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Profile

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Biology and Management of Lesser Mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in Broiler Houses

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Abstract

Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae), the lesser mealworm, is a ubiquitous generalist pest of poultry broiler and layer facilities, originating in southern Africa and now found worldwide. They spend their full life cycle within the litter and manure of poultry, causing structural damage to poultry houses, injuring birds, and acting as a reservoir for several avian pathogens, notably *Salmonella* spp. and *Escherichia coli*. Management commonly consists of spraying walls and floors of poultry houses with organophosphates, pyrethroids, neonicotinoids, or spinosyns between flocks, and periodic removal and replacement of litter. Populations have been observed to become resistant to specific insecticides after ca. 10 yr of consistent use and exhibit cross resistance to insecticides of the same mode of action. Alternative cultural and biological control methods have been identified but are not currently implemented. More research is needed regarding the economic impact of *A. diaperinus*, the mechanisms of its insecticide resistance, and patterns and mechanisms of colonization for effective integrated pest management programs to be devised and implemented.

Key words: avian pathogen, chlorpyrifos, darkling beetle, insecticide resistance, poultry

Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae: Alphitobiini) is a pest of stored grains and the poultry industry (Aalbu et al. 2002). *Alphitobius diaperinus* is believed to have originated in sub-Saharan Africa, but now occurs worldwide (Hopkins 1990). The beetle has been reported in Algeria, Argentina, Australia, Brazil, China, Denmark, France, Greece, India, Pakistan, Poland, and the United States, but likely occurs over an even broader distribution (Arshad et al. 1984, Geden et al. 1987, Bhattacharyya 1995, Salin et al. 2000, Bates et al. 2004, Skov et al. 2004, Lambkin et al. 2007, Amir and Nadir 2009, Hassemer et al. 2016, Li et al. 2016, Szczepanik et al. 2018, Arena et al. 2020, Dzik et al. 2022). Outside of anthropogenic environments *A. diaperinus* is a bird nest symbiont, feeding on scraps and detritus in the nests of a wide variety of species including pigeons and sparrows (Arshad et al. 1984, Bhattacharyya 1995). They have also been observed to have a similar life history feeding on detritus and guano among populations of bats in caves (McFarlane 1971). *Alphitobius diaperinus* is a highly resilient organism that persists despite management techniques and is a known reservoir of several avian diseases. In intensive broiler production their populations can reach staggering levels, with a single house hosting an estimated 34.7 million adults, pupae, and larvae in the litter (Singh 2011). *Alphitobius diaperinus* has been identified for use as feed in aquaculture, and is beginning to be cultivated in several European countries for this purpose (Rumbos et al. 2019). However, this profile will focus on *A. diaperinus* as a pest of the poultry industry, and will examine its life history, impacts in stored grain and poultry facilities, and current and potential controls and management techniques.

Life History

Alphitobius diaperinus has 8–11 larval instars (Fig. 1), with development time highly dependent on environmental temperature, from 10 d between instars at 20°C to 2 d between instars at 30°C (Wilson and Miner 1969). *Alphitobius diaperinus* larvae are creamy white in their early instars, later darkening to a yellow-brown hue (Figs. 2–4). They have segmented bodies measuring up to 11 mm in length during their last larval instar (Fig. 5), with three pairs of legs (Dunford and Kaufman 2006). Before pupation larvae seek isolation from other larvae and adults, dispersing and often finding substrate to burrow into. *Alphitobius diaperinus* pupae are exarate and resemble the adults, measuring 6 to 8 mm and are creamy white to tan colored (Dunford and Kaufman 2006) (Fig. 6). Adults are roughly oval in shape, measuring between 5.8 and 6.3 mm, with brown to black shiny exoskeletons (Fig. 7). The head is deeply emarginated and is often largely hidden by the pronotum. The pronotum is

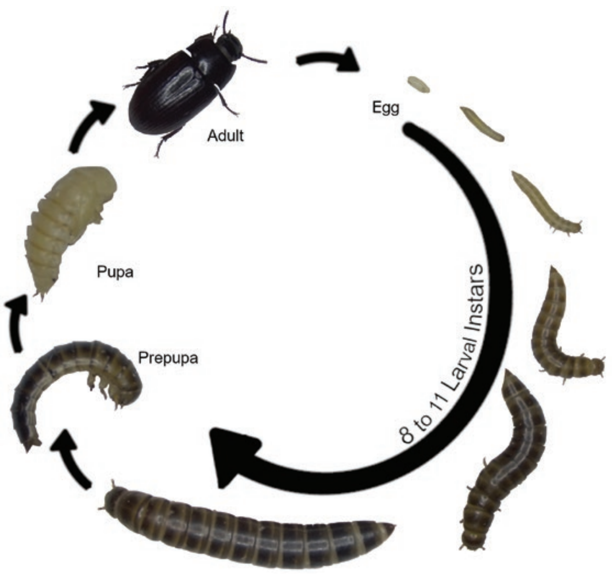

Fig. 1. Life cycle diagram of *A. diaperinus*.

