17 Biological Control: Insect Pathogens, Parasitoids, and Predators

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Introduction

Stored product insects cause millions of dollars per year in losses in stored wheat. Traditionally, stored-product pest management has relied on chemicals to control insects. In some cases, pest management has been improved by applying chemicals only when needed. An increasing effort is being made to reduce or eliminate pesticide residues in the food supply. This trend has increased since the introduction of new food safety standards required under the Food Quality Protection Act (1996), which includes stored raw commodities.

Several stored product insects, such as the lesser grain borer *Rhyzopertha dominica* (F.), are resistant to chemicals applied to stored grain for insect control. The most damaging insects of stored wheat are the lesser grain borer and the rice weevil *Sitophilus oryzae* (L.). Immature stages of these species develop inside the grain kernels, and it is very difficult to remove infested kernels from the grain. If more than 31 insect-damaged kernels are found per 100 grams of wheat, it is classified as sample grade. Biological control is the application of living organisms to control pests. Pathogens, parasitoids (insect parasites), and predators have been investigated in the context of stored product protection.

Since 1992, the addition of parasitoids and predators to stored raw commodities has been allowed under law (Anonymous 1992). The effectiveness has been studied for only a few of the 468 species of natural enemies of one or more of the 1,663 insect species associated with stored products (Hagstrum and Subramanyam 2009). There are many examples for successful biological control. For 19 species of stored product insect pests attacked by 13 species of natural enemies, 163 out of 212 estimates of pest mortality were between 70% and 100% (Hagstrum and Subramanyam 2006). For 87 of these estimates, insect pest mortality was between 90% and 100%.

Advantages of Biological Control

The use of insect parasitoids and predators to control stored product insect pests has many advantages over traditional chemical controls. These natural enemies leave no harmful chemical residues. When released in a storage facility, they continue to reproduce as long as hosts are available and environmental conditions are suitable. Unlike chemicals that need to be applied to a wide area, natural enemies can be released at a single location. They will actively spread, find, and attack pests located deep inside crevices or within a grain mass.

Parasitoids and predators that attack stored product pests are typically very small. They have a short life cycle and high reproductive capacity. They can easily be removed from bulk grain before milling using normal cleaning procedures. In many ways the stored product environment is favorable for biological control. Environmental conditions are generally favorable for natural enemies, and storage structures prevent these beneficial insects from leaving. It is likely that resistance to biological control agents will develop more slowly, or not at all, because the natural enemies are coevolving with their hosts and will tend to overcome host resistance. Insect pathogens are probably compatible with most beneficial insects and may even be spread by the activities of parasitic insects. The application of pathogens is similar to the use of residue-building insecticides. Usually, they can be stored longer than parasitoids.

Disadvantages of Biological Control

The main disadvantage of biological control is that it requires more information and careful timing compared to traditional chemical insecticides. Many beneficial insects are host-specific, which means that the right complex of parasitoids needs to be released to attack the pest insects in a particular bulk of grain. Timing of the release is also critical. For biological control to be practical, releases have to be made early enough in the pest growth cycle so that adult parasitoids outnumber the pests. If parasitoids are released too late, extremely high numbers of parasitoids will need to be released to control the pests. Unlike fumigants, beneficial insects cannot be used successfully if the manager waits until pest numbers have reached damaging levels.

Designing a biological control program for stored product insect pests requires careful planning. Many natural enemies are host-specific, so it is necessary to determine which pest species are causing the problem before releasing the appropriate parasitoid or predator species. Pathogen strains may differ significantly in their effectiveness, and pest resistance toward pathogens also may occur. A well-designed sampling program should indicate which pest species typically exceed economically damaging levels.

Parasitoids and predators can be stored and refrigerated for a short time — typically one week — and must be obtained directly from the producer as needed. In most states, little expertise and infrastructure exists to supply control agents or support the use of biological control of stored product pests. Seven species of parasitoids and predators are commercially available for stored product protection in the United States (Wilson et al. 1994; White and Johnson 2010).

