Chelonus insularis laying inside Lepidoptera eggs. Credit: Jena Johnson

Natural enemy, inherent ally: *Chelonus insularis* as a biocontrol agent against the Fall Armyworm

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Summary

The spread of the Fall Armyworm, *Spodoptera frugiperda*, to Africa and Asia from its native range in the Americas poses a great challenge. Farmers’ livelihoods all across the globe are at stake. Given the inefficacy and the toxicity of chemical pesticides, alternative means of plant protection have received more attention. Biological control, or biocontrol, is a technique in which pests are fought using their natural enemies. The effect is a ceiling on, or control of, the number of pest individuals. These enemies can be pathogens, predators or parasitoids. A parasitoid is an organism that matures at the expense of its host, but ultimately leads to its death. In its native range, the Fall Armyworm is controlled by multiple parasitoids and predators. A list of 85 Hymenopteran parasitoids of the Fall Armyworm was established through a systematic literature review. Species were found in all invaded climates. The most promising ones with regard to the rate of parasitoidism in the field were *Campoletis sonorensis*, *Chelonus* nr. *blackburni* and *Telenomus remus*. The braconid parasitoid wasp *Chelonus insularis* was listed as one of the main parasitoids of the Fall Armyworm. Could this species be effective in the recently invaded geographies? The first step in the assessment of a species for its selection as a biological control agent is to establish its host range and preference. In oviposition experiments conducted in the quarantine facilities at CABI Switzerland, the specificity of *C. insularis* towards *S. frugiperda* versus *Spodoptera exigua*, the Beet Armyworm, was evaluated. The results showed that *S. frugiperda* is preferred in a choice scenario, but not in a no-choice scenario. In the choice scenario, *C. insularis* dedicated 30% of its eggs to the alternative host. In the no-choice scenario, the rates of parasitoidism were similar at 79.6% for *S. frugiperda* versus 75.6% for the alternative host. The rate of parasitoidism was not significantly higher on fresher eggs. Larva and head capsule size were recorded to see at which host stage the parasitoid larva emerges. *S. frugiperda* was killed sometime during its late fourth or early fifth instar, as evidenced by a maximum size at death of 16mm. This shows the ability of *C. insularis* to prevent physical damage done to the crops by the current pest generation, since more than 90% of the damage is done at the fifth instar and after. Means of extrapolating laboratory results to the field by mathematical modelization were reviewed. It appeared that current models are all centered on two spatial parameters: density and patchiness. However, these parameters are difficult to estimate and field releases remain the best tool to forecast agent propagation. Important fluctuations in sex-ratio were recorded during the experiments. In one of the cages, the proportion of males attained 100% before new individuals were added. The issue was investigated through a systematic literature review and quantitative text analysis. The prevalence of different topics was measured. The results showed that temperature and diet are almost always mentioned by experimenters, but sex-ratio is seldom mentioned. No relationship was found between the prevalence of sex-ratio and that of other topics. Population-level genetics were investigated next. A simulation was run to generate a list of available alleles after three generations under arrhenotoky and single-locus complementary sex determination. The results showed that inbreeding decreases the total number of available alleles. The proportion of possible diploid male genotypes fell from 33% without outbreeding to 20% with outbreeding. Inbreeding might therefore drive male-biased sex-ratios, especially in laboratory-reared colonies. Practical suggestions are made for the rearing and handling of *C. insularis* in a laboratory setting.

Keywords	Spodoptera spp., Spodoptera frugiperda, Fall Armyworm, Chelonus insularis, Chelonus texanus, host, parasitoid, egg parasitoid, integrated pest management, biological control, host range, specificity, insect rearing, generalized linear model, population dynamics
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cocoon, which is white and hard. When ready, the adult will breach it with its mandibles. The host’s head capsule is all that remains after the skin has dried out, and can be seen attached to the cocoon. The white fringes around the cocoon are bits of the cellulose paper in which it was weaved.

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General introduction

0.1 The Fall Armyworm problem

Figure 0.1: *Spodoptera frugiperda* egg mass, larva and pupa. Credits: Rutgers University, Agro Slide Bank, Lyle J. Buss.

The Fall Armyworm *Spodoptera frugiperda* is a lepidopteran pest of crops. Native to South and Central America, it has now spread to Africa and Asia (Rwomushana 2019). The vector of propagation may have been maritime commerce or flight. The Fall Armyworm attacks hundreds of crop species, and most importantly economically vital crops such as maize, sorghum, wheat, millet, sugarcane and cotton (Rwomushana 2019). In Africa and Asia, smallholder farmers are having difficulty combating this new pest. Pesticides are showing their limits, as the pest develops resistance quite quickly and farmers are not well protected. The losses are estimated between 20 and 50% for maize (Sisay et al. 2019). In its native range, *S. frugiperda* has multiple predators and parasitoids.

0.2 Biological control

0.2.1 A brief explanation of biological control and its advantages

Biological pest control, or biocontrol, is the use of natural enemies against pests. The term has been used since 1919 (Stenberg et al. 2021). Heimpel & Cock (2018) consider 1888 to be the year of the first widely known and successful agent release. A more generic definition of biocontrol was given by the same authors as a situation involving a pest population, an agent population and a stakeholder who benefits from the control of the first by the second. This principle is much older. Van Lenteren (2005) places the oldest known example around the turn of the fourth century. Taking the stakeholder's perspective, a more functional definition is simply as the manipulation of the tritrophic system consisting of a productive species (eg. maize), a pest species (eg. a moth) and a control agent (eg. a parasitoid wasp). The manipulation itself can be that of introducing an agent from somewhere else (importation or inoculation, this is often called classical biocontrol), regularly releasing an agent already present (augmentation) or supporting it (conservation). Overall, biological control is the manipulation of the interactions between humans, resource, agent and target (Heimpel & Mills 2017, p3).

The four main mechanisms of direct control are predation and herbivory on the one hand, in which the pest is consumed as a source of energy and nutrients, and parasitoidism and pathogenicity on the other, in which the pest is used as a vehicle for reproduction. The first two remove the pest immediately upon encountering it, while the last two require a certain number of days to effectively kill the host. The term biocontrol is reserved for cases where one agent species controls the population of another pest species, or its realized impact. Organic and non-organic substances such as essential oils are not living species and therefore cannot be considered agents. Plants involved in beneficial associations with the productive species are typically not considered agents, although this distinction can be criticized (Stenberg et al. 2021). Similarly, the stakeholders themselves are never considered agents, whatever techniques and devices they may use to try to control the pest. The agent can be a predator (eg. a spider), a parasitoid (a parasite that ultimately kills its host, eg. certain wasps or flies), a pathogen (eg. a fungus or a virus) or a competitor (a species that will out-compete the target species and capture all the resources in their habitat). Stenberg et al. (2017) list more mechanisms by which the introduction of an agent might contribute to the reduction of a pest's impact. For instance, the reduction of a pest's mobility for fear of being predated, or the secretion of toxic compounds.

Biological control is at the nexus of several trends. With the global mobilization for the preservation of the environment, public action has new ethic grounds. Contemporary ethics require that practices be as sustainable as possible, especially when it comes to the environment. With time, pests become more resistant to pesticides, which increase in price as a consequence. Even with contained prices, the quantity of pesticides applied cannot grow indefinitely. As ecotoxic products by definition, they have a direct impact on human health and ecosystem functions. By putting the environment's productive capacity at risk, they also have a longer-term, indirect influence on health and the economy. Biological control aims to achieve pest suppression or destruction without interfering with ecosystem services. For the same service, the environmental cost of biological control is thus minimal when compared to that of chemical pesticides, the ecotoxicity of which can have broad and long-term effects. Many governing bodies have now integrated methods to calculate the environmental cost of their actions, which puts biological control at an advantage (Heimpel & Mills 2017, p3). Finally, biological control has lower or similar costs when compared to insecticides, and lower development costs (Bale et al. 2008). This makes it economically preferable in many cases. Biological control is well established. More than 2700 natural enemies have already been employed, and more than 440 species are sold commercially as “biopesticides” (Heimpel & Mills 2017, p8), providing the equivalent of 400 billion dollars in ecosystem services per year (Wajnberg et al. 2007, p4). Parasitoid wasps are often used to combat agricultural moth pests.

0.2.2 Biological control is not devoid of risk

Biological control comes with certain risks. A program might dedicate considerable resources for a release. The agent’s mobility and adaptability are strengths compared to chemical pesticides, but also exclude any absolute guarantees as to how it will deploy. One possible outcome is that the pest population is not controlled, but there is worse. A careless agent release can harm ecosystem functioning. So-called “classical biocontrol” uses exogenic species in order to suppress local pests (Heimpel & Mills 2017, p10). In the past, imprudent use of introduced species has led to negative consequences (Heimpel & Mills 2017, ch4), usually due to an uncontrolled host or prey range. In particular, the introduced species can shift its preference over time from a target species to a non-target species (Heimpel & Mills 2017, p124). This can lead to the extinction of non-target or even beneficial species (Heimpel & Mills 2017, p89-90), or an irrecoverable reduction in their population sizes. The cause can be direct or indirect. The introduced species can predate on or compete with native productive or otherwise beneficial species, for instance through the suppression of their food sources (Heimpel & Mills 2017, p95). In turn, species dependent on them will be affected. In the opposite, the agent can support a non-target species, which has negative consequences on other non-target species. Figure 0.2 summarizes the indirect ways by which the introduction of an agent might have adverse effects on native species. For these reasons, it is crucial to proceed to tests before an insect is released.

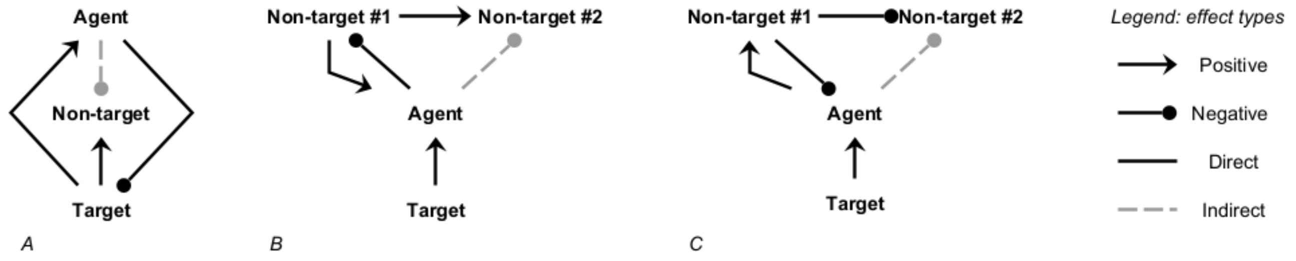

Figure 0.2: Possible indirect negative consequences of the introduction of a biological control agent. (A) The suppression of the target has negative effects on a non-target species dependent on it. (B) Predation on or competition with a non-target species, causing other non-target species to be affected. (C) The agent supports a non-target species, which has negative consequences on other species, including potentially the agent itself. In B and C, the negative effect of the agent on the host is hidden for clarity. Adapted from Heimpel & Mills, *Biological Control*, 2017, ISBN 978-0-521-84514-4.

Switzerland is part of the European and Mediterranean Plant Protection Organization (EPPO). The EPPO was created in 1951 and has 52 member countries. Species evaluated as safe biological control agents in one member country join the Positive List. In 2021, 52 species of Hymenoptera were on the list (*EPPO Bulletin*, 2021). In Switzerland, the release of non-native organisms is regulated by the Federal Office for the Environment (FOEN), while the release of insects as plant protection products is regulated by the Federal Office of Agriculture (FOAG) (Collatz & Burkhart 2015). If an organism is already on the list, a product containing it can be directly approved by the FOAG. Otherwise, it has to be evaluated. If successful, it is added to Annex 1 of the Ordinance on Plant Protection Products (PPPO).

Even then, introduced species can show different preference in a laboratory setting and in the field (Huffaker 1976, p161). The realized niche is affected by ecological filters, such as environmental cues or the spatial distribution of hosts or preys. In the field, they may be much more difficult to locate, increasing the attractiveness of alternatives. A parasitoid that only finds individuals of a secondary host species will adapt (or go extinct), thus making the field host range slightly broader. The result is lower overall efficacy against the target. In most cases, the opposite seems to happen, as the alternative host is usually more difficult to find. Besides specificity, Heimpel & Mills (2017, p131-135) add the following risks: demography (uncontrolled growth), dispersal (uncontrolled geographical migration) and pathogens (especially viruses). Dispersal can be caused by a preferred nearby habitat, even one that was not initially preferred but became preferred because of accumulating or otherwise unforeseen effects such as the saturation of the target habtat (the crop field) (Heimpel & Mills 2017, p134). An article by Howarth (1991) listed several cases of extinction due to the introduction of an agent. One example of indirect negative impact reported by the author is the extinction of *Maculina arion*, a large butterfly, in Britain. The cause was that rabbit populations had been controlled by use of the *Mixoma* virus and could no longer graze a sufficient area, and the butterfly’s habitat disappeared. The list also includes cases of pest species extinction. Indeed, in the perspective of conserving biodiversity, the loss of the host species can be considered a negative unintended consequence. One of the promises of biological control compared to chemical pesticides was the ability to limit a pest’s economic impact without eradicating it. This is less of a problem for invasive crop pests because of the economic damage caused, and because they maintain themselves in their native range anyway. However, an agent is always introduced in an ecosystem, which makes generalization impossible. Howard reports on the case of the parasitoid wasp *Glabrodorsum stokesii* which, introduced in New Zealand for biological control, had moved to natural forests and attacked native moth species there. While attack does not necessarily imply suppression, the risk on biodiversity is evident. As a result, the step from science to policy is not an easy one (McEvoy 1996). Despite this, Heimpel & Mills (2017, p126-127) note that the field realized host range is typically narrower than laboratory assays suggest. Heimpel & Cock (2018) provide a review of the outcomes of inoculative biological control against insect and weed pests from 1888 to 2011 and note that less than 1% provably resulted in environmental damage. Their method included only field studies following the effect of an introduction. In integrated pest management (IPM), the spread of pests is limited by introducing an agent that targets them. The agent must not target benign or beneficial species (must not be polyphagous). Additionally, it should not spend resources on species other than its designated target in order to maximize efficiency.

Given the risks mentioned above, simulations of the joined evolution of the pest, agent and non-target populations over time have been proposed (Heimpel & Mills 2017, p145). A striking effect is that the reduction of target host is optimal when the augmentation of parasitoids is maximal, which is precisely when non-target hosts are partially suitable. Indeed, the non-target population gives a boost to the parasitoid population, which brings the target host population below the levels possible without a non-target population. However, there is considerable difficulty in obtaining exact and reliable values for the required model parameters.

0.2.3 Specific considerations in insect-based biological control

Insects have traditionally been used as biological control agents. Insect-based biological control involves rearing an agent in sufficient quantities in a laboratory and then releasing it in the field. Heimpel & Mills (2017, p208) distinguish three different strategies:

- Inundative: the released generation does the work
- Accretive: subsequent generations do the work
- Augmentative: the present population is reinforced

The accretive and augmentative strategies imply that the agent population is established by a previous introduction or naturally present. An established population can be supported by periodical releases of laboratory-reared individuals (Heimpel & Mills 2017, p208). Additional supportive measures include food subsidies and reducing the quantities of pesticides applied (Heimpel & Mills 2017, p10-11). In another approach, a desirable characteristic for the agent is limited temporal deployment capabilities, so that it may not establish itself in the habitat. For instance, that it cannot survive seasonal shifts. In this case, it can be released again every season. Once the risks have been assessed, the ideal dispersion area and temporality for the agent can be determined (Heimpel & Mills 2017, p115 and figure p118) and suitable species can be evaluated.

Insect agents are either predators or parasitoids (Heimpel & Mills 2017, p14-17). A predator will seek and immediately destroy individuals of the target species. A parasitoid will use the pest as a vessel for its the development of its larvae. Compared to a predator, a parasitoid will not destroy the target immediately (Heimpel & Mills, p15-16). Yet they are often preferred as agents. There can be several reasons to this. (1) They can remove more pest individuals over time. A female parasitoid wasp lays eggs continuously over the course of its reproductive lifetime. This is in contrast to a predator which stops after having accumulated enough reserves. (2) They might be more selective. While a parasitoid will look for hosts with suitable characteristics, quality may be of secondary interest to a hungry predator. Both can be called mono-, oligo- or polyphagous, since parasitoid larvae also “consume” their host. This raises the question of specificity and preference. Specificity is the agent’s attachment to its target species. A parasitoid may accept a single species, or a very narrow taxonomic range, or a broad one. This is dependent on the parasitoid recognizing the host for what it is. Phylogenetically close species may often be used as alternative hosts (Heimpel et al. 2021). Even if multiple species are accepted, only the ones with certain properties may be accepted as hosts. This is the concept of preference. For instance, only certain host stages or hosts of a certain quality are attacked. Preference is the result of biotic and abiotic factors (Rezaei et al. 2019). The parasitoid might have developed a biological advantage against a host. Abiotic factors may include environment patchiness or species richness.

0.2.4 Laboratory testing versus field conditions

A parasitoid's specificity and preference are tested in laboratories. The goal is to establish its plausible efficiency and risk before it can be chosen as an agent. The advantage of laboratory testing is that the environment and the experiment parameters are controlled, and that the experiments can take place in quarantine when the species are exotic and invasive. Two kinds of experiments are typically conducted. For specificity, two tests are combined: a choice test and a no-choice test. This consists of submitting one or more hosts to female parasitoids, recording the number of parasitoidized individuals and comparing it to their total number. Regarding preference, the number of parasitized host types is compared to that of host types available (Rezaei et al. 2019). The host stage can be noted during the course of the experiments.

The main disadvantage of laboratory testing is that the conditions during experiments and the conditions in the field during the release of a biological control agent are very different, and therefore the performance or even outcome of a release may not fit the predicted results. This uncertainty increases risk. Singh & Moore (1985) listed the differences between the laboratory and the field. These include environmental parameters (temperature, light, humidity), mechanisms such as interactions with other species (eg. competition for hosts), resource quality such as food contents and host size, and spatial factors such as mate and host densities. For parasitoids, the quality of the host's food also matters. Parasitoids are guided in their search for hosts by volatile substances emitted as a result of the host's activity (herbivore-induced plant volatiles, HIPV) or detected as part the host's diet. A study by Ortiz-Carreon et al. (2019) showed that the parasitoid wasp *Chelonus insularis* is attracted to a blend of HIPV emitted by maize plants attacked by the Fall Armyworm. In a study by Hay-Roe et al. (2013), *Euplectrus platyhypenae* preferred Fall Armyworm larvae fed on corn (*Zea mays*) rather than stargrass (*Cynodon nlemfuensis*). Parasitoid mortality was higher on larvae fed stargrass. These effects were present in both host strains. Finally, a study by Bernal et al. (2015) found that parasitoidism was four times higher in Fall Armyworm larvae fed on the older *Zea mays* subspecies parviglumis, versus the modern maize subspecies mays. These results show a complexity of interactions that cannot be fully replicated in a laboratory. As a result, the realized parasitoidism rate might be an order of magnitude smaller. Sands & van Driesche (2003) list important aspects of host range testing and preconize using high-quality agents and hosts, selecting conditions that favor host development and in turn parasitoid development, and putting great care in experimental design to control for subtle effects such as learning.

Before oviposition, 3 critical conditions to success can be identified. First, the female must be able to lay female eggs. Hymenopterans are haplodiploid. Unmated females can only produce offspring of one sex. For arrhenotokous species, unfertilized eggs produce males. The female will need to mate before she can produce females. Second, the female must locate the hosts in the environment. Hosts are much harder to find in a field than in a laboratory, the main reason being their sparsity. They may also be better hidden. Some pests suppress HIPV emissions, making their activity more discreet. In the field, all olfactory cues are in competition. Wajnberg et al. (2007) note that not enough studies demonstrate that the mechanisms relied upon by the parasitoid in the laboratory are also used to the same extent in the field (p52). Third, the hosts must be suitable. Host quality is the main driver of the sex-allocation strategy. For arrhenotokous species, female eggs are more expensive. Having a limited quantity of sperm in the spermatheca, the female's strategy is to reserve them for the best-quality hosts. Indicators of host quality for egg parasitoids are egg age, texture, chemicals, and presence of other larvae or other laying females. Host quality is relative (Wajnberg et al. 2007, p15).

After oviposition, there is one final critical condition. The larvae must be successful. The host must not die. Though it does solve the immediate problem, it does not allow the parasitoid to establish itself. The parasitoid larva must not be encapsulated by the host's immune system. This mechanism can be influenced by diet (Wajnberg et al. 2007, p114). Braconids can rely on the bracovirus, a symbiotic virus of Cheloninae (among other parasitoid wasps) which reproduces in the calyx and prevents encapsulation (Jervis & Kidd 1996, p71). Its function is to suppress the host's immune system. Each bracovirus is suited to a particular host species. Even if the parasitoid larva fails and is encapsulated, the host is smaller, potentially as small as a normally parasitoidized host (personal observations), which means a much lower appetite. In the field, there can sometimes be two larvae from different mothers in one host. If the two larvae are competing for the host, this is called superparasitoidism, or multiparasitism if they are of different species. If the second larva feeds on the first, it's called hyperparsitism. Some species use ovicide to gain an advantage. They destroy the eggs of other parasitoid species inside the host egg (Godfray 1994, p142).

0.3 There are numerous parasitoid wasp species

Parasitoids are found in many species of Hymenoptera, Diptera, and can be found in other orders as well. The Hymenoptera order contains two clades: the Symphyta and the Apocrita. Species of the second have the characteristic wasp-waist. Many of them are parasitoids used as biocontrol agents against insect pests. They insert their eggs into host eggs or larvae with their ovipositor, a long needle extending from their abdomen. Hosts provide shelter and resources for the larvae to grow. Once mature, the larvae exit the host, killing it. More than 150 000 species of Hymenoptera have been described (Peters et al. 2017). The ovipositor's function is to lay eggs, but in some species it can also sting with venom. Different species have evolved different ovipositor morphologies. The laying and stinging may be performed with different parts of the ovipositor. Its shape may be suited to cutting (eg. plant tissue) or piercing (eg. host tissue). Some lay their eggs in plants, but most use insect hosts. Some lay their eggs in plants that are then eaten by their host. In some cases, the insect host is hidden inside a plant, and the laying female must manipulate her ovipositor to reach it, giving the impression that the wasp is targeting the plant. Depending on the species, the ovipositor can be shorter than the abdomen, or several times the size. A study by Sivinski et al. (2001) tested three hypotheses to explain the length of ovipositors among five Hymenopteran parasitoids found attacking fruit flies in Mexico. Namely that (1) a longer ovipositor is characteristic of species that target hard-to-reach hosts (eg. deep inside plants), (2) a longer ovipositor is more expensive and different sizes are explained by different reproductive strategies, and (3) a longer ovipositor is characteristic of host habitat, and not species. Only one correlation was found between fruit size and ovipositor length, suggesting that a longer ovipositor grants more laying opportunities. The authors conclude that ovipositor size is not the result of competition among species. This has an interesting implication for biocontrol. It means that ovipositor size is not a predictor of an introduced agent’s success in establishing itself among the other parasitoids with the same host (called the guild).

The largest superfamily in the Apocrita is the Ichneumonoidea, with over 100 000 species. The other 13 superfamilies total about 13 000 species (Mitroiu et al. 2015). In the past, the Apocrita were divided between the Parasitica which contained parasitoid wasps and the Aculeata which contained ants, bees and stinging wasps. This partition was based on observed behavior as much as morphology. As a result, there was considerable uncertainty, as shown when trying to build phylogenic supertrees from different sources (Davis et al. 2010). The division into Parasitica and Aculeata was invalidated by genomic studies (Johnson et al. 2013; Peters et al. 2017). However, the group Aculeata remains useful in the sense that all its species share a common ancestor.

