976), oocyte growth (Landers and Happ, 1980) and from the disruption of vitellogenesis (Masner et al., 1979; Wilson et al., 1983). Treating young eggs of sensitive hemimetabolous species with precocene inhibits hatch due to a disruption late in embryogenesis or results in delayed precocious metamorphosis (Bowers et al., 1976; Dorn, 1982; Aboulafia-Baginsky et al., 1984).

The predominant physiological effect of precocene is thought to be a reduction in hemolymph juvenile hormone titer since all of its actions can be reversed by application of exogenous juvenile hormone (Bowers et al., 1976; Masner et al., 1979; Bowers, 1982; Bowers, 1983; Retnakaran et al., 1985) . Evidence strongly suggests that this is a direct and not a brain-mediated response (Bowers and Martinez-Pardo, 1977; Müller et al., 1979; Bowers and Aldrich, 1980) and involves the inhibition of the secretory activity of the corpora allata (Pratt and Bowers, 1977; Masner et al., 1979; Müller et al., 1979; Unnithan and Nair, 1979; Feyereisen et al., 1981; Wilson et al., 1983). The basis of its cytotoxic effect is believed to be in its bioactivation by gland cells of insect corpora allata.

Precocene treatment causes a selective degeneration of corpora allata secretory cells (Unnithan et al., 1977; Liechty and Sedlak, 1978; Schooneveld, 1979; Unnithan et al., 1980) and it has been proposed that precocene is metabolically activated by insect corpora allata to produce the toxic effects (Brooks et al., 1979). Evidence strongly suggests that the precocene molecule undergoes epoxidation by monooxygenase enzymes to a highly reactive and unstable 3,4-epoxide intermediate that alkylates cellular macromolecules (Ohta et al., 1977; Jennings and Ottridge, 1979; Pratt et al., 1980; Soderlund et al., 1980). The monooxygenase enzymes involved in the final epoxidation step of juvenile hormone biosynthesis have been implicated as those responsible for the bioactivation of precocene (Pratt et al., 1980; Hamnett and Pratt, 1983). The organ specificity of the precocenes towards the corpora allata may result simply from a very high level of epoxidase in this gland of sensitive insects (Pratt et al., 1980). Although the differential sensitivity of hemi- and holometabolous larvae to the precocenes remains unexplained, the corpora allata of holometabolous insects have been shown to be sensitive in vitro (Bowers and Feldlaufer, 1982). The observation that precocenes are rapidly sequestered by hemolymph proteins in several insensitive insects (Soderlund et al., 1981) suggests that a toxic titer of precocene may never reach the corpora allata due to peripheral detoxification. Unfortunately, an activation pathway for precocene has been discovered in rat

liver where injection of precocene causes extensive liver damage (Hsia et al., 1981; Halpin et al., 1984).

The future application of precocene-type agents in insect control will depend on overcoming two main problems of the natural precocenes: vertebrate toxicity and inactivity in holometabolous larvae.

2.3. Chitin synthesis inhibitors

In the course of developing analogues and derivatives of the herbicide dichlobenil, scientists at Philips-Duphar B.V. in The Netherlands synthesized a series of 2,6-dichlorobenzoylphenyl ureas. These compounds had no herbicidal activity but were highly toxic to insects: larvae survived ingestion but died during the next molt (Wellinga et al., 1973). The molt disrupting action of the benzoylphenyl ureas and the discovery that they inhibited chitin synthesis in treated insects led them to be known as the chitin synthesis inhibitors. The most investigated of these compounds is diflubenzuron (Dimilin®, PH 6040, TH 6040) (Table 2-1). Numerous other companies have synthesized their own analogues, which generally consist of two substituted ring structures connected by a urea bridge (Table 2-1).

The chitin synthesis inhibitors generally induce insect death during or immediatly following the molting process, the time when chitin synthesis is particularly crucial to survival (Retnakaran et al., 1985). Characteristic abnormalities observed in treated larvae include splitting of the new cuticle, complete failure to shed the old cuticle or only partial completion of the molt with parts of the exuvium still attached (Granett and Dunbar, 1975; Lacey and Mulla, 1978a; McKague et al., 1978; Sharma et al., 1979; Retnakaran et al., 1985). Treatment during the last larval instar can result in the failure of larvae to molt successfully to the pupal stage (Lacey and Mulla, 1977; McKague et al., 1978) or prevention of adult emergence (McKague et al., 1978; Ottens and Todd, 1979). In general, young larvae are more susceptible to the benzoylphenyl ureas than older ones (Lacey and Mulla, 1977, 1978b; Sharma et al., 1979).

Chitin synthesis inhibitors have little or no effect on adult insects (Turnbull, 1982; Retnakaran et al., 1985) since the cuticle of the Pterygota is nearly formed by the adult stage (Andersen, 1979). The most pronounced effect on adults is the reduction in the number of viable offspring, the same result accomplished by treating the egg stage of susceptible species. Treatment of eggs, particularly young ones (Miura et al., 1976; Lacey and Mulla, 1977; Ottens and Todd, 1979) or parental adults (Moore and Taft, 1975; Miura et al., 1976; Wright and Spates, 1976; Ivie and Wright, 1978; Chang, 1979; Jordan et al., 1979; Ottens and Todd, 1979; Spates and Wright, 1980; Turnbull, 1982; Rup and Chopra, 1985) causes a reduction in egg hatch generally followed by a reduced survival rate of hatched larvae. Treated eggs contain fully developed larvae that are unable to escape the egg shell probably due to the possession of a weakened embryonic cuticle (Moore and Taft, 1975; Miura et al., 1976; Lacey and Mulla, 1977; Miura and Takahashi, 1979; Turnbull, 1982) . A point in embryogenesis at which mortality of treated eggs of the blackfly Simulium vittatum Zetterstedt declined sharply was considered to be representative of both an advanced deposition of embryonic and serosal cuticle with a concomitant increase in the impermeability of the chorion (Lacey and Mulla, 1977).

Studies on Diptera, particularly those of medical and veterinary importance, show them to be quite susceptible to the benzoylphenyl ureas (Jakob, 1973; Wright, 1974; Mulla and Darwazeh, 1975; Dame et al., 1976; Miura et al., 1976; Vea et al., 1976; Lacey and Mulla, 1977, 1978a; McKague et al., 1978; Chang, 1979; Jordan et al., 1979; Miura and Takahashi, 1979; Sharma et al., 1979; Ali and Lord, 1980; Spates and Wright, 1980; Turnbull, 1982; Rup and Chopra, 1985). A review of previous studies on cyclorrhaphid Diptera, such as stable flies, house flies, fruit flies and tsetse flies reveal that the benzoylphenyl ureas are ovicidal/larvicidal when eggs are exposed either by topical application or via the maternal body (Wright, 1974; Vea et al., 1976; Wright and Spates, 1976; Ivie and Wright, 1978; Chang, 1979; Jordan et al., 1979; Chang and Borkovec, 1980; Spates and Wright, 1980; Rup and Chopra, 1985) .

Chitin synthesis inhibitors disrupt the molting process of insects by interfering with the deposition of chitin into the endocuticle of the new developing cuticle after apolysis. A shortage of chitin disrupts the structural make-up of the new forming cuticle during molting and as a result of the weakened cuticle, larvae are either unable to shed the old cuticle and/or the new cuticle ruptures due to the increased muscular activity required during ecdysis (Fogal, 1977). Molted insects often die eventually from other secondary effects caused from the possession of a non-functional integument (Post et al., 1974; Ker, 1977; Vincent, 1978; Andersen, 1979; van Eck, 1979; Beeman, 1982; Retnakaran et al., 1985). The exact site of benzoylphenyl urea activity at the biochemical level that prevents the formation of chitin microfibrils is not understood but there are several theories. Two widely-accepted ones are: they directly inhibit the final stages of chitin synthesis by epidermal cells to halt the polymerization of N-acetylglucosamine units by chitin synthetase or that they inhibit a protease that activates the inactive chitin synthetase zymogen (Chen and Mayer, 1985; Retnakaran et al., 1985).

Chitin synthesis inhibitors offer good field control potential for aqueous larvae of flies that are pests in the adult stage (Mulla and Darwazeh, 1975; Dame et al., 1976; McKague et al., 1978; Sharma et al., 1979; Ali and Lord, 1980). In the U.S., Dimilin is registered for use against the gypsy moth, cotton pests and several biting fly species. In Canada, Dimilin is registered for use against the gypsy moth and mosquitoes. Their potential value against defoliating insects and agricultural pests deserves closer attention.

MATERIALS AND METHODS

3.1. Greenhouse rearing of stock colony

Two basic methods have been developed for rearing D. radicum in the laboratory. A method developed by Harris and Svec (1966) and presently employed at the Agriculture Canada Research Station in London, Ontario, requires environmental chambers to rear larvae and maintain D. radicum adults. A second method, developed and later improved by Read (1965a) (Agriculture Canada Research Branch, Experimental Farm, Charlottetown, P.E.I.), uses a heated greenhouse compartment equipped with artificial lighting to support year round rearing. After visiting both institutions, I determined Read's method to be more adaptable to available facilities. The following procedure was developed from Read's method, with minor changes and simplifications.

3.1.1. Rearing compartment

The greenhouse compartment was located at the Agriculture Canada Research Station, Brookfield Rd., St. John's. The compartment, in addition to natural daylight, received a minimum of 1400 lux from fluorescent lighting for 16 hours per day. The compartment was heated and the ambient temperature allowed to fluctuated between 15-27 C throughout the year. A ceiling fan provided the adults with a continuous flow of air.

3.1.2. Rearing procedure

Rearing of larvae was done in 5-liter plastic buckets, 3/4 filled with moist organic soil. A store-bought rutabaga (Brassica napus napobrassica L., approx. 1 kg) was peeled on its lower surface to expose the succulent root tissue and then pressed, cut surface down, into the loose soil to 2/3 its length. Three-hundred and fifty embryonated eggs (held at 20 C for 70 hours; original eggs obtained from Read's greenhouse colony of an insecticide susceptible P.E.I. strain) were distributed around the exposed portion of the planted rutabaga, covered with moist soil, and lightly watered. The buckets were watered twice more, once during the first week after planting and once in the second week; further wetting greatly increased the risk of rot which is lethal to the entire maggot population. The rutabagas were removed from the buckets 3 weeks after set-up, a time when most larvae had emerged from the rutabaga to pupate in the surrounding soil.

Adults, emerging from larval buckets, were contained by cone-shaped emergence cages (Figure 3-1A) made from 30-mesh plastic screen, machine-sewn to shape (66 x 32 cm: base circumference x height) and tapered to a 12 cm (circumference) hole at the top which was closed with an elastic band. Within 48 hours after emerging, flies were transferred to cylindrical oviposition cages (Figure 3-1B) (66 x 32 cm: circumference x height), where they mated and subsequently laid eggs. The flies were removed from the emergence cages by inserting a glass tube (opposite end closed) into the hole in the top of the cage. The negatively geotactic flies moved up into the tube and were counted, sexed (Smith, 1927) and transferred to oviposition cages. Inserting the open end of the tube into the hole in the side of an oviposition cage (Figure 3-1C) and blowing into the opposite end delivered the adults to the oviposition cages.

Adults were placed in oviposition cages at a density of 100-200 per cage, with at least 1/3 males. The flies fed from filter paper smeared with a mixture of honey, sugar, Brewer's yeast, whole wheat flour and water (approx. 8:8:4:3:1) (Read, 1965b) and placed in an inverted position on the outer surface at the top of the

Figure 3-1: Stock colony of Delia radicum in the greenhouse. (A) Emergence cage (B) Oviposition cage (C) Hole in the side of oviposition cage (D) Filter paper on the outside of cage smeared with adult food mixture (E) Plastic water tube (F) Tank filled with water


D
E
B
C
A
F

cage (Figure 3-1D). Water was presented to the adults in a plastic test tube supplied with a cotton wick and laid on top of the cage (Figure 3-1E). Cages were lightly watered each day with a fine spray nozzle hooked up to a garden hose. All oviposition cages were placed in a galvanized aluminium tank (Figure 3-1F) (120 x 88 x 20 cm) with a 12 cm water level to provide high humidity for the adults.

