Chapter VII-21

Application and evaluation of entomopathogens for control of livestock and poultry pests

Dudley E. Pinnock

Microbial Products Pty. Ltd.

Kent Town

Adelaide

South Australia 5071, Australia

Bradley A. Mullens

Department of Entomology

University of California

Riverside, CA 92521, USA

1 Introduction

The control of livestock and poultry pests presents unique challenges. The hosts are warm-blooded vertebrate animals, capable of a wide range of behaviors and movement. Range cattle and sheep in arid environments, for example, may occupy sparse grassland regions of thousands of hectares and move freely over many kilometers in search of forage. In these situations, pest management strategies must be effective and sustainable with minimal human input or supervision. At the other extreme are intensive, confined animal operations that are growing in popularity, particularly in developed countries in western Europe, Australia, and North America. In these facilities, poultry, swine, cattle, and fur-bearers such as mink are held at high density. These confinement systems may exacerbate certain pest problems, such as muscoid flies, which develop in the accumulated manure, and facilitate the spread of some permanent ectoparasites such as lice or mites.

A fundamental difference between livestock and poultry pest management and similar efforts

in plant crops relates to economic and damage thresholds. These fundamentals of integrated pest management can be much more difficult to define in animal systems. Certain production parameters, such as losses in weight gains, reduced milk yield or quality, or losses in feed utilization efficiency due to pests, can be measured. They can then be related to expected economic benefits when considering the costs and benefits of control efforts. Animals also feel discomfort and are subject to humanitarian concerns for their welfare, which may affect pest control decisions. Further, many pests associated with livestock and poultry, for example manure-breeding house flies, are less important as direct pests of the animals than they are of people nearby. This may result in very significant pest control costs to producers, who are compelled by public health authorities to control the problem or cease operations. These humanitarian and public health concerns are very difficult to define economically.

The microbial control of livestock and poultry pests, as in agricultural systems, can take the form of inoculative, augmentative, or inundative methods. There have been few inoculative

efforts in animal systems, but there is still potential for this approach as a part of integrated control programs. One example is the nematode parasite of house flies, *Paraitionchium muscadomesticae* (Geden, 1997), which has significant sublethal effects at lower infection levels and causes direct mortality at higher levels. Inoculative releases, as in classical biological control, may assist in suppressing pest levels below a nuisance or damage threshold. Certain pathogens are relatively easy to produce in fairly large quantities, but also are found naturally at low prevalence; for example, *Beauveria bassiana*. Other pathogens may not be found naturally in the area of interest, but can be very effective when applied, such as certain strains of *Bacillus thuringiensis*. In most cases, it is hoped that inoculative releases may result in establishment, or at least allow the pathogen to persist and be retransmitted within a target population. Pathogens used in augmentative or inundative programs most often are biological insecticides and are not expected to persist.

The use of entomopathogens in livestock and poultry systems is still in its infancy. Thorough surveys for naturally occurring diseases remain to be done for many livestock and poultry pests in most areas. No doubt such surveys would reveal pathogens that might be manipulated either to achieve control by direct application, or to have their natural activity enhanced and more intentionally incorporated into integrated pest management programs. To date, most attention has focused either on microbial agents which cause obvious natural mortality, such as the mycosis of house flies by the *Entomophthora muscae* complex, or on agents that can be mass-produced, such as strains of *B. thuringiensis*, *B. bassiana*, or entomopathogenic nematodes.

In this chapter we describe first the microbial control of pests of livestock in extensive rangeland systems, with examples being the sheep blow fly, *Lucilia cuprina*, and sheep lice, *Bovicola (Damalinia) ovis*. Second, we describe the microbial control of pests in intensive, confined animal husbandry systems, such as the stable fly, *Stomoxys calcitrans*, house fly, *Musca domestica*, and the lesser mealworm, *Alphitobius diaperinus*.

2 Pests of extensive rangeland systems

A Major pests of sheep

1 The "Australian" sheep blow fly, *Lucilia cuprina*

The blow fly, *L. cuprina* (Diptera: Calliphoridae), is believed to have been accidentally introduced into Australia from Africa and/or Asia during the mid-19th century. In its indigenous regions, *L. cuprina* appears to have evolved as a species feeding on carrion during its larval stages. In Australia, where the many coprophagous species create intense competition for carrion, it has adapted to exploit live animals, mainly sheep, as a larval food source.

Female *L. cuprina* lay their egg masses on the soiled or wet wool of live sheep. The neonate larvae migrate to the skin of the animal where they mass together and scrape the host's skin with their mouth hooks, creating a cutaneous myiasis, a suppurating abrasion on which they feed. Each myiasis frequently is created by several thousands of larvae and can extend over the entire back or flank of the sheep. Unless treated without delay, the sheep will die within a few days, apparently of a syndrome similar to toxic shock; the larvae do not invade into deep tissues until after the death of the host. These myiasis are called "flystrikes" by sheep farmers. It has been estimated that *L. cuprina* flystrikes cause sheep deaths and losses of wool production in Australia valued at over A\$ 280 million every year (Australian Wool Innovation, 2007).

Previous flystrike control measures in general use were a combination of traditional sheep husbandry practices such as mulesing, pizzle dropping, docking and crutching, together with the topical application of insecticides by dipping or spraying ("jetting"). The insecticides used included the organochlorines dieldrin and aldrin, the carbamate butacarb, and the organophosphates diazinon, chlorfenvinphos, fenthion ethyl and dichlorfenthion (James, 1986).

The use of organochlorines was abandoned because of the risk of residues in sheep meat, lanolin and wool, and since the 1970s, the use of organophosphates has been greatly limited because of the development of widespread

resistance of *L. cuprina* to these compounds. At the time of writing, diazinon, or diazinon combined with another toxicant, still is used as a local application directly onto an existing flystrike area to kill the *L. cuprina* larvae. Prophylactic treatment against flystrike with the triazine compound cyromazine, a chitin synthesis inhibitor, has been used widely since 1979 (James, 1986). Cyromazine does not control existing flystrikes.

2 The sheep biting louse, *Bovicola ovis*

The sheep biting louse, *Bovicola ovis* (Phthiraptera: Trichodectidae), was introduced into Australia with its host and now occurs in all sheep rearing areas. Merino sheep are especially prone to *B. ovis* infestations and untreated flocks may carry very high louse populations. The host's response to the severe irritation caused by lice is a reduction in wool production of up to 1 kg/sheep (Wilkinson, 1986), which represents a loss to the farmer of ca A\$ 8.00/sheep at time of writing. Equally serious is that the sheep will rub against any solid structure available, such as rocks, fences, and gate posts. This rubbing results in derangement of the fleece and damage to the wool fibers, so that the wool is downgraded, further increasing the losses to the farmer.

Traditional control measures for sheep lice generally were based on insecticidal plunge dips using a range of toxicants, including arsenic compounds now banned. In 1981, backline or pour-on formulations of synthetic pyrethroids were introduced for lice control and their convenience of use and early efficacy gained them wide acceptance by Australian wool growers. In 1986, control failures of some of these backline treatments were reported and resistance of sheep lice to the synthetic pyrethroids was confirmed. By 1990, resistance factors to the synthetic pyrethroids in lice from some flocks were as high as 26.1 (Johnson *et al.*, 1990) and at time of writing, resistance factors very much higher have been recorded in lice from many commercial flocks throughout the Australian wool producing areas. Current control measures include the continued use of synthetic pyrethroids where sheep lice are still susceptible to these compounds, and the use of plunge or shower dips containing the chitin synthesis inhibitor diflubenzuron or a variety

of organophosphate insecticides where synthetic pyrethroid resistance is prevalent.

B Overview of the potential of microbial control

1 The "Australian" sheep blow fly

For a microbial control method to be commercially successful, it must be cost-effective and offer distinct advantages over other, competing methods of control, or it must provide control of a pest where no other method is effective or available. For example, an increasing use of *B. thuringiensis* occurs in systems where the chemical insecticide resistance of the target pests is so high that an alternative means of control is an imperative, often as a component of an insecticide resistance management program.

There are at least two approaches to the microbial control of *L. cuprina* flystrike on sheep. The first approach is to introduce or apply a self-replicating, transmissible microbial agent which would greatly suppress the fertility of the field population of adult *L. cuprina*. Given time, this strategy would reduce the number of fertile egg masses that can be laid and so reduce the incidence of flystrike. The research with the microsporidium, *Octosporea* sp., has this objective and early results are encouraging (Smallridge *et al.*, 1995).

A second approach for control of *L. cuprina*, and which may be integrated with the first, is to deploy a microbial larvicide, for example, *B. thuringiensis*. This approach entails the solution of many new and difficult problems. The Australian sheep industry is based on flocks containing very large numbers of sheep ranging over vast tracts of land. Each farm (station) is managed with very low labor inputs. To be commercially successful in Australia, the microbial larvicide must provide reliable, cost-effective control or protection against flystrike for a period of at least 10, and preferably 12 weeks following a single application (Wilkinson, 1986). This extended period of performance must be maintained under ambient conditions of intense solar radiation and very high temperatures.