0.4 Structure and objectives

The CABI experiments planned and conducted in 2020 gave rise to additional questions that were treated over the course of the two following years. As the culmination of this effort, the present thesis comprises five aspects.

The first is a systematic approach to the question of natural enemies of the Fall Armyworm. Given the pest’s adaptability and spread to different regions, a list of current observed parasitoids attacking it in the field, along with their climatic range, would be an asset. A systematic review serves as the basis for establishing the global parasitoid complex of the Fall Armyworm.

In the second chapter, the modelization of host-parasitoid systems is reviewed. Given the limitations of laboratory testing, modelization aims to extrapolate results to open environments. Host-parasitoid models originate in older consumer-resource models. However, with parasitoids, what usage is made of a resource is a decision left to the laying female. This adds complexity. With a great number of specific differences between laboratory and field, how do current models synthesize behavior?

The third chapter covers host specificity and preference of *Chelonus insularis* for the Fall Armyworm. Oviposition tests involving the two species as well as an alternative host serve to determine if *C. insularis* would be effective in controlling the Fall Armyworm population.

In the fourth chapter, the instability of sex-ratio in the parasitoid colony reared for (and from) the experiments, and the extinction of a previous colony in 2018, motivate the use of quantitative text analysis methods to try and detect which parameters are considered essential by experimenters.

Finally, the fifth chapter reexamines the issue of sex-ratio in the perspective of population dynamics. Can the inbreeding caused by the reuse of offspring in experiments be a factor?

Table 0.1: Contents of each part of the present thesis.

Chapter	Title, hypothesis and *objective*	Pages
1	Hymenopteran parasitoids of the Fall Armyworm: a systematic review Hypothesis: Natural enemies of the Fall Armyworm are not limited to its native range. *The names, geographical ranges and parasitoidism rates of FAW natural enemies are assembled through a systematic literature review.*	23
2	A review on the modelization of host-parasitoid relationships Hypothesis: Host-parasitoid systems present singularities that considerably alter the classical consumer-resource model. *The means of forecasting the state of host and parasitoid populations in relation to one another are reviewed.*	41
3	Specificity and preference of the braconid *Chelonus insularis* for the Fall Armyworm *Spodoptera frugiperda* Hypothesis: *C. insularis* is specific to *S. frugiperda* and a good candidate for biological control. *The wasp's eligibility as an agent is gauged with a choice test, a no-choice test, the analysis of the influence of egg age and the determination of the host stage killed.*	47
4	Rearing parasitoids: what parameters are the most important? Hypothesis: The experimental parameters most critical for success will be the ones most often cited in the literature. *Text-analysis methods are used to assess which environmental parameters are most often associated with questions of sex-ratio.*	86
5	Population dynamics in a laboratory-reared colony of *C. insularis* Hypothesis: Under single-locus sex determination, inbreeding favors male-biased sex-ratios. *Irregular population sizes and sex-ratios spur the investigation of genetic causes through a computer simulation of allelic richness.*	95

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1 Hymenopteran parasitoids of the Fall Armyworm: a systematic review

Abstract

The Fall Armyworm (FAW) is the most destructive agricultural pest worldwide. In its native geographical range (Meso-America), it is partly controlled by natural enemies, mostly parasitoid wasps which use its eggs, larvae and pupae as part of their reproduction cycle. This gives hope that biocontrol could be possible in other regions (Africa, Asia). However, a natural enemy species remains to be selected as an agent. In this review, the names, geographical ranges and parasitoidism rates of FAW natural enemies were assembled through a systematic literature review. This process yielded a list of 85 species. All African biomes had native Hymenopteran parasitoids of AW, with more than 20 genera present.

1.1 Introduction

In its native range, the Fall Armyworm is controlled by a complex of natural enemies. In the regions invaded by the pest, the same species are not present. Natural enemies might exist but are evidently not as efficient. As it stands, a biological control agent for the Fall Armyworm might be found in the Hymenoptera order.

In 1979, the scientific journal *The Florida Entomologist* proposed an issue titled Fall Armyworm Symposium (Vol. 62, No. 2, Jun., 1979). In it, Ashley assembled a list of 24 Hymenopteran parasitoids, noting that their importation in the United States would reduce the population of overwintering individuals. This is a problem in the South of the country, where the winter temperatures are not low enough to suppress the pest population. Many species of Hymenoptera are already sold as biological control agents. A review by van Lenteren (2012) lists 116 Hymenopteran species available for commercial use along with their classification and estimated earliest year of use. This data was enriched using CABI's Invasive Species Compendium (accessed 2022.06.04). Each species' name and classification were checked against the database and the family was added. In the original article, three species are misreported as Hymenopterans. They were excluded from the results shown below. Figure 2.1 shows the number of new species from each Hymenopteran family according to the estimated date of earliest use. For species where a range is proposed, the earliest date was taken. The data shows a long period of punctual discoveries, followed by an explosion starting in the 1970s. The earliest reported year of use is 1902. Until 1965, only 8 species are listed. The period that follows lists 105 species, including the new families Bethylidae, Braconidae, Pteromalidae, Mymaridae, Scelionidae and Diapriidae. The most prolific decade for commercially available biocontrol agents was the 1990s, with 69 reported new species. Six species are reported by the author as "not in use anymore": *Dicyphus hesperus*, *Encarsia guadeloupae*, *Encarsia hispida*, *Encarsia protransvena*, *Eretmocerus eremicus* and *Psyttalia concolor*. With the exclusion of these species, the most represented family is Encyrtidae, with 28 species, followed by the Aphelinidae with 24. The most represented genus is *Trichogramma*, with 15 species. The author also notes the continent where each parasitoid is used. This is only a broad indication of their geographic range. The climatic range would be a better indicator of an agent's suitability. Furthermore, in inoculative biocontrol, the agent is released inside an ecosystem. A precise enough indication of location would tell if the parasitoid is already successful elsewhere in a similar ecosystem.

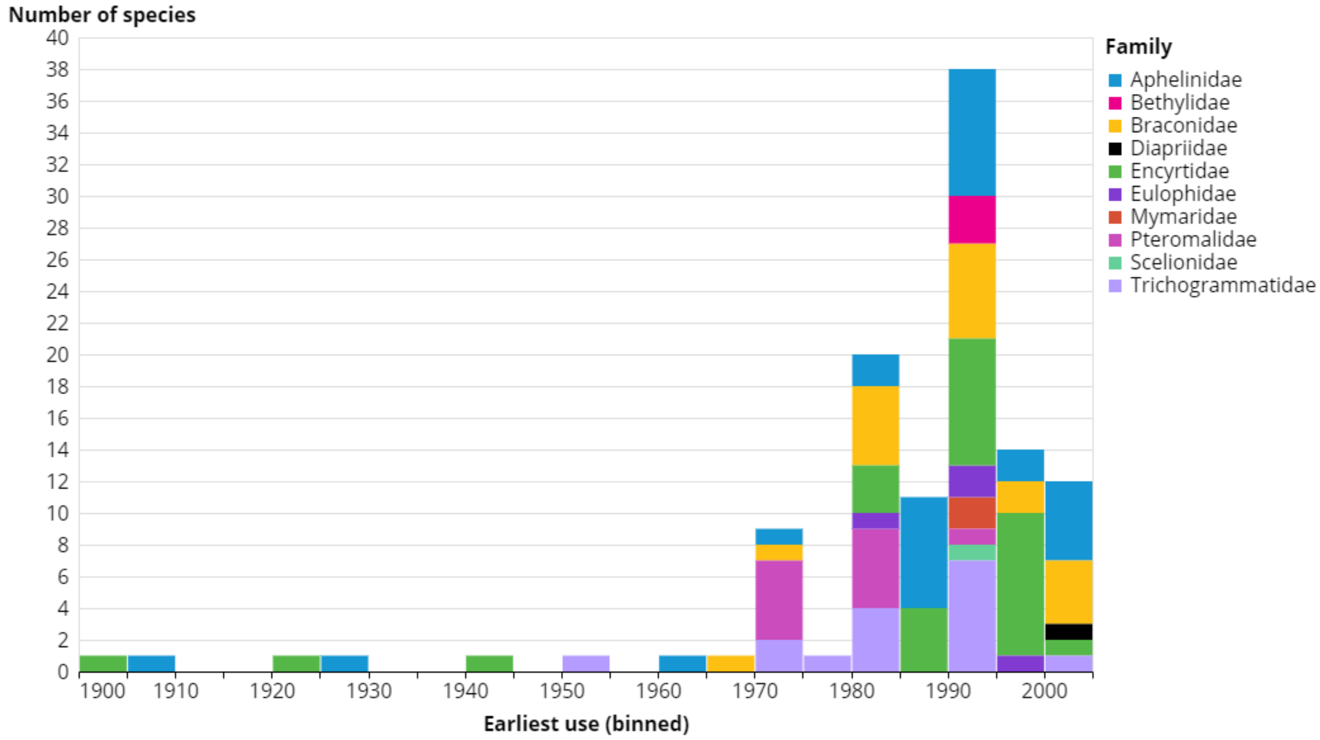

Figure 2.1: Commercially available Hymenopteran parasitoids, adapted from van Lenteren (2012).

1.1.1 Research question

The selection of a biocontrol agent is done to a set of very specific requirements regarding phylogeny, behavior, host range and habitat. The large number of species in the Hymenoptera order is both an opportunity and a challenge in this regard.

The preliminary search highlighted three main shortcomings. First, the list of species is constantly growing. In a given year, there may be any number of new parasitoids used as agents. Second, the reported geographical information is too coarse to inform the selection process. Third, effective parasitoidism rates are not always reported, when they could also help determine candidate species.

The goal of this review is to assemble a list of Hymenopteran parasitoids of the Fall Armyworm from experimental literature, along with subcontinent-level locations and recorded rates of parasitoidism.

1.2 Methods

1.2.1 Literature search

Web of Science was selected as the source for the systematic search. In preparatory searches, precise queries yielded very limited results (<10), while broader queries yielded too many (>6000). Key terms were nevertheless identified for use in parametric search. In this mode, it is possible to search in titles (TI) and abstracts (AB) separately. Abstracts were identified as a good corpus, as they contain less important yet more specific terms. The following queries were tried: `(AB = biocontrol OR AB = "biological control") AND (AB = wasp* OR AB = Hymenoptera*) NOT AB = laboratory` (2248 hits), `(TI = parasit* AND (AB = wasp* OR AB = Hymenoptera*))` (6212 hits), `TI = parasit* AND (AB = wasp* OR AB = Hymenoptera*) AND AB = success*` (957 hits) and “`TI = parasit* AND (AB = wasp* OR AB = Hymenoptera*) AND AB = success* NOT (AB = fungi OR AB = nematodes OR AB = bacteria OR AB = virus*)` (878 hits). The query was then restricted to studies on parasitoids of the Fall Armyworm with the query `AB = parasitoid* AND (TI = 'Fall armyworm' OR TI = frugiperda)` (127 hits). A cursory review of these results showed a large proportion of studies related to the pest’s biology, and not to its parasitoids. The final query was simply `AB = parasitoid* AND TI = 'Fall Armyworm'` (53 hits). All the queries were run again on 2022.06.08 with”Exact search” enabled to get an up-to-date number of hits. At the time of data extraction, on 2022.05.16, the final query listed 52 results. One additional item, obtained as grey literature through the authors directly, was a review of a specific parasitoid of Fall Armyworm, *Chelonus insularis*.

1.2.2 Inclusion and exclusion criteria

The process of including and excluding items is formalized through a decision tree. It is common to have to adjust this tree according to the search results, suggesting some cyclic aspect to the workflow. Foo et al. (2021) formalize this with a diagram where each step is preceded by one or more smaller steps of the same kind. An alternative way to proceed is to establish a set of filters in the form of conditional statements that can be true or false. The decision tree is built afterwards. This way, item evaluation and tree building are disentangled. The cyclic process becomes two consecutive phases: qualification and selection. This has several advantages.

When qualifying items, the reviewer can skim each item and quickly write down which filters it satisfies. The cost of processing the list of items is minimal and this time is spent only once. A poorly discriminating filter can simply be excluded from further consideration. The selection process (decision tree) is not affected. A strongly discriminating filter can be acted upon immediately, for instance to exclude items from further processing and save time. Filters can be added at a later time, at the cost of having to cycle through the items again. This cost is reasonable because, in this pass, only the new filters are evaluated. Furthermore, the pass can be applied to only a subset of the items (eg. those that match another filter). Instead of a binary inclusion/exclusion decision, a relevance estimate is obtained; an expression of promise or potential. The results can be stored, adding value to the bibliographic item itself, and reused in another review. When selecting which items to perform the extraction on, the filters may be stacked in any order. The decision tree is built once *en connaissance de cause*, knowing that it is appropriate for this corpus. It can be changed and the corpus will not have to be evaluated again. Multiple decision trees can be built for different purposes. This method was used to evaluate the items obtained from the literature search. Twelve filters were devised. They are shown in Table 1.1 numbered from 0 to 11, with a justification for each. In practice, evaluation results were saved in the "Extra" field of the bibliographic item.

Table 1.1: Filters used to qualify bibliographic items in the systematic review of known Fall Armyworm parasitoids.

Filter	Description and justification.
f0	Item is a scientific journal article. *A useful filter to categorize results. In the end, all results were scientific journal articles.*
f1	Abstract mentions Fall Armyworm parasitoids by name. *Even with a specific query, some of the results might fall outside of this scope.*
f2	Title mentions Fall Armyworm parasitoids by name. *A variant of the previous filter.*
f3	Some or all of the species mentioned (might) belong to the order Hymenoptera. *Titles and abstracts can mention one or several species, potentially from different orders. Sometimes, no taxonomic information is given (eg. "several parasitoids").*
f4	Title or abstract mentions a single Hymenopteran parasitoid species. *Studies focusing on only one parasitoid might include more specific information.*
f5	A full-text version was found. *Items' full-text versions may be found automatically, manually or not at all. A single filter covers all cases.*
f6	Full-text contains Hymenopteran parasitoid geographic range information. *Field surveys will contain geographical information such as country, region, municipality, farm or GPS coordinates.*
f7	Full-text contains Hymenopteran parasitoid climatic range data. *Since the research question refers to the "real-world" range, only the natural or field climatic range is considered, for instance rain season or altitude, to the exclusion of laboratory settings.*
f8	Full-text contains Hymenopteran parasitoid field efficacy data. *Only parasitoidism rates from field-collected or tent-collected larvae are considered.*
f9	Full-text contains Hymenopteran parasitoid host range information. *Studies might include multiple hosts or multiple variants of one host, for instance with different diets.*
f10	(One of) the targeted host(s) is FAW.

Filter	Description and justification.
	One of the hosts is FAW or FAW is the only host studied.
f11	Field efficacy data is in the form parasitoidized larvae over total larvae. *To get parasitoidism rate.*

The filters were evaluated in two rounds. In the first round, only the title and abstract were processed (f0 to f4), as recommended by Foo et al. (2021). A full-text version was then searched for (f5) and the retrieval method was noted. Out of 53 items, 25 full-texts were found automatically by the bibliography software, 3 were found in open access and downloaded manually, 15 were found through institutional access and downloaded manually, 7 were not found, 2 were found but could not be downloaded due to technical issues on the publisher site, and 1 was obtained directly from the authors. In the second round, full-texts were processed (f6 to f11).

A decision tree was then built, taking into account the research question and the diversity of items. The first criterion for inclusion was to exclude non-Hymenopteran parasitoids (f3) and have a full-text version available (f5). 8 items that did not concern Hymenopteran parasitoids and 6 items for which full-texts could not be located were excluded. An additional criterion was to contain geographic or climatic range information (f6 or f7). 1 item was excluded. The resulting 38 items were considered for the extraction of species names and location information. The additional criterion for the extraction of parasitoidism rates were to have it in the form $(numberofparasitoidizedlarvae)/(totalnumberoflarvae)$ (f8 and f11). 22 items were excluded and 16 items were considered. The PRISMA statement presented in Figure 2.2 summarizes this selection process.

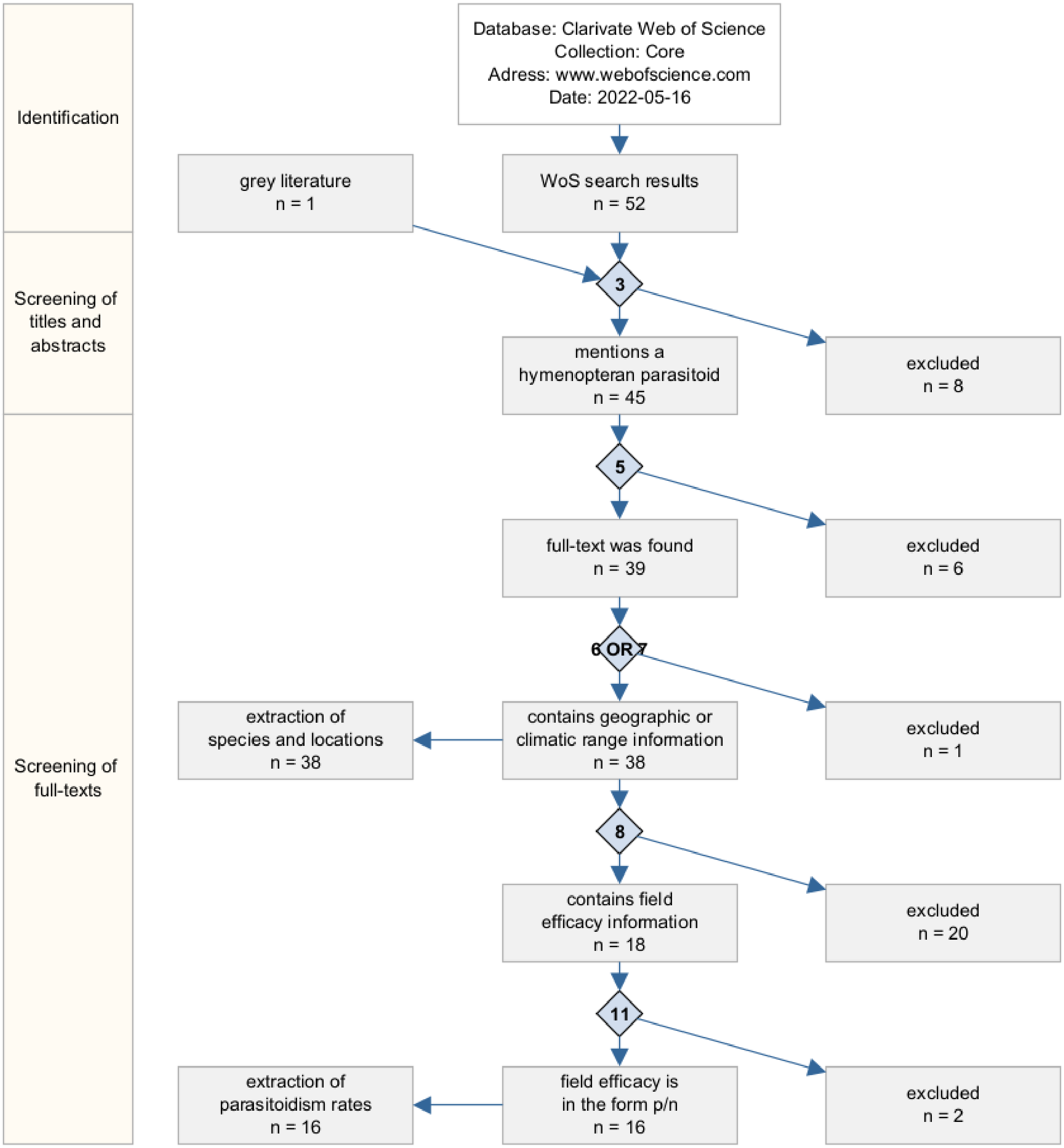

Figure 2.2: PRISMA Statement for the selection of items following a systematic search on FAW natural enemies. The numbers in blue diamonds identify the criteria satisfied by the items (eg. criterion 8 is to contain parasitoidism rates in the field). Out of 53 items, 38 were considered for the extraction of species names and their geographic or climatic ranges, and 16 were considered for the extraction of parasitoidism rates.

1.2.3 Information extraction

The process for extracting the species names was the following. The full species name, as provided by the authors, was copied to a table with the item's identifier. The species' order was checked using CABI's Invasive Species Compendium. Non-Hymenopterans were discarded. Certain authors provide the authority (eg. *Chelonus insularis* Cresson). The genus, species and authority were split into different columns and checked for spelling mistakes. In some cases, the exact species was unknown or uncertain, and the authors provided only the genus and "sp.", "spp." or added "nr.", "cf.". This information was kept as such and a more generic species name was then reconstructed. When the species was reported as a result of the study's experimental part, the record was assigned the source "Experiment". Authors frequently reported results from existing studies in the text directly. In this case, the source was noted as "In-text references".

The extraction of geographical information was more complex, because of the multiple standards for reporting location. The finest common level that could be found was subcountry. Geographic indicators were converted with the following methodology. For countries with continental landmass, the location was converted to a quadrant. Quadrants were delimited as 9 equal parts inside the country's largest contiguous area, corresponding to the 8 compass directions plus a "Central" quadrant (eg. Benin (Southwestern)). The same was done for cases where only the subcontinent is mentioned (eg. Africa (Western)). For islands, the Ocean name and hemisphere was used (eg. Pacific Ocean (South)). Authors sometimes report the altitude at which the experiments took place. This information was too sparse and was not copied.

Parasitoidism rates were copied to a separate file, with the item's identifier, the agent species name, the target species name, and all the recorded values in percentages. Treatment and season information (eg. tent, July) was also recorded when available. Since the target species name is recorded, parasitoids attacking other pests are also collected.

All the data was managed using pandas 1.4 running on Python 3.10. Charts were made with altair 4.2. Maps were made with QGIS 3.24.

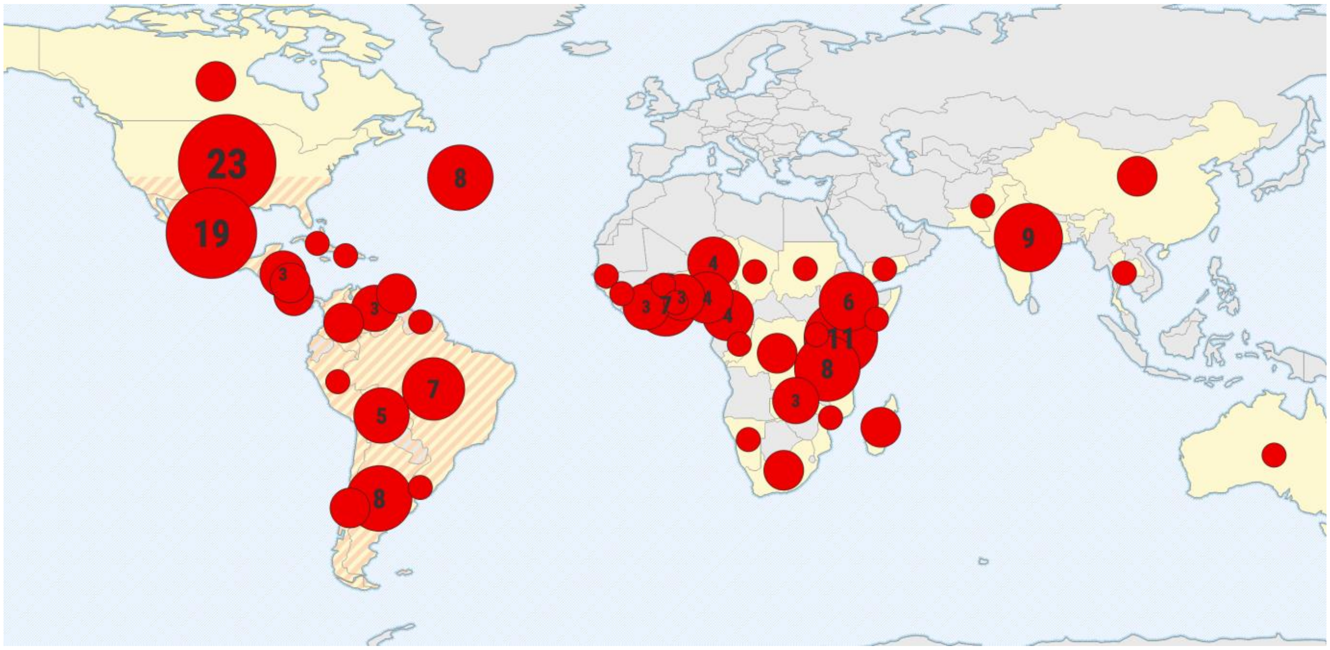

Figure 2.3: Number of corpus items mentioning each country. Countries with parasitoid species present are highlighted in yellow. The Fall Armyworm's native range is highlighted with stripes. The United States were mentioned in 23 studies, and Mexico in 19. A download link is provided in the annex.