Application Techniques

In stored product protection, generally the pest organisms have a high intrinsic rate of increase, and the pest population buildup has to be prevented. Inundative releases, using mass reared predators or parasitoids, have been used in the majority of cases. Inundative releases require mass-rearing facilities that can produce high-quality natural enemies. The timing of the releases has to be synchronized with the growth of the pest population. Monitoring with traps can help determine the best time to release mass-reared beneficial insects.

Generally, low numbers of insects initially infest commodities. Parasitoids or predators need to be released early before pests reach high numbers. Inundative releases are most effective when there are more parasitoids released than hosts, such as 2:1. If they are released too early, suitable host stages may not be available. Using wheat as an example, the first release of parasitoid insects should be made after about three weeks of storage (assuming the wheat was put into storage in the summer). Sequential releases can add additional insurance, but each additional release will add to the control cost. Sampling the grain at monthly intervals will indicate whether additional parasitoid releases are necessary. Each parasitoid can attack several host larvae each day (Flinn 1991, Smith 1992). For example, *C. waterstoni* can paralyze up to 14 rusty grain beetle larvae per day, and lay 2 to 3 eggs per day. Decision support software and population models can help to design specific release schedules.

Insect Pathogens

Many insect pathogens, including viruses, bacteria, protozoa, and fungi, infect stored product insects (Brower et al. 1996, Moore et al. 2000). Some of these organisms are highly pathogenic and kill the insect by rapid infection. Others, like the protozoa, adversely affect the development or fertility of the insect.

Bacteria

Dipel is a commercial formulation of the spore-forming bacteria, *Bacillus thuringiensis* (*Bt*). It contains an insecticidal protein that kills the insect either directly or by septicemia (blood poisoning) of the insect gut. It can be applied to grain either as a liquid or dust, as the grain is loaded into the bin. It can also be applied to the grain surface and raked into the grain to a depth of 4 inches. Current strains of *Bt* are only effective against moths and not beetles. Good control was observed in laboratory studies, but moth control was not as consistent in full-sized grain bins (McGaughey 1976). Resistance also has been reported (McGaughey and Beeman 1985).

In most cases, the toxin of *Bt* has little or no side effects on parasitoids. Studying the effect of *Bt*-infected larvae of *E. kuehniella* on the biology of *V. canescens,* Kurstak (1966) found that parasitism was not affected. In addition, *V. canescens* was shown to be a vector for *Bt,* enhancing the spread of the disease in the moth population. Kurstak (1966) and Burkholder (1981) suggested that parasitoids could improve pest control by spreading pathogens.

Viruses

Many viruses have been reported for stored product insect pests. Most of these viruses attack moths, and a few have been reported for beetles. Viruses are generally species-specific. Viruses can only be produced on living hosts or on insect cell cultures. A granulosis virus was found to be effective against the Indianmeal moth. A formulation was patented (Vail et al. 1991) and was registered for control of Indianmeal moth larvae on dried fruit and nuts and for crack and crevice treatments in the United States in 2001. The formulation is not commercially available currently.

Fungi

Several fungi that attack stored product insects have been reported. The most notable is probably *Beauveria bassiana* (Ferron and Robert 1975, Hluchy and Samsinakova 1989). It was previously thought that one of the problems of using fungi for stored product pest control is the requirement of high humidity (greater than 90%) for germination of the infective stage. However, Lord (2005) showed increased mortality of *R. dominica* by *B. bassiana* under dry conditions (45% vs. 75% relative humidity (RH). Currently, there are no fungi registered for use on stored product insects in the United States.

Protozoa

Many species of protozoa naturally infect stored-product insects and often play a major role in regulating population growth. These organisims are usually transmitted orally. In contrast to the often lethal infections caused by viruses and fungi, protozoan infections are often chronic and cause a reduction in fecundity and survivorship. Currently, there are no protozoa registered for control of stored product insect pests in the United States.

Insect Parasitoids and Predators

Insect parasitoids and predators have been used to control pest insects for a long time. In 1911, parasitic wasps were discovered in a flour mill in London, and were reported to greatly suppress the Mediterranean flour moth population. Recently, the Federal Register (Anonymous 1992) published the rule that allows the release of parasitoids and predators into stored grain, stored legumes, and warehouses. The rule makes the use of beneficials subject to regulation by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) and exempt from the requirement of a tolerance in food products. The Food and Drug Administration (FDA) will continue to use its criteria for enforcement of insect fragments in food, and the Federal Grain Inspection Service (FGIS) is still responsible for inspecting and grading the grain.

