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Toward Practical Biological Control of Parasitic Nematodes in Domestic Animals[1]

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Abstract: In a series of laboratory and field experiments where the nematophagous fungus *Arthrobotrys oligospora* was mixed directly with feces it has been demonstrated that it is possible to use nematophagous fungi for biological control of animal parasitic nematodes. A procedure used for selection of nematophagous fungi that can pass the digestive tract of ruminants, horses, and pigs is described. The selected fungus, *Duddingtonia flagrans,* has been used in further field experiments, and the results have confirmed that by the addition of *D. flagrans* to feed supplement it is possible to reduce the parasitic burden significantly.

Key words: animal parasitic nematodes, *Arthrobotrys oligospora,* biological control, *Cooperia oncophora,* Denmark, *Duddingtonia flagrans,* nematophagous fungi, *Ostertagia ostertagi.*

Initial observations and experiments: It is not unusual that experiments or ideas result from a coincidence. An example is our project initiated in Denmark in 1982 concerning the use of nematophageous fungi to control parasitic nematodes. Technicians at the Section for Parasitology, National Veterinary Laboratory, had problems with fungal contamination of fecal cultures established for *Ostertagia ostertagi* juvenile development, and it was anticipated that this contamination lowered the production of juveniles.

Inspired by an article by Pandey (9), one of us, Dr. Grønvold, hypothesized (incorrectly) that the contaminating fungus might be nematophagous. Actually, the problem showed not to be nematode-trapping fungi; however, the hypothesis nevertheless motivated valuable research. At the same time Dr. Grønvold joined a course in microbiology, and here he initiated the experiments with nematophagous fungi in Denmark.

The fungus used was *Arthrobotrys oligospora,* kindly supplied by Dr. Birgit Nordbring Hertz, The University of Lund, Sweden. From that time until now, numerous experiments have been performed, and in the following we report our research achievements, pointing out milestones in the development from an idea to a commercial product. Yet, much work still needs to be done before a commercial product is ready for marketing and practical application.

The ecology of *A. oligospora* was carefully studied in the laboratory in terms of influence of pH, temperature, oxygen tension, and other physical factors influencing growth rate. It was furthermore demonstrated that many species of animal parasitic nematode juveniles could be trapped (7). The nematode-trapping efficiency of the fungus in feces depended on the inoculation level (Table 1) (1), in that a higher concentration of conidia led to higher nematode-killing effects.

Encouraged by the results shown in Table 1 (1), preliminary field experiments were carried out in 1984 and 1985. Artificially prepared cow pats containing *Cooperia oncophora* eggs were inoculated with *A. oligospora* and placed on a parasite-free grass plot together with fungus-free control cow pats. The results were promising, as the presence of *A. oligospora* in cow pats resulted in an 86% reduction in the numbers of third-stage juveniles (J3) in grass samples. Although the results were preliminary, *A. oligospora* certainly seemed to be a

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TABLE 1. Number of third-stage juveniles of *Cooperia oncophora* isolated from fecal cultures inoculated with different levels of conidia of *Arthrobotrys oligospora*. The fecal cultures were incubated for 13 days at room temperature (95% confidence limits are shown) (1).

Conidia per g feces	Average number of third-stage juveniles per g feces
0	408 ± 96
8	437 ± 98
25	472 ± 134
250	121 ± 21
2,500	4 ± 3

potential candidate for biological control of trichostrongyle juveniles.

Additional field experiments conducted in 1986 and 1987 comprised large numbers of parasite-infected cow pats inoculated with conidia or mycelium of *A. oligospora,* and the results showed a 50 to 70% reduction in the number of infective juveniles in cow pats and their surrounding grass, compared to control fields with no fungal addition. Late in the grazing season 1987, tracer calves were turned out on the experimental and the control plot, to graze for 2 months, after which they were necropsied. The worm burdens in the calves that had grazed the plot with fungal-inoculated cow pats were 37% lower than those in the calves of the control plot (2).

Thus, the experiments performed during the first 3 to 4 years were promising. Direct application of fungal inoculum to feces was effective but is not feasible under practical conditions. Unfortunately, subsequent experiments showed that conidia and mycelium of *A. oligospora* lost their viability during passage through the digestive tract of cattle and other livestock. A protective coating of the surface of the fungus against the influence of the enzymes in the digestive tract was not available.

Selection of feasible fungi: A selection procedure for isolating fungi that were able to pass the digestive tract without losing viability and trapping efficiency was developed as a part of a Ph.D. graduate student project.

