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# Biological control of gastrointestinal nematodes and liver fluke in sheep and cattle

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# ABSTRACT

Options for the biological control of gastrointestinal nematode parasites and liver fluke of sheep and cattle were reviewed and although numerous natural enemies exist few offer potential for development as biological control agents. Toxins of *Bacillus thuringiensis* show promise for the possible future control of adult and larval stages of nematodes by genetic manipulation of pasture plants, gut microflora or faecal bacteria provided technical issues of production and delivery can be overcome. The nematophagous fungi *Duddingtonia flagrans* has been shown to reduce nematode larvae in faeces and is currently the most promising option for biological control since commercial development is being pursued by a number of research groups. Several other fungal species also show great potential for nematode control but require the development of innovative technologies to enhance survival after gut passage before practical application can occur. Research on potential biological agents for the control of liver fluke infection has focused on pathogens of the intermediate host Lymnaeid snails. Elimination of these snails may be possible through the regular inundative release of specific pathogens in fluke prone areas once methods for mass rearing are developed but environmental consequences on non-target snail species will need to be closely monitored.

# EXECUTIVE SUMMARY

Gastrointestinal nematode parasitism and liver fluke infection continue to be major problems to sheep and cattle producers in Australia due to their detrimental effects on animal production and the high cost of control measures. The rising incidence of resistance to anthelmintic chemicals and the increasing demand by consumers for livestock products free of chemical inputs has revived interest in the identification and application of non-chemotherapeutic means of control. With this in mind, options for the biological control of nematode parasites and liver fluke in sheep and cattle were reviewed.

Natural pathogens of the parasitic stages of nematodes of sheep and cattle have not yet been identified. A proposed novel approach to the control of parasitic stages by biological means requires the genetic manipulation of pasture plants or gut micro-organisms to deliver toxins, such as nematocidal *Bacillus thuringiensis* crystal proteins, to predilection sites within the host. Ethical and regulatory concerns will also need to be addressed for this approach to succeed. Numerous antagonists of the free-living stages of nematode parasites have been identified in pasture systems that favour nematode survival. Dung beetles, earthworms, predacious free-living nematodes, microarthropods and fungi have been shown to reduce numbers of nematode larvae emerging from faeces and infecting pasture and agricultural practices that conserve or enhance natural populations of these predators and pathogens will continue to assist in controlling larval populations on pasture.

Methods for the applied biological control of nematode parasites of livestock are currently being investigated. For the foreseeable future, the greatest potential seems to be through the inundative release of specific pathogens to reduce or eliminate larval stages in or near deposited faeces. Of the possible pathogenic agents identified in the review, the following three offer the greatest potential for development and application in Australian livestock systems.

***Bacillus thuringiensis*** – genetic manipulation of bacteria that colonize the gut and/or faecal material to include nematocidal *B. thuringiensis* toxins should enable reduction of the free-living larval stages of parasitic nematodes that feed on bacteria in faeces. Unfortunately, this approach may encounter problems similar to those faced by any other genetically modified organism proposed for use in agriculture.

***Arthrobotrys spp***. – recent research overseas suggests that this aggressive and effective trapper of parasitic nematode larvae deserves detailed assessment and laboratory selection of isolates with increased ability to survive gut passage. Selected isolates should then be evaluated for production of conidia/fungal biomass using methods established for other *Arthrobotrys* spp. In addition, investigation of methods developed for protecting other microbiologicals and nutrients from digestion and degradation should indicate their potential for application to *Arthrobotrys* spp. (and possibly other fungi like the egg-parasitic *Paecillomyces* spp.) for use in biological control of nematode parasites of livestock.

***Duddingtonia flagrans*** - The concentrated effort currently being directed towards *D. flagrans* will probably lead to availability of products containing this fungus in Australia within the next 2 to 3 years. Critical to the application of *D. flagrans* under industry conditions will be testing and development of strategies to maximise the effectiveness of this means of control including studies of duration and timing of application and integration with other control alternatives. Determination of the environmental impact of inundative application of *D. flagrans* will also be essential before widespread use can be recommended.

It appears likely that there will be at least one agent for biological control of nematode parasites of sheep and cattle in the near future, i.e. *D. flagrans*. The other potential biological control agents described require further development, and research should continue on these to increase the options available for application in Australian livestock production systems. It should also be emphasised that successful biological control of nematode parasites may not

require elimination of the entire parasite population, although this could be possible over time if a highly effective agent was identified. A more realistic scenario would include the strategic use of the biological control agent to lower nematode populations below the threshold where livestock production is affected by parasitism while retaining sufficient larval challenge to stimulate host immunity to infection. Careful selection and integration of all appropriate options for control (chemotherapy, grazing management, enhanced immunity by improved nutrition or genetic selection) are essential elements in the production of sheep and cattle in areas where nematode parasites are a problem. The successful development of biological control agents will add to this arsenal and may reduce the current reliance on anthelmintic chemicals.

No options for the biological control of fluke within the host have been identified and possibilities for the control of fluke eggs and larvae in faecal material remain speculative at the present time. The search for biological agents for potential application to control *Fasciola* spp. infection in livestock therefore rests with identified pathogens of the intermediate host Lymnaeid snails. Many of these pathogens have been observed to occur in native snail populations and would already exert some regulatory effect on those populations. Elimination of Lymnaeid snails may be possible within a limited area through inundative release of specific pathogenic organisms in fluke prone areas once methods for mass rearing are developed but environmental consequences on non-target snail species will need to be closely monitored. Overall, the prospect for the biological control of liver fluke infection in livestock is poor and it is expected that livestock producers will need to rely on currently recommended strategies for control for the foreseeable future.

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# 1. INTRODUCTION

Gastrointestinal nematodes and liver fluke are common problems in domestic sheep and cattle due to their impact on liveweight gain, wool growth, lactation, reproductive performance and the cost of control measures. The intensification of sheep and cattle production in Australia has resulted from the introduction of improved pasture species and increased use of fertilizers to promote pasture growth. Unfortunately, the greater stocking densities made possible through these agronomic advances have led to increases in the prevalence of helminth infections. Since the 1960's, the regular appearance and availability of a number of effective anthelmintic chemicals provided a solution to this problem. During the last ten to fifteen years however, it has become increasingly apparent that the identification and development of "new" anthelmintic compounds is not keeping pace with the rate of emergence of helminth strains resistant to chemotherapy (Hennessy, 2000). At present there are strains of sheep nematodes in Australia resistant to most of currently available anthelmintics (Besier and Love, 2003). Although probably not as widespread, a similar situation exists for resistance of strains of *Fasciola hepatica* to fasciolicide anthelmintics (Boray, 1990; Overend and Bowen, 1995). Overseas, anthelmintic resistance has been reported in parasitic nematodes of cattle (Jackson, 1993; Vermunt et al., 1995, 1996; Coles et al., 2001; Eddi et al., 2002) and although there are only two reports of resistance in cattle nematodes in Australia (Eagleson and Bowie 1986; Eagleson et al, 1992) it is likely that further resistant strains could develop in the near future.

With the reduced efficacy of anthelmintic compounds there has been increasing interest in reducing the reliance on chemotherapeutic means of controlling helminth parasites in livestock. Concerns about environmental toxicity and chemical residues in animal products have also stimulated activities aimed at reducing chemical use in livestock production and other agricultural practices. Possible alternatives for non-chemotherapeutic control of helminth parasites fall into five broad categories: vaccination, grazing management, strategic nutritional supplementation, breeding for resistance and biological control. Strategies involving manipulation of the host and its environment, as described by the first four of these categories, could theoretically be included in an assessment of biological options for control of helminth parasites but this will not be attempted here to enable concentration on biological control options which focus on direct antagonists of the parasites themselves. Nematode parasites and the liver fluke, *Fasciola hepatica,* will be reviewed separately since potential biological control options differ considerably between these two types of parasitic helminths.

## 1.1 Definition

For the purpose of this review biological control is defined as "the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it otherwise would be" (Eilenberg et al., 2001). This may involve processes to enhance the growth or reproduction of natural antagonists of the pest in its normal habitat or may require the introduction of a foreign antagonistic species into that habitat. The four different strategies for biological control applicable to agricultural systems are defined in Table 1.