Eggs were collected from oviposition cages starting 6-9 days after the adults emerged. To collect eggs an 'eggcup' (6 cm petri dish base with a piece of rutabaga pressed into moist, loose bird gravel) was placed inside each cage via the hole in the cage side. Females oviposited their eggs into holes impressed in the sand surrounding the rutabaga cube. The age span of eggs collected was controlled by manipulating the time interval in which eggcups were presented to the adults. To retrieve the eggs, the eggcup was removed from the cage and its contents emptied into a beaker containing distilled water. The beaker was swirled to float the eggs which were suction filtered onto fine black cloth. The cloth filter was removed from the funnel and placed on top of two moist filter papers in an inverted 9 cm petri dish. Eggs were counted under a stereomicroscope and sorted with a bent insect pin; wetting the absorbent layers facilitated manipulation. Eggs intended for recycling back into the stock colony were left to embryonate. Larval rearing buckets were innoculated by rinsing eggs (350 per bucket) onto the soil surrounding the exposed portion of a whole, planted rutabaga, with a stream of water from a wash bottle. The eggs were lightly covered with soil. The entire life cycle, from the time larvae hatched to oviposition of adults, took approximately 6 weeks.

3.2. Development of a petri dish rearing method for laboratory screening of insect growth regulators

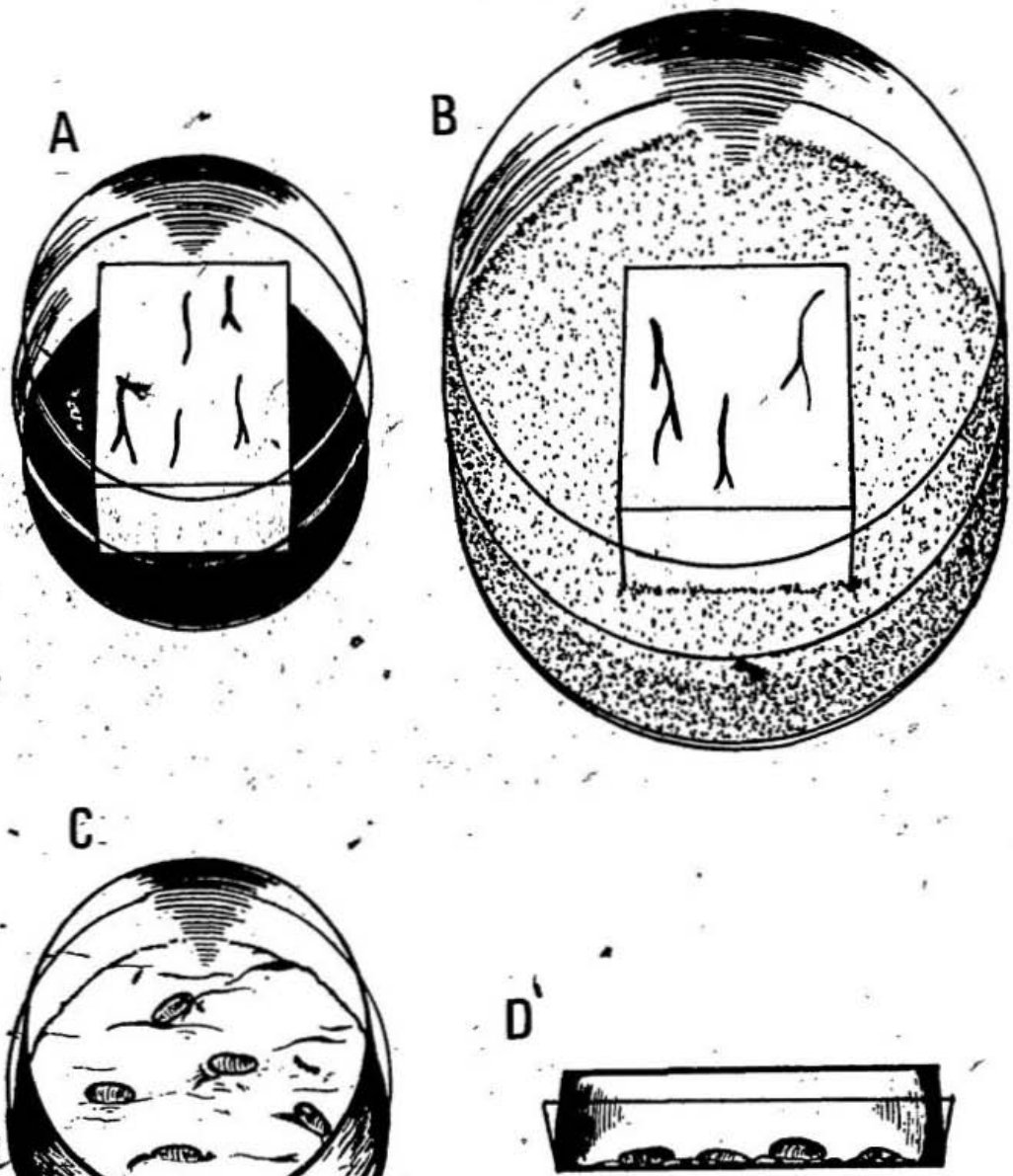
Laboratory screening of insect growth regulators against D. radicum required a small-scale rearing procedure. The criteria to be met was to provide replicate rearing units that were easily monitored and capable of supporting the life stages

through to adult emergence. In testing D. radicum for insecticide resistance, Read (1965b) placed embryonated eggs in standard 9 cm petri dishes supplied with a thin slice of rutabaga (pre-treated with insecticide) to sustain larvae for 48 hours until mortality counts were taken. I expanded his method to provide for rearing the cabbage maggot through one generation cycle. To conserve incubator space, larval and adult units were made from 6 cm petri dishes (Figure 3-2). The entire set-up was housed in a bench-top incubator at 20° C under a 16-hour daylength. Rearing D. radicum in the dark induced pupal diapause.

3.2.1. Larval rearing units

The unit base (6 cm plastic petri dish base) was supplied with an absorbent layer, consisting of two filter papers with a piece of fine black lining cloth placed on top (Figure 3-2A). The absorbent layer was moistened with approximately 1 ml of distilled water; excess water was drawn off with a pasteur pipet pressed against the absorbent layer. The absorbent layer was remoistened on the third and fifth day after set-up and once in the second and third week of development. Excessive moisture in the larval rearing units promoted bacterial growth that was highly lethal to larvae (Appendix A). Delia radicum eggs were placed to one side of the unit and a fresh piece of rutabaga (approx. 3.0 x 2.0 x 2.0 cm; 15 g) was positioned alongside the eggs. In selecting rutabaga dimensions, emphasis was placed on slice thickness to permit complete larval development and prevent overcrowding of the negatively geotactic larvae (Vereecke and Hertveldt, 1971). The extra space provided by replacing the standard petri dish cover with a petri dish base accomodated the thick rutabaga piece. The unit cover was cracked to permit air exchange and secured to the unit base with tape. Once egg hatch was recorded, all unhatched eggs were removed from the units.

Figure 3-2: Petri dish method for small-scale rearing of Delia radicum. (A) Larval rearing unit: white eggs against black cloth of absorbent layer. Rutabaga positioned next to eggs (B) Pupation unit: rutabaga containing third instar larvae pressed into moist sand (C) Adult emergence unit containing pupae (D) Adult emergence unit, side view showing space between unit base and cover


A
B
C
D

3.2.1.1. Determination of optimum larval density

The optimum number of D. radicum larvae supported per larval unit was determined by a density experiment. Embryonated eggs were distributed in groups of 10, 20, or 30 eggs/unit to six units per density level. An additional set of six units were supplied with 100 eggs each, with the intention of replacing the rutabaga at intervals to support the larval population. Units were examined on day 5 to record egg hatch and again during the last week of larval development to evaluate rutabaga consumption and the stage and activity of larvae. Cabbage maggots were reared through to the adult stage. Pupal and adult yields were recorded at each density level, up to 58 days after setting up the experiment.

3.2.2. Pupation units

In nature, mature larvae leave the host plant and burrow into the surrounding soil in search of a suitable pupation site. Most larvae emerging from the rutabaga in larval units did not pupate but died upon becoming entrapped in water droplets condensed inside the unit cover or by dessicating at the unit base. To overcome this difficulty, the rutabaga piece (containing L_3) in each larval unit was transferred to a pupation unit just prior to completion of larval feeding 16 days after set-up.

Pupation units (Figure 3-2B) consisted of two 9 cm petri dish bases, one inverted over the other to serve as the unit cover. The unit base was filled with bird gravel, moistened and water drawn off with a pipet. The rutabaga piece (containing L_3) was pressed into the sand and the unit cover cracked to permit air exchange and secured with tape around its entire edge to prevent larvae from escaping.

3.2.3. Adult emergence units

In order to record the timing of pupation and the percentage yield of pupae in toxicological experiments, the pupae were retrieved from the sand of pupation units at 24 hour intervals. The rutabaga piece was removed and the unit base filled with distilled water. The floating pupae were decanted onto filter paper and transferred with forceps to adult emergence units (Figure 3-2C). The rutabaga was throughly checked for any pupae that might have formed inside it. The sand was returned to the unit, excess moisture drawn off and the rutabaga and any post-feeding larvae returned to it.

Adult emergence units consisted of inverted 6 cm petri dishes, lined with a folded moistened Kimwipe® (Kimberly-Clark Ltd., Toronto, Canada). The units were moistened, without removing the cover, by delivering a few drops of distilled water to the space between the unit bottom and its loose fitting cover (Figure 3-2D). The cover was cracked to permit air exchange. The units were examined nearly every day for fly emergence and were viewed under a stereomicroscope to monitor the progress of pupal-adult development within the puparia. Pupae were rinsed and transferred to new emergence units upon the first sign of fungal contamination, the presence of which was lethal to the developing imagos if left unattended.

3.3. Toxicological studies: laboratory screening of several insect growth regulators against Delia radicum

Four insect growth regulators were tested at a rate of 1.0 μg to D. radicum eggs, post-feeding larvae and pupae. Diflubenzuron (Dimilin®, technical powder > 97% pure), methoprene (Altosid® 5E; 65% active), juvenile hormone I (synthetic mixture of isomers) and precocene II (crystalline; Sigma Chemical Company, St.Louis, Missouri) were topically applied with a glass micropipet, calibrated to deliver 1.0 μl of a 1.0 μg/μl solution of the compound dissolved in dimethylsulfoxide. Each insect was treated individually while being viewed under

a stereomicroscope. A solvent-treated and an untreated control group were tested against eggs, larvae and pupae. After treatment, insects were reared to adults by the petri dish method. All resulting adults were sexed. The results were subjected to a Single Classification Analysis of Variance (Sokal and Rohlf, 1969).

3.3.1. Effect of insect growth regulators on eggs

Delia radicum eggs (24-28 hrs old) were distributed at 20 eggs/unit to the absorbent layer of larval units. Under a dissecting microscope, 1 μg of compound was topically applied, one egg at a time, to the entire egg surface. Six rearing units were set-up per treatment and control groups. The absorbent layers containing the treated eggs were placed inside the rearing units and 24 hours later a rutabaga piece was positioned next to the eggs. Egg hatch was recorded on the fourth and sixth day and pupae were counted 21 and 28 days after treatment. Adult emergence commenced at 31 days and was recorded nearly every day up to 79 days after treatment.

3.3.2. Effect of insect growth regulators on post-feeding larvae

Two experiments were conducted on post-feeding larvae: the larval screening test and a second experiment to determine the minimum dose of methoprene required to suppress adult emergence when applied to late third instar larvae. For both experiments, larvae were collected as they left the rutabaga from stock and/or laboratory cultures, rinsed and blotted dry on filter paper. Only larvae that were mobile and had not yet undergone head inversion were treated. Each larva was held anteriorly, dorsal side up with forceps and the compounds were applied over the entire body surface just posterior to the larval mouth hooks. Before larvae were placed into the gravel of pupation units, they were held for 5-10 sec after treatment to aid in the penetration of the compounds.

For the larval screening test, ten larvae were tested per treatment and control groups. The experiment was replicated three times. The timing of pupation was monitored during the first replicate by recording pupal formation at 24 hour

intervals after treatment. Results of pupal and adult yields were recorded up to 52 days after treatment. Adults started emerging 16 days after treatment.

For the second experiment with methoprene, twenty larvae were treated per dose level of 1.0, 0.5, 0.1, 0.05, 0.001, 0.0001 and 0.00001 μg per larva delivered in 1 μl of dimethylsulfoxide. All concentrations were prepared from a 1 μg/μl stock solution of the compound. Results of pupal and adult yields were recorded up to 33 days after treatment.

3.3.3. Effect of insect growth regulators on pupae

Two experiments were conducted on D. radicum pupae. As a result of the pupal screening test, a second experiment to determine if methoprene, the only compound to adversely affect pupal-adult development, was effective against insects in imaginal development. For the screening test, tanned puparia in pupal-adult development were recovered from the stock colony approximately 26 days after egg hatch, rinsed in distilled water and placed in adult emergence units. Excess water was drawn from the absorbent layer so it would not interfere with topical application. Each compound was applied dorsally with respect to the developing insect. A single replicate of twenty puparia were tested per treatment and control groups. The units were remoistened after treatment and monitored nearly every day for the next two weeks.