1.3 Results

1.3.1 Species recorded

Overall, 85 Hymenopteran parasitoid species were found. Only 5 species were also cited by van Lenteren: *Trichogramma ostriniae*, *Trichogramma atopovirilia*, *Trichogramma pretiosum* (all Trichogrammatidae), *Cotesia flavipes* (Braconidae) and *Telenomus remus* (Scelionidae). This highlights the gap between known natural parasitoids and commercially available biocontrol products. The species most often cited were *Chelonus insularis* and *Telenomus remus* with 15 items mentioning them, followed by *Coccygidium luteum* and *Cotesia icipe* (13 each). More than 20 genera were recorded on the African continent and Madagascar.

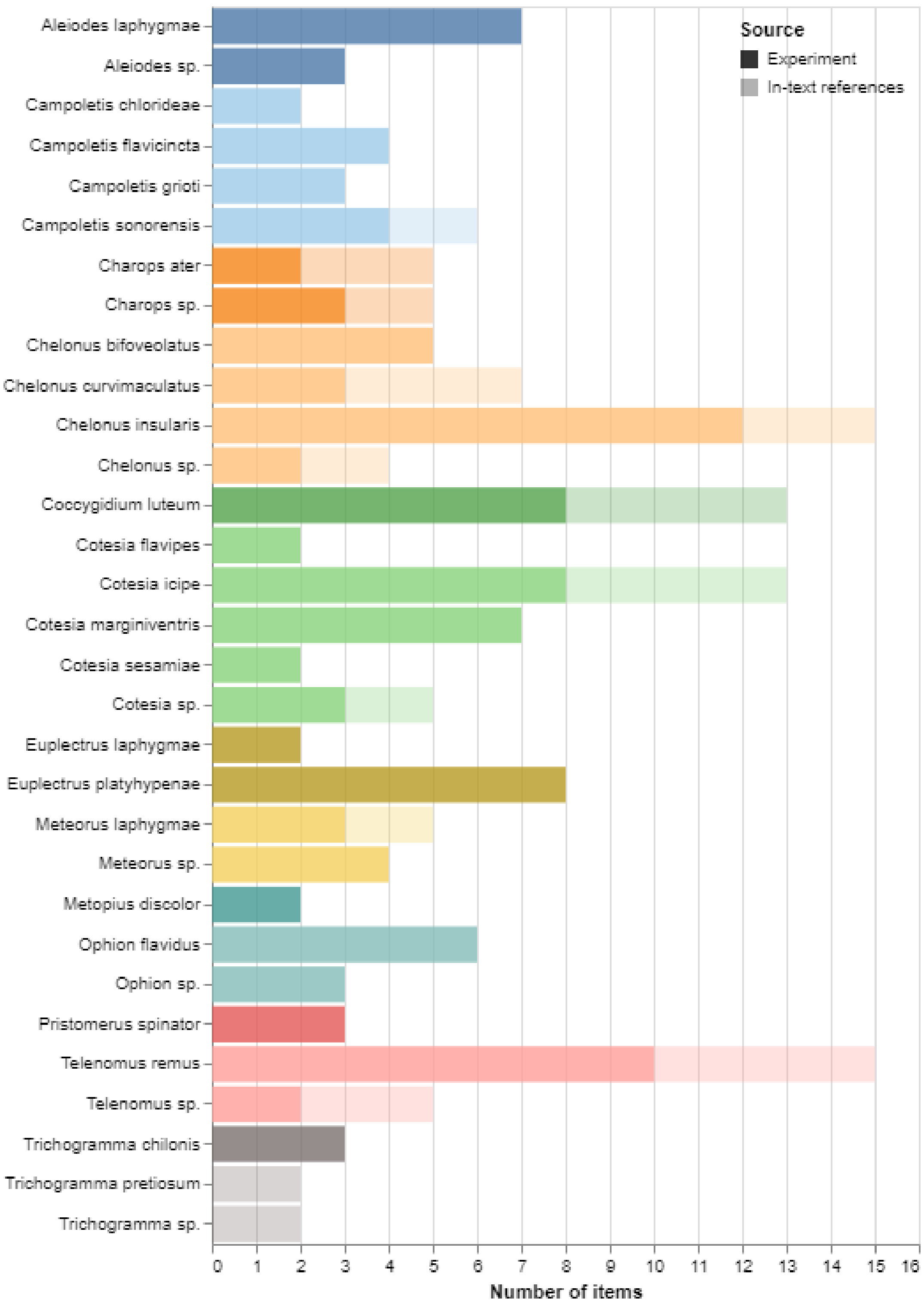

Figure 2.4: Number of items mentioning each species, for species mentioned by more than one item. 69 species were mentioned only once in the corpus. The species most often cited were *Chelonus insularis* and *Telenomus remus*, followed by *Coccygidium luteum* and *Cotesia icipe*

1.3.2 Climatic ranges

All parasitoid species are found in the Americas, where the pest originates, in Sub-Saharan Africa, in Eastern Asia and in Southern Oceania. The Köppen-Geiger classification of climates (Peel et al. 2007) can be used to deduce the parasitoids' climatic ranges from their geographic locations. The full name and assigned color for each climate can be found in annex. *S. frugiperda* is present in the following climates (CABI ISC): A, Af, Am, As, Aw, B, Bs, Bw, C, Cs, Cw, Cf. Its native range extends from Argentina to Texas. Three of these climates had parasitoid species, with 52 species present in Af, 76 in Am and 85 in Aw. For the African continent, all climates have parasitoid species reported: Af, Am, Aw, BSh, BSk, BWh, BWk, Cfa, Cfb, Csa, Csb, Cwa and Cwb. The BWk climate only had 2 species reported, while Aw and BSh had more than 30. These results could be refined with more precise location data. Still, we can conclude that there is no shortage of options for selecting a biological control agent.

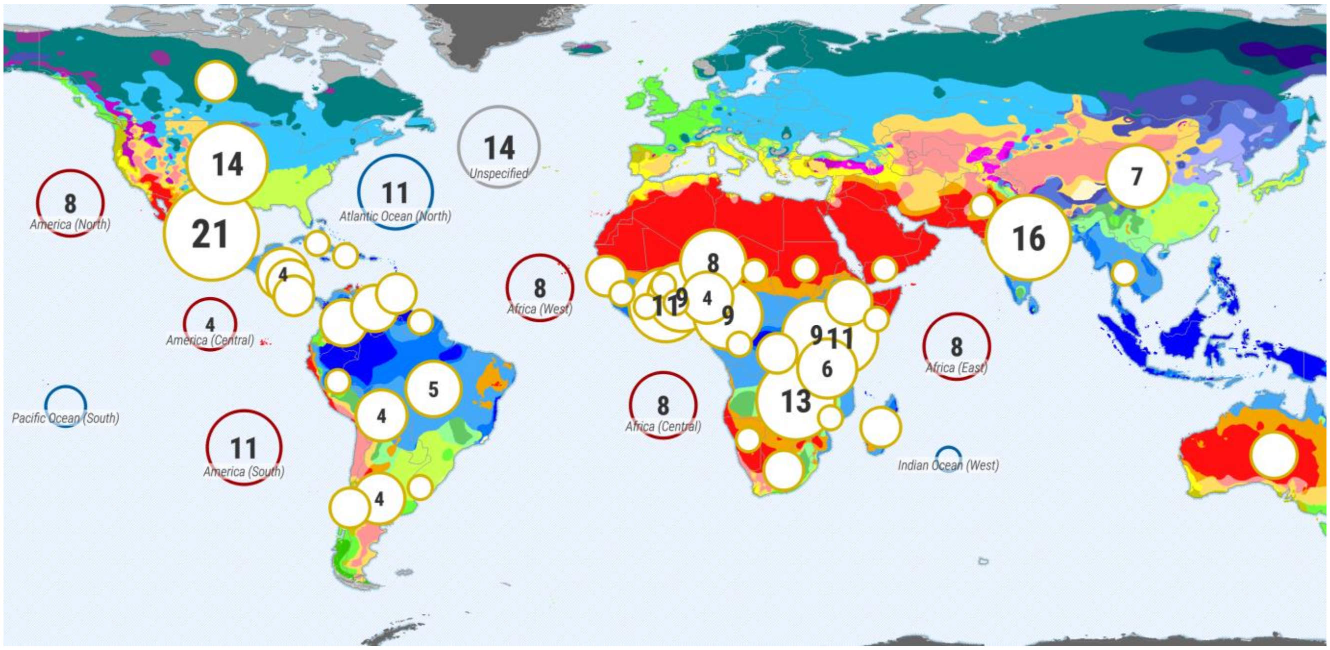

Figure 2.5: Distribution of parasitoid species per country, with the Köppen-Geiger climate classes present in these countries. A download link is provided in the annex.

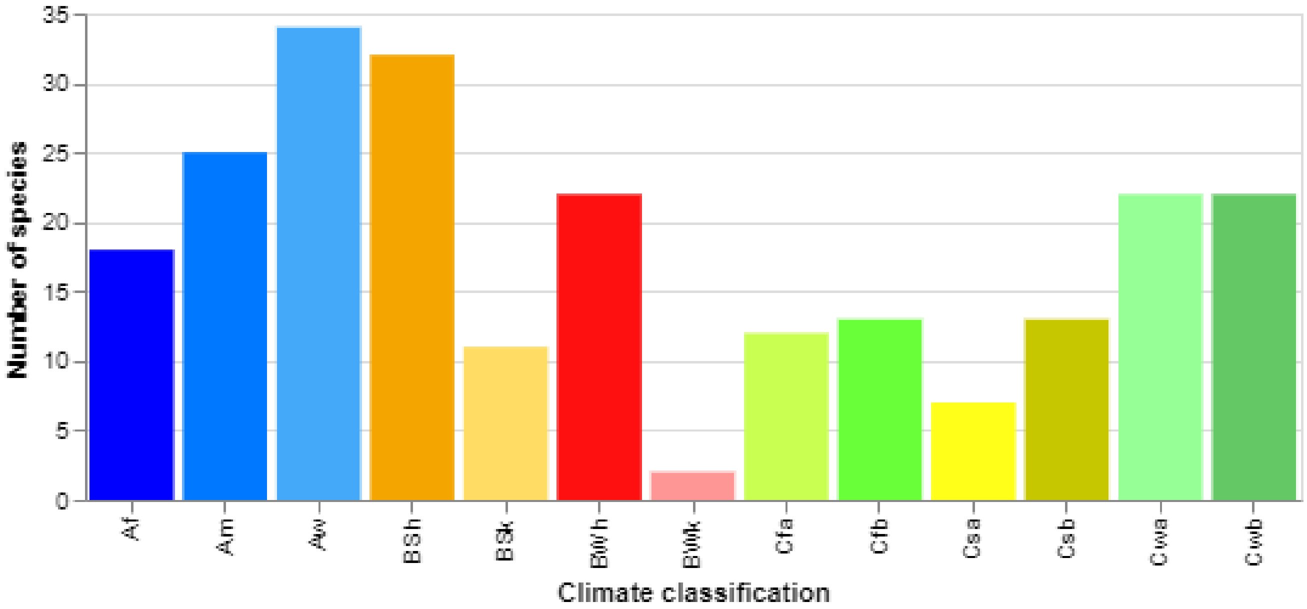

Figure 2.6: Number of parasitoid species present in African climates. The BWk climate only had 2 species reported, while Aw and BSh had more than 30.

1.3.3 Percent parasitoidism in the field

In the 16 items that contained parasitoidism rates of field-collected larvae, the rates go from less than 1% (*Coccygidium*, *Telemucha*) to 100% (*Telenomus remus*). Clearly, very different results can be obtained from experiments, since even with the same parasitoid, the rates are spread over a very wide range. This shows that effective parasitoidism in the field is not the result of host range alone, but other factors as well. Nevertheless, we can outline 3 groups: high parasitoidism rates (*Campoletis sonorensis*, *Chelonus nr. blackburni*, *Telenomus remus*), medium rates (*Aleiodes laphygmae*, *Chelonus formosanus*, other mixed species), low rates (*Chelonus bifoveolatus*, *Coccygidium luteum*, *Cotesia sp.*). Surprisingly, the rates for *Coccygidium luteum* are comprised between 0 and 10%, despite it being one of the most often cited species.

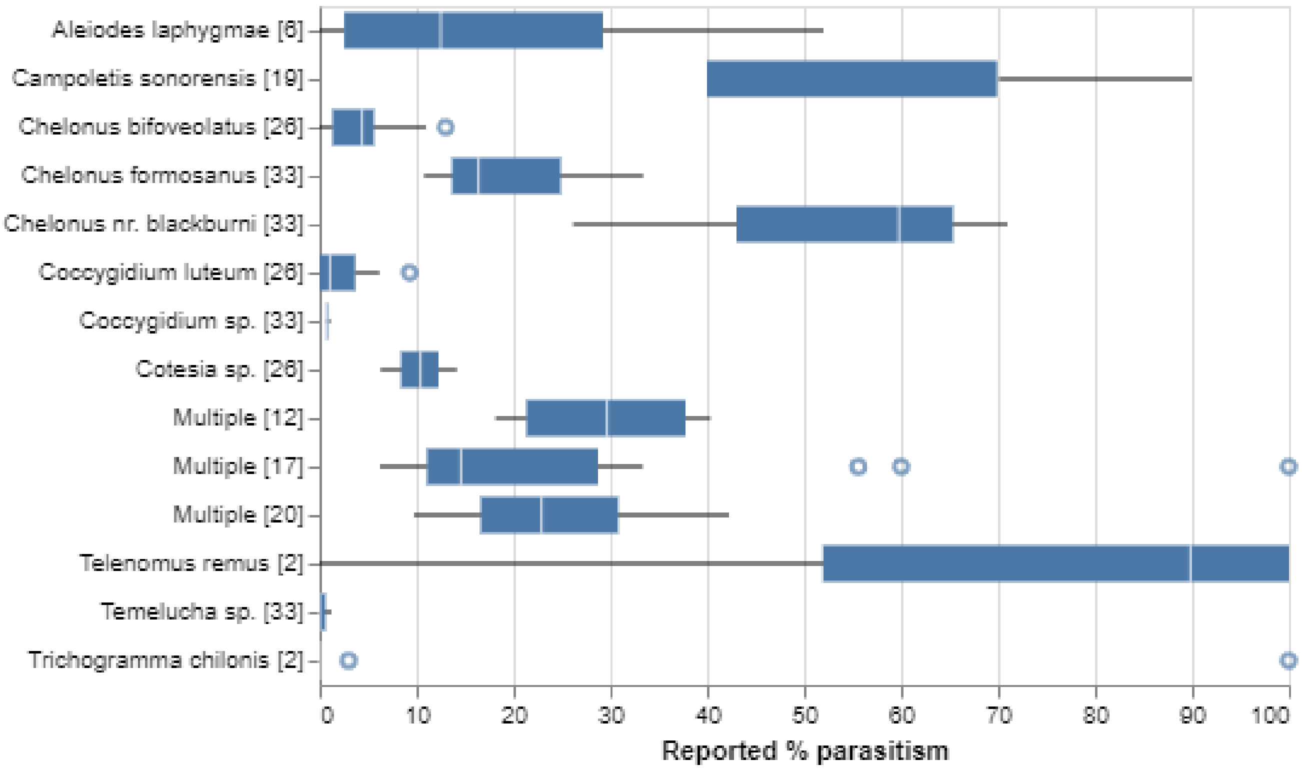

Figure 2.7: Statistical summary of parasitoidism rates found through the systematic search, per species. Only field measurements were collected. The number in brackets is the bibliographic item number found in the annex.

1.4 Discussion

The initial search yielded 53 results with a relatively lax query. Perhaps other sources could be considered. Finding information about release performance in scientific literature is difficult because what happens after the release is not really in the scope of academic research. It’s more a question of management or possibly research and development for biopesticide manufacturers or other stakeholders, who can sometimes perform minimal, cost-constrained monitoring.

The systematic approach worked well for species and geographic ranges. While certain items reported parasitoidism by certain species without reporting the location, most gave at least the country and region. More precise geographic data is often available, from region to municipality and up to GPS. The latter is now quite accessible and seems like a good candidate for a “standard” in location-reporting.

Climatic range information was rarely present as such and had to be derived from geographical descriptors using the Köppen climate classification, but there are complexifying factors. In the long term, the Köppen classification is shifting towards warmer climates, due to climate change. This will impact both host and parasitoid populations. In the short term, seasonal cycles are specific to each location. For instance, certain climates have two cropping seasons. This makes it difficult to compare parasitoidism rate or even parasitoid presence between locations. Finally, without precise location information, geospatial analysis is limited. Presently, Admin-0-level (sovereign country) was used, as it was a level of detail available for all studies. Since there can be many climates in one country, there is a mismatch. More detailed information was present for certain studies and species. However, the level of precision varied among studies. Some authors used administrative region names while others used cultural or natural region names.Simply adding conform Admin-1-level (region/state/province) information would enable more localized analysis, as Admin-1 boundaries are known and largely available geographic data. Aggregating into quadrants worked to some extent but the result could not easily be visualized on a map, as quadrants have different sizes in different countries. When GPS or municipality-level data is available, it could be aggregated by more robust methods, taking both administrative and climatic information into account.

The extraction of parasitoidism rates did not work very well. First, only 16 items contained realized rate information in the field. Second, the assembled parasitoidism rates do not constitute an insightful dataset. Each sampling had inherent particularities that cannot all be controlled for on such a small number of experiments. As a result, it is difficult to compare species. Considerably more datapoints would be needed for any kind of statistical analysis.

Therefore, a usable dataset on climatic ranges and realized parasitoidism rates should contain at least the following variables: host species, parasitoid species, collection date, collection location (GPS), crop treatments. To further improve this review, the following actions can be identified. With regard to the systematic search, the query could be modified (eg. include "frugiperda") at the cost of having more hits. To compensate, the filter methodology used to qualify items could be automatized. This is well within the capability of text analysis software. The query could also target climatic range information more directly using related terms such as "*winter*". Time did not permit including cited bibliographic references ("backwards search"), but it would certainly have augmented the corpus. With regard to data extraction, only the most precise geographic descriptors should be copied. Converting everything to subcountry-level took time and lost information. The conversion could be done later with cartography software. Finally, more recent or even forecast climate data should be used.

1.5 Conclusion

The present review helped assemble a list of potential agents. The list of species obtained is current and their geographic ranges constitute an essential complement. With a focus on field results, the list and the reported rates are representative of actual, real-world parasitoidism. This information is useful to guide the selection of species to be further evaluated. The most promising species should be selected according to their notoriety (how well studied they are), their geographic range, their performance and their specificity.

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Annex: Bibliographic items included in the systematic review

Items marked with **%** contain field parasitoidism data.

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1.6 Annex: Köppen-Geiger classification of climates

- 🟦 Af Tropical-Rainforest
- 🟦 Am Tropical-Monsoon
- 🟦 Aw Tropical-Savanna
- 🟧 BSh Arid-Steppe-Hot
- 🟨 BSk Arid-Steppe-Cold
- 🟥 BWh Arid-Desert-Hot
- 🟥 BWk Arid-Desert-Cold
- 🟩 Cfa Temperate-Withouth_dry_season-Hot_Summer
- 🟩 Cfb Temperate-Withouth_dry_season-Warm_Summer
- 🟩 Cfc Temperate-Withouth_dry_season-Cold_Summer
- 🟨 Csa Temperate-Dry_Summer-Hot_Summer
- 🟨 Csb Temperate-Dry_Summer-Warm_Summer
- 🟩 Cwa Temperate-Dry_Winter-Hot_Summer
- 🟩 Cwb Temperate-Dry_Winter-Warm_Summer
- 🟩 Cwc Temperate-Dry_Winter-Cold_Summer
- 🟦 Dfa Cold-Withouth_dry_season-Hot_Summer
- 🟦 Dfa Cold-Withouth_dry_season-Very_Cold_Winter
- 🟦 Dfb Cold-Withouth_dry_season-Warm_Summer
- 🟩 Dfc Cold-Withouth_dry_season-Cold_Summer
- 🟪 Dsa Cold-Dry_Summer-Hot_Summer
- 🟪 Dsb Cold-Dry_Summer-Warm_Summer
- 🟪 Dsc Cold-Dry_Summer-Cold_Summer
- 🟪 Dsd Cold-Dry_Summer-Very_Cold_Winter
- 🟪 Dwa Cold-Dry_Winter-Hot_Summer
- 🟦 Dwb Cold-Dry_Winter-Warm_Summer
- 🟦 Dwc Cold-Dry_Winter-Cold_Summer
- 🟪 Dwd Cold-Dry_Winter-Very_Cold_Winter
- ⬛ EF Polar-Frost
- ⬜ ET Polar-Tundra

Legend for the Köppen-Geiger climate classification.

1.7 Annex: Maps

The two maps are available online.

Figure 2.3	drive.google.com/file/d/1Ph-8og0iYXH2DylsZRhBJ76GJWWhfDML/view
Figure 2.5	drive.google.com/file/d/1v49sdknTfQVS_WnaeFgBHBTg6i5QIE_9/view

2 A review on the modelization of host-parasitoid relationships

Abstract

In order to estimate the probability of success before the release of a biological control agent, the propagation of the agent and the evolution of its population size and structure can be forecasted using a model. Historically, host-parasitoid systems have been modeled as consumer-resource systems. However, several particularities in the interaction of host and parasitoid have caused the models to include progressively more and more parameters. In this review, the emergence and history of host-parasitoid models are retraced. It appears that all current models translate species interaction as a collection of density-dependent effects, and have added spatial parameters in accordance. However, estimates for these parameters are difficult to obtain.

