Augmentative biological control using entomophagous arthropods

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Chapter 4

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1. Background and definitions

Augmentative biological control is based on the repeated introduction of biological control agents into agricultural crops. These agents are mass-produced in commercial insectaries, with the aim of eradicating pest populations in the short to medium term (van Lenteren, 2012). In this chapter, we will focus more specifically on the issues related to the use of entomophagous arthropods (insects, mites) – i.e. predators or parasitoids – and entomopathogenic nematodes used against phytophagous arthropods.

An augmentative biological control programme aims to quickly reduce the pest population or maintain low levels of infestation throughout the growing season by directly introducing natural enemies from an exogenous source into the cropping system. This type of control is particularly apt when natural enemies are absent or are unable to persist naturally in the crop and surrounding environment to prevent damage to the plants. For example, this may occur when the natural enemies are unable to survive locally between growing seasons or when their densities are too low (isolation from the crop, short growing season). The goal is therefore to artificially increase the natural enemy populations to densities that allow for satisfactory pest control (Sivinski, 2013).

1.1. The blurred line between inoculation and inundation

The term augmentative biological control traditionally encompasses two methods of introduction: inundation and inoculation (Eilenberg et al., 2001). Inundation control aims to quickly eradicate pests by releasing massive numbers of natural enemies, while inoculation control seeks a more sustainable regulation through the temporary establishment of natural enemy populations over several generations. For both methods, introductions are repeated over time when pests reappear, or according to a determined schedule. Strictly speaking, in inundation control, phytophagous populations are controlled exclusively by the introduced biological control agents, whereas in inoculation control, the offspring of the introduced agents ensure control (Eilenberg et al., 2001). In practice, the line between inundation and inoculation biological control is somewhat blurred because introduced organisms are generally capable of both reproduction and predation/parasitism. The different strategies of augmentative biological control using macroorganisms thus form a continuum ranging from seasonal inoculations of small numbers of natural enemies to intense campaigns of regular mass releases (Hajek and Eilenberg 2018). Along this continuum are various preventive practices in which biological control agents are released on a regular basis to ensure a permanent presence to keep pests in check as soon as they appear (Messelink et al., 2014; Hajek and Eilenberg 2018).

1.2. A brief history of augmentative biological control

Ancestral forms of augmentative biological control emerged as early as the second century AD in China. Chinese botanist Ji Han reported in *Nanfang caomu zhuang* ("A fourth century flora of southeast Asia") the trade in nests of predatory ants (*Oecophylla smaragdina*) that farmers bought at markets and introduced into citrus orchards for pest protection. Modern forms of augmentative biological control date back to the very beginning of the twentieth century, with the production and introduction of a hymenopteran parasitoid species (*Metaphycus lounsburyi*) and a predatory beetle species (*Chilocorus circumdatus*) to control scale insects from the Coccidae and Diaspididae families, respectively. However, it was not until the early 1970s that many new arthropod species began being used for augmentative biological control programmes (van Lenteren, 2012). Worldwide, the number of species rose from around ten to 170 by the early 2010s (Cock et al., 2010), and has nearly doubled since then (van Lenteren et al., 2018), although the market is dominated by only a few dozen species. Some that were commercially available for a while have been discontinued.

Augmentative biological control strategies are now used in many crops around the globe, such as maize, cotton, sugar cane and soya beans. However, this type of biological control is most used in crops with high added value grown in greenhouses and under cover (vegetables, ornamental plants) or open fields (strawberries, grapes). These crops account for about 80% of the steadily growing augmentative biological control market, which is today worth around US$400 to US$600 m (van Lenteren et al., 2012, 2018), while the global biocontrol market has an estimated value of US$2.8 bn. The targeted pests are mainly thrips (40% by value of commercialized macroorganisms), whiteflies (30%), spider mites (12%) and aphids (8%). Parasitoid insects, especially hymenopterans, have long comprised the majority of natural enemies used in augmentative biological control, but this has changed since the mid-2000s with the rapid development of the use of predators, mainly mites but also hemipterans, in protected or open field crops (van Lenteren et al., 2018).

Figure 4.1A. *Macrolophus pygmaeus* (predatory insect), © A. Bout/INRAE.

