Hydroprene: Mode of action, current status in stored-product pest management, insect resistance, and future prospects

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Abstract

The insect growth regulator (IGR) hydroprene is a juvenile hormone analogue used in urban and stored-product insect control programs in the United States and in other developed countries. Hydroprene can be considered as an alternative to conventional insecticides because of its specific activity against immature insect stages, low persistence in the environment, and virtually non-toxic effects on mammals. Several published records demonstrate the excellent potential of hydroprene to control many stored-product insects. However, there are concerns of insect resistance to all insecticides, including IGRs. We review the mode of action of hydroprene in insects, examine how hydroprene is used in agricultural and urban pest management systems, describe research with hydroprene in stored-product pest management, and discuss potential mechanisms by which insects could develop resistance to this chemical. We also identify potential areas of further research with hydroprene that include, but are not limited to, the estimation of effects of hydroprene on different flooring surfaces, evaluation of multiple methods of hydroprene application in a facility, identification of other control methods to be used in combination with hydroprene, identification of specific life stages of stored-product insects that are especially vulnerable to hydroprene, and inclusion of the effects of hydroprene in stored-product insect population models.

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Keyword: Hydroprene; Stored-product insects; Mode of action; Insect resistance

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1. Introduction

The laws governing pesticide registration in the United States have been revised several times in the latter part of the 20th century, in part due to the increased awareness of risk to human health and environmental safety posed by chemicals used in pest management and changing
consumer preferences (Arthur and Phillips, 2003). The most notable of these changes were the amendments made to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) as a result of passing of the Food Quality Protection Act (FQPA) in 1996. The amendments to FIFRA were made with a broader objective of providing safer pesticides that are of low risk to human health and the environment. The FQPA charged the US Environmental Protection Agency (US EPA) to re-evaluate the suitability of all currently used pesticides to meet with the changed safety expectations. As a result, some of the currently used organophosphate and carbamate insecticides have been removed from usage, while others are under threat of removal. In addition, methyl bromide, a very commonly used fumigant in stored-product pest management, is being phased out as part of an international agreement (Anonymous, 2004). There is a need for new alternatives to traditional insecticides used in stored-product pest management (Arthur, 1996; Dunkel and Sears, 1998; Mbata and Phillips, 2001; Arthur and Phillips, 2003).

Development and reproduction in insects is affected by a number of hormones, including juvenile hormones (JHs). Williams (1967) proposed that timely application of JHs could be employed to control insects because of their ability to disrupt normal physiological functions. Insect growth regulators (IGRs) are synthetic mimics and analogues which mimic naturally occurring hormones and physiological processes of insects, and are generally classified as JH analogues (JHAs), ecyhsone agonists, or molt inhibitors (Mondal and Parween, 2001). These synthetic mimics affect the normal development of immature insects. There are several recent reviews (Oberlander et al., 1997; Mondal and Parween, 2001) which review the use of IGRs in stored products. Hydroprene is a JHA that mimics the action of naturally occurring JH in insects, and is considered to be very low risk to mammals and other non-target vertebrates, because of its low toxicity and because it degrades rapidly in the environment. Although hydroprene is labeled for use in food-handling and storage establishments, it is not labeled for direct application to grains and grain-based products.

Insect resistance to IGRs such as hydroprene was considered to be unlikely because of their unique mode of action in structurally mimicking an essential hormone in the endogenous system (Williams, 1967). However, several studies have shown the development of resistance in insects to IGRs with similar modes of action as hydroprene (Horowitz and Ishaaya, 1994; Dame et al., 1998; Cornel et al., 2000, 2002; Horowitz et al., 2002). In order to make best use of hydroprene in stored-product pest management, knowledge of the mode of action and mechanisms of potential insect resistance to hydroprene are required. An excellent review on the molecular site of action of JHs and JHAs is available in Wilson (2004). Although there are several published reviews on IGRs in general for stored-products, our objective was to summarize, update, and synthesize the information specific to hydroprene currently available in the literature. We use information and data from other agricultural and pest management system to illustrate the mode of action of hydroprene, describe the uses of hydroprene in different systems, review and update published results with hydroprene, discuss the status of hydroprene in stored-product pest management, and suggest options for future research.