2.1 Introduction: the consumer-resource paradigm

In classical biological control, an agent is released into a new environment. Tests are conducted beforehand to characterize the agent's performance. However, laboratory results are limited. They must be extrapolated to the field so that a release strategy can be chosen. Mathematical models are used to forecast the evolution of host-parasitoid systems. These are a special case of consumer-resource systems. Their modelization has a long history, with different approaches debated over the last century. There are two schools: the theoricians and the empirists (Wajnberg et al., p173). The present piece aims to retrace the origins of host-parasitoid system models as well as the thinking behind current models and their inputs. For this, we can lean on 3 major books about parasitoid ecology:

- Hochberg & Ives (2000) *Parasitoid population biology*
- Wajnberg, Bernstein & van Alphen (2007) *Behavioral ecology of insect parasitoids, from theoretical approaches to field applications*
- Heimpel & Mills (2017) *Biological Control*

Hosts can be considered a resource for the parasitoid and therefore consumer-resource models could apply (Heimpel & Mills, p151). The Lotka-Volterra equations, proposed in 1925 and 1926, describe the evolution of the consumer population and the resource population over continuous time. Its simplest form is $\mathrm{g}(N) = aN$, with N resource abundance and a the consumer's efficacy. The problem is that, here, a is potentially unlimited. In reality, as a increases, there should be more competition between individuals. The consumer population would not keep increasing at the same rate. Furthermore, this model does not work for host-parasitoid systems because both species alternate between states of availability and invulnerability for the host and activity and inactivity for the parasitoid. Clearly, the modelization of host-parasitoid relationships required additional parameters (Hochberg & Ives, p28-29). The Nicholson-Bailey equations, proposed in 1935, aimed to describe a host-parasitoid system. They operate on discrete time, which takes care of periods of non-interaction. They define the population at each step as a function of H and P, the sizes of the two populations, k the host's reproductive rate and c the number of eggs laid per host. In a solitary egg parasitoid like *C. insularis*, there is only one egg per host egg, but there are many eggs per egg mass, such that this parameter c still makes sense. Superparasitoidism further complicates things. The Nicholson-Bailey equations describes a strictly synchronous system, which remains problematic as to its application in nature. Development time and periods of activity differ in each species, and individuals make decisions based on spatial disparities. In fact, neither the Lotka-Volterra nor the Nicholson-Bailey equations fit empirical observations very well (Wajnberg et al., p44). Therefore, tt is worth considering the specific implications of parasitoidism for modelization.

2.2 Literature review: modelization in three questions

2.2.1 What is the effect of host density?

In 1959, Holling described three models to account for additional interactions between consumer and resource. The response of population size to host availability can be of different types depending on the parameters that are included in the equation (Heimpel & Mills, p153; Tyutyunov & Titova 2020). The simplest approach is to consider that the population increases linearly until it reaches a plateau (Type I). This is similar to the Lotka-Volterra equation but there is an upper limit on the resource consumption rate. If the rate is limited by handling time, we have a Type II response with a hyperbolic increase over time, stopping at a plateau. In this model, as host density decreases, so does the population growth rate. The equation is $g(N) = (aN)/(1 + ahN)$, where h is the handling time. This can also describe a decreasing parasitoid egg supply. Wajnberg et al. (p53) report on Waage's 1983 experiments, which found that parasitoid aggregation and parasitism rate were density-independent. This is possible with a Type II response combined with the aggregation of parasitoids in patches with high host density. If the attack rate can vary with time or resource density, for instance as a consequence of learning, we have a sigmoid-shaped Type III response. Its formula is identical, except N is replaced with N^2. With regard to hosts, the result on their density will differ. With a Type II response, their number decreases strongly at first, then this rate will decrease until a constant number is killed each time step. With a Type III response, the killing rate is low at first, reaches a peak as parasitoid population growth rate is the strongest, then decreases again. Type II seems to better describe monophagous species while Type III is more suited to polyphagous species that can switch food sources as they become rarer. For parasitoids, this links back to the question of host specificity.

The Lotka-Volterra equations, the Nicholson-Bailey equations and the different response times proposed by Holling consider the consumer-resource system as a closed ensemble where the probability of encounter is random. The models' predictions are entirely dependent on the response Type chosen, and this choice is based on empirical observation. However, a model that cannot produce forecasts for systems that have not been fully observed before has little value. Furthermore, by manipulating the parameters, it's possible to arrive at paradoxical forecasts. These models are inherently unstable, and the explanation that this instability is counteracted in the field by environment patchiness (Heimpel & Mills, p160 and figure p161) is not satisfactory. Some models have been proven to be unable to lead to a stable situation with a low pest population. On the other hand, this translates well to reality, where the consumer population may indeed become extinct after an initial boom, because of now insufficient resources. The Arditi-Ginzburg equations were proposed as an alternative (Tyutyunov & Titova 2020). Instead of relying only on resource abundance N, they use the ratio of prey to predators N/P. Arditi and Ginzburg highlighted that both functions $\mathrm{g}(N)$ and $\mathrm{g}(N/P)$ were special cases of a function taking the number of predators and the number of preys as input, $\mathrm{g}(N,P) = (\alpha N)/(P + \alpha hN) = (\alpha N/P)/(1 + \alpha hN/P)$. This modelizes the interactions between consumers in the sense that the more consumers there are, the smaller the area where they might find resources. This is called interference.

Hochberg & Ives consider the relationship between clutch size (number of eggs laid) and host density (Hochberg & Ives, p32-39). A lower clutch size leads to more coexistence but more vulnerability to low host numbers. A higher clutch size leads to more host suppression but more vulnerability to a low egg load. Whether this applies in the case of *C. insularis* remains to be determined, as females seem to fill all host eggs. The authors propose their own model (Hochberg & Ives, p218). Population growth can be modeled as the logistic curve $rN(1 - N/K)$ with N abundance, K environment capacity and r per capita increase rate. K is the parameter defining abundance at equilibrium. However, there can be losses, such as the one incurred by the difficulty of finding mates at very low density. The term $-bNe^{(-sN)}$ can be added to the previous formula, with s mating efficiency and b per capita birth rate (Hochberg & Ives, p219). This sets a lower limit $sK - 1$ for environment capacity and a minimum starting population size $\ln(b/r) \div s$. With haplodiploidy, a 30% smaller starting population is allowed because individual fitness has a different relationship to population size (Hopper & Roush 1993). As a result, even with a smaller starting number of individuals, the population would be able to establish itself.

2.2.2 Are food and host foraging one and the same?

The female parasitoid must balance its food reserves, sperm count, egg load, host quality and make foraging, host acceptance and sex allocation decisions. For host-feeding species, the decision is whether to oviposit in a host or feed on it. For other species, the decision is whether to leave the hosts to find food. In both cases, the individual must balance time spent feeding and time spent ovipositing. A parasitoid's fitness is equal to its lifetime reproductive success. It's a function of host locating, host acceptance and patch leaving (Wajnberg et al., p34). In conditions of limited hosts or limited food, the female's behavior can indeed be summarized as the decision to stay in a patch or leave it (Wajnberg et al., ch13). The parameters are environment patchiness and richness. If there are more patches, hosts and parasitoids may coexist for a longer time (Hochberg & Ives, p48). Spatial heterogeneity has implications for the attack rate which was the main parameter in the models presented above (Hassell 2000; Hochberg & Ives, p49). Wajnberg et al. (p54) finds that patch-leaving decisions are dependent on host density. For some species, it may be more profitable to move to another patch even when there still are high-quality hosts in the patch, for example as a risk mitigating strategy: risk of patch failure, risk of superparasitoidism (Wajnberg et al., p56).

The lifespan of *C. insularis* on distilled water is two weeks. To augment it, carbohydrates, proteins and lipids are needed (Wajnberg et al., p114). Time spent searching for food is not spent searching for hosts. However, by consuming food, individuals increase their reproductive lifetime (Wajnberg et al., p131). The optimal decision for fitness is therefore to try and locate hosts as soon as possible, and only search for food if the reserves are low. If food is available in the environment, the parasitoid should spend time foraging for hosts only. This explains the role of sugar subsidies (Wajnberg et al., p20). The goal is not simply to help the agent population subsist, but to increase their reproductive lifetime by decreasing the time spent searching for food.

Bernstein & Jervis propose a model in Wajnberg et al. (ch7) for non-host-feeding synovigenic species (p157-159). The model distinguishes between mating resources and egg-production resources. The latter are considered instantly converted to eggs. There are no time or egg limitations. The authors show the predicted energy reserves of a host using both theoretical and observed parameter values. The model predicts less feeding at low and high egg loads, yet there is empirical evidence of more feeding at low egg load (Wajnberg et al., p158). In reality, insects get energy from various food sources and may direct the nutrients to either mating or egg production.

2.2.3 When should a parasitoid leave a patch?

Since patch resources can be depleted, the parasitoid must have a way to evaluate that finding another patch would return greater fitness. A model can give the optimal time to leave. In the field, the decision to leave a patch is likely to be influenced by a multitude of factors, including complex exo- and endogenic factors such as experience, the presence competitors or enemies, and chemical cues (Wajnberg et al., p13).

Charnov's marginal value theorem, proposed in 1976 (Wajnberg et al., p13), considers that a parasitoid will leave a patch when the patch value, which necessarily decreases with time as its resources are consumed, has reached the average of the neighboring ones. Another way to formulate the decrease of patch value is to say that fitness gains decrease as time in the patch extends (Wajnberg et al., p173-174). However, this makes problematic assumptions. Since the parasitoid has no knowledge of other patches, the decision to leave can only be made on the basis of information from the present patch. The theorem also assumes lone females with no competition. Yet, a correct prediction of models based on this theorem is that parasitoids remain longer in high quality patches and more remote patches.

Waage's incremental model, proposed in 1979, associates patch quality with host density, and assumes that the host density of a patch can be evaluated upon arriving in it, for instance with kairomones. The model is called incremental because for each oviposition, the parasitoid stays a bit longer. In other words, it stays as long as there are hosts left. If there are none, it leaves. This has been shown in many species. The argument is convincing, but the parameters cannot be empirically deduced (Wajnberg et al., p176). There is an apparent paradox here. If the parasitoid already knew host density upon arrival, why would it need to update this information? Many species do the opposite, and decrease time spent on the patch as the number of ovipositions increase. In 1981, Iwasa et al. posited that parasitoids should use an incremental rule or a decremental rule based on host distribution (Wajnberg et al., p177). If the hosts are aggregated, staying as long as there are hosts maximizes time spent ovipositing. If they are uniformly distributed, their local availability will soon be below that of surrounding patches and it's more efficient to leave. If they are randomly distributed, it makes no difference.

The assumption that host density in the patch can be evaluated before beginning oviposition remains a problem. Furthermore, the parasitoid has no knowledge of the quality of other patches. With this uncertainty, the optimal decision can be calculated with a bayesian model. As the patch's resources decrease, there is a threshold after which the probability that it will keep contributing to fitness is too low, and finding another patch is best. This is similar to a coin toss where if the 20 previous tosses were heads, tails is more probable at the next toss.

Waage's model is a close approximation of the bayesian optimal, but the process it describes is not formally bayesian (Wajnberg et al., p370-371). It is based on a linearly decreasing capital of motivation that is updated (incremented) with every encounter. Iwasa et al. proposed a proper bayesian model, in which the number of remaining hosts on the patch is estimated based on the number of hosts met so far. The models have a similar output but the tendency to remain in the patch decreases at a slower and slower rate, and there is a threshold after which the parasitoid leaves. But how could a parasitoid pick the bayesian optimal? Iwasa et al. concluded that Waage's model was the best the parasitoid could do to approach it.

Pierre & Green propose an elegant solution instead (Wajnberg et al., ch16). Since the optimal decision gives the evolutionary advantage, the parasitoid must achieve it in its behavior. Citing McNamara et al., they point out the difference between the theoretical optimal, calculated using prior knowledge, and the mechanism by which the parasitoid finds it, using only the information presently available. The authors propose a patience function, where patience decreases as a function of time, and increases (increments) as hosts are encountered. The formula is

$$aP - bt + \sum_{i=0^{\mathrm{n}}} (t)\, I$$

with aP the initial tendency to stay in the patch, b the rate of patience decrease, $n(t)$ the number of hosts encountered and I the size of the increment when encountering a host. I can be a function of time $\mathrm{I}(t_{\mathrm{i}})$ if two successive host encounters do not add up linearly. This is very elegant because a patience model doesn't require omniscience or memory, yet gives the bayesian optimal.

2.3 Conclusion

Host-parasitoid systems were long an object of interest for modelization efforts in ecology. Complexity varies between models depending on which factors are taken into account.

Spatial parameters are typically investigated in more recent developments. However, simple models can account for evolutions of the population structure. One parameter that is left out is the sex-ratio. The parasitoid population is considered as a monolithic entity with a varying size, always able to reproduce itself. The challenge is to find observations matching model predictions. This has already yielded interesting results, as different models seem to apply to different species. These results are also interesting for laboratory-reared colonies. The influence of patchiness on oviposition behavior seems essential for field efficacy, yet there is typically no notion of patchiness in parasitoid evaluation studies.

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3 Specificity and preference of the braconid *Chelonus insularis* for the Fall Armyworm *Spodoptera frugiperda*

Abstract

Chelonus insularis was presented with egg masses from *Spodoptera frugiperda* and *Spodoptera exigua* in both no-choice and choice tests. The results showed that *S. frugiperda* is preferred in a choice scenario, but not in a no-choice scenario. In the choice scenario, *C. insularis* dedicated 30% of its eggs to the alternative host. In the no-choice scenario, the rates of parasitoidism were similar at 79.6% for *S. frugiperda* versus 75.6% for the alternative host. The rate of parasitoidism was not significantly higher on fresher eggs. Larva and head capsule size were recorded to see at which host stage the parasitoid larva emerges. *S. frugiperda* was killed sometime during its late fourth or early fifth instar, as evidenced by a maximum size at death of 16mm. This shows the ability of *C. insularis* to prevent physical damage done to the crops by the current pest generation, since more than 90% of the damage is done at the fifth instar and after.

3.1 Introduction

3.1.1 Motivation

The Fall Armyworm, or FAW, is the most destructive agricultural pest worldwide. Given its spread to other continents, the extensive damage it causes to economically essential crops, and the disadvantages of chemical pesticides, the situation is becoming ever more urgent. In its native geographical range (Meso-America), the pest is controlled by natural enemies, mostly parasitoid wasps which use its larvae as part of their reproduction cycle. This gives hope that biocontrol could be possible in other regions (Africa, Asia). However, a natural enemy species remains to be selected as an agent. *Chelonus insularis* is one of the main parasitoids of the Fall Armyworm in its native range. However, it has also been seen parasitoidizing other hosts, raising the issue of its specificity. The present work aims to measure host specificity of the parasitoid wasp *C. insularis* to the pest *Spodoptera frugiperda*, the Fall Armyworm.

3.1.2 CABI

The Centre for Agriculture and Bioscience International, or CABI, is an international non-profit founded in 1910 and dedicated to scientific research on agricultural and environmental issues. Its headquarters are in the United Kingdom and it has centers in Brazil, China, Ghana, India, Kenya, Malaysia, Pakistan, Switzerland, Trinidad & Tobago, United Kingdom, United States of America, Wallingford and Zambia. Today, 48 countries are members of CABI.

The Swiss Center was created in 1948 and moved to Delémont in 1958. Several biological control programmes were launched in the following decades. The agency's general *modus* *operandi* is knowledge transfer (Powell 2017). It publishes and maintains several databases such as the Crop Protection Compendium and the Invasive Species Compendium. CABI was at the forefront of digitization and created an online database of scientific article abstracts as soon as 1973. In the last decade, the Plantwise programme was launched to accompany farmers in the field and collect plant health data at the same time (Cameron et al. 2016). The agency also provides training to smallholder farmers to use techniques and tools based on the latest research in integrated pest management (IPM). The Swiss Center has the following programmes: research, advocacy and implementation of biological control against weeds and arthropods, risk analysis, ecosystem and crop management, as well as academic training in partnership with the University of Neuchâtel.

Most of the agency's revenue comes from commercial publishing and donations. Its income has increased from 25M£ in 2011 (Financial Statement 2015) to 37M£ in 2019 (Annual Review 2020). Thanks to this baseline, and in contrast to organizations that work with project-based funding only, the different centers can keep their operations permanent.

The present study was funded by the UK Department for International Development (UK), the Directorate-General for International Cooperation (EU) and the Swiss Agency for Development and Cooperation (CH).

3.1.3 The pest *Spodoptera frugiperda*

The Fall Armyworm, or FAW, is a polyphagous lepidopteran pest (Rwomushana 2019). Among the 350 plants it attacks are maize and sorghum, two major food and economic crops globally. It is also present in 80 other crops. It is characterized by its appetite, its adaptability to different environments and its resistance to viruses and pesticides (Jing et al. 2021). The pest's control by chemicals and genetic engineering has been relatively unsuccessful so far. In the long run, chemical pesticides can induce resistance in the pest by selection (Agboyi et al. 2020). It has been the case for the Fall Armyworm, which has also adapted to transgenic crops (Costa et al. 2020). This has generated enormous interest in biological control. Given the damage inflicted on crops, the FAO launched a programme in December 2019 (FAO) aiming to reduce human health risks associated with pesticides and provide training and technical information to farmers. The species' biology and ecology have been extensively covered. Just for the period until December 1988, Ashley et al. (1989) list more than 1300 bibliographic references. A cursory search on Web of Science for publications since January 1989 yields 2040 hits.

Discovery, taxonomy, classification

The earliest record of this pest was in 1594, as reported in 1978 by Fernandez (Ashley et al. 1989). The first record of a *S. frugiperda* outbreak was in 1797 by Smith and Abbot (Sparks 1979). The pest was designated as "corn-bud-worm-moth". Several outbreaks were recorded in the following decades. A seminal review by Luginbill (1928) lists 25 outbreaks, reported in 34 locations.

The species was given the name *Phalaena frugiperda* by Smith and Abbot, but placed in the genus *Trigonophora*. It was then named *Laphygma macra* by Guenee in 1852. Other authors identified variants under different names. The genus *Laphygma* was regrouped to *Spodoptera*.

Distribution, natural habitat

The pest originates from Central and South America, where it is partly controlled by native natural enemies and certain farming practices. It arrived in Sub-Saharan Africa in 2016 and Southeast Asia in 2018 (Sharanabasappa et al. 2018).

This species has no diapause mechanism. In mild winter climates such as Florida's, it overwinters, continuously generating throughout the year. Further up North, freezing temperatures kill eggs and larvae, but the species migrates again every in the summer.

In East Africa, *S. frugiperda* proved unsuitable for the existing parasitoid complex (Sokame et al. 2021). However, larval mortality was higher than in the absence of those parasitoids, indicating that parasitoidism had been attempted. Unsuccessful attacks by native parasitoids could be a problem for local agro-ecosystems, as it implies that resources are taken away from the control of native pests.

Feeding habits, rearing

S. frugiperda larvae pupate at the 6th or 7th instar. They eat 94% of their larval-lifetime intake in the 5th and 6th instars, causing a shock to unprepared farmers (Sparks 1979). Furthermore, they are most active at night. Individuals get all the essential amino acids as larvae, while the adults only consume sugar.

S. frugiperda shows cannibalistic behavior in the laboratory. Parasitoidized larvae are weaker and can be swallowed by a healthy larva in a few minutes. This behavior is induced by environmental stress and notably density or frequency of encounter with other larvae. Cannibalism gives individuals an advantage when there is insufficient food. The winning larva receives up to its own weight in food (per opponent!) and keeps all the local resources to itself. Competition is decreased at the same time as hunger is remediated. However, larvae that ate conspecifics were found to develop slower than larvae that ate plant material, and to produce smaller pupae (Chapman et al. 1999).

Life cycle: organs, mating, oviposition, development stages

Like all Endopterygota, *S. frugiperda* is holometabolous. Its eggs hatch on the third day (personal observation). It takes around 4 weeks to complete its life cycle (Hardtke et al. 2015).

Parasitoid species typically attack only one stage of their host, either egg or larval. With *C. insularis* being an egg-larval parasitoid, parasitoidized Fall Armyworm eggs hatch normally. However, the parasitoidized larvae that emerge grow at a much slower pace. As a result, they do not grow as big. They are much smaller than their siblings, up to 97% when parasitoidized by *Apanteles marginiventris* and 70% with *C. insularis*, according to Ashley (1983). They have a much lower appetite as a result. They die after 10 to 15 days when the parasitoid emerges. A study by Agboyi et al. (2019) measured an 89% decrease in maize consumption for Fall Armyworm larvae when parasitoidized by the wasp *Coccygidium* *luteum*. Rezende et al. (1994) measured an 80% reduction in surface area consumed until the 12th day. The lifetime reduction was 93%. Around the 5th instar, parasitoidized larvae weighed a third of healthy larvae. There is a possibility of so-called pseudoparasitism. The host behaves as though it were parasitized, but there is no parasitoid development. This is typically because of encapsulation, where the parasitoid has been contained by the host's immune system. In case of parasitoidism failure, host larvae form a cocoon sooner, achieve the pupa stage and die.

Pictures

Figure 3.1: *Spodoptera frugiperda* eggs, larva and pupa. The species undergoes complete metamorphosis. The larval phase is voracious. *Chelonus insularis* attacks the eggs. Photos: Rutgers Agri. (eggs), Lyle J. Buss University of Florida (larva), Manitoba Agri. (pupa).

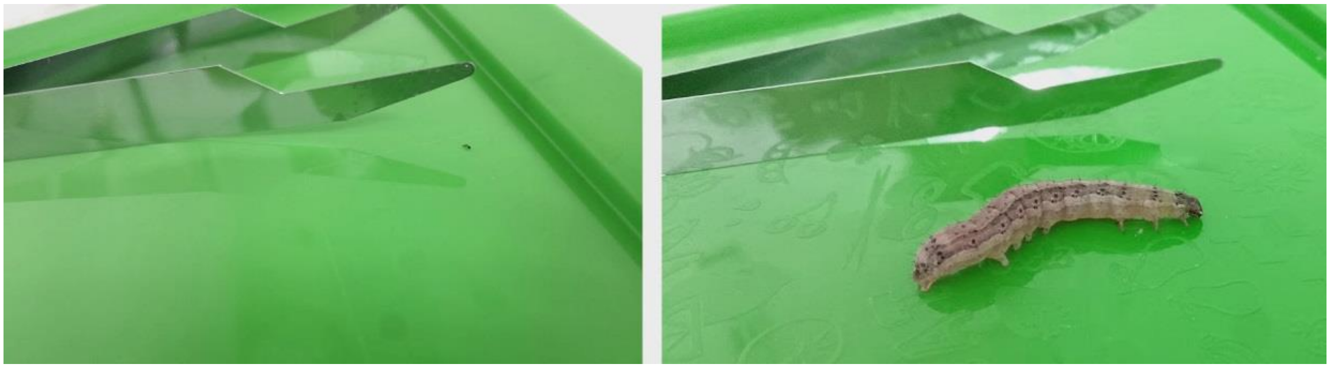


Figure 3.2: *Spodoptera frugiperda* is less than 2mm upon hatching (left), but grows consequently to several centimeters in the fifth and sixth instars (right). Only a few days

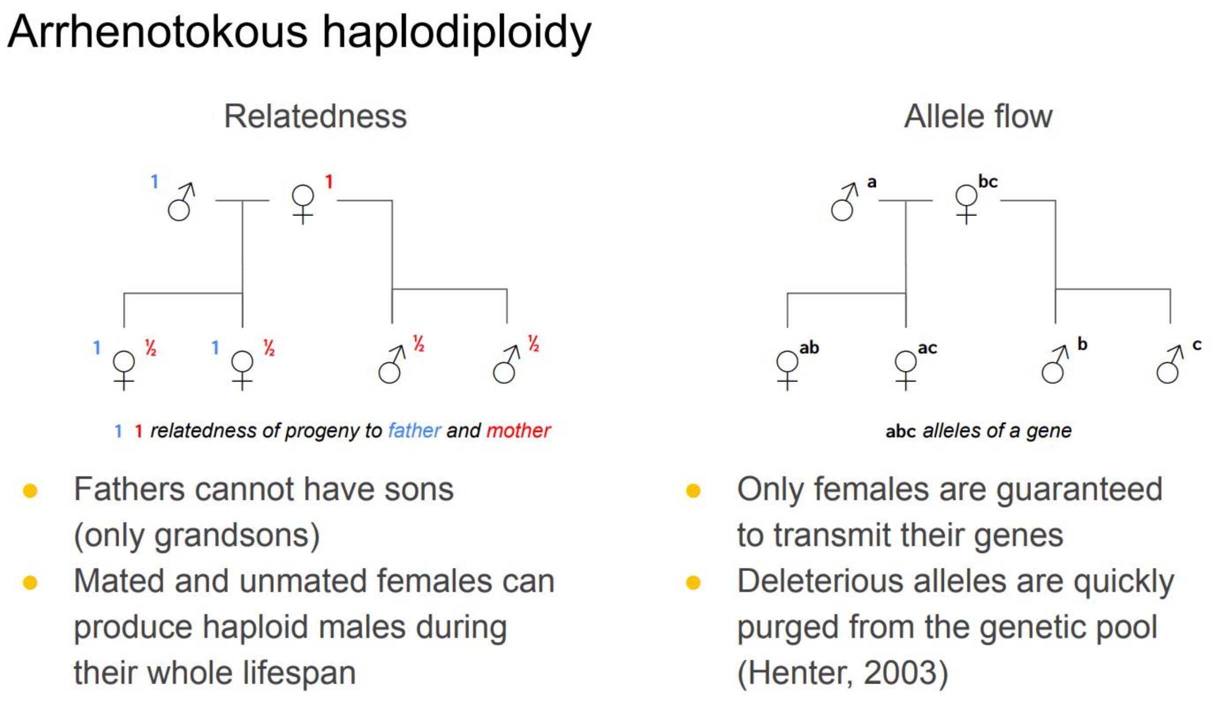
separate these two pictures. Crops are devastated in a very sudden way, leaving farmers in shock.