Parasitoids are released either as adults from small plastic containers or emerge from pupae stuck to cardboard strips placed in the storage rooms. Shipment of the natural enemies has to be quick, and cooling agents have to be added in summer (Casada et al. 2008).

Moths

For the control of stored product moths, ideally an egg parasitoid should be combined with a larval parasitoid or a predator. The larval parasitoid *Habrobracon hebetor* (Figure 1) complements the egg parasitoids *Trichogramma* spp. because one attacks the larvae, the other the eggs (Grieshop et al. 2006). When *H. hebetor* and *Trichogramma pretiosum* were released in small peanut warehouses infested with Indianmeal moths and almond moths, *Cadra cautella* (Brower and Press 1990), Indianmeal moths were reduced by 37.3% by *T. pretiosum* alone, 66.1% by *H. hebetor* alone, and 84.3% by the combination. Insect feeding damage to the peanuts was reduced to less than 0.4% by the two parasitoids, compared to 15.8% in the untreated checks.

In the United States three different species of *Trichogramma* were evaluated for their potential to suppress *P. interpunctella* in a simulated retail environment (Grieshop et al. 2007). Percentage parasitism of eggs was four times greater for *T. deion* than for *T. ostriniae* or *T. pretiosum*. A central release point for *T. deion* in the shelving units provided the best protection.

In Central Europe, stored product moths are among the most important pests in stored grain, in the retail trade, mills, the food processing industry, and private households. Since 1995, parasitoids were evaluated in Germany in private households and in commercial food-processing facilities. The most important moth species were the Indianmeal moth, *P. interpunctella*, the Mediterranean flour moth, *E. kuehniella*, the warehouse moth, *E. elutella,* and the almond moth, *C. cautella*.

Trichogramma evanescens has been released in facilities ranging from private households to industrial bakeries and the wholesale trade, and combined with *H. hebetor* mostly in commercial facilities. The parasitoids are sold in units of 3,000 *T. evanescens* and 25 *H. hebetor*. The egg parasitoids emerge from the release cards for three weeks. For *T. evanescens*, the host eggs are sterilized before parasitization to prevent the emergence of stored product moths' larvae from unparasitized eggs in the storage environment.

For private households, releasing *T. evanescens* for 9 weeks is recommended. Three *Trichogramma*-cards have to be used per release point during this time. The number of *Trichogramma*-cards required depends on the surface area of the packages that contain products susceptible to attack by the moths. Generally, two *Trichogramma*-cards are necessary for a food cupboard.

A list of studies evaluating the application of parasitoids and predators attacking the Indianmeal moth is listed in Table 1.

Beetles

Beetles cause more damage than moths to stored grain. Although there are several beetle species that attack grain, there are only five species that are the major culprits (lesser grain borer, rusty grain beetle, red flour beetle, rice weevil, and sawtoothed grain beetle). Parasitic wasps that attack stored grain beetles tend to be host specific, but there are several species that will attack more than one beetle species. For example, *Theocolax elegans* (Figure 1) will attack all of the stored grain weevils and the lesser grain borer. This is also true of the parasitic wasps *Anisopteromalus calandre* (Figure 1) and *Lariophagus distinguendus* (Förster). Other wasps — such as *Cephalonomia waterstoni*, which attacks the rusty grain beetle — only attack a single species. These parasitoids are typically small (1 to 2 mm), and do not feed on the grain. They will normally die within 5 to 10 days if no beetles are present in the grain. These parasitoids are found naturally in the grain, which suggests that after they are released they may continue to suppress pests for many years (Arbogast and Mullen 1990). Because the adult wasps are external to the grain, they can be easily removed using normal grain-cleaning processes. Table 2 shows a list of studies evaluating the application of parasitoids that attack stored product beetles.

