32

Virulence Management in Biocontrol Agents

Sam L. Elliot, Maurice W. Sabelis, and Frederick R. Adler

32.1 Introduction

Although biological control is founded upon the virulence of natural enemies to the targeted pests, there has been little effort to understand how this might change, let alone to manage it. Frank Fenner and colleagues can be credited with being the first (and last!) to monitor changes in virulence of a biological control agent, namely the myxoma virus used to control rabbits in Australia (Fenner and Fantini 1999). This is despite a body of literature showing that the virulence of natural enemies can and does change in response to selective forces, either natural or artificial. These studies cover a wide taxonomic range of organisms, including fungal pathogens of plants (Burdon and Thrall 1999; Brasier *et al.* 1999; see also Chapter 31), microsporidian parasites of daphnids (Ebert 1994), pathogens and parasites of humans (Ewald 1994; Chapters 2 and 28), malarial parasites of rodents (Chapter 12), pathogens and parasites of social *Hymenoptera* (Schmid-Hempel 1998; Boot *et al.* 1999; Oldroyd 1999), nematode parasites of fig wasps and fruit flies (Herre 1993, 1995; Jaenike 1996, 1998), and hymenopteran parasitoids of aphids (Henter 1995; Henter and Via 1995). The results of this work have suggested that the course of virulence change can be predicted and possibly even manipulated. In biocontrol, a predator, parasitoid, or pathogen is used to control a pest population, a pest being defined as an animal, plant, or microorganism that is perceived to be damaging to some human activity. In this setting, the practical aim of virulence management would usually be to increase virulence. This contrasts with the management of virulence of pathogens or parasites of humans, domestic animals, or crop plants, in which low virulence is the aim.

So how does one attempt to manage the virulence of a biocontrol agent? Theory predicts that increased virulence is selected for when a natural enemy is limited in its control over the resources contained within a victim. This occurs through competition with other natural enemies (including other strains of the same species) or through external sources of victim mortality (Van Baalen and Sabelis 1995a, 1995b; see Chapters 7, 9, 11, and 22). Both of these factors are potentially manipulable and in this chapter we present a framework in which this might be attempted.

The field of biological control employs a range of natural enemies to control pest organisms. Workers using pathogens as biocontrol agents have traditionally recognized virulence (commonly termed "aggressiveness" in plant pathology, see, e.g., Jarosz and Davelos 1995) as an important attribute to assess, but we hope to

demonstrate that virulence is also a trait possessed by arthropod natural enemies (herbivores of weeds, predators and parasitoids of arthropod pests, and fungivorous arthropods). Various biocontrol strategies can be considered, for the sake of the arguments developed in this chapter, to differ principally in the degree to which the natural enemy exploits the pest resources and converts them into new natural enemies. We use the term *inundative biocontrol* for the release of a natural enemy as a biological pesticide with a rapid and short-term effect with no reliance on subsequent generations of the biocontrol agent, *inoculative biocontrol* for the release of an agent that does not exert immediate control but acts over more than one generation, and *classical biocontrol* for the introduction of an exotic natural enemy to a new area to achieve control over many generations. These strategies form a continuum, with a further strategy being the manipulation of the environment to foster naturally occurring enemies of the pest (*conservation biocontrol*).

We begin by proposing that the virulence of a natural enemy is not necessarily assessed adequately by a consideration of the effect on individual victims, but requires consideration of how victims are distributed and exploited in space. From this standpoint, we question whether high virulence to an individual victim really is a desirable trait in a natural enemy. We then ask how stable the virulence of a biocontrol agent is likely to be in the field, how it might be manipulated in the field, and how it is affected by the mode of mass rearing (the production of large numbers of the natural enemy prior to release). We expand our treatment to include other components of pest management systems, asking why insect pathogens appear to attack herbivores more than the predators of those herbivores when they could, in principle, infect both. Throughout the chapter, our focus is on virulence from the point of view of the natural enemy under the assumption that the victim cannot adapt. The ecological and evolutionary response of the pest to its enemy's virulence is briefly discussed in the penultimate section.