Readers familiar with the use of *B. thuringiensis* products for plant protection in agricultural systems will be aware of the extreme stringency of these requirements. Even with modern formulations, it is unusual for a

B. thuringiensis spray deposit on an agricultural crop to remain larvicidal for more than a few days following application.

The successful development of a microbial *B. thuringiensis* larvicide system that meets the stringent performance specifications described above required detailed knowledge of the microbial ecology of the sheep fleece. In this, the population dynamics of the competitive displacement strategy underlying the colonization of the fleece by the *B. thuringiensis* larvicide was crucial (Pinnock, 1994; Lyness *et al.*, 1994).

2 Sheep lice

If it is to be commercially successful, a microbial agent should control lice on off-shears or short wool sheep for a period of at least 20 weeks after a single application. This duration of control is an even more stringent requirement than that for control of sheep blow fly described above. Although the *B. thuringiensis* strains and mode of action for control of sheep lice are different from those for the control of sheep blow fly, the achievement of this duration of control by *B. thuringiensis* required similar knowledge of the microbial ecology of the fleece and of the population dynamics of the colonization of the fleece by the microbial agent (Pinnock *et al.*, 1994).

C Field trial design

In general, the purpose of microbial control field trials is to test or demonstrate the pest control efficacy or some other attribute of the microbial agent under realistic industry conditions. During the development stages of the agent, often many field trials may be conducted and the scale and design of these field trials will depend on the data being sought. Ultimately, the microbial agent must undergo field trials to demonstrate and satisfy the efficacy requirements necessary for registration of the agent as a product for sale to farmers.

In some countries, no prescribed design or scale of field trials is imposed; it is the responsibility of the applicant to provide sound efficacy data from properly designed and executed field trials. In other countries, such as Australia, the design and scale of registration trials are prescribed by the

governmental registration authority. To provide the reader with examples of field trial scale and design, the Australian requirements for sheep blow fly and sheep lice product registration trials are described below.

1 Pre-treatment procedures for sheep blow fly or sheep lice trials

a Pre-selection and assignment of animals to treatment groups

Sheep are mustered and inspected and animals with abnormal physical or fleece conditions are rejected. Sheep to be used in the trial should be as uniform as possible in age, frame size, wrinkle, fleece type and wool length. As a general rule, weaners or young wethers are preferred because these are most susceptible to flystrike and lice and so provide the most sensitive test of the control agent. If a mixed commercial flock of ewes and mature wethers is to be used, these should be assigned so that there is no sexual bias in any of the dose or treatment groups. With this proviso, the sheep are assigned randomly to the different dose or treatment groups.

At this preliminary stage, it is a wise precaution to have larger numbers of sheep in the dose or treatment groups than will actually be used in the forthcoming field trial. This preserves the desired group size while allowing for any sheep which fail pretreatment inspection to be eliminated from the trial. The sheep in each dose or treatment group are then ear-tagged with plastic tags bearing an individual identification number for each animal. For ease of drafting after muster and throughout the trial, ear-tags of different colors may be used to indicate the different doses or treatments to be used.

b Pre-treatment inspections

For blow fly control trials, all sheep are given a detailed inspection immediately prior to the commencement of the trial to detect and eliminate animals that are scouring or wounded, have vaginal or urethral discharge or fleece abnormalities such as fleece rot or mycotic dermatitis, or have covert flystrikes.

For sheep lice trials, each animal is inspected as above. Sheep accepted for the lice trials are

then restrained and a pre-treatment lice count made and recorded for each animal as described below in the scoring methods.

2 Design for control of sheep blow fly

In Australia, the registration and use of pesticides are regulated by the Australia Pesticides and Veterinary Medicines Authority (APVMA). The APVMA requires, among other data, the demonstration of efficacy of a pesticide, including a microbial control agent, before it can be registered as a saleable product. The demonstration of efficacy entails the execution of prescribed field trials. For control of the sheep blow fly, the demonstration of efficacy requirements entails compliance with the following field trial design.

A minimum of 5 to 10 field trials must be conducted in a range of different geographic zones, using sheep of a representative range of age, breed, sex and wool length. In most pastoral zones of Australia, the Merino or a Merino-cross is the predominant breed of sheep, and so it is expected that this breed also would be predominant in the field trials. If breeds other than Merino are to be included, it is recommended that weaners or unclassified hoggets be used because these are more susceptible to flystrike than are older sheep.

For each trial, a minimum of 100 sheep, inspected and known to be free of covert flystrikes, must be used for each dose level or treatment group. After application, (see below for methods), all treatment groups must be kept segregated until dry, and then managed as a single flock. A similarly sized, untreated control group may be included in the trial.

a Duration of sheep blow fly field trials

There is no prescribed duration for sheep blow fly field trials, but for practical purposes, the control agent should protect sheep from flystrike for at least 10, and preferably 12 weeks. This 12 week period determines the minimum duration of the field trial. During the development of *B. thuringiensis* as a control agent for sheep blow fly, the field trials were successfully run for 20 weeks.

b Scoring method of sheep blow fly field trials

All sheep in the trials undergo an individual, detailed bodily examination daily when intense blow fly pressure - "flywave" - conditions occur, and at least every 3 days at other times. The position, size, severity and date of all flystrikes are recorded and all struck sheep must be clipped, spot-treated and re-examined the following day to ensure that the spot-treatment has been effective. This protocol is a sensible animal ethics requirement intended to prevent deaths and minimize distress and suffering of the sheep in the trial.

As a general rule, an adequate level of blow fly pressure is deemed to have occurred, and therefore the control agent properly tested, if at least 15% of the untreated control sheep are struck during the trial.

3 Pen and field trial design for control of sheep lice

The prescribed trials of products for control of sheep lice are divided into two stages. The first stage comprises initial pen trials on only a few sheep per treatment or dose. At least two initial pen trials are required. These use low numbers of sheep - as few as 5 per dose or treatment group. The pen trials provide a preliminary examination of the efficacy of the control agent at different dose levels ("dosing trials") and of any adverse effects of the agent on the test animals.

The pen trials are followed by the second stage, which entails field trials on large numbers of sheep. There are two designs for the large field trials. The design to be adopted for any given field trial is determined by the length of wool on the sheep in that trial. For off-shears or short wool sheep, which are defined as sheep with up to 42 days' wool post shearing, in addition to the pen trials at least 5 field trials are required, each with at least 1,000 sheep per dose or treatment group. At least three of these field trials must be with fine wool Merino sheep. As indicated below, these field trials run for up to 20 weeks.

For sheep with more than 42 days' wool, at least 6 field trials are required in addition to the pen trials. In each trial, at least 500 sheep are required per dose or treatment group. These long wool field trials should be at fleece lengths

equivalent to 3, 6 and 9 months' wool, with 2 field trials for each wool length.

a Duration of sheep lice field trials

If the control agent under trial is to be claimed to reduce lice numbers to undetectable levels, the field trials must run for 20 weeks or until the next shearing, whichever is the sooner. If the control agent is to have a lesser claim - for example, to reduce lice numbers by at least 95% over a period of 1 or 2 or 3 months, then the field trials must run for the claimed period plus at least 10 days, or until next shearing, whichever is the sooner.

b Scoring method of sheep lice field trials

The method of scoring sheep lice in the pen and field trials is to inspect and count lice on all sheep in the pen trials and on 25 of the sheep in each dose or treatment group in the field trials. These sheep are inspected and their lice counted before treatment, and at intervals post-treatment. The following sampling procedure is used.

The inspection and sampling procedure requires each sheep to be restrained. This may be achieved by placing the animal in a closed race, in a crutching cradle, or hog-tied on an inspection table. Once the sheep is restrained, all adult lice, nymphs and eggs are counted at 20 sites on each side - a total of 40 sites on each animal. The location of these sites has been determined by study of the distribution of lice on sheep, and the location of these sites is shown in Figure 1. A plastic mesh may be used as an aid to determining the location of the sampling sites, but with experience, the locations of these sites are learned and the mesh dispensed with. At each of the 40 sampling sites, the fleece is parted so that a line of skin 10 cm long is exposed. All lice, nymphs and eggs on this line of skin and on the exposed wool staples on either side of the parting, are counted. The efficacy of the control agent is determined by the reduction in lice numbers relative to the pre-treatment counts, as described in the preceding section.

D Application methods for sheep blow fly and sheep lice control

In general, the microbial control agent will be applied to the animal in a liquid suspension by

one of three application methods in common use in the sheep industry. These methods are backline applications, spraying (jetting) applications and application by plunge or shower dips. These methods are described below.

1 Backline application methods for sheep blow fly and sheep lice control

Backline applications rely on the translocation of the control agent from a single or a few line or strip applications along the back of the sheep to effectively treat the entire animal. This method has, and is, being used for some ready-to-use chemical treatments such as the synthetic pyrethroids. The control agent is applied in a low volume of < 1 liter of suspension per sheep along the spine of the animal using a single nozzle, hand-operated syringe-type applicator.