Table 1 Different strategies for biological control as defined by Eilenberg et al. (2001)

| Strategy | Definition | Example |
|---|---|---|
| Classical biological control | The intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control | The introduction of *Cactoblastis cactorum* moths to control *Opuntia* spp. cactus in Australia. |
| Inoculation biological control | The intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently. | The introduction of Myxoma virus for control of rabbit populations in Australia. |
| Inundation biological control | The use of living organisms to control pests when control is achieved exclusively by the released organisms themselves. | The use of *Metarhizium* spp. or *Bacillus thuringiensis* for the control of insect pests. |
| Conservation biological control | Modification of the environment or existing practices to protect and enhance specific natural enemies of other organisms to reduce the effects of pests. | Preparation or preservation of buffer zones around/between crops so the natural enemies of pests can multiply and emigrate into the crops. |

# 2. GASTROINTESTINAL NEMATODE PARASITES

The major pathogenic nematodes of sheep and cattle have a direct life cycle with a preparasitic stage on pasture and the parasitic stage in the host. Eggs passed in the faeces hatch to produce the larvae ($L_1$) which feed on bacteria, grow and then moult to progressively become $L_2$ and $L_3$ when the sheath is retained for protection against desiccation. The $L_3$ are the infective stage and do not feed but migrate from the faeces onto nearby herbage to await ingestion by a suitable host. Once inside the host, the protective sheath is cast off and the larvae are transported with the digesta to the predilection site where they remain to grow and become adult. Eggs produced by adult worms are voided into the digesta and are eventually passed in the faeces. For *Bunostomum* spp. and *Strongyloides* spp. infection is by larval penetration of the skin and migration to the predilection site via the blood stream and lungs.

In general, attempts at biological control of nematode parasites of livestock have focussed on

methods to reduce the numbers of free-living infective stages on pasture. Modification of rumen micro-organisms to enhance negative interactions with infective nematode larvae and preclude larval establishment in the host has been suggested (Waller and Faedo, 1996), but up to the present time this approach remains purely speculative and non-chemotherapeutic control of nematode species within the host remains entirely reliant on intrinsic host mechanisms. External to the host, there are numerous antagonists of both parasitic and free-living nematodes inhabiting the soil-pasture interface but few of these have been investigated as potential biological control agents. The majority of investigations into nematode antagonists have focussed on possible agents for the control of plant parasitic nematodes which cause production losses in agronomic and horticultural situations. For the purpose of this review evidence will be drawn from these studies particularly where examples of potential biological control agents do not exist for nematode parasites of livestock.

## 2.1 Natural antagonists of nematodes.

### *2.1.1 Opportunistic predators*

There is a broad range of invertebrate organisms that inhabit agricultural areas where livestock are produced. Pastures and faecal matter deposited by livestock provide a rich natural habitat for many species of arthropods and other invertebrates that may prey on parasitic nematode larvae of livestock. Abundance and diversity of these organisms is often dependent on the soil type and available pasture species as well as prevailing seasonal conditions. Some organisms that are known to have direct or indirect effects on parasitic nematode populations are dealt with below in detail. Of the remaining invertebrates, potential predators would largely be opportunistic rather than selective feeders on parasitic nematodes and include protozoans, turbellarians, tardigrades and various arthropods (Small 1988; Stirling, 1991). Of the arthropods, Collembola (springtails) and Acarina (mites) are usually abundant in healthy pastures and *in vitro* tests have shown them to be voracious predators of nematode eggs and larvae (Lysek, 1963; Epsky et al., 1988; Osman et al, 1988; Gilmore and Potter, 1993; Abou-Awad et al., 2001). Field observations also indicate significant effects of increased Acarina and Collembola populations on plant parasitic nematodes (Gilmore, 1970; Bund, 1972; Walia and Mathur, 1997). Recent evidence suggests that Collembola invade faecal matter within 7 days after deposition on pasture (Knox et al., 2002) and this would put Collembola populations in contact with nematode parasite larvae if any were emerging from the faecal mass. Other arthropod and invertebrate species observed in this and other studies may also prey on parasitic nematodes but very little published information is available on direct effects of these organisms.

### *2.1.2 Dung beetles*

In situations where large grazing herbivores are abundant, dung beetles can have a major impact on the breakdown of faecal material after deposition on the herbage. Dung beetles are coprophagous and quickly destroy the faecal pat in their foraging or during burial of balls of faeces in the ground with their eggs for consumption by larval stages (Dymock, 1993). In the USA, dung beetle activity has been shown to reduce the numbers of infective nematode parasite larvae on, or near, cattle faecal pats (Fincher, 1973). Subsequent study showed that calves grazing pastures with increased dung beetle populations had lower worm burdens than calves grazing a similar pasture with natural dung beetle populations (Fincher, 1975). This probably occurs through increased dessication of the remaining faecal material and/or by reducing the viability of buried eggs and larvae. In Australia, 45 species of exotic dung beetles were introduced between 1968 and 1982 to increase the rate of breakdown of cattle faeces to control native bush flies and the buffalo fly which breed in faeces (Dymock, 1993). Bryan (1972) showed that activity of the introduced species *Onthophagus gazella*, reduced nematode larvae numbers by up to 93% on pasture plots but suggested that seasonal variations in soil moisture may enable larval survival during wetter periods. This was confirmed in later studies

where it was observed that buried larvae could survive for up to 84 days during moist climatic conditions (Bryan, 1976). In support of this, recent studies by Waghorn et al (2002) showed in New Zealand that burial of faeces enabled greater numbers of *Ostertagia circumcincta* larvae to survive and migrate onto pasture than from unburied faeces. It is concluded that dung beetles can assist in the biological control of nematode parasites of livestock but the extent of control may be dependent on seasonal conditions and the potential for increasing control is limited.

### *2.1.3 Earthworms*

Earthworms are a very important part of most pasture systems where livestock are produced since they have a major impact on organic matter breakdown and recycling of nutrients for plant use (Russel, 1973). In burrowing through the soil in search of food, earthworms also aerate the soil and promote better penetration of water after rainfall. Animal faeces provides an excellent nutritional resource for earthworms and activity in or near faecal pats helps in the decomposition of faeces and its disappearance from pasture. Experiments in Denmark have shown that *Ostertagia ostertagi* eggs in cattle faeces can survive passage through the earthworm gut (Grønvold 1979). It has also been shown that numbers of *O. ostertagi* larvae were reduced on faecal pats on pasture possibly through reductions in egg viability by earthworm activity (Grønvold, 1987). Recent evidence indicated a significant reduction in larval numbers due to the presence of earthworms in controlled studies with *O. circumcincta* in sheep faeces in New Zealand (Waghorn et al., 2002). It appears likely that farming practices which encourage the proliferation of earthworms in grazing systems may therefore have indirect benefits in reducing the survival of nematode parasite larvae and thereby impact on nematode burdens in grazing livestock.

### *2.1.4 Predacious Nematodes*

Predatory nematodes occur in situations where protozoa, microarthropods, free-living and plant parasitic nematodes and other prey are found. Four main groups of predatory nematodes have been identified (Monochilidae, Dorylaimidae, Aphelenchidae and Diplogasteridae) and they differ in their feeding mechanisms and food preferences (Small, 1988; Stirling, 1991). Nematode predation is effected by specialised mouthparts for grasping or spearing the prey or through the injection of toxins which paralyse the prey before consumption. Little is known of the impact of predatory nematodes in pasture systems and it is considered unlikely that they will have a significant role in the biological control of parasitic nematodes of livestock in the near future. There has, however, been renewed interest in the development of predacious nematodes for the control of horticultural pests that may lead to future utilisation of these organisms in biological control (S. Jain, pers. comm.).