32.2 At What Level Should Virulence be Considered?

The archetypal parasite is an organism that lives in association with another individual organism (the host or victim) which it exploits, to the latter's detriment. The negative effect on the host (ideally measured as a loss in the host's fitness) is the parasite's *virulence* and depends to a large degree upon the exploitation strategy that the parasite adopts. For an organism such as a pathogen, its virulence is clearly definable as the effect it has on an individual host, and this we term "individual-level virulence." Arthropod herbivores are often considered as parasites of plants (e.g., Crawley and Pacala 1991; Begon *et al.* 1996) and can therefore be ascribed an individual-level virulence. We argue that this also applies to predators. In the simplest terms, a given predator–prey interaction can be lethal or nonlethal, a binary form of virulence. Intermediate shades can occur, because attacks may be nonlethal but still damaging and because the mere detectable presence of a predator can have fitness consequences for its prey, for example through antipredator

behavior (Heads 1986; Dixon and Baker 1988; Lima and Dill 1990; Stamp and Bowers 1993; Pallini *et al.* 1998).

It is at this individual level that the notion of virulence is normally applied, but we argue that selection on virulence acts at different spatial scales (Boerlijst *et al.* 1993; Miralles *et al.* 1997; Van Baalen and Sabelis 1995c; Sabelis *et al.* 1999c, 1999d). For example, many insect pests of plants have a tendency to form discrete infestation foci that cover an area ranging from part of a plant up to several neighboring plants. Such a patch of herbivores is, to an organism which invades that patch (and will reside there for more than a short period), just as much of a host as an individual is to the archetypal parasite. In this case we use the term "patch-level virulence." While we expect the properties of a host patch to have stark differences to those of an individual host – individual organisms may emigrate whereas cells cannot – there are striking similarities (Levin *et al.* 1999). For either an individual victim or a patch of victims, parallels can be found in:

- The quantity and quality of invading propagules;
- The natural enemies' ability to reproduce within and to exploit the victim(s);
- The probability of encountering other natural enemy genotypes;
- The role of host defenses;
- The amount of natural enemy propagules produced by the end of the interaction with an individual victim or with the victims in a patch.

Such analogies have been used to study the virulence strategy of a predatory mite invading a patch of herbivorous prey mites (Van Baalen and Sabelis 1995c; Pels and Sabelis 1999; Sabelis *et al.* 1999c, 1999d). Again, there is no need to restrict this to a particular class of natural enemy. To a pathogen or parasite invading a colony of eusocial arthropods, the colony is akin to an individual host (Schmid-Hempel 1998, p. 263) and the host population need not be social for this to apply (Oduor *et al.* 1997). The critical point is that the individual-level virulence in such a setting is only one of a range of variables which will contribute to an overall patch-level virulence. For example, the importance of the production of new infective propagules within a local population can be critical in determining the overall, patch-level virulence, as with the fungal pathogen *Metarhizium flavoviride* in orthopteran hosts (Thomas 1999). At this patch level of virulence, there may well be a trade-off between virulence and transmission, just as at the individual level.

At which of these (or other) levels we should consider virulence depends upon the patchiness and viscosity of the populations of the target organism and the biocontrol agent, as well as on the biocontrol strategy being employed. Individual-level virulence is more important in a purely inundative approach, as there is no dependence on reproduction, or in a nonpatchily distributed pest population. In contrast, in inoculative and classical biocontrol or in a more patchy pest population, patch-level virulence comes to the fore. It is important to realize, however, that even with the inundative use of a biocontrol agent, it may be at the patch level that selection on virulence is most influential in the natural populations from which that agent has been taken.

32.3 Is High Virulence Always Desirable?

Rather than a highly exploitative strategy, a natural enemy may adopt a strategy of restraint in exploiting its host or host patch, thereby preserving for longer its resource, exploiting its victims' capacity for growth or reproduction, and so ultimately producing more propagules than it would otherwise. This is incorporated into trade-off functions like black boxes when considering individual-level virulence (but see Nowak *et al.* 1991 for an exception), but it is only at the patch level that a mechanistic explanation for this trade-off has been attempted. The latter case considers local predator–prey dynamics in which high and low virulence strategies are termed "killer" and "milker," respectively. The trade-off revolves around the emigration of predator individuals, which relieves pressure on the victims in the patch, and thereby allows prey reproduction to be "milked" in the low virulence strategy (Van Baalen and Sabelis 1995c; Chapter 22). By producing more propagules per patch, the milker may ultimately better control metapopulations of a pest organism, which is usually the aim of biocontrol. In other words, high virulence may even compromise biocontrol (Te Beest *et al.* 1992; Thomas 1999), especially where a trade-off exists between virulence and transmission (see Bull 1994; Messenger *et al.* 1999). This is likely to become more of a concern as we move across the spectrum from inundative to classical biocontrol, that is, as the colonization of new pest patches becomes more important.