Sheep to be treated are mustered into a permanent or temporary sheepyard, from where a sheepdog works the sheep so that they move in single file through a drafting race. As each sheep passes the operator, it receives its backline treatment. This application method has the advantages of high rate of throughput of sheep and low labor input (one person and a dog). A possible disadvantage of the backline application method is that the control agent forms a concentration gradient from dorsal to ventral around the animal, which may result in only low or discriminating doses being achieved in the axils or on the ventral side of the animal. Because of this effect, the use of low volume backline application for microbial control agents is still in the experimental phase.

An alternative backline application is the use of a pressure-fed, hand spraying ("jetting") wand (see below) to apply a medium volume of 1 to 2 liters/sheep. The jetting wand is drawn through the fleece along the spine of the animal, thoroughly wetting the skin. This method has been used successfully for the application of a *B. thuringiensis* formulation as a prophylactic treatment against flystrike if the application along the backline is extended to include the breech and pizzle areas.

2 Jetting application methods

Spraying ("jetting") applications use a system of fixed, flexible or hand-held nozzles or jets

Figure 1. Sites on the body of sheep where fleece partings should be made during examination for infestations of sheep lice in pen and field trials. Diagram reprinted by kind permission from "Guidelines for Ovine Lousicides Efficacy Submissions" 1996. National Registry Authority for Agricultural and Veterinary Chemicals, Australia

to apply the control agent, for example a *B. thuringiensis* formulation, over the entire animal as a suspension in water.

The jetting nozzles are supplied with the suspension by a hydraulic pump equipped with a pressure regulating valve and drawing the suspension from a reservoir tank. Generally, a delivery pressure of 700 to 800 kPa (101 to 116 psi) is selected, and some systems have a pressure reservoir to reduce pulsing or variations in delivery pressure and so maintain a more constant flow through the nozzles. Many jetting systems have a pressure relief valve which allows a recirculating or bypass line to take suspension back to the tank or reservoir, and so keep the contents thoroughly mixed. A diagram of a hand jetting wand is given above in Figure 2.

With an experienced operator, hand jetting is the most thorough jetting method. However, hand jetting is very labor intensive, and the need to reduce costs has led to the development of automatic jetting races, where the animals are passed rapidly through a set of fixed or flexible nozzles which apply the pressure spray automatically. An example of a jetting race is shown in Figures 3 and 4.

3 Dip application methods

Plunge dipping is the traditional method of treating sheep for ectoparasite control. A traditional sheep plunge dip is a concrete-lined trough approximately 0.75 m wide, 1.5 m deep and several meters long. Plunge dip capacities range from 7,000 to over 10,500 liters. Sheep fall into

the dip from a single file race and are totally submerged for 2 to 3 sec. As they swim to the opposite end of the dip, they are submerged "dunked" twice again by operators wielding T-shaped poles. A ramp at the end of the dip allows the sheep easy exit on to a draining area where runoff is collected and returned to the dip. Research (Lund *et al.*, 1998) has shown that for effective coverage and penetration of the fleece, the dip swim length should be at least 9 m.

A recent development of the plunge dip is the mobile dip, which is a demountable, small plunge dip which can be towed behind a Land Rover® or similar four-wheel drive vehicle. These dips are operated by independent contractors and have the advantage of being able to travel to remote outback areas where the sheep are mustered. A typical mobile dip, the "Rippa Dippa S®" is shown in Figure 5. In this design, sheep from a single file race are lifted by conveyor belt and fall into an S - shaped plunge dip. This design has a swim length of approximately 12 m.

E Assessment of persistence of microbial control agents

Quantitative assessment is especially valuable during the developmental stages of the microbial agent, when different strains and/or different formulations are to be compared, and such assessments are essential if mathematical modeling is to be used as an aid to predicting dose delivery. Assessments of the persistence of a microbial agent for control of sheep ectoparasites may be made quantitatively by taking fleece samples

Figure 2. Hand jetting wands. Each wand consists of a hand piece with a cutoff valve and 3 to 6 cone nozzles. The wand is supplied with the treatment suspension by a hose and hydraulic pump – see text

Figure 3. Automatic jetting race – general view, frame omitted to show the spray bars

Figure 4. Automatic jetting race – detail of valving and pressure reservoir (1" = 2.54 cm)

Figure 5. Mobile plunge dip- the "Rippa Dippa S"® in this design, sheep approach the dip through a single file race and are lifted and delivered into the dip by a V-shaped conveyor belt. The dip is folded to an S shape for compactness, and gives a swim length of over 11 m. The sheep exit the dip via a draining ramp. This dip can be operated by two persons and has a throughput of 700 to 800 sheep per hour

(Lyness *et al.*, 1994) and applying a laboratory assay method such as viable cell counts.

Qualitative assessment of persistence often is based on the duration of efficacy of the microbial agent, and this has a direct and practical utility. The duration of efficacy also is an essential element in the validation of predictive dose models. Estimation of the duration of efficacy may be made by continual or repeated, natural challenge of the treated animals or substrate by the pests in the field. For example, the sheep may be exposed to gravid *L. cuprina* or to untreated sheep with heavy lice infestations. Alternatively, the treated animals may be artificially challenged at intervals by an implant technique (Lyness *et al.*, 1994) using *L. cuprina* neonate larvae or by "seeding" the animals with a known number, usually 50, of young adult lice.

F Assessment of efficacy of microbial control agents

The assessment of efficacy of the control agent may be made by the scoring methods described above. To be regarded as efficacious, the control agent should reliably suppress the pest in the treated area or animal below a threshold density, or limit or prevent the pest's reinfestation or reproductive capacity for a certain period of time.

For sheep blow fly prophylactics, the control agent must protect the treated sheep from flystrike for at least 10 weeks post-treatment during periods of heavy blow fly pressure. For sheep lice control, the control agent either should reduce lice numbers to undetectable levels for at least 20 weeks post-treatment, or reduce lice numbers by at least 95%, depending on the claim made for the product. The lice assessments are made using the 40 site scoring method described above.

3 Pests of intensive animal husbandry systems

A A Major pests of intensive animal husbandry

1 The common house fly, Musca domestica

The house fly is thought to have originated in eastern or central Africa, but has readily spread

around the globe with human movements and is now cosmopolitan. It is regarded as a synanthropic fly, since it develops in large numbers in organic materials accumulated near people (e.g., household garbage) or their livestock and poultry (West, 1950; Axtell and Arends, 1990). House flies readily develop in manure, especially from confined swine and poultry. They also may develop readily in other types of animal dung, but on dairies and cattle feedlots usually are most abundant in decaying foodstuffs or straw/hay contaminated with manure and urine. House flies prefer moisture levels above 60% for larval development, and cease development at temperatures below 12°C (West, 1950; Stafford and Bay, 1987).

House flies disperse readily to nearby houses and are serious human nuisance pests, and most farmers will try to keep fly numbers in check for this reason. Proper manure management, particularly moisture control, is critical for both the house fly and stable fly. Many producers use insecticides for adult control (surface residual sprays, toxic baits). There is a growing awareness of the importance of biological control agents (predacious beetles and mites, parasitic wasps), and many producers now tend to avoid treating the larval habitat (accumulated manure) with broad-spectrum pesticides because of nontarget effects on beneficial arthropods. Economic losses from fly nuisance are difficult to quantify, but regulatory agencies may require significant and expensive control efforts such as intensive insecticide use or frequent manure removal. In extreme cases producers can be fined, sued or forced to cease operations. In addition to their direct nuisance potential, house flies defecate and regurgitate on surfaces they visit. These spots are unsightly. The behavior also contributes to the potential of flies to transmit or harbor pathogens such as trachoma (which may cause blindness), and particularly bacteria or viruses that cause enteric diseases of humans or animals (Greenberg, 1971). Recently, concern for fly transmission of such pathogens has increased in accordance with development of antibiotic resistance and particularly virulent strains such as *Escherichia coli* 0157:H7 (Szalanski *et al.*, 2004; Alam and Zurek, 2004). Other flies can be common and problematic in manure, garbage, and similar organic habitats, particularly *Fannia*

canicularis. These are similar enough to the house fly for many of the same management principles to apply.

2 The stable fly, *Stomoxys calcitrans*

The biting stable fly is occasionally a human pest but is much more commonly found biting domestic livestock such as cattle or horses, and it can be a serious pest of dogs. The genus *Stomoxys* probably originated in Africa, where a number of species exist. Some species such as *S. nigra* are severe biting pests in their native range and areas where they have been introduced (e.g., islands of Mauritius and Reunion), but *S. calcitrans* is the species most widely distributed in temperate zones, including Australia, Europe and North America (Zumpt, 1973).

S. calcitrans develops in some of the same habitats as house flies. Stable flies are not common in fresh manure, but may use it after it has decomposed somewhat. They prefer rotting vegetation or feedstuffs, often if those are polluted with livestock manure or urine. Rotting straw, wet hay, or silage waste are excellent habitats for stable flies. While the largest numbers of stable flies usually are found near the developmental sites, these flies are excellent dispersers and may travel several kilometers in search of hosts or oviposition sites. Under special conditions, particularly on weather fronts, stable flies can be transported up to hundreds of kilometers. This phenomenon occurs, for example, along the Florida panhandle in the USA, where down drafts deposit large numbers of stable flies from inland farms on beaches up to 225 km away (Broce, 1993).