Anisopteromalus calandrae has been studied for biocontrol (Wen and Brower 1994a, Smith 1992). In simulated warehouse rooms that contained wheat debris with rice weevils, release of 30 to 50 pairs of

Table 1. Studies on biological control of the Indianmeal moth *Plodia interpunctella*.

Antagonist	Effect	Product	Scale	Reference
Predator *Xylocoris flavipes*	71.4% reduction	Peanuts	Semi-field	Brower and Mullen 1990
Parasitoids *Habrobracon hebetor*	74% reduction of adult moths	Grain	Lab	Press et al. 1974
Trichogramma evanescens	80% reduction of trap captures	Bakery	Field	Prozell and Schöller 1998
Trichogramma evanescens *Habrobracon hebetor* and combinations	37.3% reduction in infestation 66.1% reduction in infestation 84.3% reduction in infestation	In-shell peanuts	Semi-field	Brower and Press 1990 Grieshop, et al. 2006

Table 2. *Studies on biological control of the maize weevil, Sitophilus zeamais, rice weevil, Sitophilus oryzae, rusty grain beetle, Cryptolestes ferrugineus, and the lesser grain borer, Rhyzopertha dominica.*

Species	Antagonist	Effect	Product	Scale	Reference
Maize weevil	Parasitoids *Anisopteromalus calandrae*	For long storage periods multiple releases necessary to suppress build up of weevil population	Maize	Field	Arbogast and Mullen 1990
	Theocolax elegans	Both single and multiple releases suppressed weevil population over 90%.	Maize	Field	Wen and Brower 1994a
		At a parasitoid:host ratio of 8:1 pest population growth was reduced by 50% (semi-field) and 25% (lab)	Maize	Lab. + Semi-field	Williams and Floyd 1971
Rice weevil	Parasitoid *Anisopteromalus calandrae*	Controlled weevils >99% for 4 months	Wheat residues bagged	Semi-field	Press and Mullen 1992 Cline et al. 1985
Lesser grain borer	Parasitoids *Theocolax elegans*	Reduced populations in bins by 98%	Wheat	Field	Flinn et al. 1996
		Reduced number of insect damaged kernels by 92%, and insect fragments in flour by 89%	Grain	Field	Flinn and Hagstrum 2001
	Anisopteromalus calandrae	Parasitization rate highest at 30°C and lowest at 20°C. 69.5% parasitism at 26°C at a host parasitoid ratio of 10:1	Grain	Lab	Ahmed 1996
Rusty grain beetle	Parasitoid *Cephalonomia waterstoni*	Reduced population in bins by 50%	Wheat	Field	Flinn et al. 1996

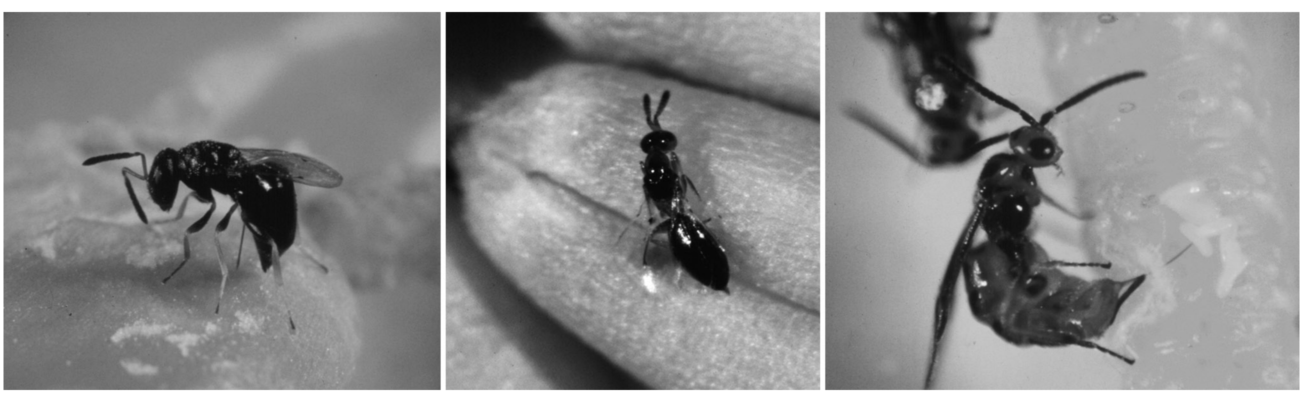

Figure 1. *Anisopteromalus calandrae, Theocolax elegans, and Habrobracon hebetor, left middle and right, respectively.*