This point can be illustrated by referring to the example of the use of predatory mites (Acari: Phytoseiidae) in the Africa-wide control of the exotic cassava green mite (*Mononychellus tanajoa* Bondar, Acari: Tetranychidae). A survey of neotropical natural enemies resulted in ten candidate species of predatory mite of which three established successfully in Africa upon release (Yaninek *et al.*, unpublished). It appeared that the two predators with the fastest predation and population growth rates hardly spread between cassava fields, whereas *Typhlodromalus aripo* DeLeon, with lower growth and predation rates, readily spread beyond the release fields and still had an impact on the pest population. The latter species stands out not only because of its lower predation rate, but also because it forages actively only at night on the upper leaves, thereby passing up opportunities for predation (A. Onzo, personal communication). During the day it sits in the growing tips of the cassava plants and waits for the few prey that walk up to feed on leaf primordia. As a result of its lower functional and numerical response, this predator does not overexploit its prey as fast as the other two. While this predator does not exterminate a patch of prey as quickly as would a more virulent one, this strategy allows it to reach larger numbers. It then has a stronger foothold from which to spread to other prey patches. Thus, the successful establishment of *T. aripo* may well represent a case in which low virulence promotes biocontrol.

Another example of low virulence being desirable in a (classical) biocontrol agent is found in weed control. Many of the successful biocontrol agents of weeds castrate their hosts, but do little harm otherwise. Castration is usually partial or temporary, which is considered the result of a host escaping from its castrator

(Minchella 1985; Hurd 1998; Hsin and Kenyon 1999). Some castrators are insects, such as pre-dispersal seed-predating weevils (Crawley 1989). Other examples are eriophyoid mites that make galls or cause other growth deformations in buds and flowers of plants, thereby blocking reproduction of the plant (Rosenthal 1996; Sabelis and Bruin 1996). Such parasites may have little immediate effect on the vigor of the host, which has been cited as a potential drawback for their use as biocontrol agents (Cromroy 1979). Such a view, however, seems shortsighted when it is remembered that the main goal is the control of *populations* of weeds, not individual plants *per se*. In their review, Te Beest *et al.* (1992) found that pathogens used in classical biocontrol of weeds are most successful where they reduce host reproductivity or cause low mortality. Simulation modeling by these authors predicted optimal weed control from an introduced pathogen when the pathogen caused 66% mortality or sterility. Increased pathogen virulence led to oscillations and increased weed biomass, but the effects on weed population dynamics are unlikely to be straightforward (Crawley 1989) and one must consider carefully what is meant by virulence. In terms of the host's fitness, the impact of castration is catastrophic and an evolutionary biologist might label this as high virulence, but in terms of host survival the parasite's impact is low and a biocontrol worker may characterize it as low virulence. By castration, a parasite may use resources that would otherwise be directed to reproduction (Baudoin 1975; Obrebski 1975; Kover 2000), thereby minimizing its effect on the longevity of the host and gaining a foothold for dispersal through a population of hosts (García-Guzmán *et al.* 1996).

Further evidence of the potential for castrators to better regulate populations of their hosts comes from a concordance of predictions from modeling with experimental evidence in the case of insect–parasitic nematodes (Jaenike 1998). Castrating parasites of animals may even prolong the host's life span, as demonstrated by artificial castration of a nematode (Hsin and Kenyon 1999) and, similarly, castration of plants by fungal pathogens has been linked to increased viability, vegetative vigor, or preferential survival under grazing (Bradshaw 1959; Clay 1990; Burdon 1991). Ebert and Herre (1996) state that it is a mystery why castration as a parasitic strategy is not more common or even universal. However, this strategy probably evolves only when single infections prevail: it is expected to be highly vulnerable to invasion by a more virulent parasite genotype that uses the resources left available by the castrator. When this happens, the best strategy for the parasite would be to switch to higher virulence as the advantage of the prolonged life span of the host is lost.