Unlike *M. domestica*, which is mainly a human nuisance pest, both sexes of stable flies are obligate blood feeders and typically feed daily. The pain associated with their bites reduces weight gains, milk yield, and feed conversion efficiency in cattle, and stable fly economic impact on the USA cattle industry exceeds \$400 million/year (Drummond *et al.*, 1988; Campbell, 1993). Economic injury levels on beef cattle are 2–3 flies/leg. Additionally, stable flies are capable of mechanical transmission of several livestock pathogens, such as Equine Infectious Anemia virus or *Trypanosoma evansi*, the causal agent of surra.

As is true for *M. domestica*, numbers of stable flies can be reduced substantially through proper sanitation, and natural biological control via predators and parasites is very important. Parasitic wasps which attack the pupal stage of the house fly and stable fly (particularly species of *Muscidifurax* and *Spalangia*) are reared commercially and sold to producers for release. Results of experimental releases have been inconsistent but may be beneficial, especially in habitats such as semi-enclosed dairy calf pens, where parasitoid releases can be focused. There also has been interest in sticky stable fly traps, which may reduce stable flies locally, provided fly numbers are not too high. At the farm level, however, it is most common for producers to try to protect their animals using insecticides such as pyrethroids applied directly to the host. Perhaps exacerbated by resistance, control is incomplete and quite temporary, usually only a few days at best.

3 Manure flies

Livestock and poultry pests can be divided into several groups, based on the degree of association between the pest and animal and the nature of the production systems. This affects our approach to sampling and potential application of a microbial agent. Permanent ectoparasites of either confined or range animals, such as lice, complete their entire life cycle of the host. In other cases only certain life stages are found on the host, but they remain there for substantial periods of time (days to weeks). This category includes adults of the horn fly and buffalo fly (*Haematobia* spp.) and to a lesser extent the face fly and bush fly (*M. autumnalis* and *M. vetustissima*). These flies spend much, but not all, of their adult life on the host, while immatures are found in dung. The common theme, however, is that both microbial control efforts and monitoring of pest and pathogen activity and prevalence often focus on the host animal.

A second category includes pests that periodically must contact the host, often to obtain blood, but do not spend much time there (a few minutes every 1–4 days). Adult stable flies would fall into this category. For these pests microbial control efforts must generally

focus on immature habitats (decaying organic materials, older manure deposits), or on resting surfaces favored by these flies in the environment (e.g., barn walls). An exception to this would be a pathogen capable of persisting on the hair or skin of a host or within a trap which utilizes host cues (e.g., kairomones) to attract and infect the target pest fairly quickly. Because the direct association between pest and host is of primary interest, monitoring often focuses on pests coming to the host or associated cues (e.g., CO₂-baited traps), while monitoring of the microbial agent often requires sampling the immatures in developmental habitats. Sampling the immatures is usually the more difficult task.

The third category includes pests closely associated with livestock or poultry, but which visit the animals only incidentally. Their economic impact is more indirect (pests of people nearby). Filth flies that develop in accumulated manure or animal feedstuffs, such as *M. domestica* or *Fannia* spp., are good examples. In these cases microbial control may be directed against either immature stages in developmental sites or adult stages in preferred resting or activity sites. Monitoring of the pathogens and target pests may require sampling adults, immatures in developmental sites, or both.

4 Lesser mealworm beetles

Confined poultry, such as caged egg-laying hens or birds raised for meat, are grown or held in buildings with variable levels of environmental control. Feed for these birds inevitably is spilled into the immediate area. This becomes a food source for larvae and adults of species such as the lesser mealworm beetle, *Alphitobius diaperinus* (Tenebrionidae). While other beetles, such as *Dermestes* spp., also can be common in the feed/feather/manure environments near birds, numbers of *A. diaperinus* often are the highest.

The lesser mealworm life cycle is approximately 50–70 days, but development is prolonged at lower temperatures, and adults may live for several months or longer (Axtell and Arends, 1990). Eggs are laid in cracks and crevices, and most of the developmental period is spent as a larva. The lesser mealworm causes serious damage to insulation, especially to polystyrene as a late instar larva burrows into

it to pupate (Vaughan *et al.*, 1984). Perhaps even more significantly, the beetle is now appreciated as a persistent source/reservoir of several pathogens, including enteric bacteria such as *Campylobacter* spp. or viruses such as turkey coronavirus (Strother *et al.*, 2005; Watson *et al.*, 2000). The longevity of the beetles and their relative inaccessibility in the environment (adults or immatures secreted in cracks and crevices, or in pupation sites) make them a challenge to control and a particular concern for harboring pathogens longer-term or between flocks. Of biological control options, pathogens and nematodes have received the most attention for control of lesser mealworm beetles.

B Overview of potential microbial control agents for manure flies or beetles

1 Fungi

Many fungi have been reported, particularly from Diptera (e.g., Steenberg *et al.*, 2001), but far fewer have been utilized for control. The *Entomophthora muscae* species complex (*E. muscae* and *E. schizophorae*) have been observed to cause epizootics in muscoid flies for many years (Mullens, 1990). Principal natural hosts include *M. domestica* and *Fannia* spp., and infection prevalence may exceed 70–80% during epizootics. A number of hosts may be infected by a given strain, although there is evidence for host specificity even within the Muscidae. More recently several attempts have been made to release infected flies and stimulate epizootics. These fungi can be maintained indefinitely *in vivo* in laboratory-reared *M. domestica* or grown (predominantly vegetatively) in tissue culture media such as Grace's medium. In the latter case, patent infections may be induced by injecting protoplasts into susceptible flies and then using cadaver-to-fly transmission to increase numbers of infected flies for release. While resting spores of *E. muscae* group fungi are reported in the literature, primary and secondary conidia are normally produced for expulsion from conidiophores; they are sticky, short-lived and almost impossible to handle or disseminate directly.

Natural *E. muscae* epizootics typically occur when host populations are high and temperatures are not too hot (maximum temperatures

below 26–28°C). Several researchers have managed to increase prevalence and stimulate earlier *M. domestica* epizootics on New York dairies through introduction of infected flies in two ways- deploying freshly killed cadavers with heads inserted into dishes of water agar in fly aggregation areas or release of laboratory-infected flies (Geden *et al.*, 1993; Steinkraus *et al.*, 1993). The level of infection was not sufficient to exert control, however. Short-term establishment of *E. schizophorae*, which is not naturally found in house flies on southern California dairies, was achieved by releasing critically ill flies in a covered calf-rearing facility. The pathogen did not persist, possibly due to behavioral fever (Six and Mullens, 1996). Enclosed habitats, such as poultry buildings, may offer more potential for control by *E. muscae* complex fungi (Kuramoto and Shimazu, 1997). Flies probably cannot bask to induce behavioral fever and are in fairly close proximity to wild flies. Kalsbeek *et al.* (2001), however, showed that house flies infected with *E. schizophorae* also exhibited behavioral fever indoors by resting on heat lamps provided for piglets in a swine barn.

The fungi *B. bassiana* and *Metarhizium anisopliae* have been screened in the laboratory against *M. domestica* and *S. calcitrans* (e.g., Barson *et al.*, 1994; Watson *et al.*, 1995), and some field testing has been done with *M. domestica* (Watson *et al.* 1996). In general the effective concentrations of conidia in these tests have been high ($> 10^6/\text{cm}^2$). The tendency of house flies to rest inside structures such as calf hutches at night makes an inside surface application of conidia an attractive prospect for control. Natural *B. bassiana* prevalence in flies is usually $< 1\%$, but prevalence was increased to 43–47% on treated farms (Watson *et al.*, 1996). The fungi, in general, have incubation periods of several days to a week or more, theoretically allowing flies to reproduce before death. Kaufman *et al.* (2005) recently tested *B. bassiana* sprayed as a coarse fog (mostly residual application to surfaces) and releases of pteromalid pupal parasitoids against house flies in caged layer houses. The results were compared with applications of pyrethrin insecticides plus the parasitoids. Fly numbers post-treatment were reduced by the fungus-parasitoid treatment. Renn *et al.* (1999) tested

M. anisopliae against *M. domestica* in large enclosures (10m^3) by using bait stations (sugar and muscalure) to attract/arrest flies and expose them to cultures of the fungus nearby. Flies did acquire many thousands of conidia, and almost all were killed in 10 days; the authors discussed how later fly-to-fly transmission could enhance the effectiveness of such an approach.

These same fungi have potential for control of *A. diaperinus*. For example, Steinkraus *et al.* (1991) tested *B. bassiana* against beetle larvae at doses of approximately 10^7 – 10^8 conidia per g of dust or ml of aqueous suspension, and achieved high infection rates ($> 60\%$) for larvae treated directly, but low to moderate infection rates ($< 30\%$ for aqueous but higher for dust treatments) for beetle larvae distributed in poultry litter. Geden and Steinkraus (2003) achieved short-lived (2 weeks) but significant control (60–90% reduction in numbers of beetle larvae) using especially corn bait or granular formulations of *B. bassiana*. Because lesser mealworms feed on grains, there is potential for making more use of consumable baits for distribution of pathogens. Selection of high-pathogenic strains and appreciation of environmental variables such as temperature (Crawford *et al.*, 1998; Geden and Steinkraus, 2003; Alexandre *et al.*, 2006) are logical and necessary exercises to improve our ability to utilize fungi for beetle control.