A. calandrae reduced the weevil population by more than 90%, and release of only five pairs reduced the pest population by about 50% (Press et al. 1984). In a similar test with larger quantities of infested grain (18 pounds) and grain in small fabric bags, *A. calandrae* significantly suppressed the weevil population (Cline et al. 1985). Suppression of the rice weevil was 76% in the loose grain, and uninfested grain in fabric bags was almost completely protected. *Lariophagus distinguendus* has been shown to disperse at least 4 m horizontally and vertically in bulk grain (Steidle and Schöller 2001).

There are two species of parasitoid wasps that attack the maize weevil and lesser grain borer (*A. calandrae* and *Theocolax elegans*). These same species will also attack the granary weevil and rice weevil. Because these two species attack the same host stages (fourth instar and early pupa), it probably is not advantageous to release both species. There is only one species of wasp, *Cephalonomia waterstoni*, that attacks the rusty grain beetle. This species is host-specific and is able to use chemical odors from the cuticle of rusty grain beetle larvae to locate their hosts.

Commercial Tests

A study by Flinn et al. (1996) showed that releasing parasitoid wasps into bins of stored wheat reduced populations of the lesser grain borer by more than 95%. Data from this study (Flinn and Hagstrum 2001) also indicates that insect fragments were greatly reduced in grain treated with parasitoid wasps. Most insect fragments in flour probably come from beetle larvae that are developing within the grain kernels. There is also potential for using biological control in the food processing industry in the United States. Moths and beetles cause millions of dollars of losses annually in packaged products. There are several species of parasitic wasps that attack all of the common stored product insect pests. Parasitoid wasps could be released to prevent serious outbreaks. However, releasing live insects into areas where food is prepared for final packaging would probably not be prudent. This is an area in which more research is needed in the United States.

For moth control, industrial applications have to be performed by specialized pest control personnel, because the period of treatment and the timing of the releases as well as the species of parasitoid depend on several factors, including the moth species. Hygiene measures at critical points in the plant have to be combined with the parasitoid release, and the compatibility of other nonbiological control measures has to be checked. For the retail trade in Germany, mainly *Trichogramma evanescens* has been released. In milling areas, bag stacks, and bulk storage, *Habrobracon hebetor* was also used. Again, the number of parasitoids to be released depends on the surface area of the commodities. In addition, data from pheromone-trap catches are used to detect moths. In Germany, *Trichogramma evanescens* were released in grocery stores to protect packaged food from infestation by moths. The moths lay eggs on the outside of packages, where they are susceptible to parasitoid attack. The moth infestation can occur at any step in the production chain. Some products are already infested when they enter grocery stores. Retailers evaluated the the biological control program's success based on the number of customer complaints due to moth infestation and the number of infested packages in the stores.

Usually, the number of parasitoids is high, but the biomass is not. For example, in a factory producing 1.5 tons of bread and breakfast cereals per year, 3 million parasitoids were released per year, with a cumulative dry weight of 6g (Prozell and Schöller 1998). In Germany, parasitoids of stored product pests have been available commercially since 1998. In Germany, Austria, and Switzerland approximately 900 million *T. evanescens* were sold to control stored product moths in 2010. The demand for *Trichogramma* can be expected almost year-round because some populations of stored product moths do not enter diapause. The species of greatest economic importance, *P. interpunctella*, enters diapause, usually from November to April.

Predators

Insect predators are different from insect parasitoids in a number of ways. A predator requires many prey during development; a parasitoid completes development on only one host. Predators also tend to be less host-specific than parasitoids. There are probably many species of predators that attack stored product insects, but most of them remain unstudied, with the exception of the warehouse pirate bug, *Xylocoris flavipes*.