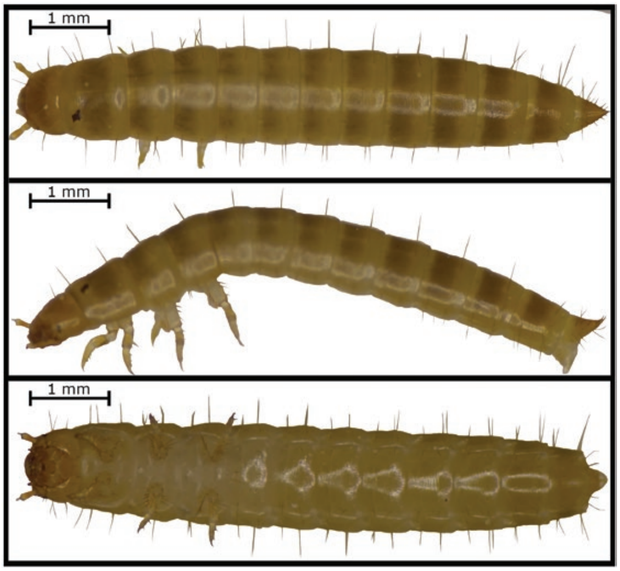

Fig. 3. Dorsal, lateral, and ventral views of an early instar *A. diaperinus* larva.

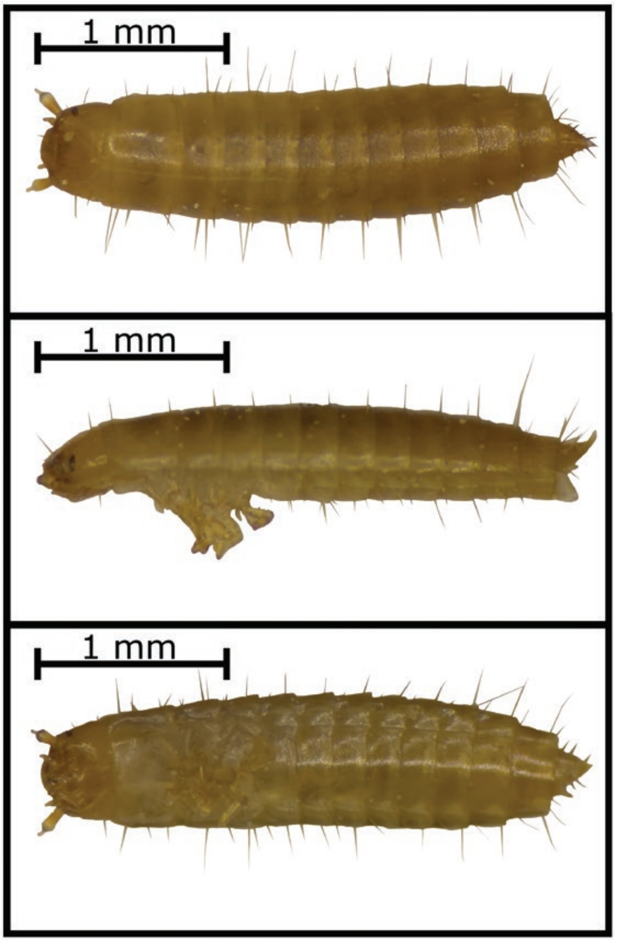

Fig. 2. Dorsal, lateral, and ventral views of a second instar *A. diaperinus* larva.