2 Nematodes

Livestock and poultry pests have a number of parasitic nematodes, particularly Mermithidae and Tylenchida. The latter infect several important flies in the genus *Musca*, including *M. autumnalis*, *M. vetustissima*, and *M. domestica*. *Paraionchium* (= *Heterotylenchus*) *autumnalae* is a well-known associate of the face fly, *M. autumnalis*, and is typical of the group (see Kaya and Moon, 1978). Mated adult females in the larval habitat (in this case, cow dung) invade the host larva. They produce a parthenogenetic female generation whose progeny invade the ovaries of the adult host. Infected female flies then deposit gamogenetic nematodes, rather than eggs (parasitic castration). Recently a new member of the genus, *P. muscadomesticae*, was discovered infecting *M. domestica* in Brazil; the potential of this agent has yet to be assessed.

Like *P. autumnalis*, *P. muscadomesticae* causes parasitic castration and can substantially reduce fly survival (Geden, 1997).

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* can be readily mass-produced and have been tested in the laboratory, where they can infect several Diptera of veterinary importance. Field trials have been variable. Early experiments generally indicated these nematodes were ineffective and did not live very long or disperse well in poultry manure, especially wet manure where *M. domestica* was common (Georgis *et al.*, 1987; Renn, 1995). Somewhat drier poultry manure (< 60% moisture) appears to be more hospitable for the nematodes, particularly if they are encapsulated in alginate (Renn, 1995). Alginate encapsulation resulted in little infection and death of adult flies feeding on the capsules, but promising laboratory results were achieved when adult flies were exposed to nematode bait pads (Renn, 1994). This raised the potential of a bait formulation. The encapsulation may serve not only to protect the nematodes from desiccation, but from ammonia and similar chemicals in a hostile habitat such as poultry manure. Other types of animal manure also generally are not as nitrogen-rich as that of poultry, and the entomopathogenic nematodes may have better prospects there. If used in a bait station type approach, the porosity of substrate can have substantial effects on how well flies may be infected by such nematodes; cotton poplin was good, while Perlite was poor, for example (Renn and Wright, 2000).

Renn (1998) demonstrated suppression of house fly in swine barns (farrowing) using *S. feltiae* or *H. megidis* incorporated into baits, or manure applications of *S. feltiae* (encapsulated or not). Control was comparable with, or better than, methomyl bait. The larvicidal application was targeted to drier areas of the manure, and it is possible that the nematodes persisted well there and attacked larvae preparing to pupate in these drier areas. Taylor *et al.* (1998) screened a number of species and strains of *Steinernema* and *Heterorhabditis* for infectivity against larvae of *M. domestica* in bovine manure in the laboratory, and nematodes significantly reduced emergence of flies added to this substrate. Some nematodes remained infective in cattle manure (detected

by *Galleria* sentinels) for up to 10 weeks after application. Nematodes similarly have been utilized against lesser mealworm beetles. Geden *et al.* (1987) applied 900,000 *S. feltiae* in 8 liters of water per 8.4 m² areas of floor soil (using a watering can) in broiler and turkey houses in North Carolina, USA. The target was particularly lesser mealworm pupae that might persist in soil between poultry flocks. Treated houses experienced slower and lower level increases in beetle populations after new flock introduction, but numbers were about equal after 10–13 weeks. They found evidence that nematodes could persist at least 7 weeks after application in the field, and one container bioassay showed persistence out to 6 months.

C Field trial design

1 Setting up plots for manure fly or beetle control

The following section refers primarily to filth flies such as *M. domestica*, *S. calcitrans*, or *Fannia* spp. The egg stage typically is short-lived, and it is assumed the primary target for control is the larvae or perhaps the pupae. Plot selection varies with the distribution of the target pest. In moist to wet substrates, the eggs of most flies hatch quickly (< 2 days), the larvae spend several days to a couple weeks maturing, and larvae move to a drier area nearby to pupate. Pupae emerge after several days to a week or two.

In confined animal systems, muscoid fly development occurs commonly at edges. The center of a feedlot, for example, is heavily disturbed by animal hooves, and flies cannot survive in numbers there. Rather, they are concentrated in relatively undisturbed manure under fencelines, or along feed bunks or water troughs. Even in undisturbed organic waste habitats, such as silo leakage or mounded manure stacks, flies are usually most common near the edges or near the surface. This may be due to fermentation heat or anoxia deeper in the manure mass. In certain situations, such as rows or areas of undisturbed manure accumulating beneath confined poultry in cages or dairy calves or swine on wooden slat floors, fly development can be extensive and relatively uniform, provided the moisture is consistently high (*e.g.*, 65–70%). Even here, however, it is much more common

for immatures to be highly aggregated. Typically, moisture variation etc. results in areas where flies are concentrated, and these areas constitute a small proportion of total available habitat. Given limited time and resources, it thus is necessary to find the areas where flies are most abundant and focus attention on them. If time and resources permit, preliminary sampling should be done in a statistically sound way, and numbers of samples required can be adjusted according to the degree of fly aggregation and level of resolution desired (e.g., Stafford and Bay, 1994).

Having thus stratified the habitat to focus sampling on suitable zones, multiples of these units are identified for treatment or control use. If at all possible, buffer (untreated) zones at least the size of treatment plots separate experimental plots. In manure, we often use sections in the range of 0.5–1 m² in surface area. These will accommodate removal of multiple small subsamples per plot over time. Pretreatment samples are taken, treatments are applied, and further samples are taken at pre-selected intervals after treatment.

If plots are indoors, such as an environmentally-controlled poultry or swine house, fly development may occur all year. Otherwise flies are quite seasonal (warm summer weather in temperate zones). In accumulated manure, they also occur in a succession. Flies are excellent colonizers and lay eggs as manure first begins to accumulate.

Relatively free of natural enemies, fly numbers are highest in the first few weeks after new manure accumulation begins. This needs to be considered in timing a control trial and in sampling. In a hen house which just had the manure cleaned out 4 weeks ago, for example, house flies would be expected to be numerous. However, a decline would be expected over the following 4–6 weeks. Good control plots or houses are critical to avoid attributing a natural decline in fly numbers to treatment effects.

As is true for fly immatures, both adult and immature beetles such as *A. diaperinus* also tend to be unevenly distributed in the environment, for example along water lines in a meat bird operation (Strother and Steelman, 2001). Determining their distribution prior to applying a biological control agent is therefore necessary both to optimize control and to allow accurate

assessment of a control effort. Once relative distributions are known, experiments can be appropriately designed, using an approach rather similar to fly trials in many cases.

2 Sampling fly or beetle substrates

A volume for sampling must be selected. In many manure substrates with fairly high fly numbers, samples of 100–200 ml in size are good. Given the high degree of spatial heterogeneity, even after selective sampling, one may need to pool several individual smaller samples prior to processing. Manure can be removed using a hand trowel or coring device shown in Figure 6. Most fly immatures tend to be fairly close to the surface of a manure type substrate (the same tends to be true for beetles), but this can vary substantially with such things as fly species and moisture, so a core type sample which crosses depths often is preferred. Samples then can be handled in one of two basic ways.

If it is necessary to extract live larvae from the samples (for example to determine infection levels with a pathogen) samples can be spread on a screen in a Berlese funnel, where the larvae will crawl away from light and heat and separate themselves into a catch receptacle. There is not a good means of detecting dead larvae; if this is necessary one must search the medium visually.

If detailed information is not needed on numbers of immatures or infection levels, successful emergence of adults can be assessed by holding the samples. It usually is not possible to place emergence traps directly over the manure in the field, since traps may be buried by new manure deposits or disturbed/destroyed by animals. Emergence trap designs incorporate some type of one way funnel to retain adult flies which enter a collecting head (see Figure 7). A small amount of toxic fly bait may be added to the collecting head to incapacitate the adult flies before counting if desired (Mullens *et al.*, 1996). The collecting head is typically a clear plastic or glass container that admits light, so flies will move into it. Containers must have ventilation to prevent accumulation of toxic gases and oxygen depletion. Most fly species develop quickly enough that maintaining manure moisture in the emergence traps is not a critical issue. If possible, we try to hold manure samples

Figure 6. Bulb planter used to take core samples for sampling flies from manure substrates. By depressing the handle spring, the sample is released without compression or disturbing the surface

on-site for fly emergence, in a location which will not impede movement of people or equipment. This ensures manure temperatures are similar to ambient.

Beetles can also be sampled by removing portions of habitat and then extracting immatures or adults, either by visual search or using

something such as a Berlese funnel. Beetles, however, are typically found in somewhat drier and looser substrates than are occupied by fly larvae. In a caged laying hen house with manure buildup beneath the hens, for example, fly larvae predominate in the upper (wetter) part of the manure accumulations, while

Figure 7. Trap used to collect flies emerging from manure samples placed into plastic dishpan. Top held on using spring clips. Small piece of screen glued on inside of collecting head may be wetted slightly to hold a small quantity of toxic fly bait, which will kill emerging flies prior to counting

beetles might be found more commonly in drier manure/feed mixtures near the base of the manure piles. In tests of pathogens, in fact, this direct search technique probably is underutilized. Direct searches and other extraction techniques might be uniquely useful in recovering pathogen-killed or moribund insects.