For the second experiment, pupae from the same generation were recovered almost a week later (32 days following egg hatch). To select a definitive stage to treat, only insects that had reached imaginal development but not yet developed cuticular hairs, or melanized wings and legs, were treated. Three replicates of twenty pupae were treated per methoprene and untreated control group. Units were checked perodically for adult emergence, which was recorded up to 50 days after treatment.

3.4. Physiological studies: effect of methoprene on hemolymph carbohydrates, proteins and amino acids in post-feeding larvae

Post-feeding larvae were individually treated with 1 μg methoprene and placed into pupation units. Thirty-six hours after treatment, larvae selected for hemolymph collection were those that had not yet undergone head inversion. Larvae that had pupated during the thirty-six hour incubation were not selected. In preparation for hemolymph collection, each larva was blotted dry on filter paper and held with forceps under a stereomicroscope. Pulsating pressure was applied posteriorly to the larva by rhythmically squeezing the forceps. The larva was punctured on its ventral side just behind the mouth hooks with a sharp point. The clear hemolymph that exuded was collected by capillary action in 1 μl micropipets. Each larva yielded approximately 1-2 μl hemolymph. Collected hemolymph was immediately blown into 5 ml trichloroacetic acid for amino acid and protein quantification or onto an activated thin-layer chromatography plate for subsequent carbohydrate separation.

3.4.1. Separation and quantification of hemolymph carbohydrates

Prior to colorimetric quantification of hemolymph carbohydrates, the specific carbohydrate components were separated by thin-layer chromatography. A method of separating sugars on silica gel by Fried and Sherma (1982) was adopted with slight modifications.

Silica gel precoated plates (Silica Gel G ; 0.25 mm, silica gel with gypsum, 20 x 20 cm, Macherey-Nagel, Düren, Germany) were impregnated with 0.1 M sodium bisulfite by dipping. The plates were air dried and columns 2 cm wide were delineated in the stationary phase by scraping with a sharp point. The plates were activated (100C; 30 min) and spotted with 5 μl of hemolymph or standard samples 2 cm from the bottom of the plate. Once the spots had dried, the plates were placed in a chromatography tank pre-saturated with 100 ml of mobile phase

(ethyl acetate):acetic acid, methanol and water; 60:15:15:10 v/v). Plates were developed to a distance of 15 cm, air dried and sprayed with an α-naphthol-sulfuric acid detection reagent. Those areas of the plate destined for further processing were covered with a glass plate to protect them from the spray reagent. The samples were detected by heating the plates at 100C for approximately 20 minutes.

After heating the sprayed thin-layer chromatography plate, areas (ca. 2x2.5 cm) corresponding in Rf values to those detected by acid treatment were scraped with a spatula from the undetected columns into 15 ml centrifuge tubes. Trehalose and glucose fractions were estimated colorimetrically by reacting the adsorbed sugars with anthrone (Roe, 1955). Three milliliters of anthrone reagent was added to each tube and the tubes vortexed, covered with aluminum foil and placed in a water bath (100 C) for 10 minutes. Samples were centrifuged (11000 g; 10 min), then absorbances read on a Shimadzu UV-visible recording (UV-260) spectrophotometer at 650 nm. The samples were quantified by reference to a standard curve of absorbance plotted against known concentrations of carbohydrate standard (Appendix B).

3.4.2. Quantification of hemolymph amino acids and proteins

Aliquots of 10 μl of pooled hemolymph were blown into 5 ml samples of 5% trichloroacetic acid. Fifteen minutes later, the samples were centrifuged (3 C; 11,000 g; 4 min), the supernatant assayed for amino nitrogen and the precipitate for protein content. Absorbances were read on a Bausch and Lomb Model 210 Spectrophotometer with a 1 cm light path.

Aliquots of the soluble fraction were assayed for amino nitrogen by reaction of hemolymph α-amino acids with ninhydrin (Rosen, 1957). To 0.5 ml supernatant, 0.25 ml of acetate-cyanide buffer was added, followed by 0.25 ml ninhydrin solution. The samples were heated (100 C water bath; 15 min) and 2.5 ml diluent (isopropyl alcohol/water; 1:1) added and immediately vortexed. The samples

were cooled to room temperature and absorbances read at 570 nm. Sample readings were compared with a standard curve prepared from serial dilutions of a glycine standard (Appendix C).

The protein precipitates remaining were assayed by reaction with the Folin-Cicocalteau reagent (Lowry et al., 1951) using Protein Assay Kit No. P5656 (Sigma Chemical Co., U.S.A.). The samples were read at 500 nm and compared with a standard curve prepared from serial dilutions of bovine serum albumin (Appendix D).

RESULTS

4.1. Development of a petri dish rearing method: determination of optimum larval density in larval rearing units

In laboratory culture during this study, D. radicum larvae supplied with ample food fed for approximately 17 days and then left the rutabaga via their feeding tunnels. At densities of 10 and 20 eggs/unit, the rutabaga was intact at the end of the feeding period and L_3 left the rutabaga in the usual way (Table 4-1). At 30 eggs/unit, some larvae had eaten through to the top of the rutabaga piece as early as 13 days into larval development. By day 16, the rutabaga was devoured and larvae were emerging from the top of the food remains; many larvae had not attained mature body size. Despite the food shortage, the percentage yield of pupae and adults was not significantly different among the three density levels. The petri dish method yielded an average of 76% adults from hatched larvae among the three density levels (Figure 4-1).

The criteria used to select the optimum larval density was rutabaga consumption and larval development. Based on the criteria, a density of 20 eggs/unit (0.75 g food per larva) was the optimum density level to be employed in toxicological studies. A density of 100 eggs/unit proved impractical because the rutabaga was quickly consumed and the young larvae were difficult to find and too easily damaged to be transferred to new units. Units were too small to allow fresh rutabaga to be added.

Table 4-1: Effect of larval density in rearing units on rutabaga consumption and the stage and activity of larvae[1]

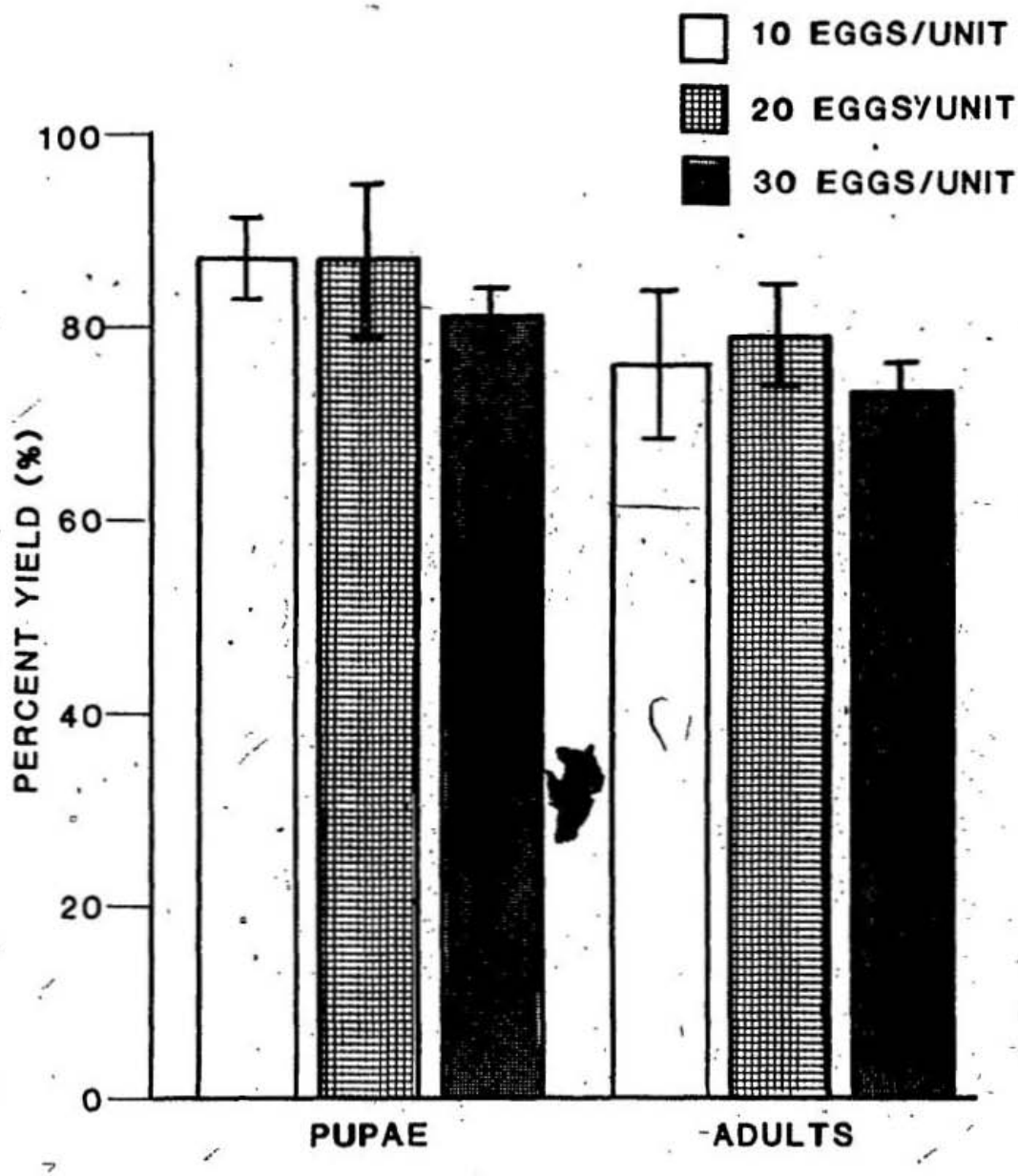
DAYS AFTER SET-UP	NO. EGGS	APPEARANCE OF RUTABAGA	LARVAL ACTIVITY AND STAGE OF DEVELOPMENT[2]
13	10	intact	immatures feeding
	20	intact	immatures feeding
	30	holes in top	immatures feeding
16	10	intact	immatures feeding
	20	intact	immatures feeding
	30	consumed	L3 leaving through top of rutabaga
18	10	intact	L3 leaving from bottom of rutabaga
	20	intact	L3 leaving from bottom of rutabaga.
	30	consumed	L2 and L3 crawling about

[1] Observations are based on 2-5 replications at each density level.

[2] L2=second instar larvae
L3=third instar larvae

Figure 4-1: Effect of larval density in rearing units on percentage yield of pupae and adults.

Values represent the mean (± S.E.M.) percentages of pupae and adults that developed from newly-hatched larvae. Number of replicates may be less than six due to dessication.


10 EGGS/UNIT
20 EGGS/UNIT
30 EGGS/UNIT
PERCENT YIELD (%)
100
80
60
40
20
0
PUPAE
ADULTS

4.2. Toxicological studies: laboratory screening of several insect growth regulators against Delia radicum

4.2.1. Effect of insect growth regulators on eggs

Topical application of 1 μg of diflubenzuron to unembryonated D. radicum eggs caused a significant ($p<.01$) reduction in hatch (Table 4-2). Six days after diflubenzuron treatment, 72.5% of eggs had hatched compared to 86% of eggs from the control groups. Diflubenzuron also caused a delay in egg hatch. Nearly all (98-100%) untreated eggs destined to hatch by day 6 had done so by day 4 but only 86% of eggs treated with diflubenzuron that were destined to hatch by day 6 had done so 4 days after treatment. Examination of treated eggs that failed to hatch revealed fully developed embryos moving inside the egg case. All unhatched embryos eventually died, apparently from dessication and/or infection. Several larvae that did initiate hatching were unable to complete the process and died partially emerged.

Treatment of eggs with methoprene, juvenile hormone I or precocene II did not affect hatching of D. radicum larvae. Untreated and solvent control groups were similar in terms of egg hatch, pupal yield and adult yield, indicating dimethylsulfoxide did not adversely affect D. radicum eggs. Two of the four compounds applied to the egg stage adversely affected post-embryonic development subsequent to hatching. Treatment of D. radicum eggs with diflubenzuron not only disrupted hatch but resulted in the death of all first instar larvae. The rutabaga in treated replicates was free of holes, suggesting that larvae hatching from treated eggs were unable to establish feeding sites. As a result of this larvicidal activity, diflubenzuron-treated replicates did not yield pupae. The other compound to adversely affect post-embryonic development was methoprene. Larvae hatching from treated eggs underwent overtly normal development to pupation. However methoprene significantly ($p<.001$) suppressed adult eclosion. In four of six replicates, not a single fly emerged (Appendix E). Dissection of unecloded puparia revealed that methoprene interfered with adult development, resulting in dead pupal-adult intermediates within the puparia.