Figure 4.1B. *Trichogramma brassicae* (parasitoid insect), © J.-C. Malausa/INRAE.

Figure 4.1C. *Neoseiulus cucumeris* (predatory mite), © L. Étienne/INRAE.

Figure 4.1. Three major augmentative biological control agents: *Macrolophus pygmaeus*, *Trichogramma brassicae* and *Neoseiulus cucumeris*.

2. Current challenges

The need to rear biological control agents on a massive and sustainable scale for later introduction into crops requires a business model and relatively large investments in production infrastructure.

2.1. Mass production of biological control agents

One of the most important prerequisites for the implementation of augmentative biological control is the capacity to produce a very large number of organisms (Morales-Ramos and Rojas, 2003). Setting up this type of facility on an industrial scale is a complex process that first requires the coordination of multiple skills and disciplines. Mass production entails major investments, both technical, with the installation and development of specific rearing equipment for the different species, and human, with the training of specialized personnel. Large-scale commercial use of biological control agents began almost sixty years ago with the production of *Phytoseiulus persimilis*, a predatory mite, against phytophagous mites of the Tetranychidae family (van Lenteren and Woets, 1988).

Mass production of most parasitoids and predators requires prior control of the production of their host(s) or prey, which are mainly phytophagous arthropods. In many cases, producing these hosts or prey is the most technically difficult aspect, and is in fact the limiting factor for biocontrol agent production. Moreover, host plants (or an alternative to them) must also be produced at this stage. In terms of profitability, these constraints will at the very least double production costs, and only the production of entomophagous organisms actually generates income (Van Driesche and Bellows, 1996). This constraint can be partly removed by searching for alternative hosts, but above all by developing rearing environments, especially for phytophagous hosts and prey. This has led to a kind of natural selection of the biological control agents that are produced and marketed: commercial insectaries have mostly opted for species that can grow on host/prey that are easy to rear in large numbers. The use of artificial environments for the direct rearing of biocontrol agents is always a major challenge. Production on artificial growth media, when possible, often appears to be of lower quality compared to production on natural hosts and prey (Grenier and De Clercq 2003; Riddick, 2009).

To simplify production, biocontrol companies sometimes select candidates with zoophytophagous tendencies, i.e. predators that can also consume plant material and therefore be easily produced in the absence of prey, on plants or in an artificial environment. Examples include Phytoseiidae mites such as *Neoseiulus californicus* or *Amblyseius swirskii* (Messelink et al., 2008), and even heteropterans such as *Macrolophus pygmaeus* or *Orius insidiosus*.

2.2. Business models

The term augmentative biological control may refer to biocontrol strategies using macroorganisms or microorganisms. The regulations concerning these two categories of agents differ. Unlike microorganisms, which are regulated at the European level (see chapter 11), macroorganisms are subject to national legislation. For instance, France is the first European country to have introduced (in 2014) a definition of biocontrol that includes the use of macroorganisms in its regulatory framework. Meanwhile, with the entry into force of the Convention on Biological Diversity (1993), access to and the use of biological resources now require benefit sharing with the country of origin. These regulations have limited, from the 2000s onwards, the diversification of commercialized species and their penetration into markets outside their country of origin (van Lenteren et al., 2018). Along with this regulatory context, a strong preference has been observed for native species, which now account for three quarters of new biological control agents placed on the market (Cock et al., 2010).

Macroorganisms accounted for about 16% of biological control products by value marketed worldwide in 2017 (IBMA France, 2017). The players holding this market share have been around a long time; more than half of the active companies to date were founded between the 1970s and the 1990s. Around 500 companies currently market macroorganisms for biocontrol applications, but only ten of them are small and medium-sized enterprises or larger. The European market for macroorganisms for biological control is dominated by three of these specialized companies: Koppert (founded in 1967 in the Netherlands), Bioline AgroSciences (resulting from the 2016 merger of Bioline, an English company founded in 1979, and the Biotop subsidiary of the French group InViVo, founded in 1991) and Biobest (founded in 1987 in Belgium). These three companies originally adopted a classic business model of selling their own production – predatory mites for Koppert, *Trichogramma* wasps and mites for Biotop and Bioline, and pollinating bumblebees for Biobest – before diversifying their approach by expanding their product portfolios, especially through distribution. In France, some crop grower cooperatives, such as Savéol and its subsidiary Savéol Nature, have set up and maintain their own insectaries to meet their needs, an organizational model that can be found in other parts of the world, especially in Latin America (van Lenteren et al., 2018). Alternative approaches to marketing macroorgansims for biological control involving public funds and sometimes combined with private capital are also available, particularly in Asia and Latin America. For example, the publicly funded Okanagan-Kootenay Sterile Insect Release (Oksir) programme, launched in 1992 in Canada, relies on a tax levied on general property owners and apple and pear growers to finance the mass production, processing and release of sterile codling moths (see chapter 5 for more on the sterile insect technique).

2.3. Non-target effects

As with any control method, the issue of possible non-target effects and their relative importance in relation to the expected and observed benefits inevitably arises. There are generally two types of non-target effects, depending on whether they occur within or outside of the relevant agricultural system.

Various non-target effects are possible within agricultural systems themselves. The biological control agent may be less specialized or more polyphagous than initially assessed and may attack other pest species. In this case, the non-target effect is positive. A more problematic case is when the biological control agent negatively impacts the crop it is meant to protect. This may occur in the case of omnivorous predatory species (Coll and Guershon, 2002). However, this is not necessarily a prohibitive characteristic as it may allow the persistence of the biological control agent if the target pest is temporarily unavailable, and can even facilitate their production. Finally, the most frequent cases of negative non-target effects within the agricultural system include cases of complex ecological processes leading to interactions between biological control agents, whether they are used to control the same or different targets. Intraguild predation occurs when several biological control species feed on a common resource as well as on each other (Rosenheim et al., 1995). These are trophic interactions that are common in natural ecosystems, but which can also occur in agricultural systems, such as when several species are deliberately introduced for initial complementarity, or when an organism introduced for biological control by augmentation interacts with natural enemies that are spontaneously present. For example, Snyder and Ives (2001) report that some predatory beetles of the genus *Pterostichus* consume both healthy aphids (*Acyrthosiphon pisum*) and parasitized aphids, which impairs the dynamics of the parasitoid wasp *Aphidius ervi*. Theoretical studies as well as laboratory and mesocosm experiments and in situ observations show highly variable consequences of intraguild predation on the population dynamics of the different organisms, and therefore ultimately on the effectiveness and durability of control.

Non-target effects outside the relevant agricultural system are related to the dispersal of the biological control agents, which depends on their own abilities (flying, walking, passive dispersal) and growing conditions (open field, open-roof or closed greenhouses). The contrast between greenhouses with favourable microclimates and resource abundance, and generally unfavourable external conditions may sometimes be enough to prevent dispersal (Hart et al., 2002). However, this compartmentalization between cultivated and other habitats cannot be ruled out by default, especially in the case of inundative releases. Among the few studies on this topic, some conducted in Switzerland have assessed possible non-target effects of inundative releases of *Trichogramma brassicae* against the European corn borer *Ostrinia nubilalis* (Kuske et al., 2003). The findings highlighted (i) the dispersal of a significant portion of the *Trichogramma* wasps outside the release plot (first 50 metres), (ii) a relative predominance of *T. brassicae* during the first days after release, and (iii) a more durable residual presence. However, the authors concluded that this will likely not seriously affect native *Trichogramma* or non-target host species. It is of course difficult to generalize from such a case study, especially as the longer-term evolutionary consequences of these introductions on natural populations of *T. brassicae* have not been estimated. The problem of non-target effects takes on a whole new dimension when the candidate biocontrol agents are exotic species (van Lenteren et al., 2003). The problem is then similar to that of classical biological control (see chapter 3).

Generally speaking, the issue of non-target effects, and more specifically those impacting non-target species, may give rise to debate within the scientific community and beyond. Indeed, although it seems quite obvious that augmentative biological control cannot be considered an ecologically neutral act, it is viewed differently depending on whether a "precautionary" or "innovation principle" is favoured. However, these debates should be put into perspective by considering the risks of current chemical-intensive practices, whose non-target effects on non-target species – including humans – are well proven.