Due in part to relative ease of use (a major advantage, to be sure), the sampling tool most commonly used for studies of *A. diaperinus* field biology or control the past 20 years has been the tube trap (Safrit and Axtell, 1984), sometimes known as the Arends trap. This trap takes advantage of the beetles' marked tendency to occupy available cracks and crevices. It consists of a PVC plastic pipe, 4–5 cm in diameter and 24 cm in length. Corrugated cardboard is rolled lengthwise inside the tube such that the openings face toward the open ends of the PVC tube. The tube traps are easy to make and deploy, for example every 10–15 m along poultry house walls, manure accumulations, or water lines. They are left in place for a defined period (e.g., a week) and then recovered and placed into individual plastic bags. Traps can be frozen prior to unrolling the cardboard and counting, categorizing and identifying the beetles, or the trap contents might also be processed alive if one wanted to determine infection levels. Trap counts are very useful in assessing relative activity (assumed to relate to density) of beetles from one time to another (e.g., before vs. after treatment).

Plot selection and sampling for testing a microbial agent applied as a larvicide might proceed as follows:

1. Presample the habitat (e.g., manure) to determine roughly where the larvae are. At this stage a simple, qualitative visual assessment is usually adequate.
2. Establish the plots. In many confined animal situations there might be rows or pits of manure that can be divided into sampling zones, with buffer areas in between. A randomized block design often is suitable here. Generally, one must take as many samples as is logistically feasible, if possible a minimum of 10.
3. Take the pretreatment samples. It often is advisable to take samples in pairs adjacent to each other in the habitat. One of these can be used for direct larval

extraction on a Berlese funnel, while its pair is held for adult fly emergence (Mullens *et al.*, 1996).

4. Apply the microbial agent. Some of these can be applied directly to the surface of the substrate after mixing in water (e.g., entomopathogenic nematodes). A low pressure hand garden sprayer (4–12 liters) is usually adequate for the purpose.
5. Take post-treatment samples. The sampling interval depends on the target pest, but, given the short life cycle of many flies, the initial post-treatment samples often are 2–5 days after treatment. Samples at 3, 7, 10, and 14 days would incorporate the expected duration of activity of many materials applied to a manure habitat which is receiving more manure daily. If at all possible, one should not depend solely on fly numbers to indicate “success” in control efforts, but take steps to determine if target insects have in fact been infected and/or killed by the pathogen. It is particularly useful to compare such infection levels to levels in the pretreatment period.

3 Sampling adult flies

Sampling adult flies to determine effectiveness of a microbial agent is challenging. For flies which frequent animals regularly, such as the horn fly *Haematobia irritans* or the stable fly *S. calcitrans*, counts on animals are frequently used. Exact estimates are very difficult, and quick visual estimates are more often used. With practice and comparison with more exact counts (e.g., comparing one's estimate with a count from an enlarged photo taken at the same time), fairly accurate estimates are possible. Often only one side of an animal is counted, and this number used in a relative way to assess control (e.g. before vs. after treatment). Binoculars are an aid under field conditions with pastured or range animals. Visual counts are somewhat subjective and thus, it is important to have the same individual do the counts over time if at all possible. For some flies which visit a host less frequently, such as *Stomoxys*, attractive traps have been designed which yield a relative estimate of activity. Alsynite fiberglass traps covered with adhesive, for example, are very attractive to *Stomoxys* (Broce, 1988). A good general reference on sampling arthropod pests of livestock and poultry, especially Diptera, is provided by Lysyk and Moon (1994).

Pest flies such as *M. domestica* or *Fannia* spp. do not preferentially frequent animals, although they may rest on them sometimes. Such flies typically are sampled at resting sites in the environment near the manure or decaying feedstuffs which produced them. Several sampling methods have been used (e.g., Lysyk and Axtell, 1986). It often is best to use two or more such methods, since each has biases. Methods 1, 2 and 4 below integrate numbers over time, while 3 and 5 depend heavily on weather conditions at a point in time.

1. Index cards (7.5 × 10 cm) can be tacked up in fly resting areas (often recognized by either large numbers of flies or fly regurgitation/fecal specks) within an enclosed animal house. Flies rest and leave specks behind which may be counted. If such cards are replaced at least weekly, they yield a relative estimate of fly activity. However, one is not certain what fly species left the speck, and this technique is useful only if one species predominates (e.g., *M. domestica*).
2. Sticky tapes also can be hung in fly aggregation areas, and both the number and identity of flies can be ascertained for relative comparisons of numbers over time. One also can walk through animal houses with such tapes and get an idea of fly numbers by how many adhere to the tape after a defined route has been walked.
3. Another sampling device is the Scudder grid, a device much like a picture frame which is thrown in designated areas over time to get a visual count of the number of flies which rest within the perimeter a few minutes later. Similarly, some workers have counted flies in designated resting areas (e.g., the walls of a swine barn) visually. Such areas can be outlined on a wall using a marker or chalk. In either case, numbers should be counted at the same time of day, in the same location and by the same researcher.
4. Baited traps, using attractant materials such as the house fly pheromone, z-9-tricosene (Muscalure), with a toxicant also are useful in some settings. Such traps attract and kill flies which can be counted regularly (e.g., weekly). A convenient trap can be fashioned from a 3.8 liter plastic milk jug (size is not critical, but such jugs are common in the USA). From 3 to 4 entrance holes 4–5 cm in diameter can be cut out in the top 1/3 of the jug and a small amount of toxic fly bait sprinkled in the bottom. Flies enter, feed on the bait and die there, where they can be

counted later. Such traps are best used under cover (e.g., hung from roof rafters) and care must be taken to keep animals and people from consuming the toxic bait accidentally.

5. Sweep net samples sometimes also are used to estimate relative fly numbers. If this is done, sweeps should be made in the same location for a set length of time or number of sweeps and at the same time of day to minimize the influence of fluctuating diurnal temperatures on fly activity.

Reducing adult fly numbers is usually the goal of control efforts, even though it is likely that application of a microbial agent might be directed at the immatures. The mobility of adult flies provides another major source of experimental error; adult flies taken in one area did not necessarily come from there. Further, it is dangerous to assume that fly numbers will be comparable even in enclosed poultry or swine houses of the same design and managed on the same schedule. Unanticipated factors may result in certain houses producing far higher numbers of adult flies than others. For these reasons it is desirable to sample the developmental substrate directly if possible, to attribute changes in numbers to a microbial treatment. This is particularly true if one is able to recover the microbial agent, or at least link it to dead hosts (e.g., dissecting fly larvae to determine presence of nematodes). Such samples, coupled with adult sampling, allow one to attribute control unequivocally to the treatment. It is assumed that sampling adult flies would most likely be in conjunction with discrete confined animal housing such as swine or poultry. Keep in mind that the treatment unit here is the house; replicate houses may be quite limited, and it therefore is quite critical that good pretreatment assessment be done. Sampling adult flies in conjunction with a microbial treatment might proceed as follows.

1. Determine by observation where the adult flies rest, and where they are most active in daylight hours. Placement of sticky fly tapes may help determine the species composition and distribution. If the target is a single dominant species such as *M. domestica*, spot cards (white index cards) are useful. Place at least 4–6 of these in each house; the ends of the houses often have larger numbers of adult flies. It is good to have some sampling tool to establish whether the fly species composition shifts over the course

of the study. Sticky fly tapes placed near the spot cards, placed and changed on the same schedule, are useful.

2. If the pathogen affects adults (*e.g.*, many fungi), a sweep net can be used to capture a sample of at least 100 flies (pooled from different regions of the house) to hold for determining pretreatment pathogen prevalence (*e.g.*, Six and Mullens, 1996). Establish baseline levels of fly activity (pretreatment sampling) over several days to a week.
3. If possible, pair the treatment and control houses according to the fly activity. Take the 2 houses with the highest numbers of flies, and randomly assign one to the treatment and the other to control. Repeat with the next pair of houses, etc.
4. Apply the microbial agent.
5. Take post-treatment samples at intervals similar to those used for the substrate sampling discussed above. Again, if the pathogen affects adults, a net sample of flies can be held or dissected to determine pathogen prevalence directly. If one expects activity of the pathogen might extend beyond 2 weeks (*e.g.*, retransmission from initially infected flies or excellent persistence of the agent), sampling longer may well be warranted. If at all possible, one should not depend only on estimated numbers of flies to assess efficacy, since fly numbers can easily fluctuate for reasons unconnected to the treatment. While reduced fly numbers are of course the goal, cause and effect is more convincing if there is direct evidence of fly mortality caused by the pathogen in question.