Table 4-2: Effect of topical application of several insect growth regulators to Delia radicum eggs[1]

TREATMENT	EGG HATCH DAY 4	EGG HATCH DAY 6	PUPAL YIELD[2]	ADULT EMERGENCE[3]
Untreated control	84.2 ± 3.0	84.2 ± 3.0	66.4 ± 11.6	83.5 ± 18.3
Solvent control	85.8 ± 0.8	87.5 ± 1.1	69.5 ± 5.4	87.7 ± 9.9
Diflubenzuron	62.5 ± 3.8***	72.5 ± 4.4**	0***	0***
Methoprene	80.0 ± 5.0	84.2 ± 4.9	65.4 ± 5.5	10.6 ± 8.9***
Juvenile Hormone I	86.6 ± 4.0	86.5 ± 4.0	70.2 ± 5.7	79.5 ± 6.9
Precocene II	84.2 ± 4.0	85.8 ± 4.2	75.7 ± 4.8	73.1 ± 7.8

* Statistically significant: ($p<.05$) compared to solvent controls
** Very significant ($p<.01$)
*** Highly significant ($p<.001$)

Statistical analysis was carried out on the raw data and descriptive data subsequently converted to percentages.

[1]All values are percentages representing the mean ± S.E.M. of up to six replicates
[2]Number of pupae expressed as a percentage of hatched larvae
[3]Number of adults emerged expressed as a percentage of the pupal yield

4.2.2. Effect of insect growth regulators on post-feeding larvae

Topical application of each of the four insect growth regulators to post-feeding L_3 did not interfere with the timing of the ensuing pupation process. In the pilot run of the experiment in which pupation was monitored, most insects pupated within 24 hours after treatment and all treated larvae formed pupae. However, methoprene at 1 μg completely suppressed adult emergence ($p<.005$); not a single fly had emerged by the end of the observation period (52 days) from the sixty larvae treated. In contrast, 75% of control insects emerged during the same period (Appendix F). Most of the methoprene-treated insects reached pupal-adult development as observed through the puparial case but died as uneclosed intermediates. Diflubenzuron, juvenile hormone I and precocene II had no significant effect on the number of emerging adults (Figure 4-2). The ratio of the sexes was unaffected by treatment (Appendix F). Dimethylsulfoxide did not adversely affect the development of post-feeding larvae.

In the response experiment, the lowest concentration of methoprene to cause complete suppression of adult emergence when applied to post-feeding larvae was 0.05 μg/larva (Figure 4-3). The next lowest concentration tested was 0.001 μg/larva which prevented 50% eclosion and represents the approximate LC50 value. Doses in the range of 0.0001 μg/larva and lower were ineffective in suppressing adult emergence (Figure 4-3).

4.2.3. Effect of insect growth regulators on pupae

In the screening experiment, topical application of methoprene to a heterogeneous population of pupae (the same age in days but at different stages of pupal-adult development) resulted in a 55% reduction in adult emergence compared to that of pupae treated with solvent only (Figure 4-4). Diflubenzuron, juvenile hormone I and precocene II had no effect on subsequent adult emergence. Dissection of methoprene-treated pupae revealed that development had stopped during pupal-adult formation.

Figure 4-2: Topical application of insect growth regulators to post-feeding Delia radicum larvae: effect on emergence of adults. Methoprene was included as a treatment but adults failed to emerged.

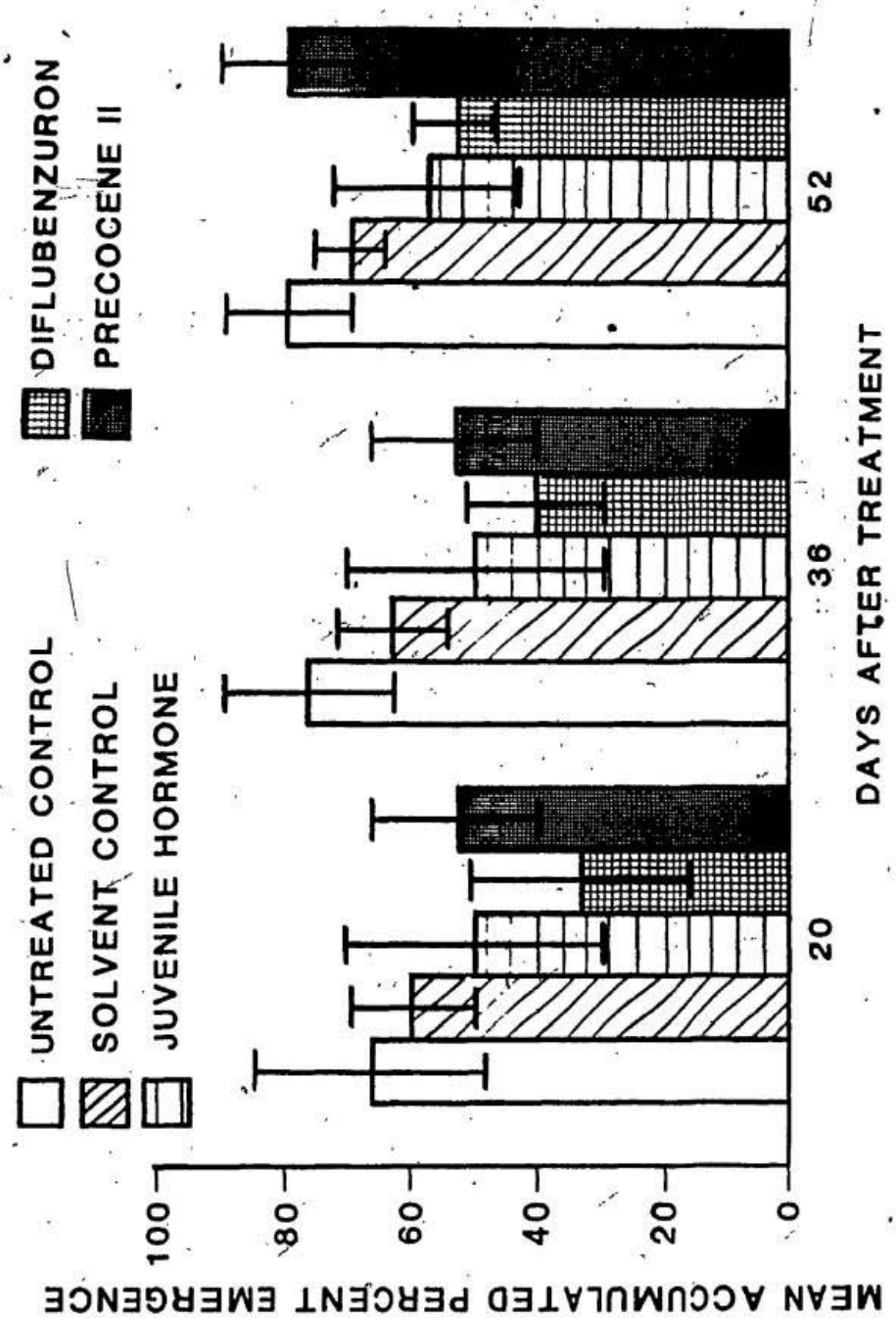

MEAN ACCUMULATED PERCENT EMERGENCE
100
80
60
40
20
0
UNTREATED CONTROL
SOLVENT CONTROL
JUVENILE HORMONE
DIFLUBENZURON
PRECOCENE II
20
36
52
DAYS AFTER TREATMENT

Figure 4-3: Dose-response of methoprene against post-feeding Delia radicum larvae: effect on adult emergence

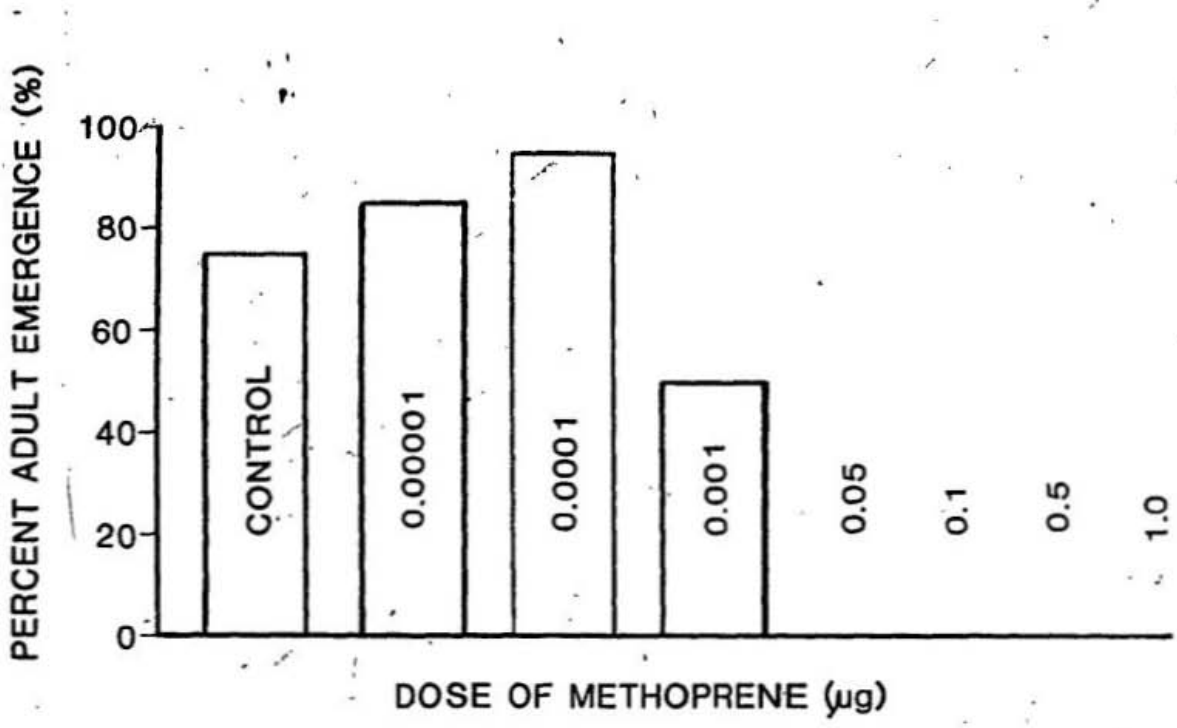
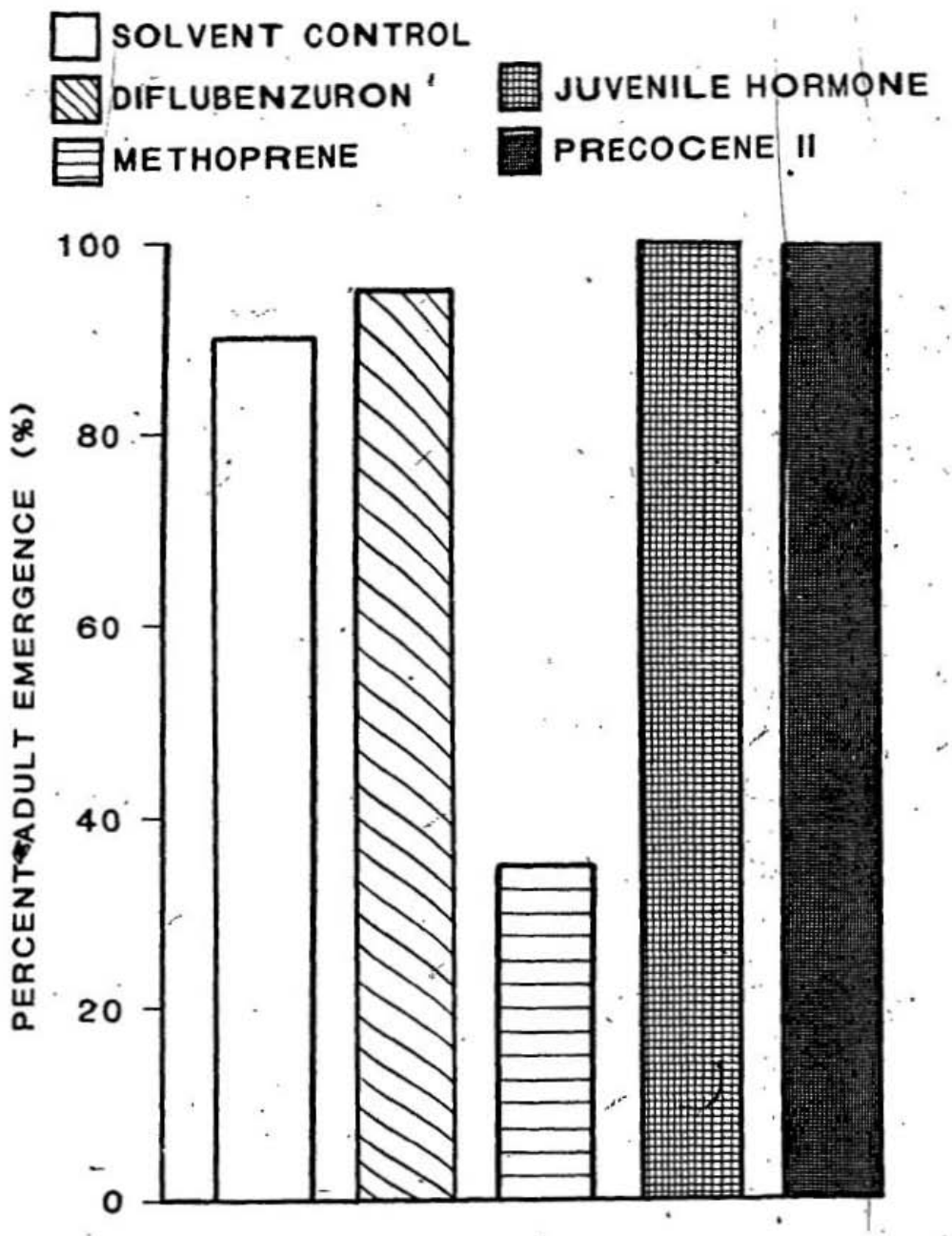

PERCENT ADULT EMERGENCE (%)
100
80
60
40
20
0
CONTROL
0.00001
0.0001
0.001
0.05
0.1
0.5
1.0
DOSE OF METHOPRENE (µg)

Figure 4-4: Effect of insect growth regulators on adult emergence when applied to a heterogeneous pupal population.