3.1.4 The parasitoid *Chelonus insularis*

The parasitoid wasp *Chelonus insularis* is one of the main natural parasitoids of the Fall Armyworm in its native range. It has been reported in Argentina, Bolivia, Brazil, Chile, Colombia, Cuba, Haiti, Honduras, Mexico, Nicaragua, Peru, Trinidad and Tobago, United States (Southern), Uruguay and Venezuela (Silva et al. 1997; Virla et al. 1999; Molina-Ochoa et al. 2003; Murúa et al. 2006; Ruíz-Nájera et al. 2007; Silva et al. 2008; García-Gutiérrez et al. 2013; Mosquera Pérez et al. unpublished). It is abundant and easy to procure. A release of *C. insularis* was attempted in South Africa between 1942 and 1954 against *Loxostege frustalis*, but rearing proved very difficult and the agent was likely not able to establish itself. A reduction in pests was observed, but then attributed to a previously unrecorded native parasitoid (van Lenteren 2012 after Greathead 2003).

Discovery, taxonomy, classification, traits

C. insularis was described by Cresson in 1865. He also described a species he named *Chelonus texanus*, which in 1978 was revealed to be the same (Medina et al. 1988). Pierce & Holloway (1912) reported that *C. insularis*’s ovipositing behavior had been recorded two times in the United States, in 1909 and 1911, both in Texas. The insect was brought to a laboratory and examined. Oviposition in the field was recorded again in 1912. The authors noted that periods of flights of moths coincided with an abundance of wasp adults. Parasitoidized egg masses were collected from as far as Arkansas. The genus *Chelonus* is part of the family of the Braconidae, one the biggest families in the Hymenoptera order. At a paraphyletic level, it is part of the Apocrita and Aculeata. It’s a holometabolous insect. It goes through multiple stages during its development, from the egg to a larva, then to a pupa, and then to the adult stage (imago). Being a parasitoid, the egg matures, and the larva develops, inside a host. An individual from another species. All insect parasitoids are holometabolous (Godfray 1994, p6). Like all Hymenoptera, *C. insularis* is haplodiploid. One sex is diploid and has two of each chromosome, while the other is haploid and has one. It is arrhenotokous, meaning males are haploid and females are diploid. Males develop from unfertilized eggs, and female from fertilized eggs. Upon laying each egg, the female decides of its sex, provided it has mated and is thus able to fertilize the egg herself with sperm from the spermatheca. With this system, deleterious mutations are quickly exposed in males and removed from the population. Eggs are formed in the calyx by meiosis. *C. insularis* is described as a pro-ovigenic species, which emerge with enough mature eggs for its entire reproductive lifetime. By comparison, in synovigenic species, eggs are produced all along the female’s adult life. In reality, different species emerge with a different proportion of mature eggs (Wajnberg et al. 2007, p115). Jervis et al. (2001) calculated this ovigeny index for more than 600 parasitoid wasp species and found that more synovigenic species are longer-lived. Strictly pro-ovigenic species represented less than 2% of all species tested and none were found in Ichneumonidae or Braconidae. *C. insularis* is a solitary endoparasitoid. It lays only one of its own eggs inside each host (Hochberg & Ives 2000, p27). It is an egg-larval parasitoid. The female lays inside the host embryo’s hemolymph. It attacks the eggs but the stage that is parasitoidized is really the larva (Ullyett 1949). It is therefore a koinobiont. The host is not paralyzed by the parasitoid, but rather lives and continues growing.



Arrhenotokous haplodiploidy

Relatedness

1 1 relatedness of progeny to father and mother

Allele flow

abc alleles of a gene

- Fathers cannot have sons (only grandsons)
- Mated and unmated females can produce haploid males during their whole lifespan

- Only females are guaranteed to transmit their genes
- Deleterious alleles are quickly purged from the genetic pool (Henter, 2003)

Figure 3.3: Arrhenotokous haplodiploidy. Under arrhenotoky, males develop from unfertilized eggs and are haploid, while females develop from fertilized eggs and are diploid. Lethal or otherwise deleterious alleles are quickly purged from the population because males that carry them are eliminated, having no viable version of the gene in question.

Distribution, natural habitat

C. insularis is found in South America, Central America and the South of the United States. This corresponds mostly to tropical and temperate climates with warm or hot summers. A study by Teppa-Yotto et al. (2021) forecasted a slight narrowing of the climatic niche for *C. insularis* due to climate change. However, this is balanced by the evolution of the Fall Armyworm's climatic niche, as well as his other main parasitoid's. Holopainen, Himanen and Poppy provide a review of the impact of climate change on insect parasitoids in Wajnberg & Colazza (2013).

Feeding habits, rearing

C. insularis adults eat honeydew and proteins. The shape of their mouth parts allows them to access concealed nectar (Wajnberg et al. 2007, p134). In captive colonies, they are given honey and water. In Hymenoptera, the bulk of the energy intake is used for either mobility or reproduction. The exact usage made by *C. insularis* of these resources is unknown.

Maturation, emergence and mating

The embryo is visible 30 hours after oviposition and the egg hatches after 36 to 39 hours (Medina et al. 1988). With some parasitoid wasps such as *Eiphosoma laphygma*, the parasitoid larva can be seen inside the young host larva through its skin with a microscope. The same did not seem possible with *C. insularis*. During the parasitoid's third instar, the host is biochemically manipulated to initiate a cocooning behavior, several days before it would if it were not parasitoidized. The parasitoid larva bursts out of the host, tail first, between segments 4 and 5 (Video 4.1 in annex). Certain signs can be observed in advance of this event (personal observations). A trick is to flip the host larva on its back. The longer it takes to right itself, the closer it is to eruption. A bit later, it freezes, curls up and takes a moult-like appearance. The parasitoid larva is maggot-like and pink-white (characteristic of Apocrita) and has 11 segments. It continues eating the host's insides, such that the carcass shrinks from 12+mm to less than 6mm (5.7mm recorded in personal observations). Protected by the chamber carved by the host, the larva makes a white, strong cocoon (Figure 3.4). Inside, a white pupa forms. Gradually, it turns black, starting with the eyes (personal observations). After a few days, the adult makes a hole in the cocoon and flies away (Video 4.2 in annex). In a study by Medina et al. (1988), *C. insularis* larvae reached the second instar after 14 days at 25˚C, the second instar lasted 2 or 3 days, the third instar lasted 1 or 2 days, the prepupal stage lasted 1 day and the pupal stage lasted about 9 days. The total time was 29 days on average (±2.5 days). Adults hatch during the day, and males hatch about 2 days before females. This is called protandry. The result is that females are inseminated immediately upon hatching, usually by multiple males. Females store the sperm in a volume called the spermatheca (Jervis & Kidd 1996, p81). It is thought that one insemination is sufficient to fill it. Therefore, protandry is more than optimal. Sexing *C. insularis* is difficult without prior experience. Females have slightly shorter antennae with 25 segments against 30 for males. Because individuals can be of different sizes, a large female may have the same antenna size as a smaller male. The abdomen of females is also broader and more rounded. Individuals of different sizes can still copulate (observation).

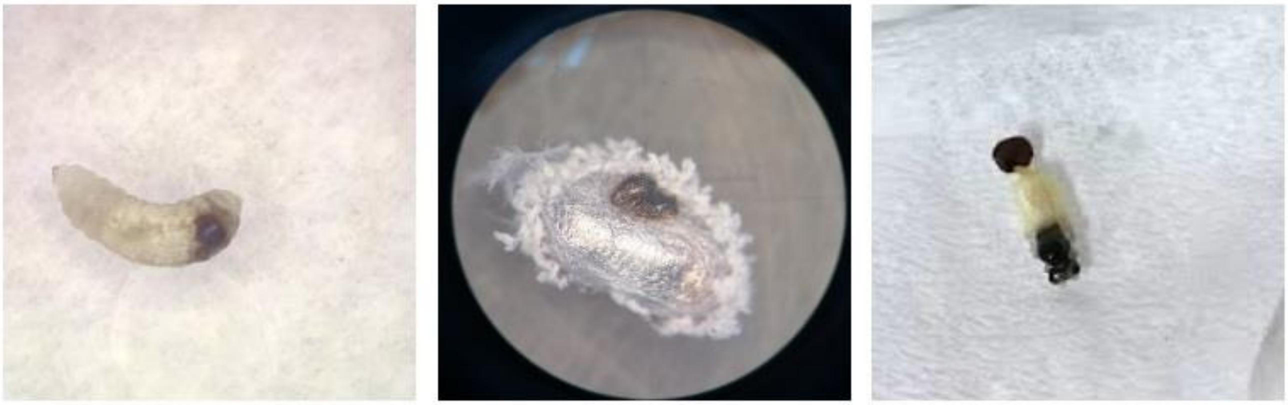


Figure 3.4: Detailed view of the transition from larva to pupa in the development stages of *Chelonus insularis*. The larva (left) emerges naked from the host. It weaves a cocoon (center). The host's head capsule is still attached to it. Inside the cocoon, the pupa gradually turns black (right).

Host locating and acceptance

C. insularis has been recorded as a natural enemy of 13 pests, according to CABI's ISC, including 8 species of *Spodoptera*. Hosts are typically hidden in the environment (eg. on the underside of leaves). Williams et al. (2003) found a significant effect in the reduction of parasitoidism when weeds were present in the plot, from 42% to less than 4%, 23 days after planting. Adults use direct and indirect cues. They are drawn to volatiles of maize plants, egg masses and hair, which carries sexual hormones (Roque-Romero 2020). Experienced females are more attracted to egg masses than to plant volatiles, but inexperienced ones aren't. When an egg mass is in the vicinity, females start tapping with their antennae until they reach it (Earl 1983). They rely on a stimuli complex and do not show a response to isolated stimuli (Godfray 1994, p27-33).

Accepting or rejecting a host is the first step in the oviposition strategy. For larval parasitoids, size is the main indicator of host quality. For egg parasitoids, the host eggs are inspected by the female using its antennae. *C. insularis* females also use their ovipositor to inspect the egg and seem to prefer 1-day old eggs (Ables et al. 1981; Glogoza 1981; Godfray 1994, p143).

Egg load, clutch size and sex allocation

A female with fewer eggs should theoretically prefer higher quality hosts (Heimpel & Mills 2017, p129), but there are differences in a species ability to distinguish quality. Some species lay into unviable or even infertile eggs (Earl 1983). As a clearly non-optimal strategy, this is surprising. Low-quality hosts are commonly accepted in parasitoids. This is because the parasitoid has limited time (Wajnberg et al. 2007, p59). However, time cannot be the main driver for behavior, because that would require the individual to have a sense of its own expirability. Neither can host density, because that would mean the parasitoid has knowledge of the whole environment. Egg load is a knowable proxy for these two theoretical quantities and could therefore be the main driver of behavioral traits. In some species, foraging abilities indeed declined as egg load decreased (Wajnberg et al. 2007, p10). However, egg load was not found to be affected by host density. According to Wajnberg et al. (2007, p58), this is because host acceptance is lower with a lower egg load. Whether this verifies for *C. insularis* remains to be determined. Egg load is easier to derive statistically from physiology than from observing oviposition (Wajnberg et al. 2007, p62). *C. insularis* parasitoidizes host eggs as they arrive, but the decision is more efficient as the time to find them increases (Earl 1983). The eggs of *C. insularis* are yolk-rich (anhydropic) (Jervis et al. 2001).

Once a host has been accepted, there is a second part to the oviposition strategy. Clutch size and sex are the parameters (Godfray 1994, p116). The female must decide how many eggs to lay and which sex to give them. The Lack clutch size theoretically gives the optimal fitness per clutch. It's equal to the progeny fitness multiplied by the number of individuals, with progeny fitness varying with clutch size (Godfray 1994, p100). Optimizing fitness per clutch is not the only option, but it's a good strategy if future reproduction is expected to be low. Alternatively, fitness per egg can optimized if future reproduction is expected to be high (eg. in the laboratory) (Wajnberg et al. 2007, p14). In practice, it is better to infer clutch size from experiments. Clutch size is entangled with other variables for parasitoids (Godfray 1994, p111 and p125), and observed clutch sizes are always smaller than the Lack clutch size. When it comes to sex allocation, the laying female can use sperm from the spermatheca to fecundate each egg individually. This means that female eggs are more expensive. The female must evaluate each egg's quality in order to assign a sex.

Parasitoidism and superparasitoidism

The female manipulates its ovipositor to place the egg inside the host egg or inside the embryo, if it is already developing. Along with its progeny, the female parasitoid injects two fluids in the host egg. One comes from the calyx and the other from the venom gland. The fluid coming from the calyx contains teratocytes and polydnaviruses like the bracovirus (Godfray 1994, p242). The transmitted viruses do not reproduce inside the host (Godfray 1994, p243). A study by Ables & Vinson (1981) demonstrated that the two fluids injected by the female are sufficient to limit host larva growth. However, this effect is temporary. After a few days, growth resumes at a normal pace, though the larvae's final weight is lower than that of the uninjected larvae. An analysis of *C. insularis*'s venom (Kaeslin et al. 2010) concluded that it increased the effect of the bracovirus. Its direct effect are temporary paralysis and higher cell membrane permeability. The venom gland is made of a reservoir and two canals. The organ appears in pupal stage 3 but contains no venom until pupal stage 6. The parasitoid larva can be present but contained by the immune system. This is called encapsulation (Godfray 1994, p232). This mechanism can be influenced by diet (Wajnberg et al. 2007, p114). Teratocytes fight encapsulation and help with the digestion of host tissue. Females leave external and internal marks on the eggs they have parasitized, including chemical cues. The chemicals have an individual composition and are produced by the alkaline gland (Dufour's gland) (Godfray 1994, p144). At the population level, marking increases resource allocation efficiency (Godfray 1994, p145). For *C. insularis*, the parasitoidism rate on *S. frugiperda* is highest three days after emergence and lowest three days before death (Rezende 1995). Oviposition is dependent on temperature (Glogoza 1981). *C. insularis* females lay the most eggs at 35°C. At 20°C, they start a day later and stop a day or two sooner than at 25-35°C. However, they also lived 3 to 5 days longer.

A host might already be parasitoidized by the same or even another species. The decision when encountering parasitoidized egg is between leaving the patch or superparasitoidizing. Superparasitoidism is common in the *Chelonus* genus. In a study by Ables et al. (1981), none of the three egg-larval parasitoid wasps *C. insularis*, *Trichogramma heliothidis* and *Trichogramma pretosium* rejected eggs that were already parasitoidized. Superparasitoidism and self-superparasitoidism can be evolutionarily advantageous (Godfray 1994, p126). In case of superparasitoidism, the larvae compete inside the host. Barring any other factors, the first (oldest) larva usually wins (Godfray 1994, p125). The optimal strategy is therefore to insert a cheap (male) egg if there are no other egg masses about. Other factors could include egg load or host density. Ullyett (1949) submitted host eggs to different densities of parasitoid females. The number of eggs laid per female increased faster than the number of hosts available to them. This amounts to more superparasitoidism with higher female density. The number of eggs laid per females peaked at around 4 females per unit area, then decreased. This is also the density at which general effective parasitoidism rate peaked. The individual effective parasitoidism rate was 60% then, while it was 90% with only one female. This highlights the advantage conferred by superparsitoidism at the species level. In the same study, the author also submitted different configurations of egg densities to *C. insularis* females. The search for suitable eggs was not systematic but random. The higher the number of host eggs per unit area, the more efficient the females were at avoiding superparasitoidism. At 20 host eggs per unit area, there were up to 8 parasitoid eggs per host egg, 2.47 on average, but at 200 eggs per unit area, there were maximum 2, and only 1.06 on average. This may seem surprising. One interpretation is that as the female encounters hosts more regularly, they seem less rare. Therefore, the risk posed by superparasitoidizing outweighs its return. Superparasitoidizing has fewer chances of returning a fitness gain versus searching for another host. In Glogoza (1981), more superparasitoidism was observed at 35°C, with up to six parasitoid eggs per host egg. Finally, Rajapakse et al. (1992) put *C. insularis*, *Cotesia marginiventris* and *Microplitis manilae* in competition for *S. frugiperda* hosts. The latter two are larval parasitoids. Though *C. marginiventris* attacked parasitoidized and non-parasitoidized larvae equally, it was only only successful on larvae parasitoidized by *C. insularis*. Additionally, it was only successful on parasitoidized larvae in a certain age range. *M. manilae* was not successful in larvae parasitoidized by *C. insularis*.

Pictures

Figure 3.5: *Chelonus insularis* female adult. Females have slightly shorter antennae than males.

3.1.5 Considerations for biological control

The fit between pest and agent is the product of a long list of factors. If the agent is to establish itself for one generation or more, success depends on the ability of a founder population to adapt and multiply in a novel environment. *C. insularis* showed high genetic diversity across 33 locations in Mexico, independently from host attacked plant (Jourdie et al. 2010). This shows that the parasitoid is effective in a variety of contexts. Related individuals generally came from hosts attacking the same plant. In this case, African and Asian climates would be favorable to the biology of *C. insularis*. Depending on the results of the specificity and preference tests, *C. insularis* could be reared in natural conditions in the target countries.

With parasitoids, there is a lag between attack and death (Heimpel & Mills 2017, p158). In this case, the lag is acceptable because the appetite of parasitoidized larvae is cut. This is not just the result of the parasitoid larva's own energy intake, or the host's shorter lifespan. The host is also manipulated to have reduced appetite, as it must have a certain weight for the parasitoid to successfully emerge. The question is agent and host synchronicity. Hosts are resources only for a short time (Heimpel & Mills 2017, p157). The relevant phenomenon is the preference of the parasitoid for eggs of a certain age range. There is some control over this, as the agent population is reared and can be released at a certain date. The problem is lessened if *S. frugiperda* adults can provide egg masses over time, but in the long run, external factors such as seasonal changes could desynchronize the two.

In the field, *C. insularis* will have to compete with other parasitoids of the Fall Armyworm (Rajapakse et al. 1991). Parasitoids interact through superparasitoidism. Essentially, the eggs are placed inside the hosts and the larvae compete each other. Following parasitoidization by *C. insularis*, exposure to *Cotesia marginiventris*, its main competitor in its native range, caused the parasitoidism rate to fall from 74% to 27% for *C. insularis*, as 46% of larvae produced the competitor. Exposure to *Microplitis manilae* made the rate fall to 64%, as only 14% of larvae produced the competitor. There were no significant differences in the number of healthy host larvae or dead larvae. The percentages of parasitoidized larvae for the competitors in the absence of *C. insularis* were 52% for *Cotesia marginiventris* and 45% for *Microplitis manilae*, though for the latter 28% of larvae died prematurely. Species may adapt their behavior when they find eggs parasitoidized by a competitor. *Microplitis manilae* reduced its number of ovipositions by about 50% when the eggs had been parasitoidized by *C. insularis*. This makes strategic sense as the percentage of emerging adults dropped to 38%, indicating a disadvantage in inter-larval competition. This was not the case for *Cotesia marginiventris*, where the rate rose from 53% to 57%. A striking result of these experiments is that in both cases, the number of healthy host larvae was drastically reduced, from 29% to 13% with *C. insularis* followed by *Microplitis manilae* and from 30% to just 5% with *C. insularis* followed by *Cotesia marginiventris*. No mentions of a double treatment were found in the literature, but the idea is nonetheless interesting. Of course, it is cheaper to evaluate, rear and release one insect with maximum efficiency. Finally, the interactions between host and parasitoid are subjected to seasonal cycles. As a result, for a given species, the actual parasitoidism rate will vary over time. Depending on the pest's state of development, different parasitoid species will use it to reproduce. There is a direct implication for performance (number of individuals removed from the environment) and an indirect one as well, in the sense that the agent might be in competition with other parasitoids at certain points in time, and not at others (Abang et al. 2021).

For different types of biological control, there will be different population objectives (Wajnberg et al. 2007, ch1). Inundative control aims for a strong initial impact thanks to a large population. In accretive and augmentative control, the establishment of the agent from one season to the next is more important. For inundative biological control, prerelease conditions matter. Host deprivation does not affect foraging and dispersal performance but egg expenditure does. Because of patchiness, some pests may persist in places while the parasitoid population goes extinct. It was thus long thought that biological control required an equilibrium. However, host extinction is also acceptable (Heimpel & Mills 2017, p162), especially for *S. frugiperda*. Heimpel et al. state that population dynamics in biological control are closer to the mainland-island model, where there are punctual encounters between host and parasitoid, rather than the open-refuge one, where hosts are kept safe simply by low parasitoid density in the patch. The authors conclude that dynamics should be studied at the metapopulation level, since there is no evidence that agent density in the patch is a key factor for biological control success (Heimpel & Mills 2017, p163).

The first step to evaluate whether *C. insularis* could make a good biological control agent against *S. frugiperda* is to estimate its host range and host stage killed. The host range is composed of host specificity, the tendency to avoid alternative hosts, and host preference, the qualities required of a host to induce oviposition.

3.1.6 Oviposition tests as part of experiment design

Choice and no-choice tests are central to parasitoid studies (van Driesche & Murray in van Driesche & Reardon 2004, p72). They both help appreciate the probability of successful control and the risk of damage to non-target species. They are a staple of host range estimation and biological control.

In the choice test, females have access to two egg masses of different species. The parasitoidism rates in each species are then compared. The choice test gives stronger positive results. It shows the propensity of the agent to pick the target instead of another, phylogenetically close, non-target species. At both individual and population levels, it indicates how much resources the parasitoid would put into the target species in the field, where alternative hosts are available.

In the no-choice test, females have access to an egg mass of only one species. The no-choice test gives stronger negative results. A low parasitoidism rate means that the parasitoid would not attack, even under constraint. The no-choice test shows the maximum physiological host range (van Driesche & Reardon 2004, p74). This may not be representative of behavior in the field (Heimpel & Mills 2017, p128), but is interesting from a risk evaluation perspective. Type II errors are more important to avoid in biological control, because an effect on non-target species is the greater risk to avoid. Therefore, no-choice test results, which are more conservative, are sometimes considered the better indicator of risk.