D Application methods for manure fly or beetle control

1 Application of nematodes

Entomopathogenic nematodes can be mixed and prepared for application as a fly larvicide or against beetles to manure or organic substrates using standard techniques (Kaya and Stock, 1997). Some work against *M. domestica* in wet, high nitrogen poultry manure habitats suggests nematode survival and infectivity for fly larvae are very poor (*e.g.*, Georgis *et al.*, 1987), but some subsequent work in other manure substrates is more promising (Taylor *et al.*, 1998). As discussed by Renn (1998), targeting drier portions of the manure (< 60% moisture),

where flies may move for pupation, may be a more effective strategy than trying to kill larvae directly in wetter manure. As a larvicidal application, this study had better efficacy using a direct nematode spray to swine manure compared with nematodes applied in calcium alginate capsules. However, the encapsulated nematodes also can be deployed in a bait station against adult flies. There is some laboratory (Renn, 1994) and field (Renn, 1998) evidence that such baits (design not yet published) can control flies in intensive swine units (or probably similar settings). Many confined animal facilities, especially poultry operations, are very dusty. This might be a serious problem in using a bait (*e.g.*, entomopathogenic nematodes) that must be delivered damp or wet.

Based on available literature it is difficult to recommend a specific nematode rate for larvicidal purposes. Much probably depends on the nematode strain selected and habitat characteristics. High application rates of 8–9 million/m² were insufficient to compensate for low infectivity on wet poultry manure (Mullens *et al.*, 1987), and such levels are economically prohibitive in most cases. Renn (1998) applied 0.5 million nematodes/sow stall (probably at least 2 m² of manure substrate below the pigs) twice weekly with results comparable to fly control using methomyl baits. Based on laboratory trials, Taylor *et al.* (1998) suggest that moderate numbers of *Steinernema* spp. also might be sufficient for fly control on bovine manure, but field trials remain to be done. Trials of nematodes against *A. diaperinus* have shown infectivity when applied to soil where beetles pupated, but control has been short-term (see Geden and Axtell, 1988).

2 Application of fungi

For purposes of laboratory infectivity screening, fungal conidia (Hypocreales) have been applied directly to adult or immature flies in aqueous or vegetable oil suspensions (Barson *et al.*, 1994). Some of these were highly infective at doses down to 10⁴ or 10⁵ conidia/ml. In selected cases, it might be possible to achieve some degree of control of a localized fly population with such an application. For example, one might spray adult flies in their overnight resting areas under the

roof or eaves of confined animal houses and hope to hit a significant proportion of them directly. Indeed, the tendency of flies to rest in stereotypical areas on a farm provides some reasonable hope of placing them in proximity with a pathogen that can infect on contact. Conidia of these fungi can be prepared and applied using standard methods (Goettel and Inglis, 1997; Kaufman *et al.*, 2005).

In many confined animal settings, however, rough surfaces such as wood are more likely to exist and be used by flies as resting sites. Liquid applications of fungi are less suitable on such surfaces than are dust formulations (Geden *et al.*, 1995; Watson *et al.*, 1995). It is also possible that conidia of *B. bassiana* may be deployed as an attractant/feeding dry bait combined with sucrose (Geden *et al.*, 1995). In either case, experimental trials suggest effective concentrations are quite high ($10^7 - 10^8$ conidia/cm²). In the field at a larger scale, application of such a conidial suspension still would require formulation to allow it to adhere well after spraying with standard equipment. While it also may be possible to infect some fly immatures within soil or manure substrates with fungi, it is likely that eventual use of fungi will be against adults.

For beetles, Geden and Steinkraus (2003) tested three fungi formulations. They included an "emulsifiable concentrate" with conidia suspended in water at 2×10^{11} conidia/ml, a coarsely ground granular corn bait with 8.5×10^9 conidia/g, and residue from fungal propagation, a granular material with 6.5×10^8 conidia/g. For the field trials the liquid was applied using a calibrated sprayer, the bait and residue applications were applied using a restaurant grated cheese shaker.

4 Conclusion

Much research and development has been done on microbial control agents for use in broadacre agriculture and forestry, but there has been far less work on the microbial control of livestock pests. Yet, livestock industries worldwide face serious problems of increasing pesticide resistance of livestock pests and of greater stringency of regulations limiting chemical residues in animal products. Effective, non-chemical means of pest control would meet an urgent need and

be welcomed. However, as we mentioned in the introduction to this chapter, the microbial control of livestock pests is, as yet, still in the pioneering stage, with various strategies showing promise but few fully operational programs. We hope that, in the years to come, microbial technologies for the control of pests will become an important part of livestock management.

5 References

- Alam, M. J. and Zurek, L. 2004. Association of *Escherichia coli* O157: H7 with houseflies on a cattle farm. *Appl. Environ. Microbiol.* **70**, 7578–7580.
- Alexandre, T. M., Alves L. F. A., Neves P. M. O. J., and Alves, S. B. 2006. Effect of temperature and poultry litter in *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch) virulence against the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). *Neotrop. Entomol.* **35**, 75–82.
- Axtell, R. C. and Arends, J. J. 1990. Ecology and management of arthropod pests of poultry. *Annu. Rev. Entomol.* **35**, 101–126.
- Barson, G., Renn, N. and Bywater, A. F. 1994. Laboratory evaluation of six species of entomopathogenic fungi for the control of the house fly (*Musca domestica* L.), a pest of intensive animal units. *J. Invertebr. Pathol.* **64**, 107–113.
- Broce, A. B. 1988. An improved alsynite trap for stable flies, *Stomoxys calcitrans* (Diptera: Muscidae). *J. Med. Entomol.* **25**, 406–409.
- Broce, A. B. 1993. Dispersal of house flies and stable flies. In "Rural Flies in the Urban Environment." (G. D. Thomas and S. R. Skoda, Eds.). Res. Bull. 317, Univ. of Nebraska, Lincoln, NE.
- Campbell, J. B. 1993. The economics of the fly problem. In "Rural Flies in the Urban Environment." (G. D. Thomas and S. R. Skoda, Eds.). Res. Bull. 317, Univ. of Nebraska, Lincoln, NE.
- Crawford, P. J., Brooks, W. M., and Arends, J. J. 1998. Efficacy of field-isolated strains of *Beauveria bassiana* (Moniliales: Moniliaceae) as microbial control agents of the lesser mealworm (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* **91**, 1295–1301.
- Division of Entomology Biennial Report. 1988. p. 41. CSIRO, Canberra, Australia.
- Drummond, R. O., George, J. E., and Kunz, S. E. 1988. "Control of Arthropod Pests of Livestock: A Review of Technology." CRC Press, Boca Raton, FL.
- Geden, C. 1997. Evaluation of *Paraionchium muscadomesticae* (Nematoda, Tylenchida: Iotonchiidae), a potential biological control agent of the house fly (Diptera: Muscidae). *Biol. Control* **10**, 42–47.