Based on a single repetition of 20 pupae. Insects in larval-pupal or pupal-adult development.


SOLVENT CONTROL
DIFLUBENZURON
METHOPRENE
JUVENILE HORMONE
PRECOCENE II
PERCENT ADULT EMERGENCE (%)
100
80
60
40
20
0

A second experiment investigated the possibility that emerging adults of methoprene-treated pupae in the screening experiment were pupae that had passed the susceptible stage to the compound. Treatment of pharate adults (adults enclosed within the puparial case) with methoprene did not affect emergence, the percentage of eclosing adults was the same for both untreated (90.0 ± 4.5) and methoprene-treated (90.0 ± 6.0) pupae (Appendix G).

4.3. Physiological studies: effect of methoprene on hemolymph metabolites in post-feeding larvae.

Separation of hemolymph carbohydrates of post-feeding larvae by thin-layer chromatography revealed trehalose as the only sugar detectable at this stage (Figure 4-5). Thirty-six hours after topical treatment with methoprene, larvae had a significantly lower trehalose level ($p<.05$) in their hemolymph compared to untreated larvae (Figure 4-6). However, there was no significant difference in hemolymph amino acid or protein concentration among treated and untreated larvae.

Figure 4-5: Separation of hemolymph carbohydrates on a thin-layer chromatography plate. Trehalose is the only carbohydrate detected in post-feeding Delia radicum larvae. Symbols: —origin, ---solvent front, G=glucose standard, T=trehalose standard, S=hemolymph sample. Hemolymph trehalose is encircled.

R_f value for trehalose in insect hemolymph is slightly less than in distilled water due to hemolymph metabolites interfering with migration on the plate.

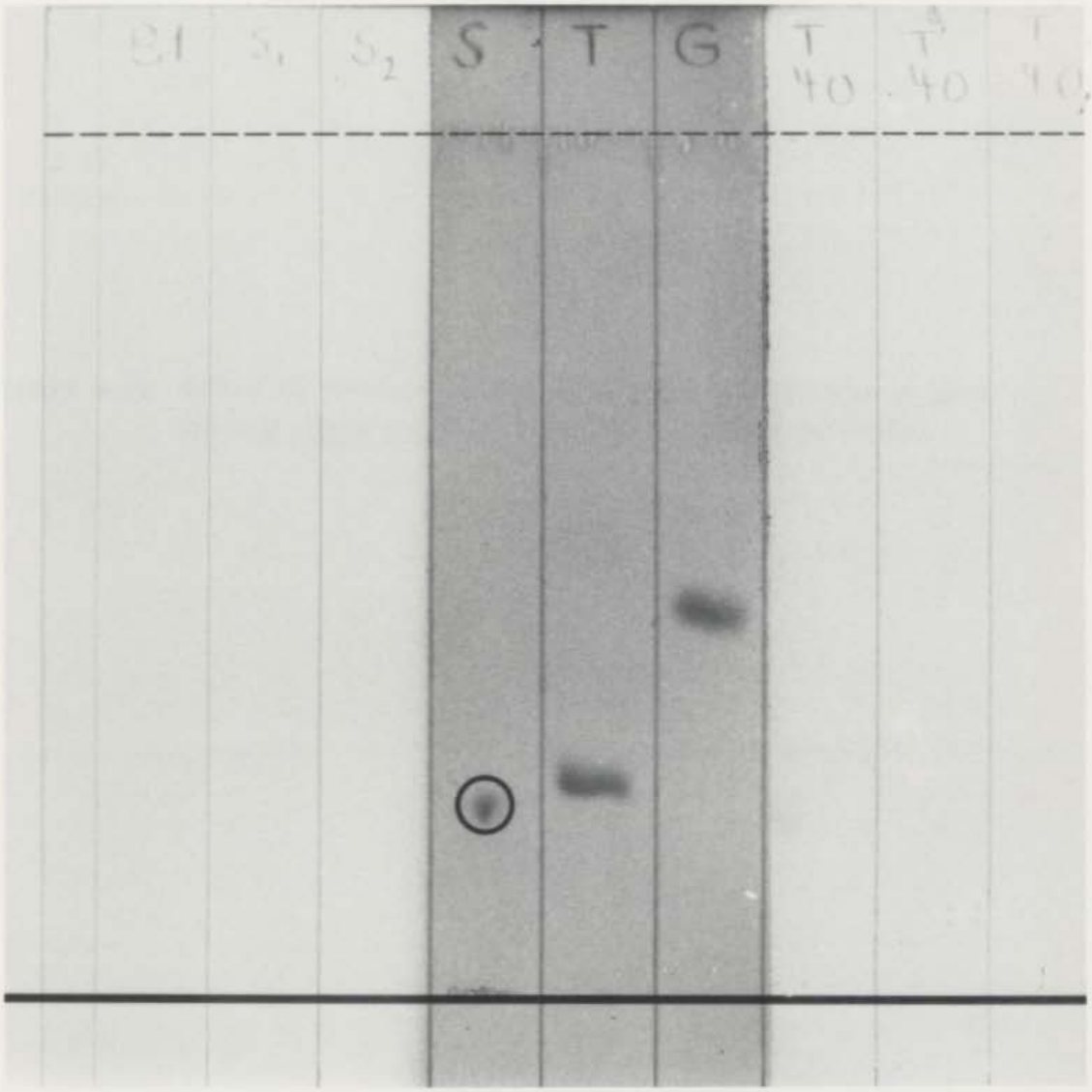
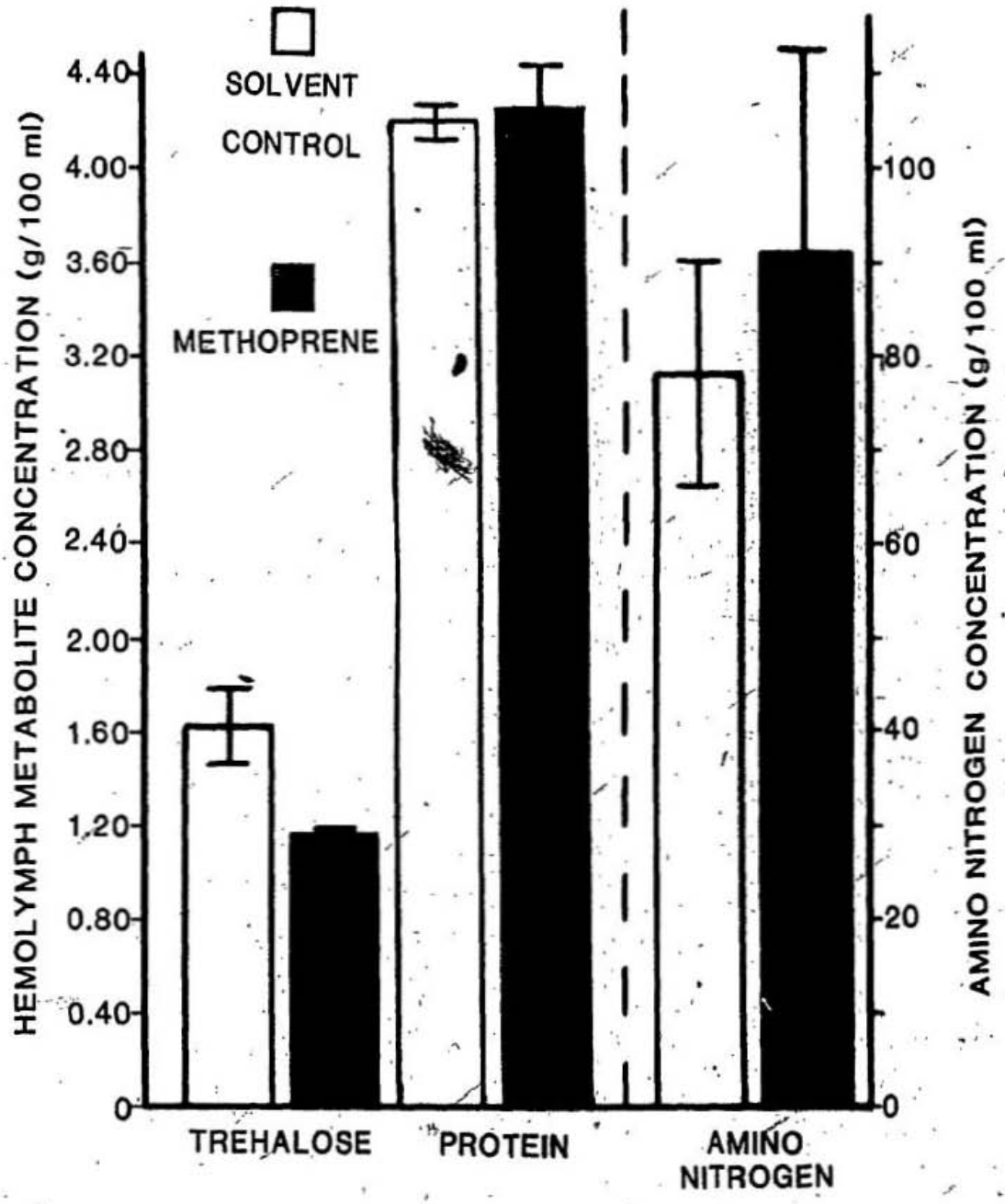

S
T
G

Figure 4-6: Effect of methoprene on hemolymph metabolites of post-feeding Delia radicum larvae 36 hours after treatment


SOLVENT CONTROL
METHOPRENE
HEMOLYMPH METABOLITE CONCENTRATION (g/100 ml)
AMINO NITROGEN CONCENTRATION (g/100 ml)
4.40
4.00
3.60
3.20
2.80
2.40
2.00
1.60
1.20
0.80
0.40
0
100
80
60
40
20
0
TREHALOSE
PROTEIN
AMINO NITROGEN

DISCUSSION

In the present study, methoprene, a juvenile hormone analogue, suppressed the emergence of D. radicum adults when eggs, larvae or young pupae were treated. Similar laboratory results were obtained with two other juvenoids against D. radicum larvae and young pupae (van de Veire and de Loof, 1973) and these compounds were subsequently reported to be effective in a field study (van de Veire and de Loof, 1974). In this study, the chitin synthesis inhibitor, diflubenzuron, possessed ovicidal activity against D. radicum and was a potent larvicide against first instars successfully hatching from treated eggs. These results support earlier findings that several benzoylphenyl ureas, including diflubenzuron, were ovicidal/larvicidal to D. radicum and the onion maggot, D. antiqua, upon injection or contact to adults or topical application to eggs (Turnbull, 1982). The success of chitin synthesis inhibitors in laboratory tests is substantiated with effective control of cabbage maggots in several field and greenhouse trials (reviewed by Harris, 1977; Philip et al., 1977; Ritcey et al., 1977; Turnbull, 1982).