### *2.1.5 Bacteria*

#### 2.1.5.1 *Pasteuria penetrans*

A considerable amount of research has focussed on *Pasteuria penetrans* as a potential biological control agent of plant parasitic nematodes, particularly *Meloidogyne* spp. and *Pratylenchus* spp. (see reviews by Sayre and Starr, 1988 and Stirling 1991). *P. penetrans* is an obligate parasite with endospores that persist in the soil in a wide range of environmental extremes (Stirling, 1991). The highly host specific spores attach to juvenile stages of the nematode reducing their infectivity to plant species. After the nematode enters the plant root, the spores germinate and vegetative stages develop that differentiate into endospores which fill the body cavity and prevent nematode reproduction (Poinar and Hansen, 1985). Although this bacteria has many characteristics essential to a successful biological control agent, difficulties in artificial mass culture (Williams et al, 1989) and overly sensitive host specificity (Stirling 1991) will need to be overcome before it can be used extensively for plant parasitic nematode control. With regard to the efficacy of *P. penetrans* against parasitic nematodes of livestock, attempts to infect larvae of sheep nematodes were unsuccessful with no evidence of spore attachment to the cuticle being observed (Waller and Faedo, 1996; Mendoza de Gives et al., 1999b).

### 2.1.5.2 *Bacillus thuringiensis*

*Bacillus thuringiensis* is a widely used microbial pest control agent with activity against a broad range of arthropod and nematode species (Lacey et al., 2001). A number of *B. thuringiensis* isolates, each with differing specificity and toxin production capability, have been commercially developed for application in agriculture and in the USA alone some 200 products have been registered (Schnepf et al., 1998). Pesticidal activity occurs through release of proteinaceous exotoxins by intact bacteria into their immediate environment or by endotoxin release after bacterial cell lysis. Application of *B. thuringiensis* for pest control may be via formulations for inundative delivery (suspensions, wettable powders, tablets, microencapsulation), development of transgenic plants that express toxins to specific pests or through genetic manipulation of toxin production capacity into endophytic bacteria (Schnepf et al., 1998; Lacey et al., 2001). Strains of *B. thuringiensis* have been shown to have activity against the plant parasitic nematodes *Meloidogyne incognita*, *Rotylenchus reniformis* and *Pratylenchus penetrans* (Zuckerman et al., 1993; Zuckerman et al, 1995) but up to the present time no commercial products have been developed for on-farm application. Activity of *B. thuringiensis* toxins has also been established against the free-living nematodes *Caenorhabditis elegans, Pristionchus pacificus, Panagrellus redivivus, Acrobeloides* spp and *Distolabrellus veechi* and the rodent parasitic nematode *Nippostrongylus brasiliensis* (Wei et al, 2003).

*Bacillus thuringiensis* has also been shown to affect the free-living stages of nematode parasites of livestock. Ciordia and Bizzell (1961) showed that by adding a commercial preparation of *B. thuringiensis* to fresh cattle faeces *Cooperia* spp and *O. ostertagi* could be eliminated after 10 days. Further studies indicated that toxins produced by *B. thuringiensis israelensis* could kill eggs and larvae of *Trichostrongylus colubriformis* (Bone et al., 1985, 1986, 1987; Bottjer et al., 1985, 1987) but *in vivo* testing of commercial preparations were not successful (Bone et al., 1989). In China, intra-venous or intra-muscular injection with *B. thuringiensis* crystal protein resulted in >90% reduction in faecal egg counts in *Haemonchus contortus* infected goats (Yao et al., 2000). A study in Mexico has also shown *B. thuringiensis* toxins can reduce the viability of *H. contortus* eggs and larvae in vitro (Lopez-Arellano et al., 2002). Recent interest in *B. thuringiensis* in Australia has led to the identification of toxins for possible development for control of ectoparasites of livestock and new isolates with potential for biological control of buffalo fly, sheep blowfly and sheep lice (Gough et al., 2002). Other workers at the same laboratory (CSIRO Livestock Industries, Indooroopilly, Australia) are presently investigating a number of *B. thuringiensis* toxins for potential anthelmintic activity and have identified two *B. thuringiensis* strains showing significant toxicity towards the larval stages of *H. contortus* and *T. colubriformis* (Kotze, personal communication). Once suitable toxins are identified, further development of specific *B. thuringiensis* isolates for the purpose of biological control could be initiated but this would depend on the ability to deliver intact *B. thuringiensis* to the faecal pat or pellet to destroy nematode eggs and larvae. Alternatively, toxin delivery could occur through genetic manipulation of pasture plants or bacteria that inhabit the gastrointestinal tract and/or those that colonize faecal material. This may enable the recombinant organism to produce/release *B. thuringiensis* toxins into the gastrointestinal tract and eliminate larval and adult stages or could possibly target the free-living stages of parasitic nematodes that feed on bacteria in faeces. Recently, Wei et al (2003) demonstrated that the health and development of *N. brasiliensis* larvae were compromised by feeding with *Escherichia coli* that were cloned to express three particular *B thuringiensis* crystal proteins. Technical processes enabling these options to be further developed for the control of livestock parasites may require considerable research.

### 2.1.5.3 Other bacteria

Other bacteria have been identified which produce compounds that have nematocidal activity and a notable example is the highly effective avermectin anthelmintics derived from the

actinomycete *Streptomyces avermitilis*. Isolates of *Agrobacterium radiobacter* and *Pseudomonas* spp. have been suggested to have potential as biological control agents of plant parasitic nematodes (Kerry, 2000). An isolate of *Bacillus subtilis* has been shown to exhibit pesticidal activity against insect and nematode infestations (Germida et al., 2000) while *Streptomyces dicklowii* has shown similar activity (Zuckerman et al., 1996). These bacteria and others have been suggested to have potential to be developed for application as biological control agents of various arthropod and nematode pests but their potential for application against livestock parasites has yet to be evaluated.

### *2.1.6 Viruses*

Of the large number of possible viral control agents of pests, the baculoviruses offer the greatest potential due to their efficacy and specificity against pest insects (Lacey et al., 2001). This has resulted in the development of a number of naturally occurring and genetically improved recombinant baculoviruses for the effective control of insect pests in agriculture and forestry (Inceoglu et al., 2001; Lacey et al., 2001). Efficacy of these insect pathogens against parasitic nematodes of livestock has not been reported but may warrant investigation. Viral pathogens have been suggested to occur in plant parasitic nematodes (Stirling, 1991) and probably exist in parasitic nematodes of livestock but little or no information exists of possible pathogenic strains that could be used for biological control applications. The technical problems involved in the identification of microscopic nematodes with viral infections prior to isolation of potential pathogenic strains have meant that very little research has been conducted on this subject.

### *2.1.7 Fungi*

There is a diverse range of micro-fungi that utilise nematodes as a food source in soil and associated live, dead and decaying plant matter (Barron, 1977; Gray 1985). Nematode parasite eggs deposited with faeces and larvae emerging from faecal material may be susceptible to the pathogenic effects of these fungi. There are three broad groupings of nematode-destroying fungi (Barron, 1977; Stirling, 1991):

**a) Nematode-trapping fungi or predatory fungi** produce trapping organs of various types on their vegetative mycelia such as sessile and stalked adhesive knobs and branches, simple and complex three-dimensional adhesive traps and constricting and non-constricting rings. When a nematode encounters a trap and becomes ensnared, the fungal hyphae penetrate the cuticle, induce paralysis of the nematode and then invade and consume the nematode using trophic hyphae (Gray, 1988; Murray and Wharton, 1990). There is some evidence to suggest that activity of nematodes near these fungi may stimulate or enhance trap formation (Jansson and Nordbring-Hertz, 1980; Grønvold, 1989) and that at least some trapping fungi use chemical attractants to increase the chance of nematode capture (Jansson and Nordbring-Hertz, 1979).

**b) Endoparasitic fungi** have a limited saprophytic phase and are often obligate parasites that infect their nematode host by spores that attach to the cuticle or are consumed by the nematode when feeding (Gray 1988; Stirling 1991). Upon infection these fungi grow and consume the body contents of the nematode and produce infective spores that are released as the nematode body breaks down or may appear outside the cuticle via evacuation tubes or conidiophores for spore release.