32.4 Is High Virulence a Stable Trait in Biocontrol Practice?

The classic case of a change in virulence in a pathogen is the introduction of the myxoma virus to control the European rabbit, *Oryctolagus cuniculus* (L.), in Australia, where subsequent evolution toward a more benign state was reported (see Kerr and Best 1998; Fenner and Fantini 1999). This observation has been criticized somewhat in terms of the bioassays used (Parer *et al.* 1994; Parer 1995)

and the possibility that virulence may subsequently have increased (Dwyer *et al.* 1990; Ewald 1994a; Fenner and Fantini 1999), but there is little doubt that virulence has changed. This is the critical feature for our discussion of the evolution of virulence, whatever the direction of the change.

For a biocontrol agent, it is highly unlikely that virulence will remain unaltered during mass rearing (see Section 32.6 below) and after introduction in the field, unless the original sample of biocontrol agents contained little relevant genetic variation and the processes creating such variation are relatively slow. This is because the evolutionarily stable virulence under these conditions is unlikely to match that under the natural conditions in which they were moulded by selection. We therefore expect a shift in virulence toward a new evolutionarily stable state. The remaining questions are then whether the changes will be toward higher or lower virulence, how far along this trajectory the natural enemy will evolve, and what the consequences are for the agent's efficiency. As we have stated, selection may not occur at the individual level, so patch-level virulence may need to be considered. Predicting the direction of change relies on an understanding of the theory of the evolution of virulence. Thus, virulence should increase by selection when there is horizontal transmission (Anderson and May 1991), transmission by vectors (Power 1992; Ewald 1994a), a high background mortality (Anderson and May 1979; May and Anderson 1983b), a high probability of multiple infections (Nowak and May 1994; Van Baalen and Sabelis 1995a, 1995b), or when parasites produce propagules for long-term survival outside the host (Hochberg 1989; Bonhoeffer *et al.* 1996; Gandon 1998). It is important to realize, however, that these factors may interact with one another. For example, low host mortality may allow more time for parasite genotypes to reproduce within the host and so compete, thereby leading to selection for higher virulence than expected if only the effect of mortality under single infections is considered (Ebert *et al.* 1997; Gandon *et al.* 2001).

Three determinants of the degree of change in virulence are the genetic variation (extant or novel) of the natural enemy, the magnitude of the selection pressure, and (of critical practical relevance) which biocontrol strategy is to be used. In classical biocontrol, the relevant time scale covers many generations of the natural enemy, so there is plenty of time for virulence to evolve. In inoculative biocontrol the time scale is shorter, but we can still expect at least some transient changes over generations. For inundative biocontrol, however, there is only one natural enemy generation and the biocontrol agent has no opportunity to evolve. The exception to this occurs when pathogens are used and they reproduce inside host individuals, in which case there will be many generations of the pathogen and there may be substantial changes in the pathogen's virulence (Sokurenko *et al.* 1999). Environmental manipulation (or conservation biocontrol) may incidentally alter the pattern of selection for virulence of naturally occurring biocontrol agents, for example by increasing background mortality of the host. An intriguing but little explored area is the extent to which the virulence expressed by an exploiter is a plastic response to a changing environment. The expression of virulence factors by pathogenic

bacteria within their animal or human host may depend on the detection of a sufficiently large population of the same species of bacterium, a phenomenon known as "quorum sensing" (Williams *et al.* 2000). Whether this sort of phenomenon also occurs in biocontrol systems is an open question. However, it is now well established that insect pathogens, especially viruses, can be more virulent when their host is stressed. It is also common for pathogens to remain at such low levels in their host that they are very difficult to detect, but under some circumstances are still lethal (e.g., Marina *et al.* 1999). Is the reason increased susceptibility of a stressed host, or within-host evolution, or could it be that the pathogen alters its strategy of host exploitation because it detects the increased likelihood of the host dying?