Of the two insect growth regulators shown to be effective in the present study, diflubenzuron would be more practical as an insecticidal agent against D. radicum. The ovicidal/larvicidal nature of diflubenzuron activity against D. radicum prevented rutabaga damage in laboratory culture whereas methoprene applied to eggs did not arrest development until after the larval stage. It appears that methoprene, or a juvenoid that demonstrated field stability, would be a suitable insecticide candidate against the second generation of D. radicum larvae in the field by preventing the emergence of the parental generation of adults. Under the conditions of this study, juvenile hormone I and precocene II had little effect against the cabbage maggot.

In the present study, the results of treating D. radicum eggs with diflubenzuron are in accordance with the findings on other cyclorrhaphids and on the cabbage maggot (van de Veire and Delcour, 1976; Turnbull, 1982) after treating the adult stage , that is a reduced egg hatch and the death of first instar larvae successfully hatching from treated eggs. Examination of eggs receiving a lethal dose of the compound revealed fully developed embryos moving within the egg case. The presence of developed larvae inside unhatched, treated D. radicum eggs was also noted by Turnbull (1982) and is typical of the action of the benzoylphenyl ureas on dipteran eggs (Miura et al., 1976; Wright and Spates, 1976; Lacey and Mulla, 1977, 1978b; Chang, 1979). These observations suggest that diflubenzuron and other chitin synthesis inhibitors do not interfere with insect embryogenesis but prevent the hatching process.

The results of diflubenzuron treatment on D. radicum eggs in this study coincide with the known mode of action of the benzoylphenyl ureas. Both the failure of treated eggs to hatch and the morbidity of hatched larvae were likely due to an abnormal and inadequate cuticle formed on first instar larvae. The disruption of chitin formation in insect cuticle due to benzoylphenyl urea treatment is thought to cause the failure of treated larvae to molt as the result of a lack of mechanical ability of the larva to escape the old cuticle (Fogal, 1977). The failure of fully formed larvae to hatch from treated eggs is likewise thought to result from an inability of larvae to escape the egg case (reveiwed by Retnakaran et al., 1985). The delay in egg hatch observed in this study was previously noted in D. radicum and D. antiqua with several benzoylphenyl ureas including diflubenzuron (van de Veire and Delcour, 1976; Turnbull, 1982) and in mosquitoes (Miura et al., 1976). Turnbull (1982) suggested that the delay in egg hatch was probably due to the reduced mechanical effort afforded by a weakened larval cuticle, requiring a longer time for the larvae to break out of the egg case. In support of this assumption, larvae that had partially worked their way out of the egg case in this study had an abnormal cuticle as determined from their soft and jelly-like appearance. As with the larvae that did hatch, death shortly ensued, probably

due to the possession of a weakened and abnormal cuticle causing dessication and/or starvation (Turnbull, 1982) or facilitating microbial attack.

Diflubenzuron had no effect when applied to late third instars or young pupae of D. radicum in this study. The activity of chitin synthesis inhibitors against late post-embryonic development in susceptible insect species is varied. The lack of activity of diflubenzuron against late larval and pupal stages has been demonstrated in other susceptible dipteran species (Wright, 1974; Lacey and Mulla, 1977; Sharma et al., 1979). However, treatment during the last larval instar of mosquitoes and blackflies resulted in disrupted larval-pupal molt and adult emergence (Jakob, 1973; Ishaaya and Casida, 1974; Wright, 1974; Lacey and Mulla, 1977; Ivie and Wright, 1978; Lacey and Mulla, 1978b; McKague et al., 1978).

Juvenile hormone and other compounds with juvenile hormone activity characteristically prevent egg hatch and normal metamorphosis in the pupal and imaginal molt of susceptible species (Williams, 1967; Staal, 1975; Sehnal, 1983). Particularly in aquatic larvae, juvenoids have proven to be quite effective in reducing adult emergence (Cumming and McKague, 1973; Garris and Adkins, Jr., 1974; Mckague and Wood, 1974; Dove and McKague, 1975; Henrick et al., 1975; Saul et al., 1983). In cyclorrhaphid Diptera, treatment of eggs with juvenile hormone-active compounds have resulted in the failure of eggs to hatch (Wright, 1970; van de Veire and de Loof, 1973). Treating last larval instars or young pupae with these compounds can disrupt pupation (Srivastava and Gilbert, 1968; Wright and Spates, 1972) but generally metamorphosis is arrested during pupal-adult development within overtly normal puparia (Srivastava and Gilbert, 1968; Wright, 1970; Wright and Spates, 1972; van de Veire and de Loof, 1973; Wright et al., 1974).

In this study, methoprene arrested the development of D. radicum during pupal-adult development inside the puparium when applied to eggs, late larvae or young pupae before the pupal molt (pharate pupae). Regardless of the stage treated,

development proceeded in an apparently normal fashion up to the time of pupal formation. However, dead pupal-adult intermediates were observed in unecloded puparia. The presence of pupal-adult intermediates in the puparium of treated, unecloded pupae has been reported in other cyclorrhaphids treated with juvenile hormone analogues (Srivastava and Gilbert, 1968; Wright, 1970; Wright and Spates, 1972). Delia radicum intermediates in this study resembled those previously reported in treated cyclorrhaphids, i.e. they characteristically had an undifferentiated pupal-like abdomen in contrast to an adult cuticle on the head and thorax (Srivastava and Gilbert, 1968; Wright, 1970; Wright and Spates, 1972). Delia radicum pupae that were already in pupal-adult development (pharate adults) were not sensitive to methoprene and were probably past the stage of determination of adult development, a time determined to occur 24 hours after pupal formation in stable flies (Wright, 1970).

The results of this and other studies on higher Diptera are in accordance with the known action of the juvenoids. The delayed effect on metamorphosis observed with D. radicum in this study is a common phenomenon amongst treated insect larvae and has been reported in late embryos of hemipterans where egg hatch was unaffected (Willis and Lawrence, 1970; Riddiford and Truman, 1972) . The period in insect development most sensitive to the disruptive action of juvenoids is late in post-embryonic development (Sláma and Williams, 1966; Wright, 1970; Staal, 1975) . Therefore, the delayed effect of juvenoids on insect metamorphosis may be caused by persistence of the analogue in the tissues that elicits an effect when the endogenous juvenile hormone titer naturally falls. Alternatively, it has been suggested that the juvenoid does not remain in the larva's tissues but interferes with the programming of the embryonic corpora allata so that it fails to cease secretion of endogenous juvenile hormone at the beginning of the last larval instar (Riddiford, 1970; Retnakaran et al., 1985).

Even though juvenoids are known to inhibit embryogenesis in a wide variety of insects (Staal, 1975; Sehnal, 1983; Vogel et al., 1979) they were not ovicidal

against the cabbage maggot in this study. Juvenoids are most effective against young embryos (Sláma and Williams, 1966; Riddiford and Williams, 1967; Wright, 1970). Even though juvenile hormone is naturally present in late insect embryos, it has detrimental effects when presented early in embryogenesis during blastokinesis (Riddiford and Williams, 1967; Staal, 1982) . The lack of ovicidal activity of juvenoids against D. radicum in this study suggests that the eggs had passed the stage that is most sensitive to juvenoid treatment. In a similar study, newly deposited D. radicum eggs were sensitive to juvenoid treatment (van de Veire and de Loof, 1973).

In this study, methoprene caused a significant reduction in the hemolymph trehalose concentration of late last instar larvae but did not affect hemolymph protein or amino acid concentration in D. radicum. In a previous study on the stable fly Stomoxys calcitrans, Wright and Rushing (1973) found that fat bodies of juvenoid-treated pupae did not utilize glycogen as rapidly as control tissues and as a result of further studies suggested that glycogenolysis in this cyclorrhaphid is probably affected indirectly by a disruption in the normal endocrine activity in the treated insect (Wright et al., 1973) . In a similar manner, the reduced hemolymph trehalose in D. radicum larvae treated with methoprene in this study may have resulted from interrupted glycogenolysis in the fat body so that trehalose could not be replaced by mobilization of glycogen stores as it was required for maintenance and preparation for pupation.

The effect of methoprene on the hemolymph of D. radicum larvae differs from that reported for last instar Aedes aegypti larvae, which showed an elevation in carbohydrate and a decrease in protein concentration of the hemolymph after exposure to the juvenoid. However, the hemolymph carbohydrate concentration of treated insects decreased significantly subsequent to pupation, as glycogenolysis by the fat body was impaired due to diminished activity of glycogen phosphorylase (Gordon and Burford, 1984). Fat body glycogenolysis and hemolymph trehalose levels are controlled by the hyperglycemic factor(s) secreted

by the corpora cardiaca (Sehnal, 1983). Thus, the reduction in the hemolymph trehalose concentration of D. radicum caused by methoprene treatment is consistent with the proposal that juvenoids function, at least in part, by disrupting the normal pattern of neuroendocrine activity in insects (Wright et al., 1973; Downer et al., 1976; Gordon and Burford, 1984; Retnakaran et al., 1985). Alternatively, methoprene may decrease hemolymph trehalose by promoting deposition of glycogen in insect fat body. The lack of activity of precocene on D. radicum larvae and pupae in this study is in accordance with the general insensitivity of holometabolous insects to the precocenes (Bowers et al., 1976; Bowers, 1982; 1985). However, there are exceptions to this general statement. In an mosquito Aedes spp., precocene inhibited larval molting of young instars without precocious development and suppressed pupation when applied to last larval instars (Cupp et al., 1977). Precocenes disrupt reproduction in treated adults of susceptible hemi- and holometabolous species (Bowers et al., 1976; Masner et al., 1979; Landers and Happ, 1980; 1983) but apparently they do not disrupt embryogenesis in the cabbage maggot.

Insect growth regulators, particularly juvenoids and chitin synthesis inhibitors, have potential as insecticidal agents against the cabbage maggot. The two compounds found to be effective against the cabbage maggot in this study, methoprene and diflubenzuron, exhibit detrimental effects on development when applied to the egg stage of D. radicum. In nature, D. radicum eggs are oviposited on the surrounding soil or on the foilage of cruciferous crops, lending themselves to insecticide treatment. Diflubenzuron applied to the egg stage has control potential as a larvicide against D. radicum as it would prevent egg hatch and kill first instar larvae before they could cause economic damage to crops. Methoprene would not be a practical choice against the first generation of larvae and thus for the cabbage maggot, as with other insect pests (Staal, 1982), it is not useful where rapid control is needed. However, methoprene has control potential in regions where this species is multivoltine and perhaps as an insecticide against the parental generation of the next years' population.

The possibility of using methoprene and diflubenzuron in the field will depend on many factors including their environmental stability. While there is little available information on the field stability of diflubenzuron it is known that methoprene has a half-life of two hours in aquatic ecosystems and a half-life of less than ten days in soil (Schaefer and Dupras, 1973; Anonymous, 1975) . Further investigations on the control potential of cabbage maggots with diflubenzuron and methoprene in the field and their physiological effects on target and non-target insects is desired.

SUMMARY

1. A procedure for rearing the cabbage maggot, Delia radicum, was established, based on previously described techniques, to accomodate available greenhouse facilities.

2. A laboratory screening method was developed to monitor the long-term effects of insect growth regulators on D. radicum. Larvae were reared on rutabaga in petri dishes and the optimum conditions for moisture and larval density were determined.

3. The effect of several insect growth regulators on the development of D. radicum: (i) the juvenoids methoprene and juvenile hormone I (ii) the anti-juvenile hormone, precocene II and (iii) diflubenzuron, a chitin synthesis inhibitor, was studied.

4. Applied topically to eggs, larvae or pupae of D. radicum, methoprene did not interfere with development up to the pupal stage, however it prevented adult eclosion due to arrested pupal-adult development within the puparium.

5. Methoprene treatment caused a reduction in hemolymph trehalose in post-feeding larvae, however, it did not affect hemolymph protein or amino acid concentrations. The implications of these physiological studies are discussed in terms of their effect on the neuroendocrine system.

6. Diflubenzuron applied to D. radicum eggs caused a reduction and a delay in egg hatch. Larvae sucessfully hatching from diflubenzuron-treated eggs died before establishing feeding sites in the rutabaga.

7. In contrast to methoprene, diflubenzuron was not effective against D. radicum post-feeding larvae or young pupae.

8. Applied to eggs, post-feeding larvae or pupae, juvenile hormone I and precocene II had no effect on D. radicum.

9. These results suggest that insect growth regulators may have potential value for the control of the cabbage maggot since a chitin synthesis inhibitor prevented development prior to feeding and a juvenoid interrupted the emergence of adult flies.

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Appendix A

Critical nature of water content in larval rearing units

Initially, the major limiting factor of the petri dish rearing method was the death of larvae overnight due to bacterial proliferation. Contaminated units were saturated with water and covered in a pinkish scum; D. radicum larvae crawled out of the rutabaga and died. This contamination was completely controlled by reducing the water content in the larval rearing units.