2. Mode of action of hydroprene

Hydroprene is a JHA which mimics the action of naturally occurring JHs (Retnakaran et al., 1985; Wilson, 2004). Various studies have shown that excessive amounts of JHs can prolong the larval stages in an insect (Edwards et al., 1995), presumably due to combinatorial actions with ecdysteroids. Application of hydroprene during the larval stages restricts and inhibits normal development, and larvae treated with hydroprene either cannot emerge as adults or they emerge as abnormal sterile adults (Ware, 2000). Hydroprene causes different toxic effects depending on the target insect species because of the diverse roles of JHs in insects. Topical application of hydroprene to Diploptera punctata (Eschscholtz), the Pacific beetle cockroach, depressed the synthesis of natural JHs at high doses, but stimulated it at lower doses (Tobe and Stay, 1979). The prothoracic glands failed to synthesize or increase the production of essential ecdysteroid hormones for the pupal molt when hydroprene was topically applied to the tobacco hornworm, Manduca sexta (L.), in a dose-dependent manner (Rountree and Bollenbacher, 1986). Application of hydroprene to last instar D. punctata prolonged the development time and caused desynchronization of ecdysteroid production (Kikukawa et al., 1989). When 10 μg of hydroprene was topically applied on larvae of the greater wax moth, Galleria mellonella (L.), the hydroprene persisted in the body for two days and resulted in delayed molting after levels were reduced to trace amounts. Normal allatotropic activity for JH biosynthesis resumed after reduction of hydroprene levels in the haemolymph to trace amounts (Bogus and Scheller, 1991).

3. Insect resistance to hydroprene

There have been no published reports to document resistance by any insect to hydroprene. However, there are several reports on resistance by insects to other JHAs, methoprene in particular. Resistance by the house fly, Musca domestica L., to various JHAs is widespread (Pospischil et al., 1996). The pasture mosquito, Oclerotatus nigromaculis (Ludlow), in central California has been found to have several thousand-fold resistance to methoprene (Cornel et al., 2002). Resistance of house fly larvae to methoprene (Hammock et al., 1977), and cross-resistance to some organophosphorus compounds and JHAs, was reported (Keiding, 1999). Methoprene resistance has also been detected in Australian populations of the lesser grain.
borer, *Rhizopertha dominica* (F.) (Collins, 1998). These reports suggest that insect resistance to JHAs could be due to either degradation of the artificially applied JHAs in the insect's body before reaching their target sites, or modification of the target site resulting in reduced affinity of the JHBPs to the JHAs. At the molecular level, insect resistance to an insecticidal chemical, including the JHAs could be mainly due to point mutations and/or upregulation or amplification of detoxification genes (Wilson and Ashok, 1998).

Degradation and elimination of JHAs from an insect is possible as is evident from the basic structure of these compounds. All six naturally occurring JHs are sesquiterpenoids with an ester linkage. JHAs such as hydroprene and methoprene are synthesized based on these structures and contain one or more ester linkages. Increased carboxylerases activity was observed following topical application of hydroprene on the Rutgers strain of *M. domestica* (Maa, 1987). Detoxification mechanisms mediated by cytochrome P450 monooxygenases could also lead to the metabolism of synthesized JHs and their elimination from the insect body. Zhang et al. (1998) showed that cytochrome P450 monooxygenase played an important role in house fly resistance to pyriproxifen.

Insect resistance to JHAs can also be due to altered JH receptors with reduced affinity to the JHAs (Wilson and Fabian, 1986; Gruntenko et al., 2000). In a methoprene-resistant *D. melanogaster* population, Shemshedini and Wilson (1993) found lower ligand affinity of a specific cytoplasmic component JHBP to both natural and synthesized JHAs. In house fly adults and larvae, Plapp et al. (1998) identified a JHBP with molecular weight of ca. 22,000 responsible for resistance to synthetic JHs. Lack of gene product at the target site due to mutations or amplification of detoxification genes are suggested as other mechanisms for resistance development by *D. melanogaster* to synthesized JHs (Wilson and Ashok, 1998).

Insecticide cross-resistance to hydroprene in insects is of critical importance in using hydroprene for insect pest management. However, there is no published evidence showing cross-resistance by insects to hydroprene. The Mediterranean climbing cutworm, *Spodoptera littoralis* (Boisd.), was resistant to endrin and aminocarb, and to two JHAs (R-20458 and ENT-34070), but was susceptible to methoprene as well. (El-Guindy et al., 1975). Malathion-resistant strains of *T. castaneum* and *T. confusum* showed no cross-resistance to hydroprene (Amos et al., 1975). Reports on cross-resistance to other JHAs, include low level resistance to methoprene by the northern house mosquito, *Culex pipens* L. (Georgihiou et al., 1975), *M. domestica* (Rupes et al., 1976), a multiresistant strain of *Tribolium castaneum* (Herbst), the red flour beetle (Hoppe, 1981), and resistance to methoprene and pyriproxifen by a pyrimiphos-methyl-resistant strain of *T. castaneum* (Kostyukovsky et al., 2000). In the laboratory, Georgihiou et al. (1978) induced resistance to methoprene in *C. pipens*, and the resistance could extend to three other JHAs. This experiment did not include hydroprene. Laboratory-induced methoprene resistance also resulted in cross-resistance to 16 pyrethroids and other JHAs (Georgihiou and Grant, 1979).