Fig. 4. Dorsal, lateral, and ventral views of an intermediate instar *A, diaperinus* larva.

roughly twice as wide as it is long and textured with minute pits or punctures. The elytra completely cover the abdomen, are striated, and can open to allow for flight (Dunford and Kaufman 2006). The full life cycle from oviposition to adult eclosion takes between 34 and 38 d at the species' optimal temperature of 30°C (Rueda and Axtell 1996). Below 30°C development slows, to a maximum oviposition to adult eclosion time of 165 d when at 20°C, with growth and development ceasing completely below this temperature (Rueda and Axtell 1996). Adults will mate as soon as exoskeleton tanning is complete, generally 5-8 days following eclosion (Hopkins et al. 1992). An adult female will live between four months and a year, laying eggs periodically throughout her adult life at an average rate of 3.5 eggs per day, laid singly on or within loose substrate, generally reaching a lifetime total of between 200 and 400 eggs, though individuals in laboratory settings have been observed to lay over 2,000 eggs in their lifetime (Hopkins et al. 1992, Rueda and Axtell 1996).

Alphitobius diaperinus adults and larvae are pests of stored grain products and chicken rearing facilities, feeding on different materials in each setting. In stored grains *A. diaperinus* is a secondary pest on cereal grains, including wheat, barley, and rye, as well as associated products such as flours, bran, and hay (Rumbos et al. 2020).

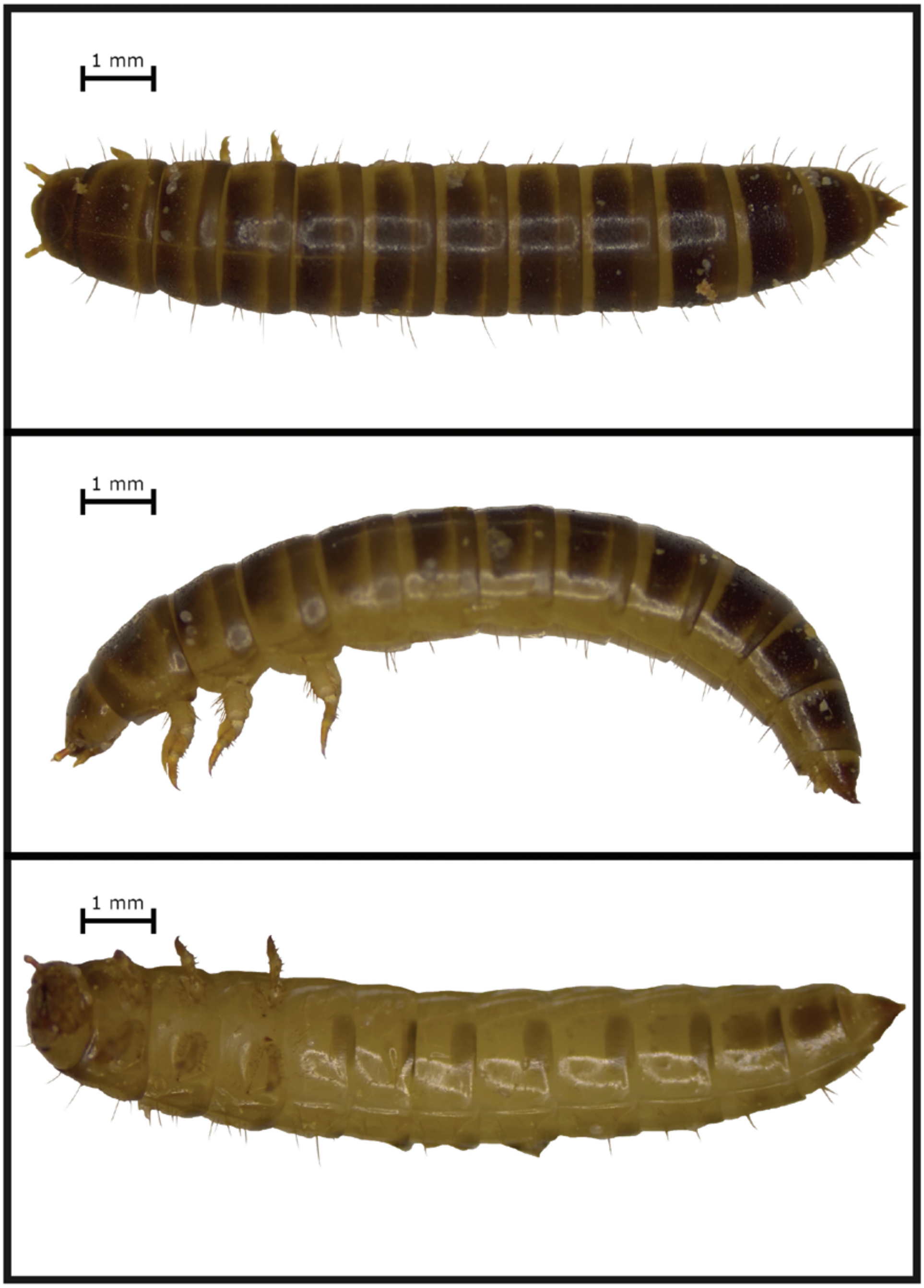

Fig. 5. Dorsal, lateral, and ventral views of a late instar *A. diaperinus* larva.