Warehouse Pirate Bug – The warehouse pirate bug will attack most immature stages of beetles and moths (Jay et al. 1968). The smaller species of beetles appear to be the preferred prey, but eggs and early larvae of most species are utilized as well. The internal grain feeders, such as the weevils and lesser grain borer, are not attacked because they are protected inside the grain kernel.

Red flour beetles were suppressed by warehouse pirate bugs in a simulated warehouse (Press et al. 1975). LeCato et al. (1977) showed that populations of the almond moth and of two beetle species did not increase in a room containing grain debris when warehouse pirate bugs were released in small numbers. All three pest populations increased greatly in the room when no predators were released. Brower and Mullen (1990) released large numbers of the warehouse pirate bug into small peanut warehouses infested with almond moths and Indianmeal moths. Moth populations were suppressed 70% to 80% during the fall storage season, and no moths were present in the biocontrol treatments during the spring.

Integration

There are many integrated pest management (IPM) examples in the literature where the combination of biological and nonbiological control methods is possible. The most promising are sanitation, modified atmospheres, modification of the storage environment (temperature), and the combination of certain species of beneficial insects and some natural insecticides. On the other hand, there are at least as many examples where integration is detrimental. Insecticidal protectants will probably not be compatible with parasitoids because beneficial insects are typically more susceptible to insecticides than their hosts. In some cases, however, parasitoids may be more resistant than their hosts (Baker and Weaver 1993). Protectants are applied at binning, which precludes releasing parasitoids at this time, and they typically last for several months. In stored grain, parasitoids could be released after the protectant had degraded to a low level. In temperate and continental climates, fall aeration would probably work as well or better to suppress pest insect population growth. Releases could be made after fumigation, if sufficient time was allowed for the fumigant to dissipate (1 to 2 weeks). Many species of parasitoids and predators are able to overwinter in the grain (Hansen and Skovgård 2010), and thus, would provide additional protection when the grain warms in the spring. This protection may carry through the marketing system.

Because natural enemies were shown to be most effective at low pest densities (Smith 1994, Zdárková 1996), the development of proper sanitation programs is a prerequisite for the application of beneficial insects. Environmental conditions should also be controlled or altered to promote growth of the beneficial insects (Haines 1984). Figure 2 shows the life cycles for two stored product insect pests and their parasitoids (sawtoothed grain beetle/*C. tarsalis* and Indianmeal moth/*H. hebetor*). In both

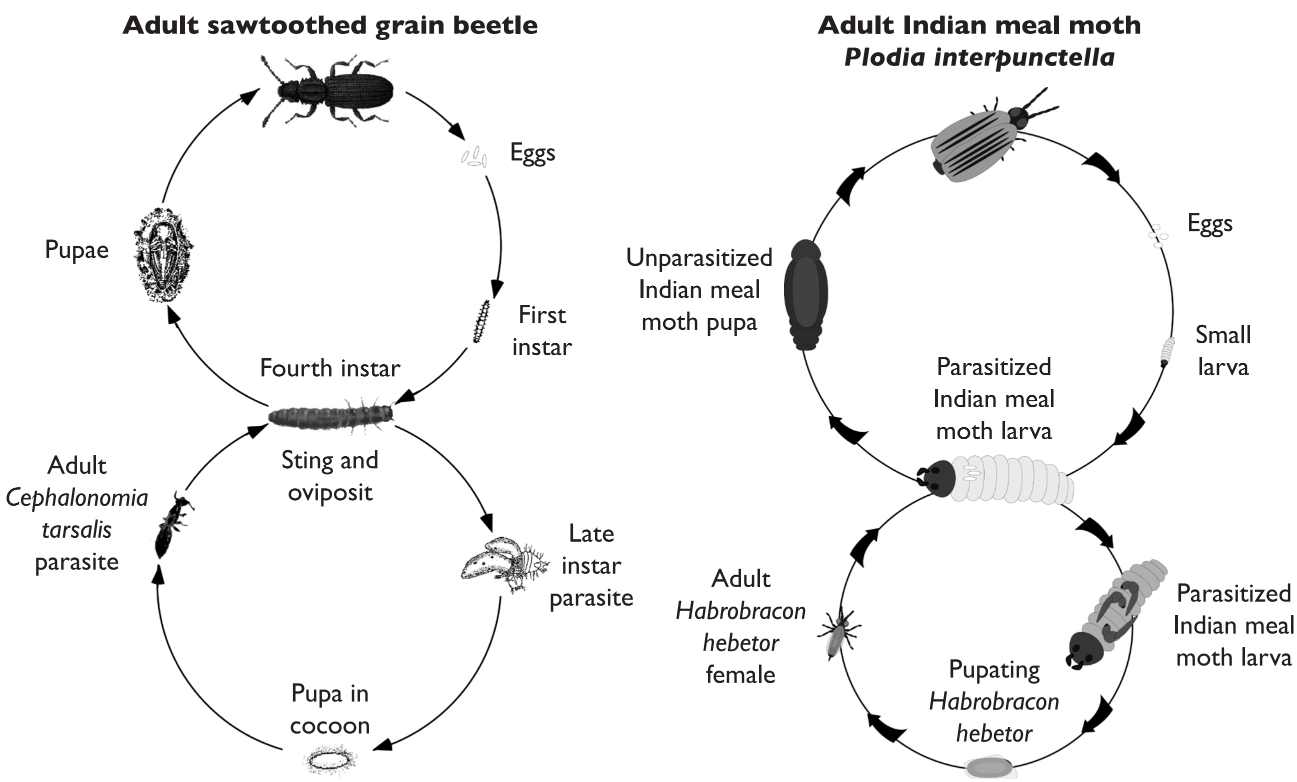

Figure 2. *Life cycle of Cephalonomia tarsalis parasitizing the sawtoothed grain beetle (left) and Habrobracon hebetor parasitizing the Indianmeal moth (right).*

of these parasitoid species, the female wasp attacks the larval stage just before pupation. At 30°C, it takes about 15 days for the wasps to complete their life cycle. At 25°C, it would take almost 30 days to complete.

An example of a perfectly compatible physical control method for biological control in wheat is cooling the grain with aeration. Parasitoids should be released in the grain about three weeks after binning. Aeration would start immediately using automatic aeration controllers. Aeration, using electric powered fans, can be used to cool the grain earlier; thus, it suppresses insect population growth sooner in the storage period (Flinn et al. 1997). In the United States, this would cool the wheat from an average of 32°C down to 25°C. The parasitoids would inhibit beetle populations from exceeding economically damaging levels during the warm summer months, until further aeration could be used to cool the grain below 15°C, which would completely inhibit insect population growth. Flinn (1998) conducted studies to assess the effectiveness of *T. elegans* for controlling the lesser grain borer in wheat at 32°C and 25°C. The two temperature regimes were used to simulate an unaerated bin of wheat and a bin with automatic aeration control starting at harvest. Suppression of the lesser grain borer population growth by *T. elegans* was 10 times greater at 25°C than at 32°C. This resulted in a very high level of population suppression; 99% in the cooled grain compared to only 50% in the warm grain.

Economics of Biological Control

In Germany, the cost for a treatment with *T. evanescens* in households is usually $19.75. In the United States, at least six suppliers have sold *H. hebetor* (Wilson et al. 1994). One release unit containing 50 adults sold for $6.50. In Germany, parasitoids could be released in 3,000 ton grain storage infested with *E. elutella* for $0.14/ton to $0.57, depending on the level of infestation (Schöller 2000). The costs of biological control for bulk-stored grain may be slightly higher than that for traditional chemical controls. Chemical protectants cost about $0.02 per bushel and biological control using predators and parasitoids is about $0.04 bushel (M. Maedgen, Biofac Inc., personal communication). The application of *Lariophagus distinguendus* to prevent infestation of *Sitophilus* sp. and *Rhyzopertha dominica* in bulk grain costs (2010) $0.93/ton in Germany (Schöller, unpublished). Currently in the United States, predators and parasitoids are not commonly used to control insect pests in stored grain. Although parasitoids and predators of stored product insects have been marketed in the United States, there are only a few companies that rear parasitoid species that specifically attack stored grain beetles. Probably the majority of the early adopters of stored product biological control are in the organic foods business. As more grain managers decide to try biological control, the number of companies offering beneficial insects for stored products will probably increase.

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