3. Ways to improve augmentative biological control

3.1. Genetic improvement of biological control agents

For most agricultural resources (crops or livestock), genetic improvement has a proven track record in improving phenotypic traits that impact performance. This potential lever was therefore quickly identified to optimize augmentative biological control methods. Genuine successes are, however, rare. While there may be many reasons for this relative failure, at least three of them deserve special attention. First of all, the market for macroorganisms used in biological control is very fragmented and profits are limited. This situation constrains investment possibilities in research and development, particularly in genetic improvement. In addition, several companies that produce biocontrol agents are reluctant to develop genetic improvement programmes because of the time required, the expected benefits and the lack of legal protection against unfair competition. Finally, at the biological level, questions arise about which traits should be selected (Hopper et al., 1993; Roderick and Navajas, 2003): classic phenotypic traits (size, potential fertility, longevity), behavioural traits (dispersal ability, exploratory tendency, resource exploitation strategies) or particular abilities (diapause allowing storage, thermal stress resistance, pesticide tolerance). However, the situation appears to be evolving (Lommen et al., 2016) and companies producing biocontrol agents are gradually acquiring skills that will enable them to address the issues involved in enhancing and protecting biological material and the related expertise.

Meanwhile, new molecular genetics and genomics methods and tools can be used to characterize biological material with a previously inaccessible degree of accuracy (Cruaud et al., 2018; Lindsey et al., 2018), resulting in unprecedented traceability capacity and the prospect of selection programmes based on coupling between molecular markers and phenotypic traits. Finally, there is a growing awareness among public and private R&D stakeholders of the suboptimal quality of historical strains used for mass rearing. One example of this is recent work carried out jointly by INRAE and Bioline AgroSciences to optimize the effectiveness of *T. brassicae* against the European corn borer *Ostrinia nubilalis* using genetic levers.

3.2. Improving mass production

Solutions must still be identified and developed to adapt the mass production of biocontrol agents to current and future needs (Leppla et al., 2004). The vast majority of current commercial insectaries rely on accumulated knowledge from relatively small-scale production. However, the biocontrol industry could benefit from other insect production sectors. For example, silk production has provided important resources for the development of biological control in China, enabling the mass production of *Trichogramma* spp. from *Antheraea* spp. eggs (silkworms) to control lepidopteran crop pests. Similarly, important developments are now expected in terms of automated production, which can leverage the technologies adopted by producers of insects for animal feed or human food. Automation should help reduce production costs while guaranteeing optimal quality monitoring and standardization of the biocontrol agents that are produced – two major challenges that must still be addressed for augmentative biological control (van Lenteren, 2012). It should be noted, however, that the quality of biocontrol agents is also determined by the transport and distribution logistics chain, and is not limited to production aspects alone.

3.3. Resource supplementation

In some cropping systems, introduced populations of biocontrol agents may have difficulty establishing or persisting because the organisms do not have all the food sources they need. For example, prey or host densities may be temporarily too low to support natural enemy populations, supplementary food sources may be absent or of poor quality, or the biocontrol agents may lack oviposition sites or shelter. Regardless of the reason, the survival or reproduction of the biocontrol agents is impacted, which reduces the effectiveness of control and requires frequent reintroductions, resulting in higher costs (Huang et al., 2011, Messelink et al., 2014). Important improvements can be made through food supplementation, i.e. providing the missing resources through crops. One of the oldest techniques is the use of banker plants, which consists in introducing companion plants that are not harvested but which support alternative prey or host populations and help maintain biocontrol agent populations (Huang et al., 2011). In addition to the phytophagous insects they harbour, these plants can also provide biocontrol agents with alternative or complementary foods, such as pollen, nectar or sap (Messelink et al., 2014). Nevertheless, in very intensive cropping systems such as greenhouse systems, the competition for productive space is such that this solution is rarely used. Methods based on food supplementation directly on crops of alternative hosts or prey or complementary foods are now being developed. For example, sterilized lepidopteran eggs or artemisia cysts are used to support natural enemy populations in different crops. The introduction of pollen, which has long been difficult because of the harvesting costs, has recently undergone a massive development, namely following the marketing by the company Biobest of broadleaf cattail pollen as an alternative food for predatory mites. Broadly speaking, the development of low-cost alternative hosts or food is seen as a major challenge to improve augmentative biological control methods (Messelink et al., 2014). Finally, other types of supplementation are based on the introduction of oviposition sites or shelters in crops that allow better reproduction and survival of juvenile natural enemies. These techniques are being developed especially for predatory mites with fibres applied to plant leaves. Combined food and shelter supplementation are perfectly compatible and even appear to produce synergistic effects in different crops (Pekas and Wäckers, 2017).