In broiler houses and caged layer facilities *A. diaperinus* lives in the litter and manure of the house, feeding on a combination of chicken manure and spilt feed (Dunford and Kaufman 2006). *Alphtiobius diaperinus* is a somewhat aggressive feeder, noted to quickly turn to cannibalism, or even to collectively attack and kill small vertebrates such as snakes when preferred food sources are unavailable (Crook et al. 1980). *Alphitobius diaperinus* produces a volatile alarm pheromone, and adult males produce an aggregation pheromone attractive to both sexes composed of limonene, (E)-β-ocimene, 2-nonanone, linalool, and daucene (Bartelt et al. 2009, Hassemer et al. 2019). Populations in Brazilian poultry facilities exhibit a sixth component to the pheromone, (E,E)-α-farnesene, indicating that there may be variation in this pheromone between broad geographic areas, though this has not been investigated in other regions (Hassemer et al. 2019).

Little is known about the movement and behavior of *A. diaperinus* outside of their preferred habitats. When litter and manure are removed and scattered across a field or buried in soil as an organic fertilizer, the beetles move upwards, out of the soil and disperse from the location, possibly in search of suitable habitat (Calibeo-Hayes et al. 2005, Kaufman et al. 2005b). This behavior has led to *A. diaperinus* being considered a nuisance pest, as dispersing beetles have been reported making their way into residential areas in large numbers, prompting complaints and possible litigation from homeowners aimed at chicken farms (Hinchey 1997). Within manure piles of layer houses or litter of broiler houses *A. diaperinus* displays a

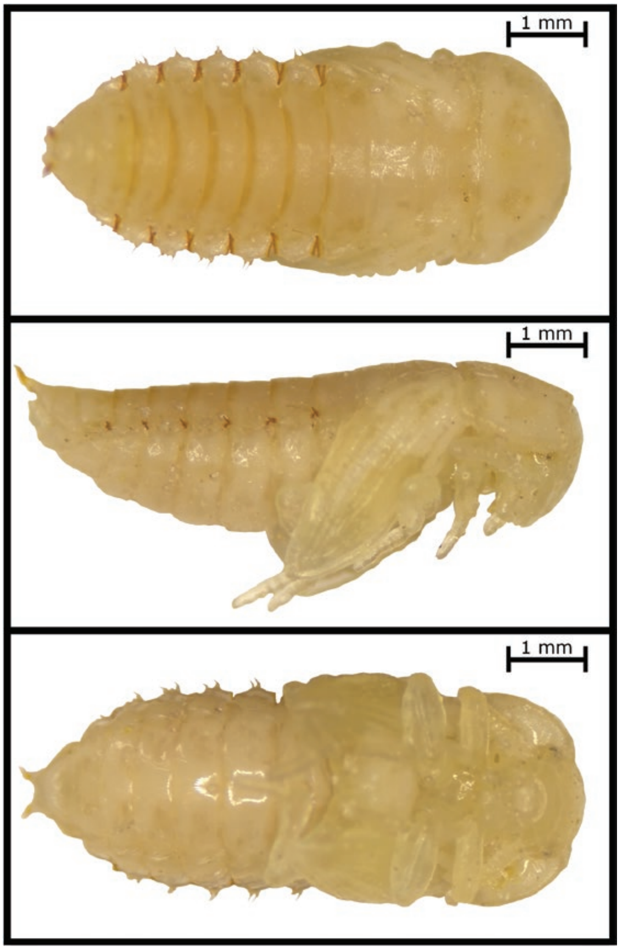

Fig. 6. Dorsal, lateral, and ventral views of an *A. diaperinus* pupa.