Given the complexity of parasitoid-pest interactions, binary results of the type 0% parasitoidism versus 100% parasitoidism are unlikely. Nevertheless, the parasitoid should generally exhibit high parasitism rates on *S. frugiperda* eggs and low parasitism rate on the alternative host species. Both tests must be considered, because a parasitoid may be forced to oviposit on species B during the no-choice test, yet it may not oviposit on species B if it has access to species A. Experience shows that host range as determined by a no-choice test will be broader than as determined by a choice test. Furthermore, true host distribution in the field is rarely known over the lifetime of the agent population. The field may be closer to a choice or no-choice scenario at different times and depending on dispersion of agents and targets. It could be akin to a no-choice scenario in the case of parasitoid expansion, or if the parasitoid population is desynchronized with its main host population. Conversely, it could be a choice scenario in the case of host expansion, when a new host arrives in environment (van Driesche & Reardon 2004, p73). In this case, the secondary species may not be sufficiently present to constitute a real possible host for the parasitoid, or it may be present but not findable.

Oviposition tests cannot modelize all possible behaviors in the field, even when combined. Host must first be located, and the decision to use an alternative host may depend on the main host being also present. These findings are summarized by van Driesche & Murray (in van Driesche & Reardon 2004, p75):

- "Host seeking was the most discriminating step in the chain of behaviors leading to host use. As a practical matter, these tests allowed some biological control agents to be introduced that might have been rejected based on no-choice tests alone…"
- "However, for several studies of parasitoids, (…) the number of species accepted for oviposition (…) increased rather than decreased under choice conditions."
- "For parasitoids, no published examples were found of attack on a nontarget species in no-choice tests where attack disappeared in choice tests containing the preferred host."

One possible explanation has to do with biochemical signals. Cues such as chemical signals may become mixed up or masked. Kairomones from the main host could be found around secondary hosts, eliciting an attack. In a laboratory setting, the effect could also be different. Signals could be more prominent or more mixed up. Chemical signals are important in biological control and in fact some methods use only chemical signals (Wajnberg & Colazza 2013). Examples include nectar subsidies (especially in conservation biological control, Wajnberg et al. 2007, p20-21), target plant compounds and pheromone confusion… The use of alternative hosts may also be subjected to strategy. A parasitoid may prefer holding back in the presence of the alternative host alone. If, however, main hosts are present but in insufficient quantity or quality, parasitoidizing the alternative may return greater fitness. This would call for further experiments. With regards to specificity, these observations give even more importance to statistical power: "negative data in choice oviposition tests would be a more robust indication of safety to nontarget species" (van Driesche & Reardon 2004, p75).

3.1.7 Statistical inference in parasitoid studies

The proper statistical treatment for parasitoidism rate has a long history. Count data does not follow a normal distribution but rather a Poisson distribution. This distribution gives the probability that we obtain k parasitoid adults in n eggs, defined as $(\lambda^k e^{-\lambda})/(k!)$ where k is an integer and λ is both the variance and the expected value of the response. In other words, λ is the true rate at which parasitoidism occurs. Unlike in a normal distribution, higher counts are found near zero. The value zero may even have a large number of counts. Therefore, effects and P-values cannot be calculated on the basis of a normal distribution. For a long time, the arcsine of the square root $sin^{-1}(\sqrt{p})$ was used to transform count data to a normal distribution, perform the analysis, and then transform the effects back. Alternatives included $\sqrt{p + 3/8}$, $\sqrt{p} + \sqrt{p+1}$ or $p^{2/3}$. Another method is to use non-parametric tests such as the Mann-Wilcoxon-Whitney test which considers the data ordinal (Barratt et al. 2007). However, transforming data can only go so far, as it leaves statistical inference tests at the mercy of the sample's properties. For instance, using transformation alone, the probability of a Type I error will increase with the sample mean (Wilson & Grenfell 1997).

A more flexible solution is to use a generalized linear model (GLM). Such a model can be summarized as $y_i = \beta_0 + \beta_1 x_i + \varepsilon_i$ where β_0 is the size of the effect independent from x, β_1 is the size of the effect dependent on x, and ε is an error with a specific distribution. Another way to describe such a model is as a stochastic part which follows the given distribution, a systematic part which depends on the input variable, and a function that links the inputs and outputs. The link function transforms the observed data by normalizing them and remapping them to a domain where linear regression is appropriate. What remains is to specify the expected distribution of the response variable. In the scope of studying effective parasitoidism, the outcome of exposure to the parasitoid can be reduced to binary levels. Either the host egg produces a parasitoid or not. The rate of parasitoidism, representing how many of the eggs produce parasitoids, follows a binomial distribution. This distribution gives the probability of obtaining k parasitoids in n eggs when the probability for one egg is p.

$$\frac{n!}{k!\,(n-k)!}p^k(1-p)^{n-k}$$

The fraction can be expressed as a binomial coefficient $\binom{n}{k}$, hence the name. All GLM implementations allow specifying the expected distribution of the stochastic part and this link function separately if necessary. In this case, the binomial distribution is used with the logit link. The logit link remaps the response from $[0;+\infty[$ to $]-\infty;+\infty[$ and remaps its mean to the mean in the expected distribution. Its name is a portmanteau of logistic unit, the unit of the values after transformation. The results are still interpretable. The probability of a value in this case is the natural log of the odds that the output variable equals one of the categories. In other words, that a given species is parasitoidized. In the model, the coefficients are given in $log(odds)$. They can be converted to odds or to probabilities. The latter is analogous to the rate of parasitoidism to expect. The formula is below, where β_0 and β_1 are the coefficients and x is 0 for the main host and 1 for the alternative.

$$probability = \frac{odds}{1 + odds}; odds = e^k; k = \sum\beta_0 + \beta_1 * x$$

The question can be reversed by asking how many eggs will be needed before a parasitoid is produced, the probability of which follows a negative binomial distribution. This is simply the binomial distribution again, but the variable and the response are switched such that the binomial coefficient can be rewritten with negative numbers as shown below (Casella & Berger 2002), this time with r the number of parasitoidized larvae, k the number of non-parasitoidized larvae and p the probability that a single egg is successfully parasitoidized.

The negative binomial distribution equals the Poisson distribution when r goes to infinity. The negative binomial distribution can be used in place of the Poisson distribution when the variance and the mean are very different, as is often the case in real-world data. For instance, mean and variance can be different when observations are not independent. Another interpretation is to consider that the true rate of parasitoidism λ, which governs the count of observed parasitoidized eggs, is a random parameter following a gamma distribution with parameters r for shape and $p(1-p)^{-1}$ for scale.

$$\binom{k+r-1}{r-1} = (-1)^k \binom{-r}{k} = \frac{(k+r-1)!}{(r-1)!\,k!}(1-p)^k p^r$$

Rezaei et al. (2019) give two indexes for evaluating preference. The first is simply defined as $c = (E_a * N_b)/(E_b * N_a)$, with E the number of parasitoidized hosts and N the number of available hosts and where a and b denote the species. This method is not ideal since the output range is assymetric. The result is bound to $[0;1[$ when species b is preferred and to $]1,+\infty[$ when species a is preferred. The Manly-Chesson preference index for species a is defined as $\left(ln(N_a/E_a)\right)/\left(ln(N_a/E_a) + ln(N_b/E_b)\right)$. The formula given by Rezaei et al. seems to have been misreported and omits the ln in the denominator. The result is contained in $]0;1]$ and is greater than ½ when species a is preferred. This index can be calculated for k species and a species is then preferred if its index is greater than $1/k$. However, both these indexes are undefined when 0 hosts are parasitoidized, which is a plausible outcome.

The more robust method is to simply use a GLM, specifying a binomial distribution, and to transform the results back from the response scale. The model can be assessed by estimating the dispersion of residuals. Overdispersion is the fact that variances are higher than expected, indicating a poor model fit. Overdispersion of residuals occurs naturally in clustered data. For binomial distributions, clustering means that probability is not constant. Instead, either outcome can be more likely depending on the trial. From a modelization standpoint, this is akin to having an unknown missing predictor. A quick estimate of dispersion is given by the total of residual deviances squared divided by the degrees of freedom. A value larger than 1 indicates overdispersion. A more precise method uses the raw residuals divided by the square root of the variance. This is appropriate for Poisson and binomial distributions, where observed data do not fit the theoretical relationship between mean and variance. A GLM can find a good fit for clustered data (overdispersed residuals) by adding predictors or by switching the distribution to a quasi-binomial or beta-binomial. A quasi-binomial distribution has an additional parameter to account for dispersion, calculated using Pearson residuals. Model performance can be assessed by estimating the likelihood ratio. Then, the parasitoidism rate to expect for each host can be calculated using estimated marginal means.

On the topic of hypotheses and P-values, transversal to almost all scientific fields, a few notes can be made (Greenland 2019). In statistical inference, a null hypothesis and an alternative hypothesis are opposed. The choice of words makes it seem as if the null hypothesis could only mean the absence of an effect. However, any hypothesis can be the tested hypothesis, the one to be rejected by statistical tests. This distinction is incidental in experiments where a treatment is applied. In this case, testing for an absence of effect is widespread, convenient for interpretation and the name of null hypothesis is then intuitive. Two probabilities are associated with testing. A Type I error is the wrongful rejection of the tested hypothesis and has a probability α. A Type II error is the failure to reject the tested hypothesis and has probability β. Both probabilities α and β are for a sample and a model. The true probability of an error is unknown, because the true population (of which we have a sample) and true process (of which we have a model) are unknown. Only if the distribution of the true population is known could the probability of the hypothesis being true or false be known, using bayesian methods. This is typically not the case in experiments. The P-value given in the analysis is an observation inherent to the sample and model chosen. It's a realization of P, a random variable at population level, and not P itself. The P-value is the percentile p to which the sample matches the chosen model under the tested hypothesis (Perezgonzalez 2015). A very small value indicates that the sample is incompatible with the data for the chosen model. At this point, the tested hypothesis can be rejected. Obtaining different P-values between samples is an expected property. They should be uniformly distributed, unless the hypothesis or the model have been incorrectly specified. They also vary according to sample size, which is again expected since a greater sample will bring more evidence in support or against a model. Additionally, the P-value is expressed in scaled units, such that the difference between p=0.01 and p=0.10 is larger than the one between p=0.90 and p=0.99 when expressed in standard deviations. The use of surprisal or S-value defined as $s = -\log_2(p)$, expressed in bits, has been proposed instead. This ties in with the concept of a distribution's tail, but interpretation may be more intuitive, because one bit is the information obtained after one coin toss. If a P-value associated with the effect of a variable is 0.001, then the S-value is 9.97 bit. In other words, the data brings almost 10 coin tosses worth of information against the tested hypothesis. If the tested hypothesis were true, obtaining a P-value of 0.001 would be as surprising as getting 10 heads in a row, which has a 0.1% probability. A smaller P-value corresponds to a greater S-value which corresponds to more evidence against the tested hypothesis. A P-value of 0.05 would not be very surprising at 4.32 bit, only slightly more than getting 4 heads in a row, and the difference between a P-value of 0.06 and 0.03 is only one bit (Rafi & Greenland 2020). With regard to linear models, the quantity of information represented by the S-value is the part of the data that doesn't fit the model under the tested hypothesis and leads to its rejection. S-values are additive. However, the use of S-values is uncommon in parasitoid studies.

3.2 Materials and methods

3.2.1 Objectives and general design

The present study comprises five experiments. Each experiment was assigned a single letter. Specificity was evaluated by a no-choice test (letter X) and a choice test (Y). Preference was evaluated by observing the influence of egg age on parasitoidism rate (A). Finally, host stage killed was determined using measurements of the parasitoidized larvae body size (S) and head capsule size at death (C).

The species selected as alternative host was *Spodoptera exigua*, the Beet Armyworm. This species is phylogenetically close (Heimpel & Mills 2017, p119-121) and available in

Switzerland. Two additional species, *Spodoptera latifascia* (Velvet Armyworm) and *Spodoptera littoralis* (Cotton Leafworm), were initially included but died during the experiments, presumably from a viral pathogen.

3.2.2 Common rearing protocol

The experiments took place at CABI Switzerland, in Delémont, Jura. Although presumed unable to survive temperate climates, the studied *Spodoptera* species are still invasive. *C. insularis* is also exogenic. The species are considered alien and all the experiments had to take place in quarantine. The conditions in the laboratory were of 26°C (±1.5) and 65% humidity (±3). The photoperiod was 14:10. After each use, all containers and tools, including, vials, tweezers, scalpels, diet trays and working surfaces were cleaned with soap and brush followed by an alcohol rub with cellulose paper. Insects were disposed of by freezing at -80°C for 72 hours. Personal protective suits were also frozen between uses and remained in quarantine.

C. insularis adults were reared in mesh cages measuring 50cm x 50cm x 50cm, small enough that the females could find the eggs easily. They were fed honey and tap water, a commonly used diet. Like natural foods, it is rich in sugar but poor in nitrogen and lipids (Wajnberg et al. 2007, p134). Dead individuals and vacated cocoons were removed from cages every few days. Depending on the species, a high number of females can introduce potential fighting and superparasitoidism as well as influence sex-ratio. On the other hand, with a lower number of females, the rate of parasitoidism is limited by global egg load. To keep this variable saturated, there were always at least three parasitoid females in a cage, and the number of host eggs per egg mass had to not be too large. *Spodoptera* eggs came from another room in the quarantine facility. *Spodoptera* rearing is outside of the scope of these experiments (Figure 3.6). Eggs were produced every night, laid on paper towels by adults. They were collected and separated in distinct masses every morning by cutting the paper with scissors. Unmated *S. frugiperda* females produce small masses with a few eggs only, all unviable. To avoid this problem, only masses of more than 10 eggs were used. The number of eggs in small and medium masses was counted, but not in big ones. There were always enough males to inseminate all the females.

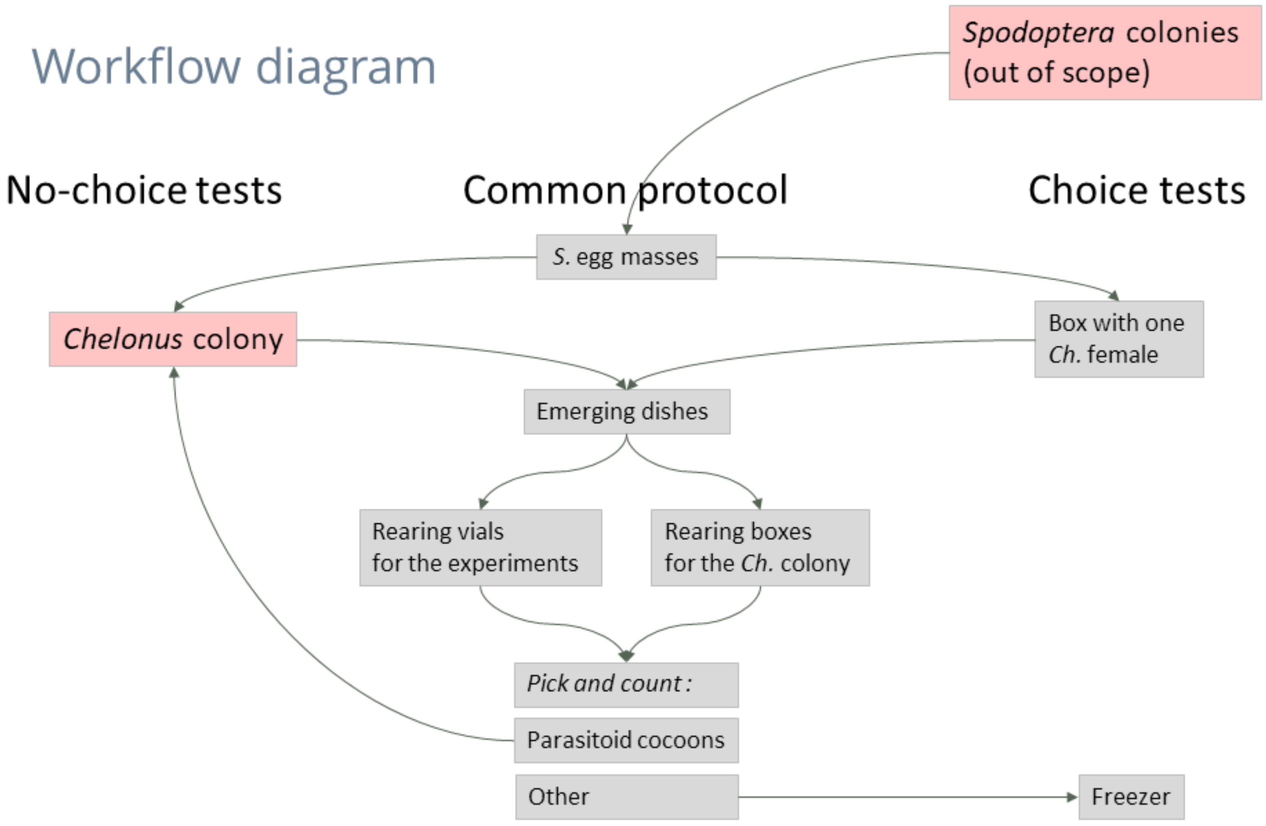


Figure 3.6: Workflow diagram of the experiments. In no-choice tests, fresh egg masses are submitted to the parasitoid colony. In choice tests, a few parasitoids are placed in a dedicated box with an egg mass of each species.

For no-choice tests, the egg masses were deposited in a big petri dish which was put in one of the cages for one night. For choice tests, the egg masses were deposited in two small petri dishes in a container with 6 parasitoid individuals (1 female and 5 males), and left for one night. Females had several hours to eat and mate between runs.

Because *S. frugiperda* and *S. exigua* exhibit cannibalism and can also eat parasitoid cocoons (personal observations), individuals had to be reared separately. Otherwise, parasitoidized individuals, which are smaller and weaker, would get eaten. Individual vials prevent cannibalism and permit individual tracking. They were furnished with cellulose paper and a cube of diet, and closed with a foam cap. The larvae were fed a modified McMorran diet. The McMorran diet has been successfully used on 101 out of 112 lepidopteran species (Hervet et al. 2016), including *S. frugiperda* and *S. exigua*. The diet was ordered from the Great Lakes Forestry Centre (GLFC) in Canada as dry ingredients and prepared at CABI. The preparation consisted in mixing the dry ingredients together, except the agar which was heated to 85°C in water separately. Everything was then blended together. After the mix had cooled, a solution containing vitamin C was added. The mix was poured into containers where it solidified into a jelly. Its surface was sprayed with an anti-fungal solution. Using tweezers, the diet and paper was changed every 5 days. A run generated 10 to 60 vials. In cases where more than 60 larvae had hatched, their total number was noted and 60 were picked at random. Larvae that were not sampled to vials were reared in boxes for *C. insularis* production. Big boxes with sufficient furnishings ensured that the encounter rate between larvae was low. Large hosts are sure to not be parasitoidized and only consume resources. They were eliminated regularly. The diet was sandwiched between two plastic tabs to prevent it from drying and prevent moisture leaking into the paper, which fosters fungi. Vacated cocoons (bright white, small hole), dead host (small, hard, brown) and parasitoid (green-grey) pupae were regularly removed, as they also harbor fungi.

Table 3.1: Composition of the modified McMorran diet fed to the *Spodoptera* larvae.

Ingredient	Quantity	Unit
Water	620	mL
Agar	17.36	g
Water	220	mL
Vitamin-free casein	35	g
4 M KOH	5	mL
Alphacel	5	g
Wesson’s salt mix	10	g
Sugar	35	g
Toasted wheat germ	30.69	g
Choline chloride	1	g
Ascorbic acid	4	g
Formalin	0.5	mL
Methyl paraben	1.5	g
Aureomycin	2.1	g
Raw linseed oil	5	mL
Vitamin solution	10	mL
Anti-fungal spray		

C. insularis cocoons retrieved from the experiment vials (Figure 3.7) and the rearing boxes were placed in smaller aerated boxes modified specifically for this purpose. Using tweezers, they were removed from the cellulose paper and deposited in a hatching box with water and honey. This was done daily. Parasitoid larvae which failed to form a cocoon were wrapped in paper and left to mature. In some rare cases, the adult had already hatched in the vial or box at collection time. After all individuals had hatched, they were aspirated in batches of four to seven, sexed, and put in one of the cages depending on the host they hatched from.

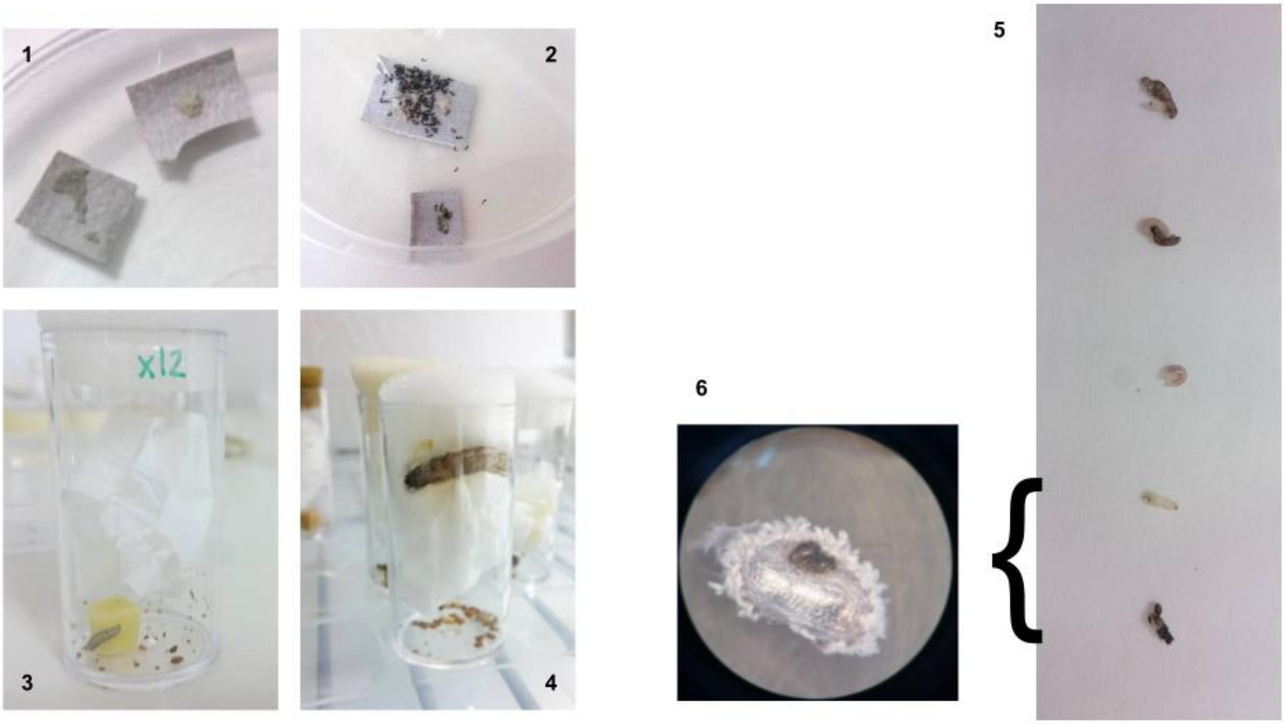


Figure 3.7: Hosts and parasitoids at different stages of rearing. (1) *Spodoptera exigua* (left) and *Spodoptera frugiperda* (right) egg masses right after exposure to the parasitoid. (2) *S. exigua* larvae just after hatching. (3) A parasitoidized larva. (4) A non-parasitoidized larva of the same age. (5) Development stages of the parasitoid larva and pupa. From top to bottom: after having eaten all its insides, the parasitoid larva breaches the host's skin; the host's remains are exited by wiggling; the larva starts building a cocoon out of silk; the pupa forms, Hymenopteran pupae are inactive; the pupa progressively becomes darker. (6) The pupal stage (last two steps) happens inside the cocoon, which is white and hard. When ready, the adult will breach it with its mandibles. The host's head capsule is all that remains after the skin has dried out, and can be seen attached to the cocoon. The white fringes around the cocoon are bits of the cellulose paper in which it was weaved.