Behavioral adaptations are one method by which insects can develop resistance to insecticide treatments, and this could include resistance to hydroprene as well. Studies with stored-product insects show that there may be some avoidance behavior on treated surfaces or treated grains (Prickett and Ratcliffe, 1977; Collins et al., 1988; Cox et al., 1989; Barson et al., 1992; Mason, 1996). These studies mainly involved adult stored-product beetles and their behavior towards conventional insecticides. Watson and Barson (1996) noted that a strain of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), with high resistance to permethrin readily detected arenas treated with permethrin and avoided making contact. The authors suggested that this avoidance behavior by two insecticide-resistant strains of *O. surinamensis* to contact insecticides, pirimiphos-methyl, etrimfos, permethrin, and an insect repellent might enhance their survival due to biochemical or physiological resistance. Refuge-seeking behavior of two insecticide-resistant strains of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), significantly increased their survival when exposed to surfaces treated with fenetrothion and malathion (Cox et al., 1997). In all these studies, stored-product beetles that were already resistant to certain chemicals, or ones that had prior exposure to such chemicals, were used in the assays. Behavioral avoidance and refugee-seeking behavior by stored-product insects towards contact insecticides could be secondarily derived characters. Nevertheless, the potential to develop such resistance towards contact insecticides exists, and therefore one can assume the same might apply to hydroprene as well.

**4. Hydroprene in stored-product pest management**

Hydroprene is primarily used to control urban and stored-product pests in the US, but it is used in other countries to control field crop insect pests. Various lethal and sub-lethal effects of hydroprene on different insects are found in these reports, including morphological disorders (Mathai and Nair, 1991; Vijayalakshmi and Ramaraj, 1991), prolongation of stage-specific development times (Chakravorty et al., 1990), adult sterility (Nair and Muraleedharan, 1998), and reduced fecundity (Gelbie and Matolin, 1984; Mathai and Nair, 1991). There are several reports on the effects of hydroprene on the general morphology and population dynamics of *B. germanica* (Bennett et al., 1986; King and Bennett, 1988, 1989, 1990; Stoltzman and Stay, 1997; Bell et al., 1999). In these studies, hydroprene caused twisted wing formation, stunt growth, morphogenetic and gonadotrophic effects, vitellogenesis in the basal oocytes of the larval ovary, maintenance of juvenile characters, and reduction in cockroach populations over a period of time. The experiences with IGRs in household pest management could help promote...
the use of these chemicals as alternatives to conventional pesticides in stored-product pest management (Oberlander et al., 1997; Bell and Edwards, 1999; Arthur and Phillips, 2003).

A listing of published references describing some aspect of research with hydroprene on stored-product insects is given in Tables 1 and 2. Much of the earlier research in the 1970s and 1980s on the use of hydroprene in stored-product pest management mainly focused on beetles (Loschiavo, 1975, 1976; McGregor and Kramer, 1975; Amos and Williams, 1977; Rup and Chopra, 1984), and relatively fewer studies were conducted on stored-product lepidopteran pests (Nickle 1979; Stockel and Edwards, 1981). There are several examples of the successful use of hydroprene in field crop lepidopteran insects (Skuhravy and Hochmut, 1975; Mandal and Chouduri, 1984; Chakravorty and Roychoudhury, 1986), and tests have also been conducted on stored-product lepidopteran pests, including the almond moth, Cadra (=Ephestia) cautella (Walker) (Shaaya and Pisarev, 1986), the Indian meal moth, Plodia interpunctella (Hübner) (Mohandass et al., 2006a, b), and the rice moth, Corcyra cephalonica (Stainton) (Samui et al., 1981, 1982; Deb and Chakravorthy, 1982; Bhargava and Urs, 1993).