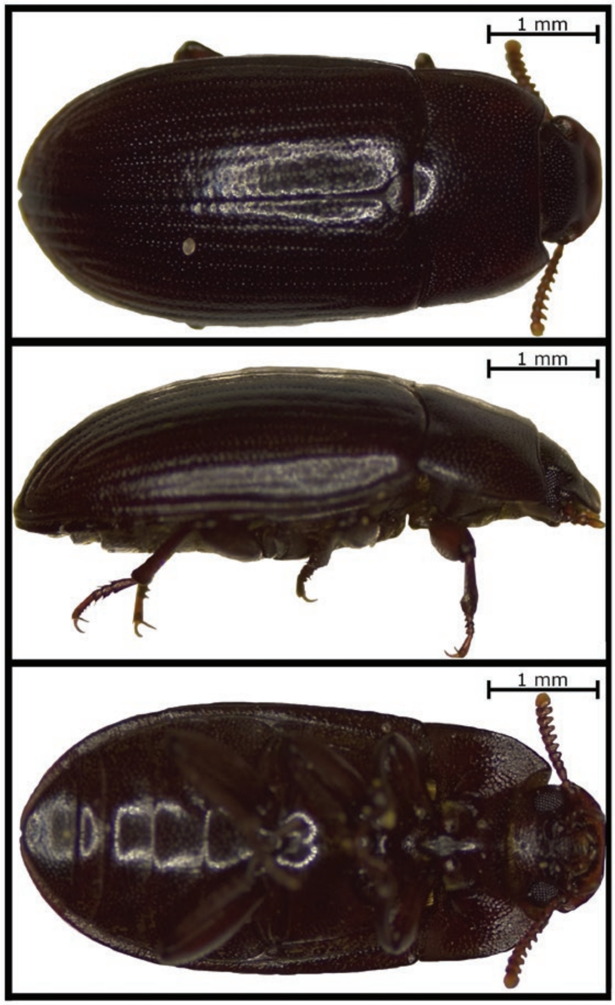

Fig. 7. Dorsal, lateral, and ventral views an *A. diaperinus* adult.

tendency to aggregate along the walls of the facility and beneath feeder and drinker lines (Lambkin et al. 2007, Amir and Nadir 2009). These locations are thought to be preferred by the beetle as sources of shelter from predation by poultry, as experimental shelters placed on the litter surface also result in beetle aggregation (Lambkin et al. 2008). Within the litter substrate *A. diaperinus* displays vertical movement, burrowing deeper into substrate when unsheltered by other material and when ambient temperature decreases (Salin et al. 2000). Both adults and larvae display similar patterns of movement, with the exception of the climbing and boring behavior of pre-pupal larvae (Salin et al. 2000, Dunford and Kaufman 2006).

Pest Impacts

Alphitobius diaperinus is near omnipresent in intensive chicken rearing facilities. At low population levels their impacts are considered negligible, but their high fecundity means that an uncontrolled population can quickly grow large enough to cause significant damage to birds and structures. Of major concern in broiler houses is the capacity for *A. diaperinus* to act as a reservoir for a variety of avian diseases, including *Salmonella typhimurium, Escherichia coli,* avian leucosis, Marek's disease, and Turkey Coronavirus (Eidson et al. 1966, de las Casas et al. 1968, Goodwin and Waltman 1996). *Alphitobius diaperinus* is especially effective as a reservoir in broiler houses because they live within the litter and manure on which the chickens spend their entire lives, consuming the feces of the chickens and in turn being consumed by the chickens. Beetles with pathogens on their exoskeleton or within their guts can transmit those pathogens to chickens when ingested, especially to young chicks, which have been documented to consume between 300 and 500 beetle larvae in a single day (Despins and Axtell 1995, Skov et al. 2004, Hazeleger et al. 2008, Roche et al. 2009). Microbial communities can persist in the gut of *A. diaperinus* for 5 wk or more when removed from contaminated environments, potentially allowing them to vector pathogens between flocks (de las Casas et al. 1968, Crippen et al. 2022).

In cases of extreme infestation *A. diaperinus* has been known to burrow into and partially feed on vertebrates, including dead and moribund chicks (Crook et al. 1980). Furthermore, chicks feeding on *A. diaperinus* are incapable of properly digesting the insect's exoskeleton, resulting in watery feces containing masses of undigested beetles and larvae. Chicks feeding on *A. diaperinus* consume less feed, which together with the digestive stress of consuming darkling beetles results in decreased growth and weight gain (Despins and Axtell 1995). These losses in weight and impacts to health remain even if chicks later switch to an all-feed diet, resulting in delayed growth compared to chicks fed exclusively starter feed their entire lives (Despins and Axtell 1995). However, turkey poults of a similar age can seemingly be fed *A. diaperinus* larvae without significant impact to growth and feed efficiency (Despins and Axtell 1994). The reduced growth and feed efficiency of broilers feeding on darkling beetles lead to more feed being required to bring a flock to salable weight, resulting in estimated losses of between US$2,649.90 and US$4,262.90 per 100,000 birds, with exact numbers varying based on the price of feed being used (Karen et al. 2018).

Extensive infestation can also lead to damage to insulation materials due to the burrowing of larvae seeking pupation sites, the initial tunneling exacerbated by the wider adults leaving following emergence (Fairchild et al. 2005). This damage can greatly reduce the effectiveness of foam board insulation and compromise the carefully controlled airflow in and out of the house, forcing heating and venting mechanisms to be more active and increasing costs for the grower. However, feeding damage done to fiberglass insulation by *A. diaperinus* tends to be less problematic due to the presence of plywood or a thick vapor barrier covering the fiberglass which tends to maintain a houses tightness and therefore the effectiveness of the houses environmental control system (M. Czarick, personal communication). *Alphitobius diaperinus* also occurs in caged layer houses in manure piles accumulating below the hens. In these houses the beetle is a structural pest, with prepupal larvae climbing and boring into wooden support beams and pupating inside them, degrading the beam as they do insulation and over time threatening the structural integrity of the house (Kaufman et al. 2005a). In both layer and broiler houses beetle activity results in reduced efficacy of house structures and eventually a need for replacement of structural components. Ultimately, because of its pervasive presence in poultry production, it is difficult to exactly quantify the economic impacts of *A. diaperinus*. Parsing losses of birds, feed, and airflow, and temperature regulation due to beetle activity from losses due to other factors is a worthy goal that would nonetheless require an intensive and far-reaching study.

Monitoring

Infestations of *A. diaperinus* are all but impossible to fully remove from a broiler house, and the benefits of complete eradication are not worth the cost, especially when the vast majority of issues arising from infestations are the result of massive populations and become negligible with smaller infestations. Populations of *A. diaperinus* are rarely intensively monitored by farmers, but when they are checked, the method generally involves setting traps. The most effective traps for the collection of *A. diaperinus* use corrugated cardboard as a substrate for beetles to climb and seek shelter within (Safrit and Axtell 1984). The Arends tube trap, consisting of sections of cardboard rolled up and placed within short lengths of PVC pipe, is a commonly used design that captures large numbers of beetles while still being simple to place and retrieve. Sampling efforts for *A. diaperinus* typically consist of traps being distributed across the litter of the house and left for 1 wk, after which they are collected and frozen to kill the captured beetles, which are then removed and counted. Due to the general lack of monitoring practice, control methods are applied identically over time, with management changing only when issues begin to occur. A recent improvement to monitoring practice involves deployment of the synthetic aggregation pheromone in conjunction with pitfall traps, increasing their efficacy (Hassemer et al. 2019). With more precise monitoring techniques, more targeted or adjusted controls could be implemented, potentially saving growers control expenditure.

Chemical Management

Treatment for *A. diaperinus* is typically conducted proactively, seeking to keep populations low instead of responding to problematic population levels. Frequency of treatment varies among growers, up to treating after every flock, but most will conduct treatment once every 3–4 flock cycles. Chemical treatment for *A. diaperinus* consists of spraying of litter, floors, and walls with insecticides between flock cycles, when no chickens are present in the house. A variety of insecticides are available for use: pyrethroids (bifenthrin, cyfluthrin, β-cyfluthrin, γ-cyhalothrin, cypermethrin, permethrin), organophosphates (fenitrothion, tetrachlorvinphos), neonicotinoids (acetamiprid, imidacloprid), insect growth regulators (methoxyfenozide, pyriproxyfen, teflubenzuron), and spinosyns (spinosad) (Lambkin 2005, Lambkin et al. 2007, Kozaki et al. 2008, Tomberlin et al. 2008, Boozer 2011, Lambkin and Furlong 2011, Zorzetti et al. 2015, Hickmann et al. 2018, Renault and Colinet 2021). The organophosphate chlorpyrifos was often used, but as of February 2022, it is no longer registered for use in poultry in the U.S. (EPA 2021). Organic farms restricted from the use of synthetic pesticides will use boric acid instead (G. Cartanza, personal communication). *Alphitobius diaperinus* has been shown to develop resistance to most types of insecticide after prolonged use (Lambkin 2005; Hamm et al. 2006; Lambkin and Rice 2006; Lambkin and Rice 2007; Kaufman et al. 2008; Steelman 2008; Tomberlin et al. 2008; Lambkin et al. 2010; Lambkin and Furlong 2011, 2014; Hickmann et al. 2018; Renault and Colinet 2021).

Alphitobius diaperinus has also been found to develop cross resistance to insecticides exhibiting the same mode of action. For example, populations in commercial broiler houses in Australia that had evolved field resistance to cyfluthrin also exhibited resistance to γ-cyhalothrin, despite never being exposed to the latter chemical before testing (Lambkin et al. 2010). Populations have been shown to become highly resistant to fenitrothion after 10 to 20 yr of consistent use (Lambkin 2005). *Alphitobius diaperinus* has not yet exhibited cross-resistance between different chemical modes of action, such as pyrethroids and spinosad (Lambkin et al. 2007, Singh and Johnson 2015). Rotating modes of action could therefore be expected to delay resistance and maintain control of beetle populations. The mechanisms of resistance to various insecticides are not fully understood. Metabolic mechanisms appear to play at least a partial role in pyrethroid resistance, but are not the only factor, evidenced by incomplete sensitivity restored to resistant beetles following addition of the enzymatic inhibitor piperonyl butoxide (Lambkin and Furlong 2011). Analysis of acetylcholinesterase genes in tetrachlorvinphos-resistant and susceptible populations of *A. diaperinus* showed that point mutations in the gene are insufficient to explain resistance (Kozaki et al. 2008). Issues of insecticide resistance may be exacerbated by lack of monitoring practice, as lack of insecticide efficacy may go unnoticed until visible issues occur. Some control methods, such as insect growth regulators, go underutilized despite demonstrated efficacy, likely due to the delayed effect on beetle mortality resulting in a perception that these chemicals are less effective (Zorzetti et al. 2015). Combinations of insecticides may have the potential for synergistic effects that increase effectiveness. For example, spinosad has been shown to increase sensitivity to cyfluthrin and other pyrethroids (Lambkin and Furlong 2014). Alternative methods of insecticide application, such as applying insecticide to the materials of house walls, may provide effective control while using less material, reducing management costs (Kaufman et al. 2008).

Alternative chemical control methods to synthetic insecticides include application of diatomaceous earth and plant essential oils. Diatomaceous earth can decrease population growth of *A. diaperinus* compared to untreated houses but does not display the lethality or population reduction of synthetic insecticides and may be more suited to maintaining control of already low populations (Oliveira et al. 2017). Select essential oils such as lemon and citronella have been shown to repel (but not directly kill) *A. diaperinus* and could be paired with trapping methods that utilize a push-pull system (Francikowski et al. 2019). The options available to control *A. diaperinus* are limited and as resistance to synthetic insecticides grows more common, more diverse and effective methods are becoming necessary.

Cultural Management

Removal and replacement of litter is one of the most effective means of controlling *A. diaperinus* populations in broiler houses. Changing the litter removes the bulk of the population, including larvae, adults, and eggs deposited in the litter. However, frequent litter removal (e.g., after each flock cycle) is expensive, both in material and labor, and is not considered financially viable by some growers. The practice of windrowing, piling the litter in rows to allow for fermentation and heating of manure for sanitation purposes, has an additional benefit of causing beetles to vacate the manure, reducing the resident population in the litter between flocks (Barker et al. 2013). However, this reduction is less than that of fully removing and replacing litter, as a portion of the population may take refuge by burrowing or hiding in crevices. Manipulation of house and litter temperature between flocks can be a means of reducing the population directly. Warming litter to 45°C or hotter successfully kills beetles and can be used in conjunction with insecticides and litter drying for greater effect (Wolf et al. 2015). Exposing the interior of the house to winter temperatures by opening doors between flocks, lowering the temperature below 20°C, can help reduce beetle population sizes as well (Singh 2011). When litter is spread in fields, mechanical incorporation of manure into soil via plowing can decrease beetle re-emergence by over 50%, though manure spreading is generally not performed with cognizance of darkling beetle presence (Calibeo-Hayes et al. 2005).