3.2.3 Experiment details

No-choice test (X): An egg mass of each *Spodoptera* species was submitted to the *C. insularis* colony. One egg mass was placed in each cage containing at least three females. The egg masses remained for 24 hours (±30mn). The eggs were left to hatch. The larvae were reared following the common protocol, until they reached a final state. The number of individuals in each state was then counted. The test was replicated as many times as possible. This amounted to 45 runs, though not all were successful.

Choice test (Y): *C. insularis* females were presented with two egg masses of different species. The individuals were reared in the same way. In total, 14 runs were completed.

Influence of egg age (A): Egg masses of *S. frugiperda* collected either 2 days before, 1 day before or the same day were presented to *C. insularis* females in the no-choice test, so that the parasitoidism rates could be compared. Out of all no-choice test runs, 7 had 1-day old eggs and 3 had 2-day old eggs.

Host stage killed (S, C): Two experiments were grouped in order to find at which point the parasitoid kills its host. On the one side, 20 parasitoidized *S. frugiperda* larvae were measured every day with a precision of 0.5mm until they formed a cocoon (S). On the other side, 40 head capsules, left after the parasitoid larva has consumed the host larva's remains, were collected and measured (C) with a precision of 0.1mm.

3.2.4 Statistical analysis

The experiments were conducted with natural and practical constraints which have implications for analysis. We must note that the measures are not truly independent, since the same individuals are tested multiple times. This is not true replication, but rather pseudoreplication (Wajnberg et al. 2007, p423-424). In this context, increasing the number of runs increases the probability of committing a Type I error and finding an effect where only stochasticity is at work. This is common in insect studies. To compensate, individuals are usually "reset" between runs. This was the case here, as parasitoids were left to feed, mate and were exposed to the main host in-between each exposition to the secondary host. In order to test the effect of host species, statistical testing was conducted with these hypotheses:

- H1: one of the hosts is favored
- H0: no host is favored
- Type I error: we reject H0 but no host is actually favored
- Type II error: we don't reject H0 even though a host is favored

A Type II error has more consequences in biological control, as it implies damage to the ecosystem. Increasing power (1-β) corresponds to decreasing risk. The analysis of parasitoidism rate was based on counts of parasitoidized (par) and non-parasitoidized individuals (npar). At the end of one run, all larvae were in either one of the possible states. All parasitoid results (P_) were counted as parasitoidized. All host results (H_) were counted as non-parasitoidized. Missing individuals (DIS) were removed from the experiment. The parasitoidism rate is $par/(par + npar)$ with par = H_IMA + H_PUP + H_BIG + H_SML + H_DED and $npar$ = P_IMA + P_COC + P_PUP + P_LAR + P_DED. This suits both choice and no-choice tests. The data can be downloaded at the link given in the annex.

Table 3.2: Exhaustive list of the possible results of rearing. Individuals can be in either one of these states at the end of a run.

Code	Description
H_IMA	Adult host (imago)
H_PUP	Healthy host pupa
H_BIG	Big host larva (healthy or pseudoparasitoidized)
H_SML	Small host larva (parasitoidized or pseudoparasitoidized)
H_DED	Dead host (any stage) (could be because of parasitoidism or something else)

Code	Description
P_IMA	Adult parasitoid
P_COC	Parasitoid cocoon
P_PUP	Parasitoid pupa (did not manage to create a cocoon)
P_LAR	Parasitoid larva (did not manage to create a cocoon)
P_DED	Dead parasitoid (any stage)
DIS	Individual has disappeared!

Table 3.3: In addition to the counts presented in Table 3.2, these variables were collected for choice and no-choice tests.

Name	Description
`id`	Unique identifier for the run, starting with X or Y
`run`	Run number, starting at 1
`host`	Species of the egg mass presented to the parasitoid
`cage`	Cage identifier, starting at c1
`host_of_origin`	Hosts the parasitoid in this cage were reared on
`egg_collected`	Date the egg mass was collected
`expo_begin`	Date the egg mass was placed in the cage
`egg_age`	Number of nights before the egg mass was placed in the cage
`expo_end`	Date the egg mass was removed from the cage
`boxed`	Date the larvae were transferred to individual vials
`failed`	Boolean equal to 1 if no eggs hatched
`ended`	Date the individuals were removed from their vials and counted
`checksum`	Checksum to detect whether all the individuals had been counted
`n_box`	Number of individuals placed in vials
`n_hatched`	Number of individuals hatched
`sampled`	Percentage of individuals placed in vials
`sum_p`	Number of parasitoidized individuals
`sum_np`	Number of non-parasitoidized individuals
`n_end`	Number of individuals counted
`proportion`	Proportion of parasitoidized individuals
`quality`	Ternary value depending on the proportion being positive, null or undefined

To evaluate the specificity of *C. insularis* towards *S. frugiperda* versus the alternative host *S. exigua*, a GLM model was fitted, assuming the binomial distribution with the logit link. This was applied to both oviposition tests. The models were checked for overdispersion and either a quasi-binomial distribution was used or an observation-level random effect was added if appropriate. For runs with *S. frugiperda*, the same method was used with egg age as a predictor. Finally, to evaluate the host stage killed, the size of parasitoidized larvae at death and the size of their head capsules were statistically described and compared with *S. frugiperda*'s biology.

3.3 Results

3.3.1 No-choice test (X)

The no-choice tests yielded the proportion of parasitoidized larvae in 14 runs with *S. exigua* and 23 runs with *S. frugiperda*. The proportion was zero for 1 run with *S. exigua* and 7 runs with *S. frugiperda*. This is likely to indicate a problem with the parasitoid during these runs, such as an absence of fertile females. These runs were excluded from the analysis, as they resulted from limitations inherent to laboratory work and therefore did not represent *C. insularis*'s capability in the field. The parasitoidism rates are shown in Figure 3.8. One outlier is present in runs with *S. exigua*, and two are present in runs with *S. frugiperda*. Excluding these, the parasitoidism rates were between 44.9% and 96.3% for *S. exigua* and between 45.8% and 100.0% for *S. frugiperda*. The mean rate was only slightly higher for the main host with 79.6% against 75.6% for the alternative host. The median was slightly higher for the alternative host.

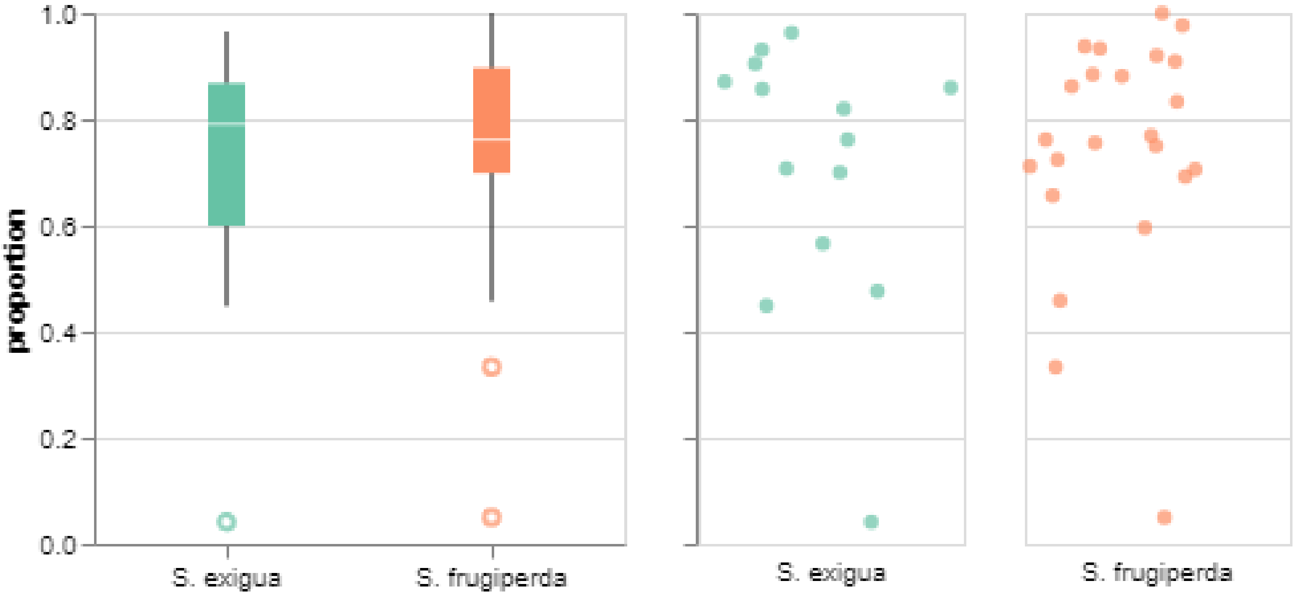


Figure 3.8: Proportion of parasitoidized larvae for each host species in no-choice tests. The result of each individual run is shown on the right. Points located more than 1.5 times the interquantile range were considered outliers.

A first model was fitted using the counts of parasitoidized (`sum_p`) and non-parasitoidized (`sum_np`) larvae as the response and the host species (`host`) as the predictor. The outliers were kept. A positive effect was found when *S. frugiperda* was the host. However, residual dispersion for this model was estimated at 9.54 >> 1, a case of important overdispersion. Another model was fitted using a quasi-binomial distribution. With this new model, the effect found for host species had a P-value of 0.484, meaning the data does not support an explanatory role for host species in a no-choice scenario. A chi-square test on the likelihood ratio yielded a P-value of 0.481, showing that the model could not reliably predict parasitoidism. The rate of parasitoidism to expect for each species was obtained with estimated marginal means, which yielded 69.8% (±6.3) for *S. exigua* and 75.1 (±4.2) for *S. frugiperda*. Given the standard errors, the two are indistinguishable.

The host of origin was recorded as a potential blocking factor. However, parasitoids reared on *S. latifascia* and *S. littoralis*, were only present in the first few runs, as these species quickly became unavailable. A model using `host_of_origin` as a predictor found no discernable effect. The number of females as well as the proportion of males in the cage at the beginning of exposure were retrieved from sexing data, but their relationship with the rate of parasitoidism was random.

3.3.2 Choice test (Y)

The choice tests yielded the proportion of larvae of each species parasitoidized in 12 runs. *S. frugiperda* was preferred to *S. exigua* in 10 out of 12 runs (Figure 3.9). At one point, *S. exigua* eggs became unavailable and the opportunity was taken to submit two *S. frugiperda* egg masses. This is technically a control for the side of the box chosen by the parasitoid, which is not expected to have an effect. However, it does give an idea of heterogeneity of the parasitoidism rate in one patch. In other words, what difference can be expected barring the effect of host species. When the parasitoid had no real choice, the rate differed by 7 to 13 percentage points between the two egg masses, and was always between 80% and 97%. In 2 runs, no parasitoids emerged from *S. exigua* eggs. In 1 run, parasitoids emerged from 100% of the *S. exigua* eggs, while 86% of *S. frugiperda* larvae also produced parasitoids. Excluding outliers, the parasitoidism rate was always at least 39% for the alternative host and 60% or more for the main host (Figure 3.10).

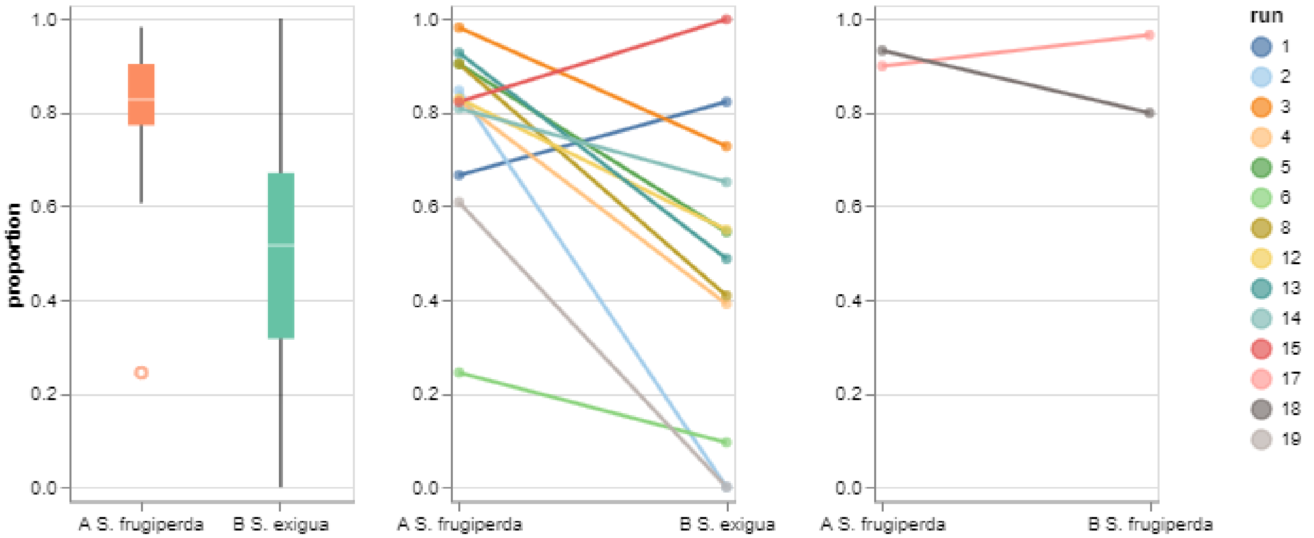


Figure 3.9: Left: Rates of parasitism were higher in *Spodoptera frugiperda*. Center: Rates of parasitoidism in each species in the choice test. The slope indicates the strength of the preference. The rate was higher in *S. frugiperda* than in *Spodoptera exigua* in 10 out of 12 runs. Right: Rates of parasitoidism in *S. frugiperda* egg masses submitted together.

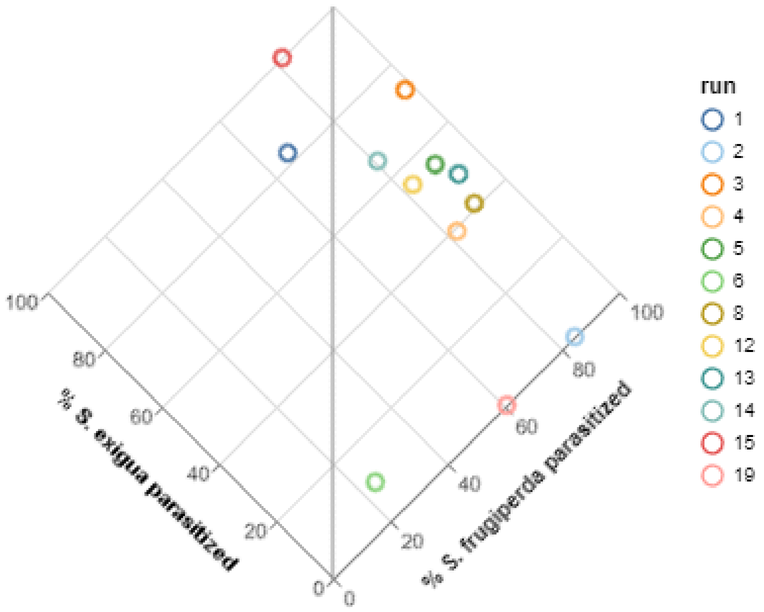


Figure 3.10: Proportion of larvae parasitoidized in each species in choice tests. Parasitoidism rates are plotted against one another. The center line represents perfect equal preference. In three runs (2, 6, 19), the proportion of parasitoidized *Spodoptera exigua* was 10% or less. In all the other runs, it was at least 39%. The proportion of parasitoidized *Spodoptera frugiperda* was always 60% or more, except in one run (5) where it was 25%.

A model was fitted using the host species as a predictor and a run-level random effect. Since there was one female in each run, this accounts for variation among female individuals. The residuals were overdispersed, and an observation-level random effect was added. A strong positive effect (β > 1.8) was found when the host was *S. frugiperda*. The likelihood ratio was 13.137 with a P-value of 0.0003, indicating that host species is a good predictor of parasitoidism in a choice scenario. The rates of parasitoidism to expect were 45.2% (±11.0) for the alternative host and 83.5% (±6.1) for the main host. Despite large standard errors, the rate to expect is 1.85 times higher with *S. frugiperda* in a choice scenario.

3.3.3 Egg age (A)

The main host had egg masses of different ages presented to the parasitoid. Intuitively, egg age seemed to have a negative effect on oviposition rate (Figure 3.11). However, a model fitted with egg age and an observation-level random effect found no effect. Chi-square analysis confirmed that egg age was not a good predictor of parasitoidism rate. Marginal means showed that the rates were comparable, with large standard errors.

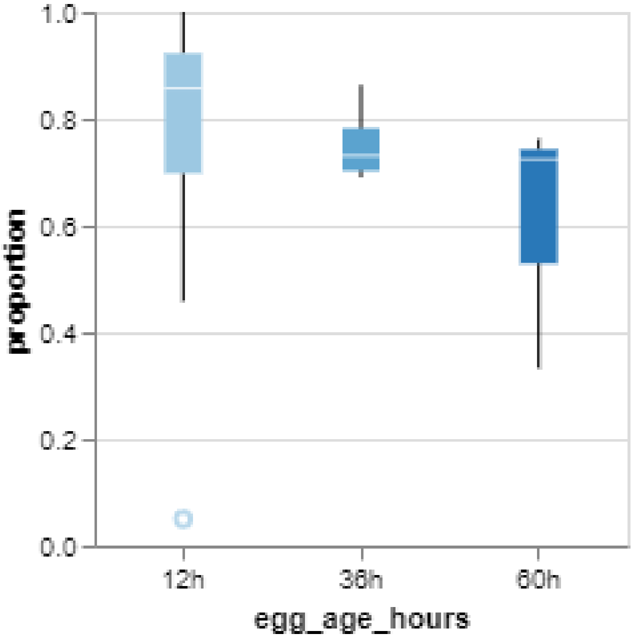


Figure 3.11: Proportion of parasitoidized larvae depending on the age of the egg mass.

3.3.4 Host stage killed (S, C)

S. frugiperda larvae attained an average size of 14.2mm (σ = 0.97mm) at time-of-death (Figure 3.12). The smallest larva was 12mm, and the biggest was 16mm.

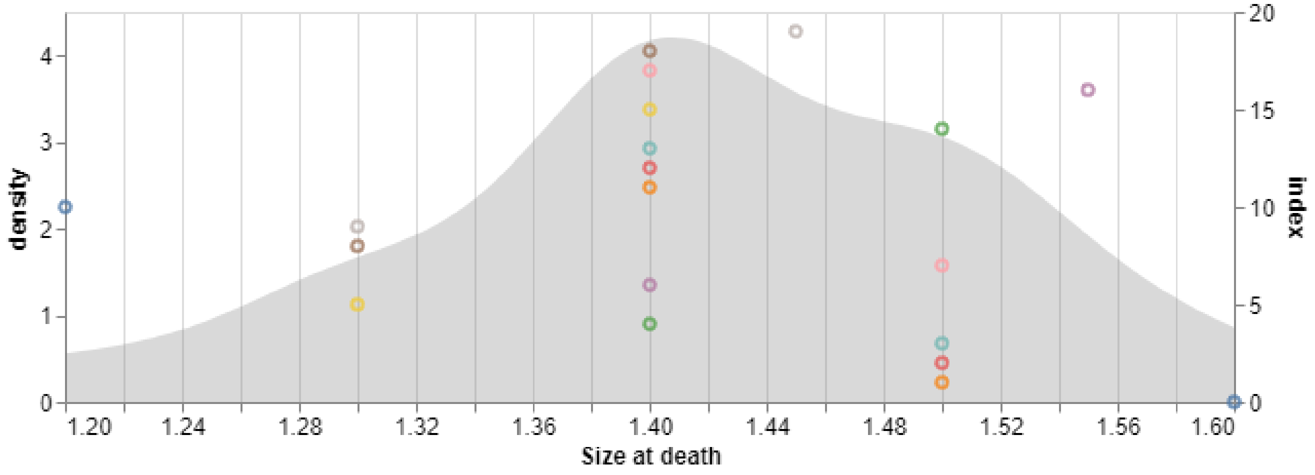


Figure 3.12: Size of parasitoidized *Spodoptera frugiperda* larvae at the time of their death. No larvae attained a size greater than 16mm. A density estimate is provided in grey.

Head capsule sizes at time-of-death were normally distributed. *S. frugiperda* larvae are killed when their head cap is around an average 1.39mm (σ = 0.10mm), versus 1.02mm for *S. exigua* (σ = 0.06mm). The sizes varied from 1.1mm to 1.6mm for *S. frugiperda* and from 0.9mm to 1.1mm for *S. exigua*.

Table 3.4: Head capsule sizes for larvae of different species of *Spodoptera* parasitoidized by *C. insularis*. The maximum head capsule size for *S. frugiperda* was 1.6mm.

species	count	mean	std	min	max
S. exigua	54	1.030	0.060	0.9	1.1
S. frugiperda	40	1.390	0.101	1.1	1.6
S. latifascia	15	1.453	0.083	1.4	1.6

3.4 Discussion

3.4.1 No-choice test (X)

Parasitoidism rates between the two hosts were very similar, and indeed no effect could be attributed to host species. The rates were clustered, as evidenced by high residuals dispersion when using a simple binomial distribution. In other words, both hosts were “preferred” in the no-choice scenario. No-choice tests give strong negative result, and in this case the test failed. *C. insularis* will attack the alternative host.

In addition to 8 runs where no parasitoidism occurred and which were excluded from analysis, 3 runs produced outlier results. In particular, the rate was 5% or less in 2 runs. No recorded variable could explain these punctual fluctuations, though host quality makes a compelling suspect. Earl (1983) found that the rate of parasitoidism from *C. insularis* could vary between 0 and 90% on infertile *S. exigua* eggs.

3.4.2 Choice test (Y)

Parasitoidism seemed higher in *S. frugiperda* in the choice test. Statistical analysis showed a strong effect backed by low uncertainty. The rate was 1.85 times higher for the main host than for the alternative. This strong positive result shows that *C. insularis* will allocate the majority of its resources to *S. frugiperda* even if an alternative host is present. The no-choice test showed that the parasitoid is capable of high parasitoidism rates on this alternative host, and so the realized rate must be close to the rate intended by the laying female. In the present experiment, 65% of the resources were dedicated to the main host. However, the reason for allocating the remaining resources the alternative host is unknown. Some of the main host eggs may have been of low quality, leading the female to prefer alternative host eggs. Or all the main host eggs may have been parasitoidized, leaving the female with an egg excedent to spend on the alternative host. In this case, the rate for the main host would not necessarily be 100%, as encapsulation and other failures are still possible. It would be pertinent to run the same test again with much larger egg masses in order to exhaust parasitoid egg load. Then, possibly, only a few males would emerge from the alternative host.

3.4.3 Egg age (A)

No effect could be attributed to egg age. Statistical analysis was hindered by an insufficient number of runs. The experiment would have to be repeated. The egg masses were collected only daily. Further oviposition tests with egg age varying in steps of 12 or even 6 hours could lead to a more precise analysis. However, this would require many replicates and a way to tell why parasitoidism failed. For instance, dissection would permit differentiating between egg rejection by the laying female and encapsulation by the host. Host quality is not a well understood topic for egg parasitoids. There might be other important variables besides age.

3.4.4 Host stage killed (S, C)

The measurements of both head capsule and larva sizes at death indicate that *S. frugiperda* is killed at the end of the 4th instar. The same results were found by Medina et al. (1988). This is encouraging since the 5th and 6th instars cause most of the damage to crops. It's also possible that parasitoidism affects this scale, such that a parasitoidized larva at the 5th instar has the same size as a healthy larva at the 4th instar. In this case, the larvae's appetite would still be reduced. In practice, the reduction in pest population size has a greater effect than the reduction in individual appetites.

Table 3.3: Larva and head capsule sizes in mm at successive instars of *S. frugiperda*'s larval phase (Capinera 1999).