3.4. Population dynamics

In contrast to classical biological control, which aims to achieve a long-term equilibrium between pest and natural enemy populations (see chapters 2 and 3), augmentative biological control raises questions about the unbalanced dynamics of systems that are regularly disturbed by introductions of biological control agents. Several theoretical studies have thus highlighted interactions between the introduction strategies of natural enemies over space and time, and the intrinsic biological characteristics of these populations. For instance, the presence of positive or negative density dependence (i.e. the influence of the abundance of natural enemies on their population growth) or the type of dispersal modulate the effectiveness of a given introduction strategy. Thus, when natural enemies interfere with each other – a common occurrence in predatory mites – the most effective strategies are based on frequent introductions of small numbers of agents (Nundloll et al., 2010). More generally, these theoretical studies underscore that the successful implementation of augmentative biological control hinges on detailed knowledge of the biological processes in the populations involved. They can also guide users towards better strategies for releasing a particular natural enemy or, when technical or cropping constraints come into play help users choose the most suitable biological control agents. For example, the high dispersal capacity of biological control agents has long been considered as a selection criterion. This is now being called into question by studies highlighting the potentially deleterious nature of excessive dispersal (Heimpel and Asplen, 2011).

3.5. Entomovectoring

One last way to improve the use of biological control agents, or their usefulness in crop protection, is to develop strategies based on a technique known as entomovectoring. This technique consists of having one or more elements transported or distributed by an insect. This element may be another arthropod (insect or mite), a bacterium, or a natural or synthetic substance involved in or unrelated to the biocontrol solutions. Current practices entail distributing plant protection or pollination solutions using pollinating insects, such as bumblebees that are commonly introduced for pollination of different crops grown under cover or in orchards. The best known examples are the distribution of an antifungal against *Botrytis cinerea* by bumblebees introduced in strawberry crops (solution proposed by the Lallemand group) or a *Bt* biopesticide (Biobest solution). One of the advantages is that these solutions can be distributed quickly, easily and specifically to the targeted locations, all in small quantities. Recent work has also focused on leveraging the zoophytophagous characteristics of certain predators, such as *M. pygmaeus*, to distribute biocontrol microorganism-based solutions, bacterial toxins or natural defence stimulators directly into the plant's tissues. These predators would then provide a second layer of protection against fungal pathogens, without compromising their primary function. In addition to increasing the benefits of these enhanced predators, these developments also help reinforce the interest of natural defence stimulators, which are sometimes still too costly and can induce phytotoxicity. These approaches thus offer prospects for a multi-layered biocontrol approach.

Figure 4.2. Workflow diagram for developing an augmentative biological control programme.

The activities carried out are divided into three phases for which the main actors and their levels of involvement are indicated.

4. Conclusion

The development and promotion of augmentative biological control methods depends not only on scientific and technical considerations, but also on social (training, advice), economic (absolute or relative costs compared to competing practices), regulatory (authorization/withdrawal of plant protection products, legislation on exotic organisms) and even legal considerations (protection of know-how and biological material) (see figure 4.2). Accordingly, an ambitious development of this strategy must involve concerted efforts at different levels. For example, at the scientific level, sufficient time and financial means must be allocated to first correctly identify/evaluate candidate biocontrol agents, and then verify their effectiveness and harmlessness in real-world use. From a zootechnical standpoint, it would make sense to improve mass production techniques, which are still highly dependent on human labour and therefore expensive. In this respect, progress could be achieved through converging interests and generic innovations, not only in terms of other biocontrol strategies – especially the various autocidal control methods, which also require mass production (see chapter 5) – but also with other insect production activities for animal or human consumption. Finally, further consideration must be given to the business models underpinning the production and marketing of biological control agents. Recent merger and acquisitions in the private sector will hopefully result in more substantial investment in research and development operations. Regional initiatives, based on joint partnerships (public and private organizations) and efforts to go beyond the usual responsibilities of certain traditional stakeholders, could be a complementary or alternative solution.