Control is more difficult while a flock is present in the house, as the use of insecticides and the manipulation of temperature and litter are more restricted. During a flock cycle proper maintenance of litter and house conditions, such as keeping litter dry, can slow the growth of *A. diaperinus* populations (Wolf et al. 2015). Some animal welfare standards, such as those recommended by the Global Animal Partnership, stipulate that broiler house interiors be illuminated by lamps or sunlight via windows (Global Animal Partnership 2020). It is unknown what effect this has on overall beetle populations, but there is some evidence that *A. diaperinus* is repelled by certain wavelengths of light (unpublished data). In caged layer houses, damage to support beams by boring larvae and emerging adults can be prevented by wrapping strips of polyethylene around the beams (Kaufman et al. 2005a). The beetles cannot grip and climb the material, and so are prevented from climbing and burrowing into the beam, protecting the structure. Another potential avenue of cultural control is the use of attractants and repellants to influence the movement and aggregation of *A. diaperinus* within houses. A push and pull system using synthetic *A. diaperinus* alarm and aggregation pheromones to direct beetles and larvae into pitfall traps dramatically improved the beetle yield compared to un-baited traps (Hassemer et al. 2019). Lemon and citronella essential oils have also been found to have a repelling effect on *A. diaperinus* and could serve a similar function (Francikowski et al. 2019).

Natural Enemies

Biological control is a less common method of controlling *A. diaperinus,* with only one biological control agent, the entomopathogenic fungus *Beauveria bassiana*, currently marketed as a treatment for *A. diaperinus* populations. *B. bassiana* has been shown to be capable of infecting and killing *A. diaperinus* and has the potential to be even more effective as a control agent when deployed in an attract-and-kill strategy (Santoro et al. 2008). Pitfall traps baited with aggregation pheromone and designed to inoculate *A. diaperinus* with spores of *B. bassiana* have shown success in inducing lateral spread of *B. bassiana* across *A. diaperinus* populations, creating a more efficient method of application for longer-term control than sprays of *B. bassiana* spores alone (Hassemer et al. 2020). However, several other natural enemies have been identified and are being investigated as potential control agents. The parasitic mite *Acarophenax mahunkai* Steinkraus & Cross is found with *A. diaperinus* in wild bird nests and feeds on the eggs of *A. diaperinus* (Steinkraus and Cross 1993). Introduction of this mite species to broiler houses could potentially serve as a long-term passive control method that increases the pressure on broiler house *A. diaperinus* populations. In addition to parasitic mites, entomopathogenic nematodes in the genus *Steinernema* may potentially serve as biological control agents (Szalanski et al. 2004). In laboratory settings, *Steinernema* species have been shown to cause between 60 and 95% mortality in populations of larvae and adults within a few days of exposure (Szalanski et al. 2004, del Valle et al. 2016, Kucharska et al. 2016). In field trials in broiler litter, *Steinernema* caused a ca. 70% mortality rate for up to 7 wk, after which mortality fell to between 0 and 30%, as the nematode population was potentially disrupted by litter manipulations between flocks (Geden et al. 1987). Without repeated applications, beetle populations quickly recovered to peak size.

Closing Remarks

The impacts of *Alphitobius diaperinus* are numerous but generally minor, amounting to an economic drain on a grower rather than a pressing concern that will ruin a given flock. This is further emphasized by the prevalence and near omnipresence of *A. diaperinus*, as few farms will be entirely devoid of the species. As such, management of the species must be conducted with the intent to mitigate these losses, and management regimes must remain affordable and viable over the long-term. One of the largest obstacles to creating a true integrated pest management regimen for *A. diaperinus* is our lack of ability to fully assess the economic damage inflicted by the beetle, preventing the determination of an economic injury level and economic threshold. To surmount this issue more research is needed on the economic costs of bird injury, feed loss, and insulation damage. Another obstacle is the looming specter of insecticide resistance. Long term integrated pest management programs will need to account for changes in insecticide efficacy, and new, field-tested control methods such as cultural and biological control need to be developed to augment or replace insecticide regimes and slow or manage the growth of resistance. These alternative control methods may be discovered or augmented by a greater understanding of the patterns and behavioral drivers of movement of *A. diaperinus* within houses and on regional scales, as well as the mechanism of colonization of new and cleared houses. With better informed and more effective management strategies, *A. diaperinus* can be greatly diminished as an economic pest of poultry production.

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