**c) Fungal parasites of eggs and cysts** are a diverse group of fungi that invade nematode eggs and cysts of root-knot nematodes by penetration with vegetative hyphae (Morgan-Jones and Rodriguez-Kabana, 1988). Some species in this taxonomically and ecologically broad group are opportunistic invaders, normally being soil saprophytes, but other species are quite host-specific (Stirling, 1991).

### 2.1.7.1 Fungal agents for biological control of plant parasitic nematodes.

Despite the considerable effort devoted to the development and application of fungal agents for the control of plant parasitic nematodes these efforts have largely gone unrewarded (see reviews by Kerry 1987; Stirling 1991) with only two commercial products noted in a recent review (Butt et al., 2001). Application of fungal agents in field situations have often produced inconsistent results when compared to laboratory and small-scale field trials even when commercial preparations have been used. Possible reasons for this inconsistency include poor competition with other soil biota, poor germination due to fungi stasis and lysis in some soils and inappropriate selection of innately saprophytic species over predatory species of fungi (Stirling 1988). Technical issues in production, storage and delivery have also hindered successful product development and application (G.R. Stirling, pers. comm.). More recently, work has concentrated on the interactions of fungal agents within the plant rhizosphere and this approach may offer more optimism for future applications of possible fungal agents for the biological control of plant parasitic nematodes (Kerry, 2000).

### 2.7.1.2 Potential fungal agents for biological control of livestock parasites.

The use of nematophagous fungi for the biological control of nematode parasites of livestock was first investigated in a study where a pasture plot treated with spores of *Dactylella bembicoides, Dactylella ellipsospora* and *Arthrobotrys oligospora* reduced nematode infections in two lambs grazed on those plots when compared to similar lambs from a fungi free control plot (Roubaud and Deschiens, 1941). No follow up to this study has been reported and it is assumed that the prospect of treating pasture areas with large quantities of fungus precluded continuation of this approach on economic and/or technical grounds.

The observations that *Acrostalagmus* (syn. *Verticillium*) spp. (Parnell and Gordon, 1963) and *A. oligospora* (Soprunov, 1966) caused reduced numbers of nematode larvae in faecal culture showed that there was potential to control parasitic nematodes if sufficient fungal spores could be present in faecal material when voided by the host animal. In doing this, the fungi would have considerable advantage in establishment and entrapment of nematode larvae in the faeces prior to invasion by other fungi and fungal antagonists from the surrounding soil or pasture. Inclusion of the fungal spores in the animal's diet would enable this to occur provided the spores are able to survive gastrointestinal transit. A number of fungal species are known to utilise parasitic nematode larvae of livestock as a source of nutrients.

#### *2.1.7.2.1 Arthrobotrys spp.*

Early experiments indicated that *A. oligospora* could trap and destroy *Trichostrongylus axei* and *O. ostertagi* of cattle (Pandey, 1973) and *H. contortus* of sheep (Virat and Peloille, 1977). More intensive studies commenced in the 1980's in Denmark with in vitro studies on agar showing that *A. oligospora* readily trapped *O. ostertagi, Cooperia oncophora* from cattle*, Cooperia curticei* and *H. contortus* from sheep, *Cyathostoma* spp. from horses and *Oesophagostomum* spp. from pigs (Nansen et al., 1986; 1988). Further studies established that by combining conidia or mycelia of *A. oligospora* with cattle faeces a significant reduction in the numbers of *O. ostertagi* and *C. oncophora* larvae occurred in faecal cultures (Grønvold et al., 1985) or in herbage near faecal pats on pasture (Grønvold et al., 1987; 1988; 1989). In the latter study worm burdens in grazing calves were also shown to be 37% lower in pasture plots where fungi treated faecal pats had been distributed compared to plots where untreated faeces had been placed.

Contrary to the observations of *Arthrobotrys* spp. surviving gastrointestinal transit and germinating in faecal material (Gruner et al., 1985; Hashmi and Connan, 1989; Larsen et al., 1994; Araujo et al., 1996; Manueli et al., 1999; Chandrawathani 1998; 2002; Saumell et al., 1999; Sanyal, 2000c) the *A. oligospora* strain used in the Danish studies did not survive passage through the alimentary tract of cattle, goats and pigs (Grønvold et al., 1993). This led to the development of an *in vitro* stress selection process to identify strains of fungi that could survive transit through the gut of livestock based on standard methods for nutritional evaluation of ruminant forages (Larsen et al., 1991). This process yielded 6 viable isolates of *Arthrobotrys* spp. and 7 viable isolates of *Duddingtonia flagrans* after treatment whereas spores of *Harposporium anguillulae*, *Drechmeria coniospora*, *Verticillium* spp., *Dactylella* spp. and *Monacrosporium* spp. did not remain viable. Further assessment of the surviving strains by passaging them through calves confirmed the *in vitro* result and suggested that *D. flagrans* was likely to be the better candidate for development as a biological control agent due to greater survival through the gut and substantial ability to trap larvae (Larsen et al., 1992).

Similar studies to those described above were conducted in Australia where initial screening of 94 species of nematophagous fungi (from the Centraalbureau Voor Schimmelcultures, Baarn, The Netherlands) for trapping ability identified 10 species with comparable activity to *A. oligospora* (Waller and Faedo, 1993). Of these 4 *Arthrobotrys* (*A. javanica, A. oligospora, A. oviformis, A. polychphala*), 2 *Geniculifera* (*G. bogoriensis, G. eudermata*) and 2 *Monacrosporium* (*M. rutgeriense, M. thaumasium*) species were then subjected to selection for survival of gastrointestinal passage through ruminant livestock and *A. oligospora, A. oviformis* and *G. eudermata* were shown to have potential for further development for biological control (Waller et al., 1994). A number of nematophagous species including 6 *Arthrobotrys* spp. had been previously observed to occur in Australia (McCulloch, 1977) and a survey of compost, soil and fresh and aged faecal samples enabled isolation of *Arhtrobotrys* spp, *Geniculifera* spp, *Monacrosporium* spp, *Dactylaria* spp and *D. flagrans* along with several endoparasitic species (Larsen et al., 1994). Since this time, Australian research has concentrated on the development of local *D. flagrans* isolates for biological control of nematode parasites of livestock.

A number of other studies have established that *Arthrobotrys* spp. effectively control the larval stages of nematode parasites of livestock. In vitro assessments have been reported for *H. contortus* (Mendoza de Gives et al., 1992, 1994, 1999a; Sanyal 2000d; Flores Crespo, 2001), *Haemonchus placei* (Araujo et al., 1993,1994; Gomes et al., 2000), *Cooperia punctata* (Araujo et al., 1998; Gomes et al., 2001), *T. axei* (Mendoza de Gives et al., 1999a), *O. ostertagi* (Xu et al., 1999) and *O. circumcincta* (Mendoza de Gives et al., 1999a). After feeding *Arthrobotrys* spp. conidia to livestock, numbers of *H. placei* (Araujo et al, 1996), *C. punctata* (Araujo et al, 1998) and *H. contortus* (Sanyal, 2000d; Flores Crespo et al., 2001; Chauhan et al., 2002) larvae have been significantly reduced. It has also been proposed that the application of *Arthrobotrys*

spp. to areas where *Strongyloides* spp. are a problem in intensively reared livestock production systems may reduce infection rates since in vitro studies indicate substantial reduction in *Strongyloides* spp. can be achieved with *Arthrobotrys* spp. (Mendoza de Gives and Vasquez Prats, 1994; Chandrawathani et al., 1998; Gonzales et al., 1998; Sanyal, 2000b).

Little development of *Arthrobotrys* spp. for the biological control of parasitic nematodes has occurred beyond the studies described above. This is largely due to the apparent low survival rates of this fungi during passage through the gastrointestinal tract of livestock and the high numbers of conidia required to be given to achieve significant reductions in larval emergence from faecal material. Methods for the mass culture of *Arthrobotrys* spp. have been developed for horticultural applications (Stirling et al., 1998a) that could possibly be applied to isolates selected for livestock applications. Recent work by Araujo et al (1998) is also very encouraging since application of *A. robusta* conidia twice per week to grazing calves over 4 months resulted in reductions in faecal egg counts and *C. punctata* worm burdens by 54% and 70%, respectively. If self administration of conidia through a feed supplement block or feed premix can achieve a similar level of control then wider scale field applications may be a possibility and further research activity is certainly warranted. Additional support to further study of *Arthrobotrys* spp. comes from recent evidence showing that when *Arthrobotrys* spp. and *D. flagrans* were administered to sheep together, the reduction in *H. contortus* larvae emerging from faecal culture 4 days after treatment was greater than for either fungi species alone (Flores Crespo et al., 2001).