- Geden, C. J., Arends, J. J., and Axtell, R. C. 1987. Field trials of *Steinernema feltiae* (Nematoda: Steinernematidae) for control of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in commercial broiler and turkey houses. *J. Econ. Entomol.* **80**, 136–141.
- Geden, C. J. and Axtell, R. C. 1988. Effect of temperature on nematode (*Steinernema feltiae*) treatment of soil for control of lesser mealworm in turkey houses. *J. Econ. Entomol.* **81**, 800–803.
- Geden, C. J., Steinkraus D. C., and Rutz, A. 1993. Evaluation of two methods of release of *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) to infect house flies (Diptera: Muscidae) on dairy farms. *Environ. Entomol.* **20**, 1202–1208.
- Geden, C. J., Rutz, D. A., and Steinkraus, D. C. 1995. Virulence of different isolates and formulations of *Beauveria bassiana* for house flies and the parasitoid *Muscidifurax raptor*. *Biol. Control* **5**, 615–621.
- Geden C. J. and Steinkraus D. C. 2003. Evaluation of three formulations of *Beauveria bassiana* for control of lesser mealworm and hide beetle in Georgia poultry houses. *J. Econ. Entomol.* **96**, 1602–1607.
- Georgis, R., Mullens, B. A., and Meyer, J. A. 1987. Survival and movement of insect parasitic nematodes in poultry manure and their infectivity against *Musca domestica*. *J. Nematol.* **19**, 292–295.
- Goettel, M. S. and Inglis, G. D. 1997. Fungi: Hyphomycetes. In “Manual of Techniques in Insect Pathology.” (L. A. Lacey, Ed.) pp. 213–249. Academic Press, New York.
- Greenberg, B. 1971. “Flies and Disease.” Princeton University Press, Princeton, NJ.
- James, P. J. 1986. Current practices of blow fly control and future possibilities. In “Proceedings of the Sheep and Wool Refresher Course, Adelaide, February, 1986.” South Australian Department of Agriculture and Australian Wool Corporation..
- Johnson, P. W., Boray, J. C., and Dawson, R. L. 1990. Synthetic pyrethroid resistance *Damalinea ovis*. In “Resistance Management in Parasites of Sheep.” (J. A. McKenzie, P. J. Martin and J. H. Arundal, Eds.) p. 21. Australian Wool Corporation, Melbourne, Australia.
- Kalsbeek, V., Mullens, B. A., and Jespersen, J. B. 2001. Field studies of *Entomophthora* (Zygomycetes: Entomophthorales)-induced behavioral fever in *Musca domestica* (Diptera: Muscidae) in Denmark. *Biol. Control* **21**, 264–273.
- Kaufman, P. E., Reasor, C., Rutz, D. A., Ketzis, J. K., and Arends, J. J. 2005. Evaluation of *Beauveria bassiana* applications against the house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York State. *Biol. Control* **33**, 360–367.
- Kaya, H. K. and Moon, R. D. 1978. The nematode *Heterotylenchus autumnalis* and the face fly, *Musca autumnalis*: a field study in northern California. *J. Nematol.* **10**, 331–341.
- Kaya, H. K. and Stock, S. P. 1997. Techniques in insect nematology. In “Manual of Techniques in Insect Pathology.” (L. A. Lacey, Ed.) pp. 281–324. Academic Press, New York.
- Kuramoto, H. and Shimazu, M. 1997. Control of house fly populations by *Entomophthora muscae* (Zygomycotina: Entomophthorales) in a poultry house. *Appl. Entomol. Zool.* **32**, 325–331.
- Lund, R. D., Johnson, P. W., Gould, N. S. and van de Ven, R. J. 1998. “Improved Design and Use of Dipping Equipment for Sheep Lice Eradication.” NSW Agriculture, Trangie, NSW, Australia
- Lyness, E. W., Pinnock, D. E., and Cooper, D. J. 1994. Microbial ecology of sheep fleece. *Agric. Ecosys. Environ.* **49**, 103–112.
- Lysyk, T. J. and Axtell, R. C. 1986. Field evaluation of three methods for monitoring populations of house flies *Musca domestica* (Diptera: Muscidae) and other filth flies in three types of poultry housing systems. *J. Econ. Entomol.* **79**, 144–151.
- Lysyk, T. J. and Moon, R. D. 1994. Sampling arthropods in livestock management systems. In “Handbook of Sampling Methods for Arthropods in Agriculture.” pp. 515–538, CRC Press, Boca Raton, FL.
- Mullens, B. A. 1990. *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) as a pathogen of filth flies. In “Biocontrol of Arthropods Affecting Livestock and Poultry.” (D. A. Rutz and R. S. Patterson, Eds.) pp. 231–245. Westview Press, Boulder, CO.
- Mullens, B. A., Meyer, J. A., and Georgis, R. 1987. Field tests of insect-parasitic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against larvae of manure-breeding flies (Diptera: Muscidae) in caged-layer poultry facilities. *J. Econ. Entomol.* **80**, 438–442.
- Mullens, B. A., Hinkle, N. C., and Szijj, C. E. 1996. Impact of alternating manure removal schedules on pest flies (Diptera: Muscidae) and associated predators (Coleoptera: Histeridae, Staphylinidae; Acarina: Macrochelidae) in caged-layer poultry manure in southern California. *J. Econ. Entomol.* **89**, 1406–1417.
- Pinnock, D. E. 1994. The use of *Bacillus thuringiensis* for control of pests of livestock. *Agric. Ecosys. Environ.* **49**, 59–63.
- Pinnock, D. E., Lyness, E. W., and Cooper, D. J. 1994. Microbial control by colonisation: the development of *Bacillus thuringiensis* for control of sheep ectoparasites. In “Proceedings of the Second Canberra Meeting on *Bacillus thuringiensis*.” (R. J. Akhurst, Ed.) pp. 153–158. CSIRO, Canberra, Australia.
- Renn, N. 1994. A comparison of baits containing different isolates of entomopathogenic nematodes for the control of *Musca domestica* using a large-scale

- laboratory facility. *Proc. Brighton Crop Prot. Conf.* pp. 1061–1066.
- Renn, N. 1995. Mortality of immature houseflies (*Musca domestica* L.) in artificial diet and chicken manure after exposure to encapsulated entomopathogenic nematodes (Rhabditidae: Steinernematidae, Heterorhabditidae). *Biocontrol Sci. Technol.* **5**, 349–359.
- Renn, N. 1998. The efficacy of entomopathogenic nematodes for controlling housefly infestations of intensive pig units. *Med. Vet. Entomol.* **12**, 46–51.
- Renn, N., Bywater, A. F., and Barson, G. 1999. A bait formulated with *Metarhizium anisopliae* for the control of *Musca domestica* L. (Dipt., Muscidae) assessed in large-scale laboratory enclosures. *J. Appl. Entomol.* **123**, 309–314.
- Renn, N. and Wright, E. 2000. The effect of artificial substrates on the pathogenicity of *Steinernema feltiae* (Rhabditida: Steinernematidae) to adult *Musca domestica*. *Nematology* **2**, 217–222.
- Safrit, R. D. and Axtell, R. C. 1984. Evaluations of sampling methods for darkling beetles (*Alphitobius diaperinus*) in the litter of turkey and broiler houses. *Poultry Sci.* **63**, 2368–2375.
- Six, D. L. and Mullens, B. A. 1996. Seasonal prevalence of *Entomophthora muscae* and introduction of *Entomophthora schizophorae* (Zygomycotina: Entomophthorales) in *Musca domestica* (Diptera: Muscidae) populations on California dairies. *Biol. Control* **6**, 315–323.
- Smallridge, C. J., Cooper, D. J., and Pinnock, D. E. 1995. The effect of the microsporidian *Octospora muscaedomesticae* on adult *Lucilia cuprina* (Diptera: Calliphoridae). *J. Invertebr. Pathol.* **66**, 196–197.
- Stafford, K. C., III and Bay, D. E. 1987. Dispersion pattern and association of house fly, *Musca domestica* (Diptera: Muscidae), larvae and both sexes of *Macrocheles muscaedomesticae* (Acari: Macrochelidae) in response to poultry manure moisture temperature, and accumulation. *Environ. Entomol.* **16**, 159–164.
- Stafford, K. C., III and Bay, D. E. 1994. Dispersion statistics and sample size estimates for house fly (Diptera: Muscidae) larvae and *Macrocheles muscaedomesticae* (Acari: Macrochelidae) in poultry manure. *J. Med. Entomol.* **31**, 732–737.
- Steenberg, T., Jespersen, J. B., Jensen, K.-M. V., Nielsen, B. O., and Humber, R. A. 2001. Entomopathogenic fungi in flies associated with pastured cattle in Denmark. *J. Invertebr. Pathol.* **77**, 186–197.
- Steinkraus, D. C., Geden, C. J., and Rutz, R. A. 1992. Susceptibility of lesser mealworm (Coleoptera: Tenebrionidae) to *Beauveria bassiana* (Moniliales: Moniliaceae): effects of host stage, substrate, formulation and host passage. *J. Med. Entomol.* **28**, 314–321.
- Steinkraus, D. C., Geden, C. J., and Rutz, D. A. 1993. Prevalence of *Entomophthora muscae* (Cohn) Fresenius (Zygomycetes: Entomophthorales) in house flies (Diptera: Muscidae) on dairy farms in New York and induction of epizootics. *Biol. Control* **3**, 93–100.
- Strother, K. O. and Steelman, C. D. 2001. Spatial analysis of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in broiler production facilities. *Environ. Entomol.* **30**, 556–561.
- Strother K. O., Steelman C. D., and Gbur E. E. 2005. Reservoir competence of lesser mealworm (Coleoptera: Tenebrionidae) for *Campylobacter jejuni* (Campylobacterales: Campylobacteraceae). *J. Med. Entomol.* **42**, 42–47.
- Szalanski, A. L., Owens, C. B., McKay, T., and Steelman, C. D. 2004. Detection of *Campylobacter* and *Escherichia coli* O157: H7 from filth flies by polymerase chain reaction. *Med. Vet. Entomol.* **18**, 241–246.
- Taylor, D. B., Szalanski, A. L., Adams, B. J., and Peterson, R. D. II. 1998. Susceptibility of house fly (Diptera: Muscidae) larvae to entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae). *Environ. Entomol.* **27**, 1514–1519.
- Vaughan, J. A., Turner, E. C., Jr., and Ruszler, P. L. 1984. Infestation and damage of poultry house insulation by the lesser mealworm, *Alphitobius diaperinus* (Panzer). *Poultry Sci.* **63**, 1094–1100.
- Watson, D. W., Geden, C. J., Long, S. J., and Rutz, D. A. 1995. Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). *Biol. Control* **5**, 405–411.
- Watson, D. W., Rutz, D. A. and Long, S. J. 1996. *Beauveria bassiana* and sawdust bedding for the management of the house fly, *Musca domestica* (Diptera: Muscidae) in calf hutches. *Biol. Control* **7**, 221–227.
- Watson, D. W., Guy, J. S., and Stringham, S. H. 2000. Limited transmission of turkey coronavirus in young turkeys by adult *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *J. Med. Entomol.* **37**, 480–483.
- West, L. S. 1950. "The Housefly." Cornell Univ. Press, Ithaca, NY.
- Wilkinson, F. C. 1986. Economics of sheep lice control. In "Proceedings of the Sheep and Wool Refresher Course, Adelaide, February, 1986". pp. 70–74 South Australian Department of Agriculture and The Australian Wool Corporation, Adelaide, Australia.
- Zumt, F. 1973. "The Stomoxysine Biting Flies of the World." Gustav Fisher Verlag, Stuttgart, Germany.