32.5 How Can Virulence be Manipulated in the Field?

If we have some understanding of what determines virulence we are a little way down the road to manipulating it. We have so far discussed the spatial scale at which virulence should be assessed, whether individual-level or patch-level virulences are desirable features of a biocontrol agent, and how likely they are to change in the field. The theory on the evolution of virulence provides pointers as to how virulence management may be undertaken in a given system. The first step is to elucidate the principal transmission route(s) of the natural enemy (horizontal versus vertical, the role of vectors). Then whether there is a trade-off between transmission and disease-induced mortality must be determined (otherwise avirulence of the natural enemy is expected to evolve). Having established these basic features, the key point is to consider the degree to which a given genotype is able to keep control over the exploitation of the victim's resources in the face of competition with other genotypes or other sources of victim mortality. Thus virulence can be increased in the field by any measure by which the background mortality or the probability of multiple infections is increased (Nowak and May 1994; Van Baalen and Sabelis 1995a, 1995b), or by any measure that reduces the cost to the natural enemy of overexploiting the victim's resources.

One possible route to manage virulence is via vectors of the biocontrol agent, if such exist. Alternatively, chemical SOS signals of the plant, supplementary food (extrafloral nectar), or refuges (domatia) that attract or maintain natural enemies may be manipulated to increase the chances of multiple infection of the herbivores and so promote the virulence of natural enemies (Sabelis *et al.* 1999a, 1999c, 1999d; Chapter 22). The ability of a plant to foster such "bodyguards" may also provide an explanation for the high virulence of the insect pathogen and saprophyte *Bacillus thuringiensis* to insects. The bacterium is commonly found on the plant phylloplane. If it is the plant which maintains this population, independent of potential insect hosts, then there is no cost to the bacterium of overexploiting the victim, and so no constraint on evolving higher levels of virulence (Elliot *et al.* 2000). In this case, breeding for mutualistic plants will not only directly benefit the insect pathogen but may also indirectly trigger selection for increased virulence of these pathogens.

The integration of different pest control strategies may also allow management of virulence. Thus, selective pesticides or, most interestingly, other natural enemies will provide an alternative source of background mortality of the pest, which causes the biocontrol agent to "compete" with them and generates selection for high virulence. If high virulence is desired, then the clear implication is that an integration of control strategies should maintain high virulence in the biocontrol agents. When strains of biocontrol agents are screened for high virulence to the target pest, factors can be included that will contribute to the maintenance of that virulence, such as having long-lived propagules (Hochberg 1989; Bonhoeffer *et al.* 1996; Gandon 1998). For any biocontrol agent, the potential for its virulence to be a plastic trait should be borne in mind. We have discussed the possibility of pathogens displaying plasticity within their host (Marina *et al.* 1999; Williams *et al.* 2000), but it is also known that organisms exploiting a patch of victims can adjust their strategy according to the availability of victims or the presence of competitors (Janssen *et al.* 1998). It is possible that the response of an exploiter to potential competitors is to reallocate its resources from within-host reproduction to some form of interference competition, such as the production of toxins (Chao *et al.* 2000). In this case, multiple infections would lead to the capacity for virulence of the biocontrol agents to be "wasted", from the point of view of effective biocontrol.

32.6 Does Mass Rearing Affect Field Virulence?

A common concern in the mass rearing of biocontrol agents is the maintenance of their effectiveness in biocontrol (Hopper *et al.* 1993; Thompson 1999; van Lenteren and Nicoli 1999). The selective pressures in a mass rearing can be quite different from those in the field and routine procedures are usually in place to limit the loss of virulence. Pathogens are normally cultured on artificial media, which can lead to a loss in virulence to its original host as the trade-off between virulence and transmission rate is removed or even reversed. This effect can be ameliorated by using a media that more closely resembles the host nutritionally (e.g., Hayden *et al.* 1992). It is known that small populations of parasitoids may contain sufficient genetic variation to allow selection for higher or lower virulence (Henter 1995). In mass rearings, in which parasitoids are commonly reared in factitious hosts (i.e., different hosts from the target hosts, and ones which can themselves be reared more easily or cheaply), the loss of virulence toward the target pest is a practical problem. A routine procedure with pathogens or parasitoids is to pass them through the target host species to restore virulence, but this is done with little scientific understanding of the mechanisms which restore virulence. Such periodical selection is for high virulence at the individual level and one may question the desirability of selection for this single trait. Even for some pathogens used inundatively, survival in the field is usually as important for effective pest control as is virulence. For arthropod natural enemies, behavioral traits are also important and it is unlikely that single traits measured in the laboratory will predict the efficacy of the biocontrol agent in the field (Bigler 1994).