#### *2.1.7.2.2 Duddingtonia flagrans*

*Duddingtonia flagrans* produces a thick walled chlamydospore which is resistant to gastrointestinal digestion by sheep (M. Knox, P. Josh and L. Anderson, unpublished results) and it survives passage through the gut of livestock species better than other species of nematophagous fungi. In the years since *D. flagrans* was confirmed to have the greatest potential for development as a biological control agent of nematode parasites of livestock (Peloille, 1991; Larsen, 1991,1992) a considerable amount of research has concentrated on this species. Studies carried out *in vitro* or by mixing *D. flagrans* chlamydospores with faecal material have confirmed a high degree of trapping efficacy against a broad range of nematode parasites from several livestock species as shown in Table 2. Rate of growth of *D. flagrans* has been shown to be influenced by temperature (Fernandez et al., 1999) and its growth compared to some other fungi species is considered to be slow (Grønvold et al, 1996).

Studies have shown that feeding cereal grains on which *D. flag*rans has been cultured to livestock results in significant reductions in larval availability in laboratory faecal cultures and near faecal pats on pasture and can substantially reduce nematode parasite burdens of animals grazing pastures where *D. flagrans* has been used. The species of nematodes shown to be successfully controlled are: *H. contortus, T. colubriformis, O. circumcincta*, and *Nematodirus* spp. in sheep (Peloille, 1991; Larsen et al, 1994, 1998; Faedo et al., 1997, 1998, 2000; Githigia et al., 1997; Llerandi-Juarez and Mendoza de Gives, 1998; Mendoza de Gives et al., 1998; Manueli, 1998; Knox and Faedo, 2001; Flores Crespo et al., 2001; Fontenot et al., 2003; Chandrawathani et al., 2002; Pena et al., 2002; Waller et al., 2001a, b; Waller et al., 2002; Sanyal 2000d, 2001; Khan et at., 2002), *C. oncophora, O. ostertagi, D. viviparous, Mecistocirrus* spp., *Haemonchus* spp. and *Oesophagostomum* spp. in cattle (Larsen et al., 1992, 1995b; Grønvold et al., 1993a; Wolstrop et al., 1994; Nansen et al 1995; Fernandez et al., 1998, 1999 a, b; Sanyal, 2000 b, d; Faedo et al., 2002 b; Dimander et al., 2002), *H. contortus* in buffalo (Sanyal, 2000d), *H. contortus, O. circumcincta and T. colubriformis* in goats (Gawor et al., 1999; Sanyal, 2000d; Sanyal and Mukhopadhyaya, 2002; Terrill et al., 2002; Chartier and Pors, 2002), *O. dentatum* and *Hyostrongylus rubidus* in pigs (Nansen et al., 1996; Petkevicius et al., 1998) and cyathostomes, *Strongylus edentatus* and *S. vulgaris* in horses (Larsen et al., 1995a, 1996; Fernandez et al., 1997, 1999a; Baudena et al., 2000).

A number of review articles have discussed the potential for using *D. flagrans* as a biological control agent of livestock nematode parasites and have put forward some logical steps towards the development of commercial formulations of this fungus (Waller and Faedo, 1996; Larsen, 1999, 2000, 2002; Thamsborg et al 1999). The current situation regarding the issues of production, dose rate determination, delivery and environmental impact assessment of *D. flagrans* use for livestock will now be considered.

Table 2 Studies conducted on the control of nematode parasites with *D. flagrans* in vitro or where fungi was mixed with faeces before assessment of trapping efficiency.

| Host and Parasite species | In vitro or fungi addded to faeces |
|---|---|
| ***Cattle*** | |
| *Ostertagia ostertagi* | Larsen et al (1991)<br>Grønvold et al. (1996)<br>Fernandez et al. (1999d) |
| *Cooperia oncophora* | Grønvold et al. (1999) |
| *Dictyocaulus viviparous* | Henricksen et al (1997)<br>Fernandez et al. (1999c) |
| ***Sheep/Goat*** | |
| *Haemonchus contortus* | Mendoza de Gives et al. (1999a)<br>Sanyal (2000d) |
| *Ostertagia circumcincta* | Mendoza de Gives et al. (1999a) |
| *Trichostrongylus axei* | Mendoza de Gives et al. (1999a) |
| ***Pigs*** | |
| *Oesophagostomum dentatum* | Petkevicius et al. (1998) |
| ***Horses*** | |
| Cyathostomes | Bird and Herd (1994, 1995) |

Production of *D. flagrans* has progressed from laboratory scale culture methods (Larsen, 1991; Grønvold et al., 1993; Larsen and Faedo, 1998) to commercial scale preparation of large quantities in Denmark and Australia. Effective dose rates of *D. flagrans* chlamydospores used in the above studies vary from $1x10^6$/kg liveweight for cattle, horses and pigs to $0.3\text{-}5x10^5$/kg liveweight for small ruminants but may be influenced by the source of chlamydospores and their viability both pre- and post-gastrointestinal transit. Cereal grains remain the preferred substrate for culture and this is fortunate since daily inclusion in supplementary feeds has been shown to be the most effective form of delivery (Larsen, 2002). There has been some research on delivery through feed supplement blocks (Waller et al., 2001b; Chandrawathani et al., 2002) and intra-ruminal controlled release devices (Waller et al., 2001a) but further development of these methods is necessary before field application will be possible due to inadequate retention of chlamydospore viability over time when included in some block and controlled release device formulations. Recent research in our laboratory has shown *D. flagrans* can be delivered to sheep in some specially designed feed pre-mix formulations, which will facilitate introduction into Australian livestock feeding systems (M. Knox, unpublished). Application in field situations will largely be governed by knowledge of parasite epidemiology, other strategies for parasite and livestock management and the prevailing seasonal conditions. Some authors have speculated on likely scenarios for *D. flagrans* use in the field (Waller, 2002; Larsen, 2002) but it will be essential to conduct field evaluations in representative environments before firm recommendations are developed for extension purposes.

One of the major barriers to the release of any microbial biological control agent into agricultural systems is the detrimental environmental consequence of upsetting the natural balance of non-target organisms (Cook et al., 1996). The majority of research effort on *D. flagrans* has concentrated on measuring its effectiveness in controlling nematode parasites and, to the present time, the small amount of research directed towards determining the environmental effects of inundative introduction of this fungus into soil-pasture ecosystems have not revealed any negative impacts. Initial studies in Australia showed no effects on soil nematode populations where sheep were offered feed blocks containing chlamydospores of Australian strains of *D. flagrans* while grazing an improved temperate pasture (Yeates et al., 1997). Further investigation showed that there were no detectable negative environmental impacts from using selected Australian strains of *D. flagrans* in a typical improved pasture system since dissipation of the fungus was limited to the area near the point of faeces deposition, persistence was short-term and there appeared to be no negative impacts of use on free-living nematodes

and microarthropods associated with the soil/pasture interface (Knox et al., 2002). In Europe, no effects of Danish strains of *D. flagrans* were observed in earthworms living in soil and consuming faecal matter from cattle containing high numbers of chlamydospores (Grønvold et al., 2000). When applied in cattle or sheep faeces in Sweden and Denmark, the Danish *D. flagrans* showed little growth beyond the faecal environment and had no impact on soil nematode populations (Faedo et al., 2002).