The first selection step involved direct incubation of various fungus-containing soil types and compost samples in diluted rumen fluid at 39°C for 24 hours, imitating the conditions in the rumen. This treatment was intended to select nematophagous fungi that could survive exposure to rumen enzymes and metabolites. Only part of the natural nematophagous fungal population in soils and composts survived this first selection step.

In the next selection step, the fungi selected in the first selection were incubated in rumen fluid and subsequently in fluid containing pepsin and hydrochloric acid to mimic conditions in the acid stomach (abomasum). Two isolates of the genus *Arthrobotrys* and six of the genus *Duddingtonia* survived exposure to these fluids. In vivo experiments showed that these isolates were able to pass the alimentary tract of calves and reduce numbers of juveniles (J3) of the cattle nematode *Ostertagia ostertagi* (J3) by approximately 85% (4,5) in the dung.

Field experiments: In a field experiment (3), feces from *O. ostertagi*-infected calves were mixed (1:1) with feces from calves fed *D. flagrans* and placed as cow pats on a parasite-free grass plot together with parasite-infected control cow pats. Herbage juvenile infection was reduced by 75 to 85%. These results were confirmed in a later field experiment in 1992 (10), where an experimental group of *O. ostertagi*-infected calves grazing on a plot was fed *D. flagrans.* Feeding with fungal material during the first 2 months of the season showed a significant lowering of pasture infection, although the weather that particular summer was unusually dry. Similarly successful results were obtained in field experiments in both 1993 and 1994 (6,8).

In field experiments with horses and pigs conducted in 1994, chlamydospores of *D. flagrans* were fed to these animal species, which were naturally infected with gastrointestinal nematodes. This treatment reduced herbage infectivity and reduced parasite burdens as monitored by

experimental "tracer" foals and pigs (unpubl.).

In conclusion, the experimental part of the project has confirmed that it is possible to use nematophagous fungi for biological control of important parasites in outdoor-reared domestic animals.

Until now, the fungal material has been offered as feed additives to the animals. This practice can be used for horses and pigs since these are commonly given a daily supplementary feeding. However, this procedure is not applicable for grazing calves or sheep. Ruminal slow-release devices, lick stones, or addition to water could be alternative methods.

For experimental use, chlamydospores have been produced by cultivation of the fungus on barley grains. However, the most economical procedure for production of the microorganisms is no doubt mass cultivation in fermentors. This procedure is under development for production of chlamydospores of *D. flagrans*.

Patent and company partners: A patent has been applied for the selection and isolation procedure of nematophageous fungi useful in biological control of parasitic nematodes in animals. The patent has until now been approved in Australia. It is our experience that the research group must allocate the necessary resources and time needed to inform the patent authorities. The expertise of the research group is strongly needed for the description of the patent as well as for the development and testing of the product.

The development from a research project to a commercial product has been made in cooperation with Chr. Hansen's Bio Systems, Denmark. This company is responsible for the mass production of the chlamydospores as well as patenting and marketing. It also has partly funded the project in 1993, 1994, and 1995.

The research policy of a commercial company is different from that of a university. In general, commercial companies require a rapid return from their investments, which means that basic research is limited in many situations. Universities need stable funding in order to conduct long-term fundamental research. However, the combination of the expertise in basic and in applied research is fruitful and essential to the development of a commercial product. Nevertheless, private funding can never be the only financial support for the research.

The many failed attempts at rapid implementation of biological control have indirectly reduced financial support from governmental research councils, private funds, and industry. In our case this has been reflected in fluctuating and short-term funding that does not permit basic studies on the cow pat environment and the dynamic interaction between nematodes, fungus, and other biotic factors. Limited funding has possibly prevented us from basic insight studies, which, in the long run, will be needed for optimal implementation of the biological control principle and selection of fungal candidates that could show even more potency.

Conclusion

The relative success of our project was based on the early formation of a collaborative group of scientists, i.e., scientists with different specialties but with a common interest in biological control. The Royal Veterinary and Agricultural University was and is an excellent place for this work, as disciplines in relation to veterinary and agricultural subjects are located on the same campus.

Concern for the environment and increasing drug resistance problems have stimulated a growing interest for biological control. However, the efficiency of biological control is often more variable than that of chemical control. One major reason for variable results is a limited insight into the underlying biology and the complexity of physical and biological factors. Yet, it appears to us that biological control of animal parasitic nematodes by nematophagous fungi is rather reproducible.

Basic scientific knowledge, enthusiasm, commercial knowledge, cooperation, pa-

tience, and good luck are important ingredients in the work toward practical biological control.

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