Biocontrol agents may be reared on the target pest, as with the phytoseiid mite *Phytoseiulus persimilis* Athias-Henriot, a predator of the two-spotted spider mite. In this instance, virulence of *P. persimilis* to a patch of prey is determined by its rate of conversion of prey into eggs and its retention in the prey patch (Sabelis and van der Meer 1986). Both of these traits are selected for in commercial mass rearings because there is selection for increased growth rate and dispersing mites are lost. Thus, the high patch-level virulence observed in the field (Pels and Sabelis 1999; Chapter 22) is expected to be conserved in a mass rearing. A converse example can also be found among predatory mites: *Hypoaspis aculeifer* (Canestrini) is used in the biocontrol of the bulb mite *Rhizoglyphus robini* Claparède, a pest of lily and freesia corms, but is reared on a nontarget mite, *Tyrophagus putrescentiae* (Schrank). In the rearing, a genetic polymorphism in preference for these two prey and associated reproductive success is maintained by hybrid advantage (Lesna and Sabelis 1999). This means that those genotypes with a preference for the target pest are maintained, but are diluted in a mixture of other genotypes that perform less well on the target pest. Thus, the mass-reared predators have a lower virulence with respect to the target pest than does the specialist genotype.

32.7 Pathogen Virulence Toward Herbivores and Their Predators

A quick glance at the field of invertebrate pathology shows that many more pathogens are known from arthropod herbivores than from arthropod predators or parasitoids (their natural enemies). There is a clear bias as the majority of invertebrate pathologists are interested principally in controlling herbivorous pests using microbes. However, this may also be a genuine biological pattern that begs an evolutionary explanation. Carnivores may vector the pathogen between local herbivore populations (see Brooks 1993), for example following ingestion of infected prey and passage through the gut of a predator (Vasconcelos *et al.* 1996) or following the external pick-up of propagules (Pell *et al.* 1997; Roy *et al.* 1998). Ewald's (1987b, 1994a) explanation is that high virulence to the carnivore would be counterproductive for the pathogen. Assuming that predator and prey can be infected only once, recent modeling work showed that the pathogen should evolve to be relatively mild to the predator, provided it is sufficiently more mobile than its prey (Elliot *et al.*, unpublished).

Multiple infections may also play a role in explaining lower virulence in carnivores than in their victims. Carnivores may well be able to avoid infected prey, so reducing the likelihood of multiple infections, while it is hard for a prey patch to prevent invasions by infected carnivores. The long-term consequence of this is a lower relative virulence to the carnivore. A parallel can be seen with arthropod vectors of animal parasites, for which the conventional explanation of low virulence to the vector is that of Ewald (1987b, 1994a; Koella 1999). A more powerful explanation, however, is that the vector takes only a few blood meals in its life (and so is more likely to be infected singly), whereas the host may receive much unwelcome attention from vectors (and so is more likely to be multiply infected; Macdonald 1957; Molineaux and Gramiccia 1980; Anderson and May 1991). Thus, pathogens

and carnivores are expected to be compatible biocontrol agents where multiple infections of the carnivore are sufficiently rare that the pathogen's virulence to them does not impede their acting as vectors.

32.8 Ecological and Evolutionary Response of the Pest

So far we have only considered evolution of the natural enemy, ignoring the consequences of decreased pest density and the pest's evolution of resistance to its enemies. Introducing a biocontrol agent ultimately leads to a decrease in pest density and a natural enemy density lower than the initial one, which in turn decreases the probability of multiple infection and thereby the optimal virulence. This is an explanation for the observed reduction in virulence of the myxoma virus used for the classical biocontrol of rabbits (Fenner and Fantini 1999). It represents an alternative to the explanation given by Anderson and May (1991), which is also based on trade-offs but assumes single infections.

The use of biocontrol agents also generates selection pressure on the target pest to evolve resistance. This has been observed in the case of rabbits that developed resistance to myxoma (Fenner and Fantini 1999). Such a pest response opens the possibility for coevolution between exploiter and victim. The end result of this process is not immediately obvious, because pest density may increase and the mean susceptibility may decrease, so it is unclear how all of this affects the probability of multiple infections. Clearly, population dynamics determines densities and the probability of multiple infection, whereas the direction of evolution is determined by the probability of multiple infection. Thus, the interplay between evolution and population dynamics determines the outcome.