A possible concern regarding environmental impact derives from the major program currently being undertaken to introduce one particular isolate of *D. flagrans* into agricultural systems in many countries throughout the world. Introduction is occurring through sponsored programs with the Food and Agriculture Organisation of the United Nations (Brazil, China, Malaysia, South Africa) and the European Union (Sweden, France, The Netherlands, Great Britain, Spain, Greece) as well as proposed introductions into Australia, New Zealand and the USA through contracted agents. It is not clear whether these introductions are being undertaken with appropriate monitoring of the effects of the exotic strain on locally adapted *D. flagrans* or on other species of native nematophagous fungi in terms of displacement of local isolates. Such monitoring is essential to ensuring minimal risk of environmental damage by exotic introductions (FAO, 1996; Goettel et al, 2001). Reduced genetic diversity and/or abundance of local strains and species of fungi and other organisms may have dramatic consequences in the agricultural systems where application of the exotic *D. flagrans* is likely to occur, particularly in relation to soils organisms responsible for mineral and nutrient cycling and availability to pasture and also to potential pests. Detailed environmental assessment, including further development and application of molecular methods for the identification of individual isolates of *D. flagrans* (M. Faedo and A. Brownlee, unpublished; Sanyal and Mukhopadhyaya, 2002), is therefore recommended before any widespread introduction of non-native isolates occurs.

#### *2.1.7.2.3 Other nematophagous fungi species*

Only a limited number of other species of nematophagous fungi have been studied for their potential to control nematode parasites of livestock. *Pleurotus pulmonaris* was shown to reduce numbers of *O. ostertagi, C. oncophora, O. quadrispinulatum* and equine cyathostomes *in vitro* (Larsen and Nansen, 1990; 1991). Numbers of *H. placei* larvae on agar plates were controlled by *Monacrosporium ellypsosporum* (Araujo et al, 1992). Gonzales-Cruz et al (1998) showed *Monacrosporium gephyropagum* reduced the numbers of *Strongyloides papillosus* on agar plates. Using nine isolates of *Monacrosporium* spp. infective larvae of *C. punctata* and *H. placei* were reduced on agar plates (Gomes et al., 1999). Similarly, *Monacrosporium* spp. was shown to reduce numbers of *H. contortus, O. circumcincta* and *T. axei* in vitro (Mendoza de Gives et al., 1999). After *in vitro* and *in vivo* selection for survival of gastrointestinal passage through ruminant livestock *G. eudermata* were shown to have potential for further development for biological control (Waller et al., 1994). The comparatively low trapping efficiency of these fungi and their limited viability after passage through the gut of livestock suggests that, at the present time, other species offer greater hope for development and application for biological control of livestock parasites.

#### *2.1.7.2.4 Endoparasitic species of fungi*

Only two genera of endoparasitic fungi have been investigated for their ability to control nematode parasites of livestock. Studies by Jansson et al. (1985) showed that conidia of *Drechmeria coniospora* would only attach to parasite larvae after exsheathment removed the third stage larvae cuticle. The low level of infection of intact third stage larvae was later overcome for *H. contortus* by applying $10^8$ conidia per gram of faeces in culture (Santos and Charles, 1995), a level that is probably impractical for application in livestock production situations. Another investigation by these researchers showed that application of 300,000 conidia per gram of faeces of *Harposporium anguillulae* significantly reduced the numbers of *H. contortus* larvae in faecal cultures (Charles et al., 1996). In New Zealand, *Harposporium*

*helicoides* was shown to reduce the number of *O. circumcincta* larvae recovered from pots of pasture (Waghorn et al., 2002) and *Harposporium* spp. has been observed to quickly establish in faecal deposits on pasture from the surrounding soil and herbage (Hay et al., 1997; 1998; 2002). Despite the positive results of the latter studies it is difficult to imagine how endoparasitic fungi can be delivered to livestock for biological control purposes since they do not survive and remain viable after gut passage (Larsen et al., 1991). The remaining hope for the use of endoparasitic species of fungi for biological control of livestock parasites may therefore rest with pastures where the build up of naturally occurring organisms is encouraged through conservation farming practices.

#### *2.1.7.2.5 Egg penetrating species of fungi*

A number species of fungi known for their ability to penetrate and parasitise egg of free-living and plant parasitic nematodes have been investigated for their ability to invade the eggs of nematode parasites of livestock. Eggs of *Ascaris lumbricoides* were infected and destroyed by the fungus *Verticillium chlamydosporium* (Lysek, 1978; Lysek and Krajci, 1987). This species of fungus was also shown to attack and destroy eggs of *Ascaris galli* and *Parascaris equorum* (Chien, cited by Larsen 1999). An in vitro study investigated the effects of *Verticillium suchlasporium* (3 isolates), *V. chlamydosporium* (2 isolates), *Verticillium leptobactrum, Paecilomyces lilacinus* (2 isolates) and *Gliocladium roseum* and showed that *G. roseum* had no effect whereas the other species infected *Nematodirus* spp eggs although there were differences between fungal isolates in their egg penetrating ability (Larsen et al., 1999). Some activity of these species against *Ascaris suum* has also been suggested (Larsen, 1999). *P. lilacinus* has also been shown to attack and destroy eggs of *Toxocara canis* (Araujo et al., 1995b). The above results are encouraging but similar to the case for endoparasitic fungi it is difficult to see progress being made towards using egg parasitic fungi for the control of nematode parasites of livestock since delivery of sufficient spores to the faecal pat will be problematic for the foreseeable future. Mass culture techniques have been developed for *V. chlamydosporium* (Stirling et al., 1998b) and *P. lilacinus* (Davide and Williams 1999) for use against plant parasitic nematodes in horticultural situations that could possibly be applied to isolates of potential use for livestock if delivery issues can be resolved.

## 2.2 Conservation biological control and nematode parasites.

In any livestock enterprise based on the productive use of pasture, the livestock producer is continually faced with decisions regarding stocking pressure (i.e. numbers of animals grazed per unit area) since this will have a major impact on his economic situation and on the environmental sustainability of his grazing enterprise. In Australian pastures, a stocking rate greater than that able to be sustained by the pasture will have substantial impact on animal nutrition and productivity (Langlands et al, 1984) and also have major impact on organic matter and the abundance of microorganisms inhabiting the herbage and underlying soil (King and Hutchinson, 1983). Both these factors may influence nematode parasite populations since the ability to mount an effective immune response to infection is dependent on nutritional resources available to the animal and the presence of potential antagonists of larval nematodes will be determined by pasture abundance. Nematode parasitism is frequently less problematic in grazing systems where overstocking is avoided, and in may not be merely coincidence that in these systems populations of soil organisms, including potential antagonists of parasitic nematode larvae, are often greater. A greater understanding of the limits imposed by grazing practices on invertebrate and microbial abundance in pastures is required and should include an assessment of the impact of such practices on nematode parasite larval survival. It may eventuate that dramatic results can be achieved as noted in agricultural systems where organic amendments have had significant impact on reducing plant parasitic species of nematodes (Akhtar and Malik, 2000; Oka et al., 2000).

## 2.3 Future prospects for biological control of nematode parasites

Potential pathogens of the infective stages of nematode parasites within the host are yet to be identified and the only potential means for the control of these stages by biological means is through genetic manipulation of pastures or gut microflora to deliver toxins as has been suggested above with nematocidal *Bacillus thuringiensis* crystal proteins. At the present time, it is thought that the development of effective means of biological control will most probably rest with methods to reduce or eliminate free-living larval stages on or near the faecal pat. Although highly desirable, it is not likely that any pathogen of nematode parasites will be identified that will enable effective implementation of biological control in the traditional sense as defined earlier in this review. Inoculative control is also unlikely to succeed since the persistence of potential pathogens in the general environment has not been observed and even if persistence is achieved, it will not necessarily mean that the pathogen will remain effective against the target parasite species in the longer term. As discussed earlier, agricultural practices that conserve or enhance natural populations of predators and pathogens of parasitic nematode larvae will continue to assist in controlling this problem in grazing situations. Improved definition of the parasite control benefits and demonstration of clear economic benefits of their implementation will be essential before wider scale promotion and adoption of conservation biological control practices can be pursued.

For the foreseeable future the most probable means of biological control of nematode parasites of livestock will be the inundative application of specific pathogens to reduce or eliminate larval stages in or near deposited faeces. Of the possible pathogenic agents identified above the following three offer the greatest potential for development and application in Australian livestock systems.

***Bacillus thuringiensis*** – isolates have been identified with high level activity against parasitic nematodes but methods need to be developed to apply toxin producing isolates for biological control. Possibilities for application include genetic manipulation of bacteria that colonize faecal material to include nematocidal *B. thuringiensis* toxins and thereby reduce free-living larval stages that feed on bacteria in faeces. Problems with this approach would be similar to those faced by any other genetically modified organism proposed for use in agriculture.

***Arthrobotrys spp***. – this nematophagous fungus is widespread in prevalence in livestock systems throughout the world and is an aggressive and effective trapper of parasitic nematode larvae. Methods for mass production of conidia have been developed for some isolates that could easily be applied, or further developed, for alternative isolates. A number of isolates have been shown to substantially reduce nematode larvae in faeces after gut passage. Further selection of isolates for ability to survive gut passage or the development of methods to increase survival through the gut will enable *Arthrobotrys* spp. to be utilised for biological control. Considerable research has been applied to the successful development of methods for protecting microbiologicals and nutrients from digestion and degradation until delivered to specific regions of the gastrointestinal tract. Research should therefore be initiated to investigate the potential for extension of these technologies for application to *Arthrobotrys* spp. (and possibly other fungi like the egg-parasitic *Paecillomyces* spp.) for use in biological control of nematode parasites of livestock.

***Duddingtonia flagrans*** - The concentrated effort currently being directed towards *D. flagrans* seems likely to lead to commercial availability of products containing this fungus in the near future. Delivery of *D. flagrans* will probably be via feed supplements fed to livestock on a daily basis. There is also scope for other delivery options through feed blocks and controlled release devices after further development of these technologies. Critical to the application of *D. flagrans* under industry conditions will be testing and development of strategies to maximise the effectiveness of this means of control including studies of duration and timing of application and integration with other control alternatives.

Continued evaluation of the environmental impact of inundative application of *D. flagrans* will be essential before widespread use can be recommended.

Past efforts at biological control of mainly arthropod pests have desired "elimination" of the pest whereas lowering the threshold of nematode parasite availability may enable livestock production to continue without significant losses due to parasitism. It has been shown through use of a mathematical model of *T. colubriformis* population dynamics that provided a biological control agent could achieve at least 75% reduction in larval numbers from deposited faeces over a 90 day treatment period, there will be a dramatic decrease in anticipated deaths due to clinical disease within the modelled sheep flock (Barnes et al., 1995). It will be interesting to see if these predictions are validated in future field applications of *D. flagrans* and/or other possible biological control agents since this will be essential for high level acceptance and adoption by livestock producers.

# 3. LIVER FLUKE INFECTIONS IN SHEEP AND CATTLE.

Liver fluke disease, caused by *Fasciola hepatica* infection, in sheep and cattle in Australia results in significant economic losses to livestock producers in endemic areas. Costs of control include approximately $10 million per year on fluke drenches and a further $50-80 million in lost production (Boray, 1999). The life cycle of *F. hepatica* involves a primary and intermediate host and is dependent on the availability of suitable habitat for egg survival and habitation by the indigenous intermediate host snail *Lymnaea tomentosa* (Boray, 1964) and some introduced exotic Lymnaeid snail species (Boray, 1978). Briefly, adult flukes in the bile ducts of the liver produce eggs which are transported to the gut in bile and then passed in the faeces. In wet areas, the eggs hatch and the first larval stage, or miracidia, emerges to infect the immediate host snail in which they develop and multiply as sporocyst, rediae and cercariae. The cercariae leave the snail and attach and encyst as infective metacercariae on grass and other vegetation near the water until consumed by sheep, cattle or other hosts. Immature flukes then penetrate the gut wall, find the liver and migrate through the liver tissue to the bile ducts where they grow to become adults (Boray, 1999).

In Australia, effective control of liver fluke infections relies on coordinated application of the following three approaches (Boray, 1999). Firstly, strategic use of effective anthelmintics will reduce fluke populations in the host and thereby the number of eggs passed to the pasture. This has led to regional recommendations for routine treatment of sheep and cattle based on epidemiological knowledge of liver fluke infection. Secondly, reduction of the number of intermediate host snails through application of molluscicides or by reduction in snail habitat through improved drainage of wet, low-lying areas. This approach is usually only effective in small areas due to difficulties in accessibility and the potential for environmental damage. Finally, modification of grazing practices to reduce fluke infection through fencing off snail-infested pastures or by selective grazing with more resistant animals (eg. mature cattle) or those about to be treated with effective anthelmintics. Options for the biological control of *Fasciola* spp. infection have not been developed to a stage where application in the field can be recommended but possible options are discussed below.

## 3.1 Biological control of flukes within the host.

Although bacterial or viral pathogens of larval and adult fluke may exist within the ruminant host, the technical issues involved in the identification, isolation and application of possible pathogens render this approach to biological control of *Fasciola* spp. largely speculative at least for the near future. Extension of current research into fungi and other pathogens of parasitic nematode eggs and larvae in livestock faeces may offer possible options for reducing the viability of fluke eggs in the faecal pat. If systems can

be developed to deliver viable fungal spores to the faeces after gut passage, the egg penetrating species of fungi described earlier may offer some hope in this regard.

Opportunities for the biological control of *Fasciola* spp. lie primarily with the application of methods to decrease the population of the intermediate host snails or methods for decreasing the infection rates of snails by miracidia. A large proportion of research on possible agents for biological control of the intermediate host snails has been directed towards species which act as the intermediate host of trematodes that are pathogenic to humans, particularly *Schistosoma* spp. This information will be reviewed to demonstrate possible avenues for the control of intermediate hosts of *Fasciola* spp. along with research focussing on this specific problem where it exists.

## 3.2 Biological control of the intermediate host of liver fluke.

### *3.2.1 Predators of fluke snails*

The aquatic and semiaquatic habitats preferred by Lymnaeid snails provide a rich environment for vertebrate and invertebrate organisms that may utilise snails as a food resource. The predators and parasites of snails and slugs include flatworms and nematodes, leeches and other annelid worms, rotifers, arthropods (insects, arachnids and crustacea), molluscs, amphibians and reptiles, fish, birds and mammals (Michelson, 1957; Stephenson and Knutson, 1966; Berg, 1973). The majority of these potential predators are considered opportunistic feeders on fresh-water snails and little quantitative information exists regarding their impact on snail populations in their natural habitats (Michelson, 1957). It is thought that in most situations snail populations exist in natural balance with predator populations with losses due to predation being compensated for by the ability to reproduce rapidly when conditions are favourable. The impact of predation of Lymnaeid snails by Sciomyzid fly larvae and ducks has been the subject of more detailed study.

Observation that the larvae of some species of marsh flies (Diptera: Sciomyzidae) preferentially utilised aquatic, pulmonate snails as a food resource led to a number of studies to determine the possibility for use in the biological control of intermediate hosts of human and livestock disease (Berg, 1964). Early research was directed at the control of the intermediate hosts of *Schistosoma* spp. and laboratory tests indicated some success against the hosts of *Schistosoma mansoni* and *S. haematobium* (Berg, 1973). In seeking a possible biological agent to control liver fluke infection in livestock, a field trial in Hawaii showed that introductions of the Sciomyzid *Sepedon macropus* substantially reduced numbers of *Lymnaea ollula*, the local intermediate host of *Fasciola gigantica* (Berg 1964). Despite reductions in the snail populations there was however, no indication that transmission and infection rates of cattle had been affected. Biological control of *Fasciola* spp. was thought feasible and efforts were then directed towards mass rearing of Sciomyzid larvae for use in field applications (Gormally, 1985; McLaughlin and Dame, 1989). Investigation of the effects of native Sciomyzid larvae on *L. tomentosa* in Eastern Australia suggested that the fly population fluctuated considerably over time, as did the population of snails, but the effects on incidence of fasciolosis appeared insignificant (Boray, 1969). High costs associated with mass rearing, the necessity for regular inundative application and negative environmental impacts on non-target snail species make this option for the biological control of *Fasciola* spp. unlikely to proceed to wide-scale use.

It has been suggested that domesticated and wild ducks and other waterfowl may have potential for use in the biological control of Lymnaeid snails. In Germany, intensive duck and goose husbandry eliminated Lymnaeid snails in the field and controlled *F. hepatica* infection (Levine, 1970). Confinement of domesticated ducks in a portable pen in Arizona, USA reduced *L. palustris* numbers by 40 to 93% (Samson and Wilson, 1973). More recently, the use of foraging flocks of domesticated ducks in Indonesia has been shown to reduce the numbers of *L. rubiginosa* in fallow rice fields while increasing the incidence of *Echinostoma revolutum* infection

of the snails and both these factors can reduce *F. gigantica* infection rates in cattle and buffalo (Waller, 1996). Except in these more intensive systems it is thought that the potential for biological control of Lymnaeid snails by ducks or other waterfowl remains limited.

### 3.2.2 Competition with and predation by other snail species

When a number of species of snails inhabit the same environment there is potential for negative interactions between the species. The introduction of predatory snails was once considered the most promising possibility for biological control of Schistosome bearing snails (Madsen 1990). A number of species of predatory snails were introduced to the Martinique Island over a number of years and successfully reduced numbers of the intermediate host snails of *S. mansoni* and the incidence of the disease (Pointier, 2001). Similar introductions to other areas were however not as successful due to low adaptation of introduced species to the local environment (Madsen, 1990). Introduction of *Marisa cornuarietis* to a man-made dam in Tanzania resulted in the elimination of *L. natalensis,* the local intermediate host of *F. gigantica* (Nguma et al., 1982). Ximenes et al. (1993) introduced three species of predatory snails during an integrated program to eliminate *L. truncatula* and reduced the incidence of fasciolosis in cattle. The potential for using introduced predatory snails to control *L. tomentosa* in Australia has not been investigated and modern-day concerns over possible negative impacts on non-target species (Cowie, 2001; Strong and Pemberton, 2001) would suggest that future activity in this area is unlikely.

### 3.2.3 Effects of parasitic infection of snails

Trematode larvae that infest snails live as true parasites in their host, causing pathological changes that can influence survival depending on the level of infection and number of species involved (Boray, 1969). Parasitism by trematodes can also lead to diminution of the reproductive capacity of the snail host or result in complete sterilisation due to the utilisation of host nutrient resources by the parasite (Combes, 1982; Madsen, 1990). During *F. hepatica* infection of *L. tomentosa*, normal development of larval stages proceeds without damage to the internal organs of the snail except after heavy infections with miracidia. Death of the snail was thought to be most likely when severe tissue damage occurs around the time when the cercariae were being shed (Boray, 1964).

A number of parasite species may infect aquatic snails at any one time and in some instances interspecies antagonism has been observed to occur. The antagonism between different trematode species within the intermediate host snails of Schistosoma spp. has been the subject of detailed study in the quest for potential biological control agents of this medically important parasite (see reviews by Lim and Heyneman, 1972; Combes, 1982). Reviews of this topic conclude however, that control by this means may be restricted to small localities with indigenous species since variation exists in the degree of antagonism between isolates and the high cost of production of infective stages would make this means of control untenable on a wider scale (Lim and Heyneman, 1972).

Boray (1964) noted that a number of species of Echinostomidae frequently infect *L. tomentosa* causing reduced infectivity with *F. hepatica* and less pathological effects in the snails. Subsequent laboratory and field studies confirmed that mixed infections with echinostomes and *F. hepatica* were extremely rare with *F. hepatica* being absent when echinostome infections were present (Boray, 1969) suggesting biological control through interspecies antagonism. In Indonesia, the negative interactions between echinostomes and *Fasciola* spp. has been utilised in a simple method for reducing fluke infections in the intermediate host snail *L. rubiginosa*. Suhardono et al (1995) showed that by combining faeces from echinostome infected ducks with faeces from *F. gigantica* infected cattle/buffalo prior to distribution in rice fields, infection rates of *L. rubiginosa* could be significantly reduced. Trematodes are not the only parasites that interact negatively with *Fasciola* spp. Infection of *L. truncatula* with the microsporidian parasite *Nosema eurytremae* has been shown to reduce the viability of *F. hepatica* rediae and depress the

cercarial output from infected snails (Canning et al., 1979). Interspecies antagonism frequently occurs between parasite species of snails but the potential to harness this interaction for improved biological control is limited. There may be some options for practical application of antagonistic species within local areas, as cited above for Indonesia, but generally application is not considered feasible for implementation on a broader scale as would be necessary in Australian grazing systems.

### *3.2.4 Other pathogens of fluke snails*

Free-living nematodes are frequently found in the environments inhabited by Lymnaeid and other aquatic snails but little is known about their interactions with snails although some species may be occasional pathogens (Berg, 1973). Boray (1964) observed a freshwater Dorylaimid species closely associated with *L. tomentosa* which was often found in the snail egg mass, in the body of dead snails and on two occasions in the gut of live snails. He concluded that these nematodes only occasionally penetrate live snails and that infestation occurred more frequently after death of the snail from other causes. Recently, the dauer larvae of Phasmarhabditis nematodes reared in monoxenic culture with the growth promoting bacterium *Moraxella osloensis* have been shown to be highly pathogenic to a number of molluscs including several slug species and the fluke snail *L. stagnalis* (Wilson et al., 1993;1995; 1998). This method looks very promising for biological control of horticultural pests and may have application for *Fasciola* spp. control in situations where snail habitat is confined to a small area, possibly as a replacement for use of molluscicides. The likely cost of this method of control and impact on non-target molluscs is unknown at present.

The Oligochaete worm *Chaetogaster limnaei* has been observed to attach to the soft tissue underneath the shell of Lymnaeid snails but is thought to be a true mutualist rather than a parasite of the snail. Laboratory (Khalil, 1961) and field (Dimatulac and Pinto, 1983) studies have shown that snails with *C. limnaei* tend to have lower infection rates with *F. gigantica* miracidia than those without *C. limnaei*. This coupled with observations of *F. gigantica* miracidia in the gut of *C. limnaei* suggest the latter to be an opportunistic predator on fluke larvae (Khalil, 1961). As *C. limnaei* is frequently observed in *L. tomentosa* in Eastern Australia (Boray, 1964) it is thought that these worms probably play a role in reducing infections with *F. hepatica.* However, it is thought that it would be very difficult to exploit this predacious capacity for improved biological control of fluke infections.

Microbial pathogens of fluke snails probably exist in nature but attempts to isolate these have had little success (Michelson, 1957; Berg, 1973; Madsen, 1990). Bacteria, fungi and protozoa have been shown to affect snails in laboratory culture but attempts at applying these in field situations have been largely unsuccessful (see review by Madsen, 1990). It appears that very little research has been conducted on this subject and although microbial pathogens may be identified at some future date their application in current agricultural systems seems a long way off.

## 3.3 Prospects for the biological control of liver fluke.

No options for the biological control of fluke within the host have been identified and possibilities for the control of fluke eggs and larvae in faecal material remain speculative at the present time. The search for biological agents for potential application to control *Fasciola* spp. infection in livestock therefore rests with identified pathogens of the intermediate host Lymnaeid snails. Many of these pathogens have been observed to occur in native snail populations and would already exert some regulatory effect on those populations. Elimination of Lymnaeid snails may be possible within a limited area through inundative release of specific pathogenic organisms. In order for this to be possible, considerable research would be required to develop economically viable methods for mass rearing of the pathogen and for its delivery to fluke prone areas. Regular release may also be necessary since Lymnaeid snails have a tremendous reproductive capacity and recolonization of fluke prone areas would be relatively rapid. Consideration of the effects of inundative release of pathogens on non-target species would be an essential component of future research activity, particularly if non-native species were being considered. Overall, the prospect for the biological control of liver fluke infection in livestock is poor and it is expected that livestock producers will need to rely on currently recommended strategies for control for the foreseeable future.

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