In larval development, the two critical periods that require a moist substrate are egg hatch and larval exodus at the end of the feeding period. To accomodate these two critical periods, the larval units were watered twice during the period of egg hatch and the rutabaga slices, containing mature L_3, were removed and placed into the moist sand of pupation units before the end of larval feeding. Between these two critical periods the units were watered only once to prevent the rutabaga from drying out. During the feeding period, larvae were protected from dessication inside the rutabaga.

Appendix B

Quantification of hemolymph trehalose

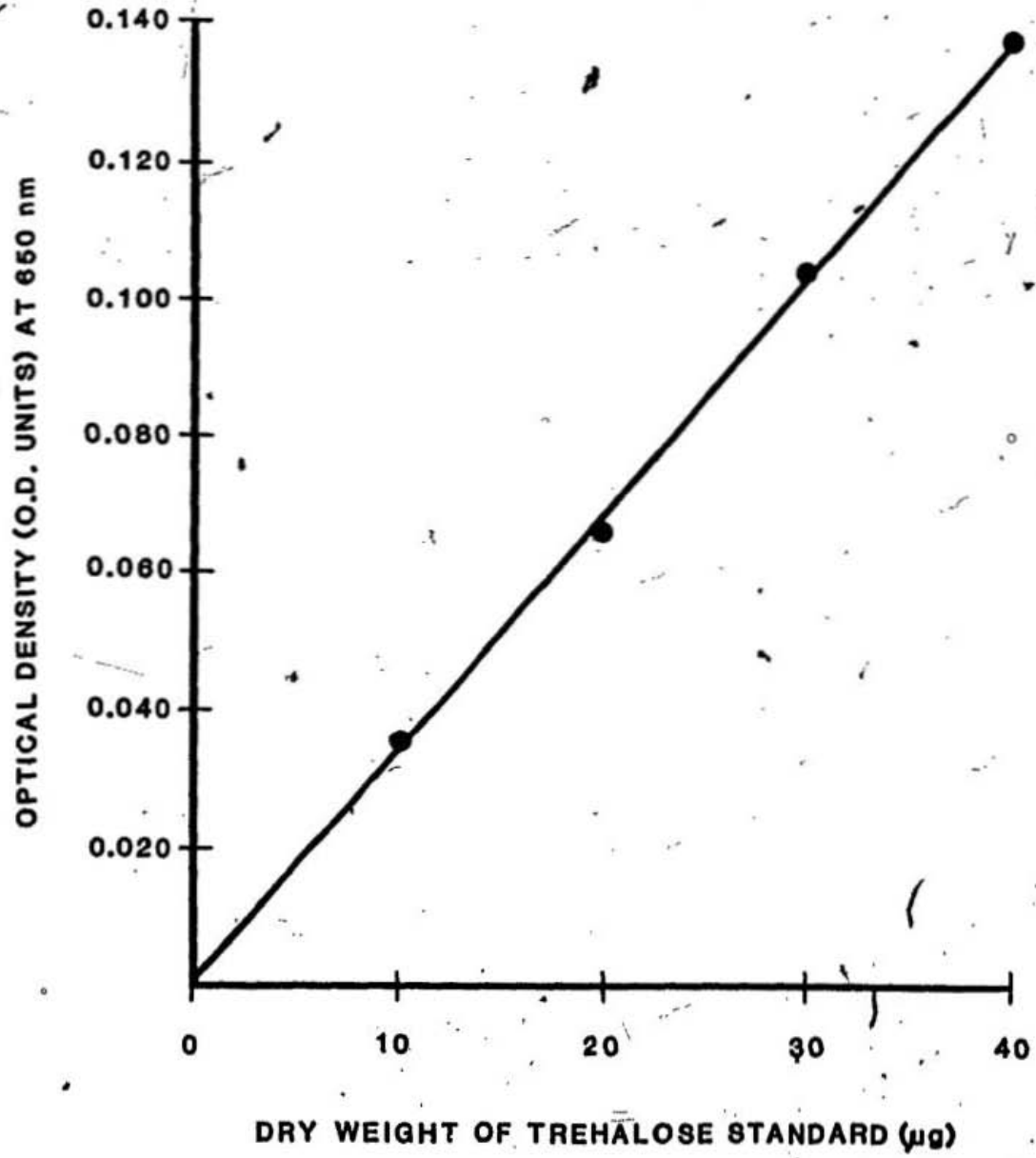
Separation of hemolymph carbohydrates by thin-layer chromatography revealed trehalose to be the only detectable sugar in the hemolymph of post-feeding D. radicum larvae. Scraping of the area corresponding to the R_f value for glucose and subsequent colorimetric analysis confirmed that the level of glucose was too low to be detected.

Trehalose was determined colorimetrically with the anthrone reagent (Roe, 1955). The anthrone reagent causes carbohydrates to be dehydrated by concentrated sulfuric acid to form furfurals and a variety of other degradation products. Furfurals condense with anthrone to form a green-coloured complex.

A curve, plotting optical density against dry weight of trehalose standard (Figure B-1), was constructed by treating dilutions of a stock solution (20 μg/μl) as per hemolymph sample. Five microliters of trehalose standard was applied to the thin-layer chromatography plates per standard sample. It is noted from the curve that Beer's law applies to the production of color.

The trehalose content of hemolymph samples was derived from the standard curve by relating optical density (O.D.) units of a sample to its corresponding dry weight of trehalose standard (Table B-1). The trehalose concentration was expressed per 100 ml of hemolymph.

Figure B-1: Trehalose standard curve: optical density plotted against dry weight of trehalose standard. Values are the means of 4 determinations.


0.140
0.120
0.100
0.080
0.060
0.040
0.020
OPTICAL DENSITY (O.D. UNITS) AT 650 nm
0
10
20
30
40
DRY WEIGHT OF TREHALOSE STANDARD (µg)

SAMPLE CALCULATION

From the curve (Figure B-1), an O.D. value of 0.103 corresponds to a trehalose concentration of 30 μg. To express this per 100 ml hemolymph:

30 μg/5μl hemolymph=0.6 g/100 ml

Control 81.2 μg/5 μl=16.2 g/l=1.62 g/100ml

Methoprene 57.8 μg/5 μl=11.6 g/l=1.16 g/100ml

Table B-1: Effect of methoprene on hemolymph trehalose concentration in Delia radicum post-feeding larvae

OPTICAL DENSITY AT 650 nm ABSORBANCE VALUES					
Treatment	Rep 1	Rep 2	Rep 3	Mean ± S.E.M.	Dry wt. from curve (μg)
Control	0.256	0.331	0.247	0.278 ± .0266	81.2 ± 7.766
Methoprene	0.200	0.192	0.201	0.198 ± .0028*	57.8 ± .8175

* Statistically significant: $p<0.05$ compared to controls

Appendix C
Quantification of hemolymph amino acids

Amino acids were quantified by reacting soluble amino acids with triketohydrindenehydrate (reduced ninhydrin) to form a violet coloured product that was estimated colorimetrically (Rosen, 1957). All amino acids, except proline and hydroxyproline (secondary amines) give a purple colour with ninhydrin. Proline and hydroxyproline are imino acids and give a different reaction product.

A standard curve was prepared from dilutions of a stock glycine solution (0.01 g/100 ml 5% trichloroacetic acid) (FigureC-1). The standard curve adheres to Beer's law. The amino acid content was determined from the curve by relating optical density (O.D.) units of a sample to its corresponding dry weight of glycine standard. The amino acid concentration was then expressed in microgram equivalents of glycine per 100 ml of hemolymph.

HEMOLYMPH VOLUME:
Equals 1.0 μl since 0.5 ml aliquots were removed from a solution of 10 μl hemolymph added to 5 ml trichloroacetic acid.

Amino acid concentration is expressed in mg amino nitrogen/100 ml hemolymph:

1 μg glycine=.186 μg N

Figure C-1: Standard curve for amino acid determination: optical density plotted against dry weight of glycine standard. Values are the means of 2-3 determinations.

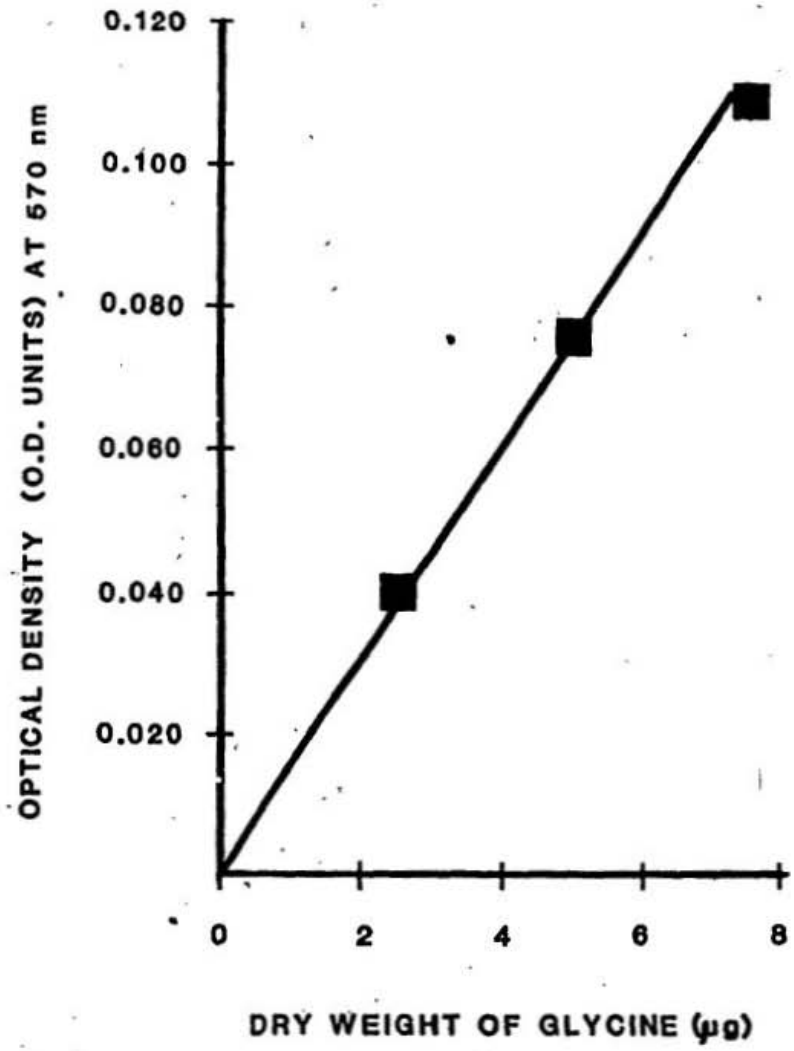

0.120
0.100
0.080
0.060
0.040
0.020
OPTICAL DENSITY (O.D. UNITS) AT 670 nm
0
2
4
6
8
DRY WEIGHT OF GLYCINE (µg)

Table C-1: Effect of methoprene on hemolymph amino acid concentration in Delia radicum post-feeding larvae

OPTICAL DENSITY AT 570 nm ABSORBANCE VALUES							
Treatment	Rep. no.	Aliquot	Aliquot	Aliquot	Aliquot Mean	Mean ± S.E.M	µg Eqv. Glycine
Control	1	.082	.081	.086	.083	.064 ± .0102	4.2 ± .66
	2	.050	.060	.035	.048		
	3	.071	.042	.071	.061		
Methoprene	1	.099	.144	.103	.115	.075 ± .0198	4.9 ± 1.29
	2	.048	.071	.046	.055		
	3	.057	.044	.067	.056		

Converting units:

Control: 78 ± 12.3 mg amino N/100 ml hemolymph

Methoprene: 91 ± 24.0[1] mg amino N/100 ml hemolymph

[1]Not statistically significant

Appendix D

Quantification of hemolymph proteins

The colorimetric quantitation of proteins by the Lowry method (Lowry et al., 1951) is based on the principle that the peptide (C-N) linkage of a protein complexes with copper in alkali. The resultant copper-protein complex then reduces the Folin reagent (phosphomolybdic-phosphotungstic reagent) to form a purple-blue colored product.

A standard curve was constructed with dilutions of crystalline bovine serum albumin (BSA) by plotting optical density (O.D.) against concentrations of BSA (figure D-1). The protein content of the hemolymph samples was determined from the curve by relating optical density (O.D.) units of a sample (table D-1) to its corresponding dry weight of BSA standard. This represented the protein concentration in 10 μl of hemolymph. The protein concentration was then expressed in microgram equivalents of BSA per 100 ml of hemolymph.

Control 4.20 μg equiv. BSA/100 ml hemolymph
Methoprene 4.25 μg equiv. BSA/100 ml hemolymph

Figure D-1: Standard curve for protein determination: optical density plotted against dry weight of BSA standard. Except at 300 μg, values are means of 3 determinations.

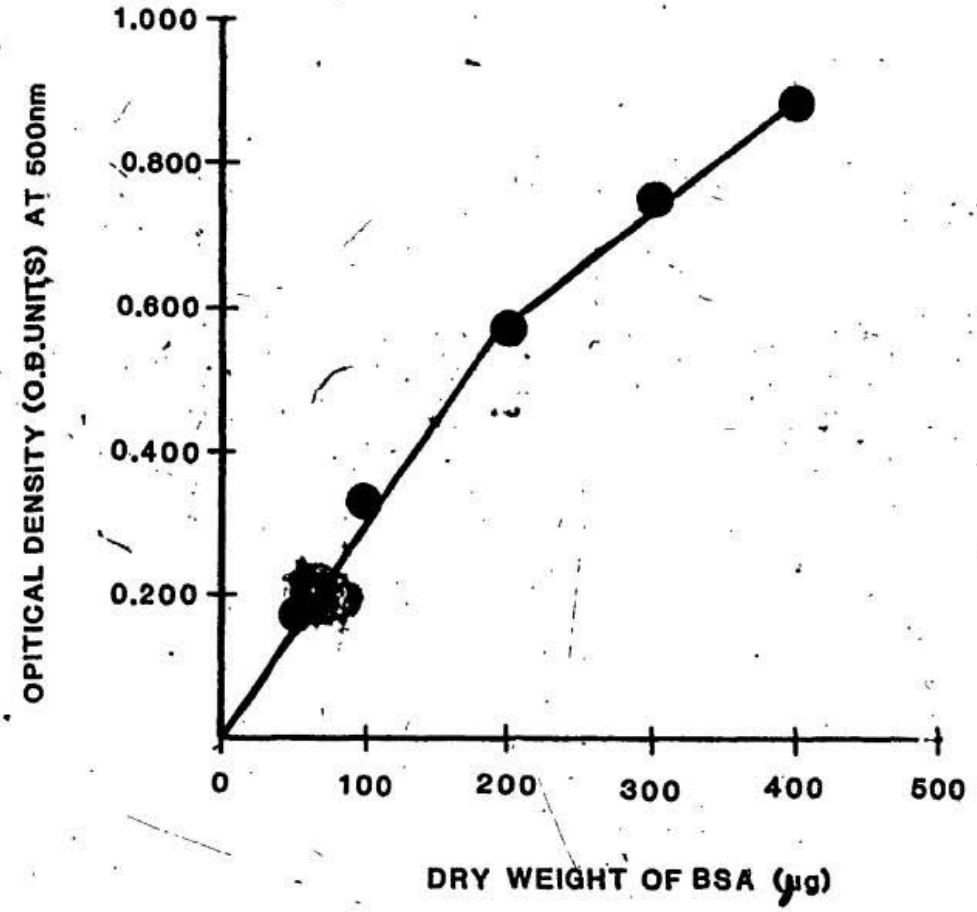

1.000
0.800
0.600
0.400
0.200
OPITICAL DENSITY (O.D.UNITS) AT 500nm
0
100
200
300
400
500
DRY WEIGHT OF BSA (µg)

Table D-1: Effect of methoprene on hemolymph protein of Delia radicum post-feeding larvae

OPTICAL DENSITY AT 500 nm ABSORBANCE VALUES				
Treatment	Rep 1	Rep 2	Rep 3	Mean ± S.E.M.
Control	.917	.888	*	.902 ± .0145
Methoprene	.968	.845	.895	.903 ± .0357[1]

[1] Not statistically significant

Appendix E

Effect of insect growth regulators on *Delia radicum* eggs: experiment record

Table E-1: Record of hatch, pupal yield and adult emergence after treatment of Delia radicum eggs with four insect growth regulators

Treatment	Rep. No.	Egg hatch[1] Day 4	Egg hatch[1] Day 6	No. Pupae Day 21	No. Pupae Day 28	Emergence x Days After Treatment[2] 32 35 36 37 44 45 50 54 57 59 61 66 70 71 72 79	Total F/M[3]	Total Adults
	1	18	18	5	5	10 00 00 00 00 00 00 00 00 00 11 01 00 00 00 00	2/2	4
	2	17	17	6	6	00 21 00 00 00 01 00 00 00 00 00 00 00 00 00 00	2/2	4
Control	3	19	19	16	16	15 32 00 00 00 00 00 00 00 01 00 02 00 00 00 00	4/10	14
(Untreated)	4	16	16	14	16	00 41 10 10 32 00 00 00 00 00 00 02 00 10 10 00	11/5	16
	5	15	15	11	11	10 43 00 00 00 00 00 00 00 00 00 00 00 00 00 00	5/3	8
	6	16	16	12	13	00 25 10 00 00 00 00 00 00 10 00 01 00 00 00 00	4/6	10
	1	17	18	9	10	01 31 01 00 00 00 00 00 00 00 00 11 00 00 00 00	4/4	8
	2	18	18	7	9	02 12 10 00 00 00 10 00 00 00 00 00 00 00 00 00	3/4	7
Control	3	17	17	12	12	11 05 00 00 00 00 00 00 00 01 00 20 00 00 00 00	3/7	10
(Solvent	4	17	17	15	15	01 82 00 00 00 00 00 00 00 00 00 00 01 01 01 00 01	8/7	15
-treated)	5	17	18	13	13	20 22 00 00 00 10 00 00 00 01 00 02 01 00 00 10	6/6	12
	6	17	17	14	14	13 11 00 00 00 11 00 00 01 00 01 11 00 00 00 00	4/8	12

TABLE E-1 CONTINUED...

Treatment	Rep. no.	Egg hatch[1] Day 4	Egg hatch[1] Day 6	No. Pupae Day 21	No. Pupae Day 28	Emergence x Days After Treatment[2] 32 35 36 37 44 45 50 54 57 59 61 66 70 71 72 79	Total F/M[3]	Total Adults
Diflubenzuron	1	10	13	0	0			
	2	15	18	0	0			
	3	11	12	0	0			
	4	12	14	0	0			
	5	14	16	0	0			
	6	13	14	0	0			
Methoprene	1	18	19	9	10	00 00 00 00 00 00 00 00 00 00 00 00 00 00 00 00	0/0	0
	2	18	18	15	15	00 00 00 00 00 00 00 00 00 00 00 00 00 00 00 00	0/0	0
	3	18	19	10	11	00 00 00 10 00 00 00 00 00 00 00 00 00 00 00 00	1/0	1
	4	13	13	9	11	00 00 00 00 00 00 00 00 00 00 00 00 00 00 00 00	0/0	0
	5	16	17	10	11	00 00 00 00 21 00 00 00 00 00 00 21 00 00 00 00	4/2	6
	6	13	15	7	8	00 00 00 00 00 00 00 00 00 00 00 00 00 00 00 00	0/0	0

TABLE E-1 CONTINUED..

Treatment	Rep. No.	Egg hatch[1] Day 4	Egg hatch[1] Day 6	No. Pupae Day 21	No. Pupae Day 28	Emergence x Days After Treatment[2] 32 35 36 37 44 45 50 54 57 59 61 66 70 71 72 79	Total F/M[3]	Total Adults
	1	16	16	11	12	30 32 00 00 20 00 00 00 00 00 00 00 00 00 00 00	8/2	10
	2	16	16	10	10	10 21 00 00 00 00 00 00 00 00 00 11 00 00 00 00	4/2	6
Juvenile	3	19	19	11	11	11 23 10 00 00 00 00 00 01 00 00 00 00 00 00 00	4/5	9
Hormone I	4	15	15	10	10	01 34 00 00 00 00 00 00 00 00 00 11 00 00 00 00	4/6	10
	5	20	20	13	16	00 11 20 01 20 00 00 00 00 01 00 12 00 00 00 00	5/6	11
	6	18	18	13	14	11 43 00 00 00 00 00 00 01 01 00 10 00 00 00 00	6/6	12
	1	19	20	11	11	22 20 00 00 00 00 00 00 00 00 00 01 10 00 00 00	5/3	8
	2	17	17	14	14	00 22 00 00 00 00 00 00 00 03 10 02 00 00 00 10	4/7	11
Precocene II	3	18	18	15	16	00 15 00 00 00 00 00 00 00 00 00 01 00 00 00 00	1/6	7
	4	18	18	11	12	00 23 20 00 00 00 00 10 01 00 11 00 00 00 00 00	6/5	11
	5	15	16	13	14	02 62 30 00 00 00 00 00 00 00 00 00 00 00 00 00	9/4	13
	6	14	14	10	11	00 32 10 01 00 00 00 00 00 00 00 00 00 00 00 00	4/3	7

[1]Number of eggs hatching out of twenty

[2]The two digits represent the number of females and males, respectively

[3]F=females, M=males

Appendix F

Effect of insect growth regulators on post-feeding larvae: experiment record.

Table F-1: Record of pupal formation and adult emergence after treatment of post-feeding Delia radicum larvae with four insect growth regulators

Treatment	Rep. No.	No. Pupae/10 24hrs a b c[1]	48hrs a b c	96hrs a b c	Adult emergence x days post-treatment 17	18	19	20	33	35	36	39	40	41	45	52	Total F/M^2	Total Adults
Control (Untreated)	1	1 2 7	0 0 10	0 0 10	01	11	00	00	01	00	01	00	01	00	00	00	1/5	6
	2				13	00	00	22	00	01	00	00	00	00	00	00	3/6	9
	3				00	00	44	10	00	00	00	00	00	00	00	00	5/4	9
Control (Solvent-treated)	1	1 1 8	0 1 9	0 0 10	01	02	00	11	01	00	00	00	01	00	00	00	1/6	7
	2				00	00	00	32	00	00	00	00	00	01	00	00	3/3	6
	3				01	00	33	10	00	00	00	00	00	00	00	00	4/4	8
Diflubenzuron	1	4 1 5	1 0 9	0 0 10	00	00	00	00	02	00	00	01	03	00	00	00	0/6	6
	2				30	00	00	30	00	00	00	00	00	00	00	00	6/0	6
	3				00	00	30	10	00	00	00	00	00	00	00	00	4/0	4

TABLE F-1 CONTINUED...

Treatment	Rep. No.	No. Pupae/10 24hrs a b c[1]	48hrs a b c	96hrs a b c	Adult emergence x days post-treatment 17	18	19	20	33	35	36	39	40	41	45	52	Total F/M[2]	Total Adults
Methoprene	1	1 1 8	0 0 10	0 0 10	00	00	00	00	00	00	00	00	00	00	00	00	0/0	0
	2				00	00	00	00	00	00	00	00	00	00	00	00	0/0	0
	3				00	00	00	00	00	00	00	00	00	00	00	00	0/0	0
Juvenile	1	4 0 6	0 0 10	0 0 10	10	00	00	00	00	00	00	01	00	00	00	10	2/1	3
Hormone	2				01	00	00	43	00	00	00	00	00	00	00	00	4/4	8
I	3				00	00	33	00	00	00	00	00	00	00	00	00	3/3	6
Precocene II	1	3 2 5	0 0 10	0 0 10	11	00	00	11	00	00	00	11	10	00	12	00	5/5	10
	2				00	00	00	40	00	00	00	00	00	20	00	00	6/0	6
	3				00	00	44	00	00	00	00	00	00	00	00	00	4/4	8

[1] a Number of wandering larvae, b Number of untanned pupae .
c, Number of tanned pupae

[2] F=females, M=males

Appendix G
Effect of methoprene on pharate adults

Table G-1: Record of D. radicum emergence after topical application of 1 µg methoprene to pharate adults

Treatment	Rep No.	Adult emergence x days post-treatment x=4	x=7	x=8	x=9	x=11	x=14	Sex Totals F/M[1]	Total Adults
Untreated	1	1 2	8 6	0 0	0 0	0 0	0 0	9/8	17
	2	1 3	7 7	0 1	0 0	1 0	0 0	9/11	20
	3	0 2	7 7	2 0	0 0	0 0	0 0	9/9	18
	MEAN	3	14	1	0	0.33	0	27/28	55
Methoprene	1	0 1	6 6	1 2	0 0	1 0	0 0	8,	17
	2	0 0	6 5	1 1	3 0	0 0	0 0	10/6	16
	3	2 2	5 10	0 1	0 0	0 0	0 0	7/13	20
	MEAN	1.7	12.7	2	1	0.33	0	25/28	53

[1] F=females, M=males