In many of the earlier studies, tests were conducted in which hydroprene and other IGRs were incorporated into the insect diet (Strong and Diekman, 1973; El-Sayed, 1988). There are several tests conducted with hydroprene on stored grains, with often conflicting results. In general, internal feeders of stored grains such as Sitophilus oryzae (L.), the rice weevil would appear to have limited susceptibility to IGRs because females oviposit directly into the kernels. Gupta and Mkhize (1983) reported complete suppression of S. oryzae on wheat treated with high doses of 20, 50, and 100 ppm hydroprene, however, these are extremely high doses of an insecticide applied directly to a grain commodity. In other tests with a more realistic application rate of 10 ppm, progeny of S. oryzae were able to develop in wheat treated with hydropren and methoprene (McGregor and Kramer, 1975).

Surface spray treatments and spot or crack-and-crevice applications are common management practices used in indoor facilities (Arthur and Phillips, 2003). In the USA, hydroprene is labeled for use as an aerosol fog, surface spray, or as an impregnated disc. Spot application of hydroprene as a surface spray reduced populations of several stored-product insect species in a botanical warehouse, including P. interpunctella, the cigarette beetle, Lasioderma serricorne (F.), the merchant grain beetle, Orzyzaephilus mercator (Fauvel), T. castaneum, and the flat grain beetle, Cryptolestes pusillus (Schönherr) (Arbogast et al., 2002). Hydroprene has volatile activity and can be used for crack-and-crevice treatments in confined spaces (Atkinson et al., 1992). Under laboratory conditions, Arthur (2000, 2003) and Arthur and Hoernemann (2004) showed inhibition of development and reproduction in T. castaneum and the confused flour beetle T. confusum, Jacqueline Du Val, by applying the labeled rate of commercial formulation of hydroprene (Gentrol®). A volatile formulation of hydroprene (Pointsource™) also arrested larval growth of T. castaneum and T. confusum (Arthur, 2003).

The effects of hydroprene on insects differ with the type of surface on which hydroprene is sprayed. Atkinson et al. (1992) sprayed hydroprene on various types of absorbent (unfinished plywood, fiberboard, vinyl tile) and non-absorbent surfaces (glass, stainless steel, ceramic tile, and formica). The survival, number of oothecae, and percentage egg hatch of B. germanica were more affected by hydroprene when sprayed on non-absorbent surfaces than on absorbent surfaces. Similarly, Kaakeh et al. (1997b) found juvenoid activity on stainless steel was significantly higher than on tempered masonite and unpainted plywood. Temperature is often an important factor in the response of insect species to insecticides, but there are limited data regarding the effect of temperature on efficacy of hydroprene, particularly for stored-product insects. Arthur and Dowdy (2003) treated concrete surfaces with hydroprene and held them at 45 and 55 °C for 4, 8, and 16 h and found no significant difference in T. castaneum mortality due to heating. The environmental persistency of hydroprene when sprayed as a surface treatment could be influenced

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<th>Table 1</th>
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<td>Agricultural insects</td>
<td>Rountree and Bollenbacker (1986)</td>
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<td>Medical-veterinary insects</td>
<td>McGregor and Kramer (1975)</td>
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<td>Stored-product insects</td>
<td>Amos and Williams (1977)</td>
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<td>Urban insects</td>
<td>Nickle (1979)</td>
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<td>Other insects</td>
<td>Gupta and Mkhize (1983)</td>
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<th>Table 2</th>
<th>Specific references for hydroprene and stored-product insects</th>
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<td>Research topic</td>
<td>Reference</td>
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<td>Aerosols/volatiles</td>
<td>Bell and Edwards (1999)</td>
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<td>Commodity treatments</td>
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<td>Dietary incorporation studies</td>
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<td>Mode of action/toxicity</td>
<td>Amos et al. (1975)</td>
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<td>Surface treatments</td>
<td>Loschiavo (1975, 1976)</td>
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<td>Other</td>
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by the spillage and food residues in storage facilities. The presence of wheat flour on concrete surfaces treated with hydroprene reduced the residual effect, and most late-instar *T. confusum* larvae emerged as adults with few deformities (Arthur and Hoernemann, 2004). Sanitation of food-storage facilities may be therefore required in order to achieve effective control.

Hydroprene application in storage facilities, retail stores, flour mills, and warehouses, can potentially affect population dynamics of stored-product insect pests and may lead to their eventual eradication from these facilities. In a computer simulation study by Zeeman (1993), *B. germanica* populations were unable to re-establish after 90–120 day when hydroprene and fenoxycarb baits were used at the early phase of population growth. A predicted control level of at least 80% of the population was necessary to prevent re-establishment of the population. Applying ultra low volume cold mist of hydroprene every 3 or 4 months reduced Australian cockroach, *Periplaneta australasiae* (F.), populations by 81% (Bell et al., 1999).