Holt and Hochberg (1997) highlighted an apparent discrepancy in the persistence of classical biocontrol versus chemical control. Under chemical control pests may rapidly become resistant (Roush and Tabashnik 1990; Gould 1991) whereas under classical biological control such resistance has not been reported (Croft 1992). This discrepancy may result from genetic constraints on selection, from differences in selection pressure, or from differences between the control agent and the pest in their capacity to respond evolutionarily. It remains an open question why the inundative use of pathogens has led to resistance (Tabashnik 1994; Moscardi 1999), whereas inundatively released arthropod natural enemies have not, as in the case of greenhouse biocontrol. This pattern is confirmed by an elegant experiment carried out with pea aphids *Acyrthosiphon pisum* (Harris), and a parasitoid *Aphidius ervi* Haliday (Henter 1995; Henter and Via 1995). Here, increased host resistance to parasitization and increased parasitoid virulence were obtained by selection in the laboratory, but increased host resistance did not arise under strong parasitization pressure in the field. This suggests that resistance to parasitoids (and predators) is more costly than resistance to pathogens or chemical pesticides. This is understandable, since barriers to chemicals, or their breakdown (and pathogens are quite reliant on chemical means of overcoming host defenses), are often based on changes in the expression of a small set of genes (Roush and Tabashnik 1990; Sayyed *et al.* 2000). This contrasts with the costs of polygenic

traits involved in establishing morphological defenses to arthropod natural enemies (Tollrian and Harvell 1999) and perhaps to the costs of immune responses (Kraaijeveld and Godfray 1997; Fellowes *et al.* 1998). We suspect a gradient in costs from relatively cheap biochemical defenses through immune responses to costly morphological defenses. As the development of resistance is likely to depend upon the costs to the pest organism, it should be more rapid when defenses are less costly (Sasaki and Godfray 1999), for example against chemicals or pathogens. For models incorporating more costly resistance, Sasaki and Godfray (1999) showed that resistance may either not arise or may develop and break down in cycles. This may explain why Henter and Via (1995) did not find an immediate development of resistance after a sudden increase in parasitization pressure and possibly also why biological control with arthropod predators has rarely resulted in resistance when compared with control by pesticides or pathogens.

32.9 Discussion

In this chapter we question the assumption that high individual-level virulence is the Holy Grail of biological control. We argue that virulence at the individual level may be but one component of virulence toward a patch of victims. It may be at the patch level that selection on virulence has occurred in the natural setting, and it may be at this level that virulence will be most relevant in biocontrol. After all, it is in the control of (meta)populations of a pest that biocontrol workers are ultimately interested. Whenever the agent is expected to produce more than one generation, its patch-level virulence is composed of its ability to find and attack new victims, convert these into offspring, and disperse these locally. Thus, a suite of traits becomes important as one moves from inundative via inoculative to classical biocontrol. In the first case, a rapid kill of pests in a localized area is desired, whereas in the last case the aim is to achieve establishment, spread, and long-term persistence of the biocontrol agent over a large geographical area.

When interacting with an individual pest or with a local population of pests, a biocontrol agent faces the possibility that its resource will become unavailable because of incidental pest mortality or competing exploiters. The key to understanding changes in virulence is to understand the degree to which the exploiter is able to maintain control over the victim's resource. Any means by which this control can be reduced increases the virulence of the natural enemy and perhaps its effectiveness for biocontrol.

As a final word of caution, changes of virulence have both population dynamical effects and effects on victim resistance. These will in turn alter the selective pressures on virulence. If virulence increases, pest numbers are likely to decrease and pest resistance increase. As these processes feed back into the population dynamics of the biocontrol agents, they will further affect the probability of multiple infections and so alter the pattern of selection on virulence. Thus, the processes and options for virulence management discussed here must be put into a broader coevolutionary and population dynamical context.

Acknowledgments We thank Arne Janssen and his graduate student discussion group, Bas Pels, and Minus van Baalen for their helpful comments on the manuscript of this chapter. Sam L. Elliot was supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO).