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Chapter 6 Turning the tables. Using fungi to control other pests

Just about everything we do is affected by pests of one form or another and over the years we have invented an armoury of chemical pesticides which have permitted enormous improvements in agricultural and horticultural yields. But most of these chemicals are powerful and indiscriminate poisons and worries over the adverse environmental impact of heavy usage of chemicals like these are increasing. As we have seen so far in this book, fungi are very effective pests of other creatures in their own right so it's not surprising that attention is turning to the use of fungi as control agents by harnessing their natural antagonisms to pests of our crops - and there is potential for us to use fungi in controlling other fungi that cause diseases of crops, as well as insect pests, nematode worms and even weeds.

Over two thousand years ago, the Chinese were writing about the fungus diseases of silkworms and cicadas. So the importance of fungi that infect insects (they're called entomogenous fungi) in natural populations of insects has been recognized for a long time. It's interesting that early support for the germ theory of disease came from a fungus disease of silkworms. The disease was called muscadine disease and it killed silkworms in Europe so effectively that the future of the silk industry there was in peril. A lawyer-turned farmer named Agostino Bassi found that the silkworm corpses produced masses of fungus spores. The fungus, now called *Beauveria bassiana* in Bassi's honour, is one of today's prime candidates as an alternative to chemical pesticides. Bassi is worthy of honour. The people who are remembered by histories of biology and medicine as establishing the germ theory of disease are Pasteur, Lister, and Koch. Great men indeed, but they did their work in the 1870s, and Bassi had already settled the issue in 1835/36 (ten years before Berkeley's work on the Late Blight of Potato discussed in chapter 2). Unfortunately, choosing to work on insects and fungi is a sure way of being forgotten, then as now, and Bassi never got the credit he deserved. Still, they did name the fungus after him! The idea that these fungi might be useful as biological control agents was first explored by two pioneering scientists in the Ukraine, Metchnikoff and Krassilstschik, in the 1880s. These two visionaries mass-produced the spores of an insect-attacking fungus (called *Metarhizium*) and tested it against insect pests of wheat and sugar beet. Throughout the twentieth century insect-infecting fungi have been assessed as possible control agents for a variety of insect pests. There's a repetitive pattern. Field experiments are carried out, products are developed, and may even be successfully marketed for several years. But then the fungal products are replaced by more effective chemical insecticides. The sequence of development, commercialization and later withdrawal has been repeated with a number of other fungal products. Currently, there are no fungal products which are widely used for insect control, although in particular parts of the world (including Russia, China, Brazil, and the UK) and, more importantly, for particular insect pests in very particular circumstances, there are fungal products which are in use.

During recent years, of course, there has been a renewed interest in these insect-attacking fungi because of increasing insecticide resistance and environmental concerns over pesticide use. New strains of fungi have been isolated from a wide range of hosts, and these have even further emphasized the potential that exists for the use of fungi in insect control. But the continuously repeated cycle of development of a promising fungal product followed by its eventual failure has emphasized the importance of ecological features in determining their success as biological control agents. The problems encountered relate to the biology and life style demands of the fungi which we try to use as control agents. Over one hundred different sorts of fungi (representing all the main fungal subdivisions) have been shown to parasitize living insects. They are more common in tropical areas where temperature and humidity favor their growth. But they occur in most ecosystems, ranging from natural water habitats to the high technology horticultural production systems we create around the world. The host range of individual fungi is variable, with some attacking only one insect whilst others, and *Metarhizium* is an example, have a broad host range.

The key controlling feature that determines the usefulness of these natural control agents is their dependence on humidity for germination and growth. For them to attack their insect prey their spores must germinate on the insect and the processes of spore germination and growth of the spores on the surface of the insect are highly dependent on both available moisture and temperature. Even ninety percent relative humidity might result in only half the spores surviving. In the real world this means that the microclimate in the tiny area that the host insect prefers might determine the success or otherwise of the control agent. For example, in attempts to control aphids in glasshouses there was a major difference in efficiency against aphids which fed on the undersides of leaves (very effective control) where the humidity is high, compared to the more exposed stem (virtually no control). In similar experiments the spread of infection through aphid populations was maximal when free water was present but completely prevented at ninety-three percent relative humidity. Still rather damp to you and me, but dry as a desert to the fungal spores. Temperature also affects germination and growth. Both are markedly reduced below fifteen degrees Celsius and above thirty-five degrees, so the window of effectiveness is fairly narrow.

Once the fungus spore has germinated and grown through the insect's skin it produces cells which circulate within the insect and proliferate by budding. Insect death usually occurs three to fourteen days after the fungus spore alighted on it. Death results from a combination of mechanical damage resulting from tissue invasion by the fungus, loss of nutrients to the fungus and reaction to toxins generated by the fungus. After the death of the insect, the fungus grows on its corpse, eventually producing a new crop of spores. So the initial application of fungus spores results in the production of even more spores after the first successful infections. It is this amplification of the control agent that makes the biological control mechanism so attractive. A chemical agent will be diluted, washed away in the rain or used up. But as long as there are hosts to infect the biological control agent will grow stronger and stronger, producing an epidemic that can destroy the target insect completely. Unfortunately, this promise is rarely realized, though there are sufficient successes on the record for us to keep trying.

Rice pests are potentially good targets for these insect-attacking fungi; the high humidity in rice paddies and warm temperatures in rice growing areas combine to produce close to ideal conditions for epidemic infection of the insects. Attempts to control rice pests in the field by applications of fungal spores have indeed been relatively successful. Pests of glasshouse crops can also be controlled well by biocontrol agents. This is probably the most ideal circumstance because, being within the glasshouse, the environment can be controlled to optimize the infestation. Even here, though, the environmental demands of the parasites can cause difficulties. For example, control of whitefly on cucumber crops is effective only after the crop has reached four weeks old. This is because the smaller leaves of younger crops greatly reduce the humidity around the plants and the fungal spores cannot germinate. The age of the insect pest can influence results, too. Ninety-four percent kill was obtained when eggs of the white fly were treated (because the first larvae are then infected as soon as they emerge from the egg), but only twenty-eight percent of older larvae were killed when the treatment was delayed. The fungus spore preparation has to be applied several times to maximize and the spray must cover the entire plant to get good spread through the whitefly populations because the larvae themselves are not sufficiently mobile to spread the fungus disease. If all these requirements can be met, the development of fungal infections in the whitefly when humidity is high is very rapid and it is possible that a single night of high humidity in a glasshouse would be enough to destroy a whitefly infestation. There are no chemicals and no chemical residues, so biocontrol of whitefly, although difficult and demanding, is very promising.

Another successful application of fungi is in the biocontrol of the Lucerne aphid in Australia. This pest was introduced to Australia with none of its natural enemies and so it rapidly became a major problem. A search for natural Lucerne aphid disease agents found several likely pathogens in Israel. One of these was introduced into Australia; it spread rapidly and now helps to maintain the pest population at a low level.

Beauveria bassiana, that belated monument to Agostino Bassi, has been tried as a microbial control agent of several insect pests in different parts of the world, although most development work on this organism was done in the then Soviet Union where it was mainly used for control of Colorado potato beetle. Field trial showed that it was more active against weakened insects and the standard treatment developed was to use the fungus in conjunction with a quarter of the usual rate of insecticide application. The reduced dose of insecticide weakens the insects to such an extent that the fungus can easily infect and kill virtually all of them. In the 1970s through to the 1990s preparations of *Beauveria bassiana* spores were used on several crops over an area of at least half a million hectares in China. The fungus was used to help control pests like the corn borer, pine caterpillars and leafhoppers. Biocontrol agents of this sort are particularly useful in peasant farming and commune-based agricultural systems because the peasants can produce their own supplies of fungal spores. In this case, spores were produced by growing the fungus on boiled rice. This puts production of the control agent into the hands of the community which needs it in a way which simply cannot be done with chemical insecticides, which usually have to be bought with hard-earned cash from multinational chemical companies. This ‘empowerment’ of the community is such an attractive proposition that it may outweigh any lesser effectiveness of biocontrol agent when compared against the chemical treatment. There’s a similarly successful example from Brazil. The fungus known as *Metarhizium* is used commercially in Brazil to control spittlebug of cane sugar plants. The fungus spores are produced by growing the fungus on sterilized rice and once a good crop of spores have appeared the rice grains are dried and grains and spores are ground up and the powder sold under a variety of trade names. Again, this process is very simple and is well suited to production on a local basis by grower co-operatives. The yield of spores is very good, about a million-million spores per kilogram of rice, which is enough to treat a whole hectare of cane sugar. Spittlebug control is one of the few major success stories in biocontrol. Conversely, trials of the organism *Beauveria bassiana* in the USA have been less successful. During a three-year test, control of the potato beetle was highly variable. In only eight out of twenty-four trials was potato yield from fungus-treated plots significantly greater than yield from control (untreated) fields, but only two of these gave yields on a par with insecticide treatment. It seems likely that the more industrial farming practices result in high pest numbers at the time of fungal application and that this reduces the effectiveness of the fungus.

As the stories above show, there have been several major successes in particular places, under particular circumstances and with particular pests. These successes are a spur to continued research, but there has been no really dramatic breakthrough. Nothing has been found that has wide effectiveness and can be used under a range of conditions. What is most urgently needed to set this topic on fire is a fungal formulation that is not only safe to use (the common characteristic of these agents) but also cheap to produce, easy to apply, not too dependent on specific environmental conditions and that can consistently control the target pest.

Unfortunately, we are not very close to being able to achieve that. There are no commercially available ‘mycoinsecticides’ in the USA or Europe which get anywhere near this ideal. The history of the topic is littered with products being launched in great hope and then being withdrawn because they failed to provide reliable pest control. Astonishingly, despite this disappointing experience, commercial interest continues and it is remarkably widely accepted that fungi have the potential to control insect, aphid and other pests. It seems to be a topic in which blind optimism wins out over bitter experience. I suppose that to a great extent what drives this blind optimism are the possible advantages of mycoinsecticides compared to conventional chemical insecticides. Fungi infect most sorts of insect, aphid and mite, but individual strains of fungus can be very specific and may only infect one type of host. So, you can imagine fungi being used to control important pests without affecting innocuous insects in the same environment - that's part of their inherent safety. Of course, insect-attacking fungi cannot infect any other type of animal (or plant, for that matter), so unlike some of the more toxic chemical treatments the environmental impact of a mycoinsecticide is strictly limited to the host species it was developed to control. Another advantage over chemicals is that there is no evidence so far of fungal resistance occurring in insect populations. Now, this might be due to the fact that they've been used to only a limited extent so far, but it is a sharp contrast with chemicals which seem to select out resistance strains of the pest very quickly. The holy grail, therefore, is that widespread mycoinsecticide use could provide great benefits to us and our environment. Like the real holy grail, the problem is to find it.

The generally disappointing experiences of the past hundred years or so, and to be fair the few successes, do give some indications as to what directions we need to take in the future to realize the high hopes which so many people have for mycoinsecticides. The first point is the crucial importance of selecting the right strain of fungus. There are an enormous number of fungi out there and just about all features of the pathogen-host relationship can vary greatly between strains of any one species. By searching through a large collection of different strains, one with the desired combination of characters will be found. Eventually! Molecular biology might help. Genetically-modified pathogens of pest insects might be designed which bring together toxins, virulence or other pathogen functions from organisms which cannot be mated together conventionally. Progress is being made in understanding pathogenicity mechanisms, particularly things like how the fungus first penetrates the outer defences of the host, so it might become possible to increase the speed of insect kill by genetic manipulation of the fungus. This is important because most mycoinsecticides can only be applied after the crop has become infested by the pest. That being the case it is essential that the pest is killed rapidly, otherwise you'll end up with the unsatisfactory situation of killing the pest after it's done its damage.

Once you've selected, engineered and genetically modified a really mean insect pathogen, you need to be able to produce it on a large scale if it is to become a commercial proposition. The most attractive production method is liquid fermentation using stirred tank reactors. The engineering of this technology is well understood because it is used for other useful products like citric acid and antibiotics. However, as I've explained above, two of the most successful examples used a method using cereal grains as a semi-solid medium to produce the fungus spores. This is a traditional way of producing several human food products so it is a type of production that offers possibilities, but each new product requires new research so it can be a slow development program. Another commercial requirement is that the product should be easy to store. One expert has suggested that a storage life of more than18 months is necessary for a commercial product. Few farms around the world can afford refrigerated and/or humidity-controlled storage, so we're talking about storage in a barn or shed with no special precautions. These aspects of the 'recipe' can be controlled by the nature of the formulation with which the fungus is mixed to make the commercial product. The formulation can also assist initial infection if it includes materials which help maintain favourable humidities while the spore germinates on the host. There is a limit to this, of course, and then the product is at the mercy of the real world, depending on atmospheric factors and the density of the host population to achieve the desired catastrophic infection of the target organism, prolonged pest control, reduced risk of resistance and a high degree of safety to non-target organisms with all the associated environmental benefits. It's a long job.

It's not only insects that are pests. Weeds pose a large problem in both agricultural and natural ecosystems, not just in gardens and amenity sites in urban areas. Most people would agree that an insect which eats its way through the leaves of a plant is something of a pest, but weeds are not that easy to spot. What is a weed to one person is a wild flower to another. A plant might be designated as a weed because it has a detrimental affect on agriculture or amenity plantings, but the same plant may actually benefit beekeepers, wild flower and fruit gatherers, herbalists and others. Not surprisingly, attempts to control the 'weed' may generate heated arguments! There's an interesting story about a plant called *Chromolaena odorata* from Brazil which became what is known as a pantropical weed causing a biologically devastating invasion of Ghana and the Côte d'Ivoire in 1993. In Brazil, where the plant was native, it was not considered a weed, and though classed as common in Brazil, it never formed the dense, continuous stands which characterized its growth pattern in West Africa (and Asia). The plant caused significant agricultural and environmental damage over a large area of West Africa and Asia, and was suggested as a good candidate for a biocontrol programme. This led to an outcry, however. Some people suggested using chemical herbicides, despite the enormous area that would have to be sprayed. But others argued that the weed should not be controlled at all because the plant had medicinal properties and because it formed a rapid plant cover in the 'slash and burn' agriculture of the region. The counter arguments were put so strongly that funding was withdrawn from the proposed biocontrol project.

Agricultural losses due to weeds are difficult to estimate but there is broad agreement that at least ten percent of the world's agricultural production is lost each year. For the US this means that annual crop losses due to weeds range up to thirty billion US-dollars. Estimates like this do not include the negative effects of environmental weeds in natural ecosystems. Another measure of the importance of weeds is to estimate how much people are willing to spend to get rid of them by using herbicides. Herbicide sales account for about seventy percent of all pesticides sold (the proportion was only twenty percent in the early 1950s) and have a retail value of around twenty billion US-dollars. Invasions by weeds which are 'foreign' to a region have become a particular problem as international travel and commerce have increased. Such introductions (usually unwitting and unintentional) can invade native vegetation and out-compete the resident plants so disrupting the balance of the whole ecosystem.

The recognition of fungi as important natural enemies of weeds which might be safely exploited to our benefit is not new, but it wasn't until the 1970s that they began to be used in serious attempts at weed biological control. The approaches used for control of insect pests are just as valid for biocontrol of weeds with fungi. Where the target is a plant which has become a pest outside its natural range by being introduced into some 'exotic' area, then the best strategy is to introduce a fungal natural enemy of a target weed from its native range into the exotic area. An alternative strategy is to use fungal pathogen from the exotic area but to mass produce them and apply at such a high concentration that the weed population is inundated with fungal disease. Just as with attempts to control insect pests, it is a long job and is dependent for success on enormous amounts of scientific research. It is also essential to make proper economic evaluations of the project. No matter how attractive the idea might be from scientific and conservation points of view, someone will have to foot the bill and economic feasibility and cost/benefit analyses must be done.

At first glance the use of fungi in weed biocontrol seems like enlightened management which is likely to solve important problems and contribute towards sustainable agricultural development. But the truth is not quite so clear cut. Yes, you can find numerous examples of weed infestations which have caused major problems like disrupting fishing activities, harbouring disease vectors, and major crop losses. In some instances the effects have overtaken whole cultures and led to agriculture being abandoned or major population movements to escape the problem. No one can deny that weed control (with fungi or with chemicals) in such dramatic cases is desirable in social and cultural terms. But these are the dramatic extremes which are, thankfully, rare. In most places, most weeds are just a few plants growing out of place in a field or other cultivated plot. Careful husbandry should be able to cope with them. In fact, weeding is still one of the most common activities of a large part of the human population. So think of it this way: in a sense, weeds could be seen as being beneficial because they generate honest employment. You might counter that by pointing out that physically removing weeds is such a demeaning human activity that it is a waste of human resources. Freeing the human population from this burden then becomes a noble goal for scientists and technologists alike. The attractions of the 'honest toil' argument are deceptive, indeed illusory. There's a direct analogy with the development of industrial machines which change the nature of the industrial shop floor. Supplementation, and eventual replacement, of human toil is the desirable aim and herbicides have the capacity to do that. When mycoherbicides are common in the marketplace they will expand the options available to major users of herbicides.

Control of weeds with mycoherbicides is an ecologically 'clean' method. It uses a natural agent and leaves no chemical residues. There are concerns that fungal plant diseases which are used as mycoherbicides might also pose a threat to crops or native plant species. Knowledge is the key, here; particularly knowledge about host specificity, disease severity and possible tolerance to the disease. Paradoxically, a fungus that is capable of attacking a native or even a crop plant may have potential as a biocontrol agent under particular circumstances. It's then a matter of weighing the cost of causing disease in native plants against the benefit of using that same disease to control the weed. If the weed population is less tolerant of the disease then the balance shifts in favour of using the fungus to control the weed. This type of consideration applies to chemicals, too. Most pest control chemicals are toxic to a wide range of organisms - the useful ones are more toxic to the pest than to the crop. It's a matter of scientific research again. The risks of plants other than the target weed being affected by an introduced mycoherbicide fungus can only be judged if appropriate scientific knowledge about the mechanisms of fungal host-specificity is available.

The potential economic value of mycoherbicide is becoming easier to judge as more trials are completed. Introduction of a rust fungus into Australia to a weed which originated from the Mediterranean region was certainly biologically effective. It resulted in better than ninety-nine percent reduction in infestations by the weed. But the estimated *annual* saving due to increased crop yields and reduced chemical herbicide use was sixteen million Australian dollars. Since the cost of the whole project was only three million Australian dollars, the return on this initial scientific investment has been enormous. Unfortunately, benefits on this scale are not often seen. The mycoherbicide 'business' has a history, similar to the mycoinsecticide business, of products being researched, brought to the market and sold apparently successfully for several years, but then being withdrawn. Often the market is too limited to repay the high initial development costs and then support the ongoing costs of further development to cope with competition. Several products which were successfully sold in the 1980s were withdrawn in the 1990s because of the costs of registration procedures imposed by environmental protection agencies.

This last point brings home the fact that a major obstacle to the introduction of biological control is (unjustified) fear of disease spread. This 'pathophobia' has led to overzealous rules and seemingly endless tests and trials. Oddly enough, this bias against introduction of fungal plant diseases as biocontrol agents is not also applied to introductions of insect pests and most countries pay little attention to precautions against new weed introductions. About half of the weeds in United States and thirteen of the top fifteen weeds are introduced species. Similarly, seventy-eight of the one hundred and seven most noxious weeds in Canada were introduced to the country. The best way to control such 'aliens' is to introduce diseases from their home territories. But the regulatory authorities make that process unreasonably difficult (and unrealistically costly). They do so despite the fact that the overall rate of effectiveness that has been achieved with the fungal diseases which *have* been introduced in the past, is sixty-seven percent. We're often quite good at shooting ourselves in the foot!

Plant parasitic nematode worms are also candidates as targets for using fungi as biological control agents. These nematode worms spend part of their life cycle in soil or on the root surface where they are exposed to nematode-attacking (called nematophagous) fungi that could provide useful alternatives to chemical nematicides. Current evidence is that trying to enhance the activities of resident nematophagous fungi in soils requires too much material to be generally acceptable. On the other hand, adding biological control fungi to soils where they are scarce or absent could lead to commercial products. But many problems remain to be solved before predictable control can be achieved at practical rates of application. If nothing else, greater knowledge of the pest and its interactions with soil, host and fungal disease agent might lead to identification of more acceptable chemical nematicides.

One thorny problem that all biocontrol projects face is that of what to do about patenting the biocontrol fungus. Commercial companies would expect to be able to get some patent protection for any agent in which they have invested. A chemical is relatively easy - you can patent the chemical itself, the way you produce it and whole families of chemical derivatives. You can then put the material on the market, confident that even if a competitor buys a supply of the material they cannot make more of it very easily. When the agent is a live organism, though, any competitor with a half-way competent biologist would be able to grow more of that organism from even the smallest sample of the commercial material. If the biocontrol agent is a genetically modified organism their identity is not likely to be difficult to establish and the company which first created them will, therefore, be able to identify and claim what rightly belongs to them. But suppose the biocontrol agent is a fungus which was isolated from nature. Say, your staff collected fungi from a particular site and found one of them to be especially virulent in controlling a specific pest. What's to stop a competitor going out to the same place and finding a related fungus that's equally virulent? What's to stop an unscrupulous competitor growing up your fungus and simply *claiming* that it's something they've newly found? There's no difficulty in writing patents, registrations or other legal devices which assign ownership. The real difficulty is in having a catalogue of identifying features of the commercialized organism which is sufficiently good to ensure that you could recognize it in any competitive product. Genetic markers help a lot with organisms, like fungi, that have few distinguishing features of their own. That's why there's so much interest in 'genetic fingerprinting'. These techniques really would allow you to identify that a competitor had stolen your organism. Of course, you can never stop competitors going out to find a biocontrol organism of their own. The thing to do in that case is to license your patented product for your competitor to sell at a price less than the cost of developing a separate competing organism. Badge-engineering for fungi!