5. Recommendations for future studies with hydroprene

1. Surface treatment of hydroprene is a recommended practice for stored-product pest control in the USA. Many of the retail stores, pet stores, warehouses, and food storage and packaging facilities employ surface treatment with hydroprene for pest management. Different types of floor surfaces found in these facilities differ in chemical absorbance, which could affect longevity of hydroprene sprayed on the surface and residual efficacy on insect pests (Kaakeh et al., 1997a). Studies to quantify effects of such differences on the mortality and/or development time of stored-product insects as a result of hydroprene sprayed on different surfaces are required. Carpeted surfaces are also commonly found in some retail stores and smaller food-handling facilities. The effect of hydroprene sprayed on carpet surfaces should also be determined.

2. Identification of hot-spots or places of high occurrence of insects by monitoring pest populations in stored-product environments (Arbogast et al., 2000, 2002; Campbell et al., 2002) and application of hydroprene in such areas may help control insect populations. Arbogast et al. (2002) reduced the population of several stored-products pests by spot application of hydroprene. However, the use of spatial data derived from pheromone-baited trap records in making management decisions is still poorly understood in stored-product pest management (Campbell et al., 2002). Pheromone-baited trap records alone may not provide adequate information on the exact distribution of insects (Arbogast and Mankin, 1999), and, if possible, other sampling methods should be employed to get a clearer knowledge of their distribution.

3. Multiple methods of hydroprene application could improve stored-product pest management. Surface application of hydroprene and aerosols are common methods of hydroprene application employed in many smaller storage facilities, such as packaging units and retail stores. However, insects may breed or find refuges in places where spraying might not reach, such as underneath storage units and shelves or near packaged food. Therefore, a single method of hydroprene application may not yield desirable results in all situations. Studies to quantify the effects of multiple methods of application of hydroprene, such as the use of volatile formulations (Arthur and Hoernemann, 2004), placement of impregnated discs, and aerosol sprays must be conducted in order to evaluate the potential of such methods and also to optimize the use of hydroprene in stored-product pest management.

4. Stored-product pest management in the future will consist of more integrated approaches, as opposed to strict reliance on chemical control methods. Such integrated approaches are receiving more attention (Johnson et al., 1997, 1998, 2002) and have benefits such as sustainable control and slowing resistance development by insects to newer chemicals (White, 1992; White and Leesch, 1995; Arthur and Phillips, 2003). Hydroprene has potential to be used along with heat treatments (Arthur and Dowdy, 2003) and such potential may exist for combination with other management practices as well. Studies to evaluate control methods compatible with hydroprene application, such as the application of conventional insecticides, chemical fumigation, and other management methods, are also necessary for the development of integrated control programs.

5. Stored-product insects could develop behavioral adaptations to resist hydroprene application, although there are currently no published records documenting this phenomenon. Many of the studies that were conducted in the past have verified such behavioral adaptations of stored-product beetles to conventional insecticides but not IGRs. However, there are few published studies on lepidopteran insect pests. Studies with lepidopterans may elucidate new mechanisms by which insects develop behavioral resistance to IGRs and other insecticides.

6. Modeling the population dynamics of several stored-product insects inside food storage facilities has the potential to assist in timing of management practices (Hagstrum and Flinn, 1990; Flinn and Hagstrum, 1990; Flinn et al., 1997). Unlike many conventional chemicals which mainly cause mortality, hydroprene in addition to mortality also delays the development time of the younger life stages of an insect. Therefore, bioassays to quantify such effects could yield data that can be used in simulation models. At present, the data regarding the effects of hydroprene on stored-products insects are limited. However, when such data becomes available, simulation models for stored-product insects (Throne, 1995; Throne et al., 1998, 2000) that include the effects of hydroprene have the potential to help optimize.
hydroprene application and also can act as an evaluation tool for hydroprene applied at different rates and/or different modes of application. Identification of life stages of stored-product pests that are especially vulnerable to hydroprene, and quantification of mortality and/or other effects will help in modeling the overall population dynamics of stored-product insects more precisely. In addition, this will help in insecticide resistance management, because timing of application of hydroprene can be targeted towards a vulnerable life stage, at least in storage facilities where generations of stored-product pests do not overlap.

6. Conclusions

Hydroprene has the potential to affect the population dynamics of several stored-product insect pests and could potentially lead to their eventual eradication in a facility when used appropriately. Lessons can be learned from records of insect resistance to compounds such as methoprene and pyriproxifen. Safer management practices, including rotation with other conventional chemicals and other management options, will help in slowing resistance development by insects to hydroprene. Simulation models to predict the effects of hydroprene on stored-product insect populations can help in making management decisions.

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