Instar	Larva size	Head capsule size
1	1.7	0.35
2	3.5	0.45
3	6.4	0.75
4	10.0	1.3
5	17.2	2.0
6	34.2	2.6

3.4.5 Experiment analysis

Choice and no-choice test results are dependent on several factors inherent to insect agents that are often left out (Withers & Mansfield 2005). In the present case, this includes female experience, host deprivation and egg load. Host deprivation has been shown to lead to higher host acceptance. Egg load is related to host deprivation for synovigenic species since the female increases its egg load while not ovipositing, which can potentially reach a maximum. For pro-ovigenic species, egg load remains constant between ovipositions. Spatial factors, notably those related to host locating such as aggregation (Jervis & Kidd 1996, p51) and encounter rate, were not controlled for. However, since oviposition tests were done in small containers with many parasitoids, they should not play a direct role. *C. insularis* females were attracted to eggs right away, showing that variables related to host locating were indeed saturated.

The results of the choice and no-choice tests are in accordance with the general consensus that the host range is broader in no-choice tests (Withers & Mansfield 2005). The average parasitoidism rate for the alternative host was 67%, higher than that found by Earl (1983) which was 51%. It is possible that the alternative host was accepted for oviposition but that the parasitoids did not develop, because of encapsulation or other reasons. The only ways to detect encapsulation are to dissect the host larva or use a PCR test. Another method is to apply a pathogen to the ovipositor (McNeill et al. 2000). This will reveal which larvae have been probed, but requires state-of-the-art equipment and procedures as the pathogen must be strictly contained.

The main concern when designing experiments is balancing their robustness with the quantity of work involved. There were no controls for oviposition tests, as this would have doubled the quantity of work. Replication can quickly lead to infeasible protocols. In the present case, the number of host egg masses that could be exposed to the parasitoid each day was limited by the number of vials that could be processed (checked, cleaned, resupplied and refurbished) by one person in a day. All the work that went into rearing was put at risk by several random factors. For instance, on a given day, the host egg masses could be non-viable. This is because unmated female *Spodoptera* still lay (unfertilized) eggs. A verification step could perhaps be implemented to avoid unnecessary work, as could other quality control mechanisms. Situations where no meaningful data can be produced can go on for several days. This constitutes an important threat because the parasitoid colony relies on successful parasitoidism. This also implies host deprivation. Perhaps some emergency protocols could be devised in advance (what-if? scenarios). Finally, the random sampling of individual larvae to be reared in vials from the hatching box is not really random if it is done by hand. If cannibalism could be eliminated, all larvae could be kept together, and the random selection step eliminated.

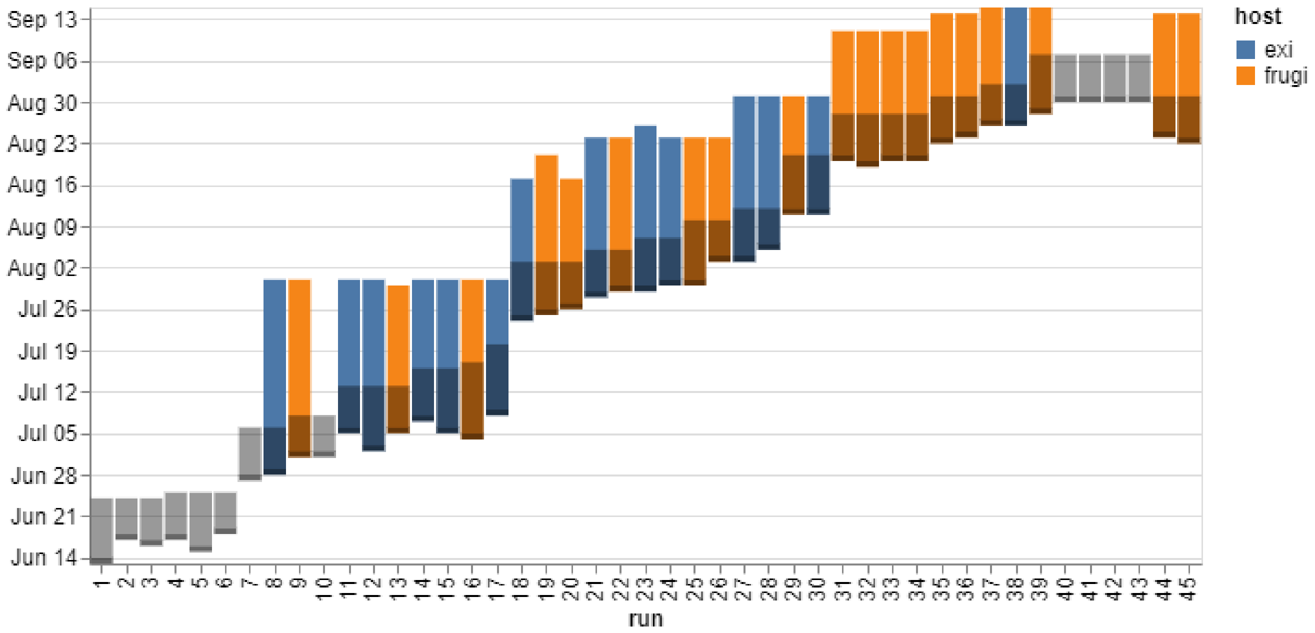


Figure 3.13: Duration of each run of the no-choice test over the course of the experiments. The darkest part of each bar represents the exposure time, the less dark part represents the duration where the larvae are left to hatch, and the bright part is the time spent in individual vials. Runs for which the ending date was not recorded appear in grey.

3.4.6 Rearing

The techniques proposed by Silva & Parra (2013) to reduce cannibalism worked well. A compartmentalized box, well ventilated and furbished with many layers of brown paper, reduced cannibalism to a negligible rate. The cause is probably lower perceived host density. A helping factor is that parasitized larvae bury themselves deep in the paper soon enough, thereby escaping hungry non-parasitized larva. Many parasitoid cocoons were retrieved from the rearing boxes. Frequently, the cocoon was formed in the foam cap, sometimes without even leaving a hole, or in the diet from where it was difficult to extract without causing damage. Survival rates for these individuals varied.

The time separating the end of the oviposition window and the point where all individuals had formed cocoons was between 17 and 29 days. The average was 22 days (σ = 3 days). Although development was not recorded with the same precision as Medina et al. (1988), this shows that some variability can occur. The higher temperature in the laboratory would certainly be a contributing factor. Temperature during development has a positive influence on parasitoid larva size and growth rate as well as adult size, but no influence on fitness (Colinet et al. 2007).

Conclusion: usefulness for biological control

The experiments yielded rich results. In choice tests, *C. insularis* showed strong preference for *Spodoptera frugiperda*, its main host, including with egg masses up to 60 hours old. The pest was killed at the late 4th or early 5th instar. In a no-choice scenario, the parasitoid attacked the alternative host and successfully reproduced. It is therefore not very specific. Further host range tests should be conducted with species native to the invaded geographies, especially in Sub-Saharan Africa where biological control could have the biggest economic impact. If *C. insularis* does not establish itself and the risk to non-pest species is low, periodic releases are still possible. The question of competition between parasitoids is also interesting. Local Fall Armyworm parasitoids are ineffective in controlling population size, but they may still rely on this host for a portion of their reproductive effort. In this case, the introduction of a very capable parasitoid could reduce their effectiveness against other pests (Agboyi 2020).

Should *C. insularis* prove incompatible with a release in these agro-ecosystems, other agents may be found in the Fall Armyworm's large parasitoid complex. Additional success factors will be found in the strategy and implementation (Meagher 2016). Some parasitoids have shown greater dispersal upon release after having been deprived of hosts for several days (Wajnberg et al. 2007, p10). The release agent populations could be supported with flowering plants or other subsidies. Pesticides are detrimental to the rate of parasitism (Meagher 2016) and are generally considered incompatible with biocontrol, since the agent relies on the pest to reproduce. Zenner et al. (2006) found that parasitoidized hosts were more susceptible to chemical insecticides and biopesticides. With appropriate target and temporal separation, certain strategies that combine biocontrol with other targeted pesticides may be possible, if the parasitoid larva can be kept safe inside the host (Meagher 2016).

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Annex: Statistical analysis commented

The output of the statistical analysis is available online at https://pastebin.com/raw/qsmKqLqw

```
library(tidyverse)
library(openxlsx)

# No-choice test (X-NC)

# Load data and exclude null and missing results
counts_x = as_tibble(read.xlsx('Data.xlsx', sheet=5)) %>%
  filter(quality == "is positive")
# > We have 37 good observations

# Fit a GLM with a binomial distribution and the host species as predictor
model_x = glm(cbind(sum_p, sum_np) ~ host, "binomial", counts_x)
summary(model_x)
# > An effect is found when the host is *Spodoptera frugiperda*

# Estimate overdispersion
```

```
print_f = function(x, y) {
  # Print aligned
  cat(str_pad(x, 14), y, fill=TRUE)
}
dispersion = function(x) {
  # Calculate dispersion statistics
  deviance = sum(residuals(x, type="deviance")^2)
  pearson = sum(residuals(x, type="pearson")^2)
  dof = df.residual(x)
  p_value = pchisq(pearson, df=dof, lower.tail=FALSE)
  print_f(           "dof", round(dof)            )
  print_f(      "deviance", round(deviance, 3)    )
  print_f("deviance_ratio", round(deviance/dof, 3))
  print_f(       "pearson", round(pearson, 3)     )
  print_f( "pearson_ratio", round(pearson/dof, 3) )
  print_f(          "pval", p_value               )
}
dispersion(model_x)
# > The ratios are close to 10:1 and the P-value is very small, we have overdispersion

# Overdispersion can also be tested using the package DHARMa, which outputs plots
library(DHARMa)
sim = simulateResiduals(model_x, refit=TRUE)
testDispersion(sim)
plot(sim)

# Compensate for overdispersion by using a quasibinomial distribution
model_x_quasi = glm(cbind(sum_p, sum_np) ~ host, "quasibinomial", counts_x)
summary(model_x_quasi)
# > The effect is accompanied by an extremely large P-value

# Another method is to add an observation-level random effect
model_x_olre = glmer(cbind(sum_p, sum_np) ~ host + (1|id), family="binomial", data=counts_
x)
summary(model_x_olre)
# > Same conclusion

# Another method is to use the beta-binomial distribution
library(aod)
model_x_beta = betabin(cbind(sum_p, sum_np) ~ host, ~ 1, data=counts_x)
summary(model_x_beta)
# > Same conclusion

# Another implementation of the same approach
library(dispmod)
model_x_dispersed = glm.binomial.disp(model_x)
summary(model_x_dispersed)
# > Same conclusion

# Calculate the likelihood ratio to determine model relevance
```

```
library(car)
Anova(model_x_quasi)
# > The data does not support host species as a predictor of parasitoidism rate

# Use a pairwise comparison
library(emmeans)
emmeans(model_x_quasi, "host", type="response", adjust="sidak")
# > The parasitoidism rate to expect is sensibly the same for both species

# Choice test (Y)

# Load data and delete dummy runs
counts_y = as_tibble(read.xlsx('Data.xlsx', sheet=3)) %>%
  filter(!id %in% c('y17', 'y18'))
# > We have 24 observations

# Fit a binomial model with a run-level random effect
library(lme4)
model_y = glmer(cbind(sum_p, sum_np) ~ host + (1|id), family="binomial", data=counts_y)
dispersion(model_y)
# > There is overdispersion

# Add an observation-level random effect
model_y_olre = glmer(cbind(sum_p, sum_np) ~ host + (1|id) + (1|observation), family="binom
ial", data=counts_y)
dispersion(model_y_olre)
# > Overdispersion has been corrected
summary(model_y_olre)
# > A strong positive effect is found when the host is *Spodoptera frugiperda*

# Calculate the likelihood ratio
Anova(model_y_olre)
# > The analysis is conclusive, host species is a good predictor of parasitoidism rate

# Use pairwise comparisons
emmeans(model_y_olre, "host", type="response", adjust="sidak")
# > The parasitoidism rate to expect is twice as high for *Spodoptera frugiperda*

# Egg age (A)

# Consider only *Spodoptera frugiperda* hosts
counts_a = counts_x %>% filter(host == "frugi")
# > We have 23 observations

# Fit a binomial model with egg age as a predictor
model_a = glm(cbind(sum_p, sum_np) ~ factor(egg_age), "binomial", counts_a)
dispersion(model_a)
# > There is overdispersion

# Add an observation-level random effect
```

```
model_a_olre = glmer(cbind(sum_p, sum_np) ~ factor(egg_age) + (1|id), family="binomial", d
ata=counts_a)
summary(model_a_olre)
# > The data is compatible with an absence of effect of egg age

# Calculate the likelihood ratio
Anova(model_a_olre)
# > Inconclusive, egg age is not a good predictor of the differences in parasitoidism rate

# Use pairwise comparisons
emmeans(model_a_olre, "egg_age", type="response", adjust="sidak")
# > Parasitoidism rates are very similar
```

Annex: Experiment data

The data produced by the experiments can be downloaded at docs.google.com/spreadsheets/d/1mMNcUx8yP0D55G3tvNUD9O05EcR6gN-4/edit

The file contains 9 pages:

- c-caps: host larvae head capsule measurements
- s-stage: host larvae size measurements
- y-ch: choice test
- x-nc: no-choice test
- sex: sexed counts of individuals in each cage
- failed: egg masses that produced no individuals
- help: definitions
- discarded: discarded data

Annex: Videos

Two videos are available online.

Video 4.1	*Chelonus insularis* bursts out from a *Spodoptera frugiperda* host larva. drive.google.com/file/d/187A5H5MQP-lvvt6poJyMExV6clcZ5c9H/view
Video 4.2	*Chelonus insularis* uses its mandibles to emerge from its cocoon. drive.google.com/file/d/1721ElT_97LxNT1pWssoeUZ0R2LOy0y7w/view

4 Rearing parasitoids: what parameters are the most important?

Abstract

Parasitoid population dynamics are sensitive and colonies can become extinct unexpectedly and for reasons unclear. A systematic word count in abstracts as well as full texts allowed us to determine which environmental parameters were seen as important by researchers in experiments where parasitoids are reared. The experimental parameters investigated were sex-ratio, temperature, humidity, pressure, diet, environment structure, host quality and population dynamics. Temperature and diet were by far the most often mentioned parameters. Sex-ratio yielded less than a quarter as many hits as temperature, despite its importance for colony survival. Physical properties like pressure and humidity were seldom mentioned. Parameters like temperature, diet, environment structure and sex-ratio appeared together more frequently. No relationship was found between the number of mentions of sex-ratio, or the presence of sex-ratio (at least 1 mention), and the mentions or presence of other parameters. A more complete search and analysis method could yield more decisive results. Researchers working with parasitoids should take note of these parameters and perform sex-ratio counts whenever practical. As evidenced by the complexity of parasitoid population ecology, further studies would be needed for each parasitoid.

Keywords: parasitoid, rearing, parameters, factors, experiment, laboratory, text mining

4.1 Introduction

Biological control has been a topic of scientific investigation for over a century. Yet, given the number of different techniques (predation, parasitism) and the sensitivity in application (different climates, trophic interactions), research continues at pace. A recent subject of investigation is the Fall Armyworm (*Spodoptera frugiperda*, a devastating pest of crops, see Rwomushana et al. 2018, p23-27) and its control using natural enemies, in this case parasitoid wasps. Part of the quarantined facilities at CABI Switzerland were recently dedicated to studying the parasitism rate of *S. frugiperda* by two wasps: the ichneumonid *Eiphosoma laphygmae* (Allen et al. 2020) and the braconid *Chelonus insularis*. However, during the course of the experiments, the parasitoid populations fluctuated greatly. In particular, sex-ratio was very unstable, even leading to the extinction of one of the *C. insularis* colonies.

A precursory review of the related literature revealed that sex-ratio is known to be unstable in parasitoids. Sex-ratio is expected to follow a 1:1 average since that would give the best evolutionary fitness, yet for parasitoids this is not the case. Godfray (1994) describes several experiments where sex-ratio was skewed without explanation. Since it is the female's decision whether to lay a male or female egg, factors that influence behavior can be suspected. For instance, one paper showed that rapid changes in barometric pressure discouraged flight in female of two Trichogramma species (Fournier et al. 2005), which has consequences on parasitoid density in a patch. A review of sex-ratio theory and possible explaining factors is found in Wajnberg et al. (2007). The authors also propose models for optimal behavior. Quicke (1997) warns that primary sex-ratio is difficult to determine in parasitoids because there might be differential mortality between male and female eggs or larvae. Finally, a review on sex-ratio in ichneumonoids was done by Smart & Mayhew (2008). Sex-determining mechanisms, differential mortality, superparasitoidism and sperm limitation are put forward as potential explanatory parameters. The authors conclude that among all the rules having an influence on sex-ratio, each species must use a specific mix. Indeed, in the case of CABI, different items were investigated, including the climate regulation appliances, but no cause could clearly be identified for the collapse of the *C. insularis* colony. In any case, some of these parameters could be controlled relatively easily (temperature) while others would have required more advanced equipment (pressure). Some other parameters would be beyond control entirely, given the way the experiment was set up.

Therefore, it was decided to proceed to a systematic review of papers that include parasitoid rearing as part of their experimental protocol, following the idea that experimental parameters which are the most frequently mentioned are generally perceived as the most essential to control for the success of experiments. With this information, researchers who are working with parasitoids could implement measures to better control population dynamics in their colonies.

In this review, we establish the frequencies of occurrence of certain **rearing-related parameters** in the text of selected Open Access articles. We then compare their frequencies depending on the occurrence of terms related to sex-ratio.

4.2 Methods

Web of Science was selected to search for papers. The search string targeted papers in which a parasitoid was reared, either to study it, study its interactions with another species, or for the sake of finding the best rearing methods. This narrowing down was done by searching the titles and abstracts for specific terms: rear and all its forms (reared, mass-rearing), parasitoid, words that might denote experimental parameters (factor, effect). The `OR` clause on Web of Science is not exclusive. Variations on parasitoid such as parasite, hyperparasitoid or superparasitoidism did not improve the quality of matches and were discarded. The same was done for words targeting specific topics of research such as diet or factitious hosts. The query was iteratively refined, manually assessing the relevance and specificity of results until the best compromise was achieved. Less relevant results were excluded by enabling ‘exact search’. This functionality prevents so-called ‘fuzzy matching’ in which related matches are injected into the query according to unknown and uncontrollable rules. The final search was performed on February 10, 2021 with the query:

```
( TI=(reared) OR AB=(rearing) OR AB=("to rear") ) AND AB=(parasitoid*) AND ( AB=(mass) OR
AB=(population*) OR AB=(method*) OR AB=(factor*) OR AB=(effect*) OR ( AB=(environment*)
AND AB=(parameter*) ) )
```

This search yielded 913 highly relevant hits. The records were imported into Citavi and the full texts were downloaded automatically wherever possible. Of the 495 with the full text available online, 201 were downloaded. For the remaining 712 papers, the abstracts were collected into a secondary corpus.

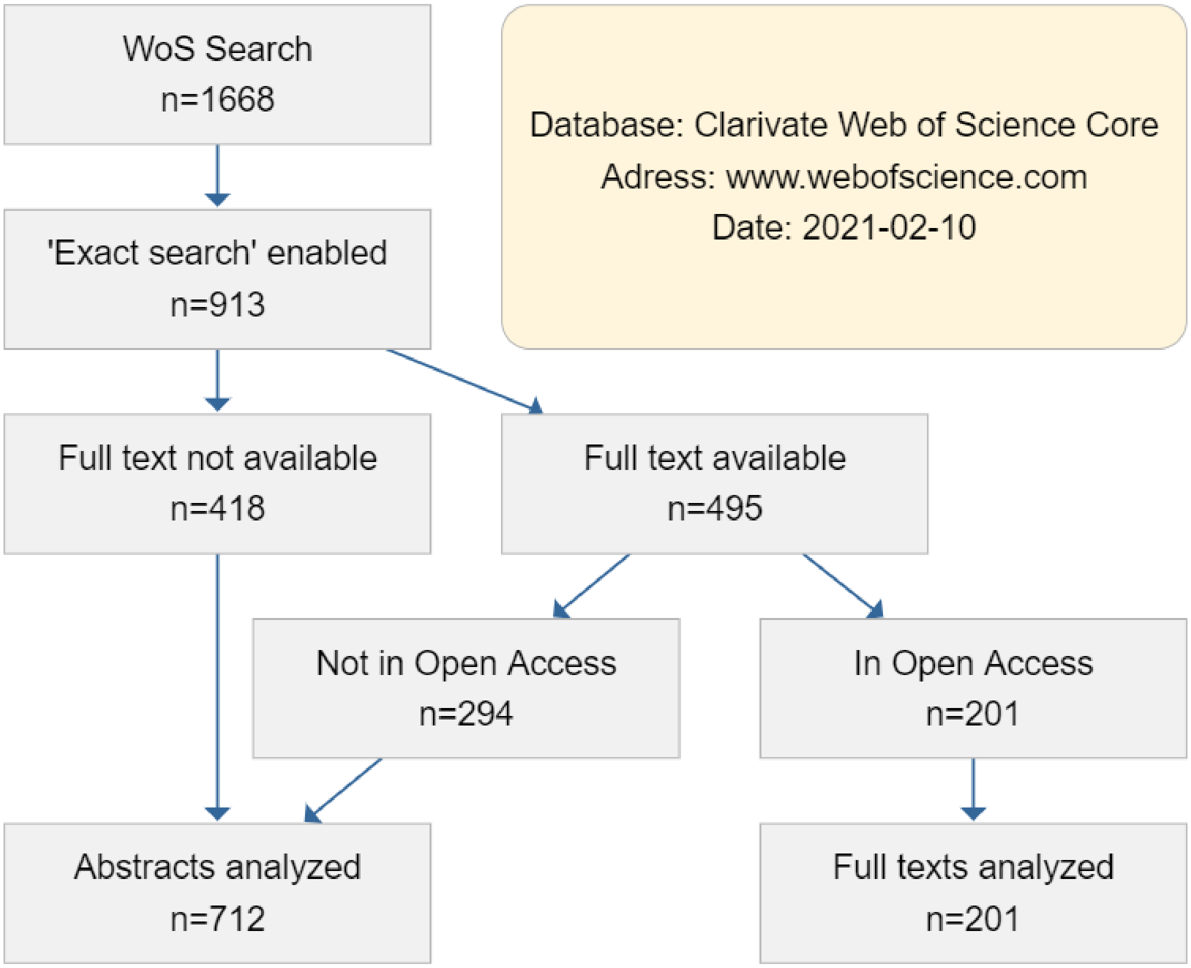

Figure 4.1: PRISMA diagram of the selection process.

In each corpus, the presence of an experimental parameter of interest was evaluated through the number of occurrences of related words. In text-analysis terms, the parameters are called **topics**, and the words were looked for with search expressions. These expressions might use wildcards (*). The algorithm returns all matching tokens (units of text). A list of parameters to be investigated was established, based on Schneider (2009). Temperature is a determining parameter for the development of insects. Humidity, pressure and other climate-related parameters are also important in that they must match the studied species' habitat. Other parameters, not based on the measure of ambient physical properties, constitute further parameters that researchers may have taken note of. According to Wajnberg et al. (2007), the female parasitoid's host search strategy is informed by cues about certain environmental factors. While the experiments set up at CABI were not really concerned with host search (the parasitoids were confined in cages), the spatial characteristics of the environment such as its patchiness (a measure of heterogeneity) have an influence on parasitoid behavior. A host's quality, mostly determined by its size, has also been shown to influence egg-laying decision, as well as search strategy. Although *C. insularis* is an egg parasitoid, and thus host size is not a concern, some papers may include it for their insect. The respective diets of the parasitoid (usually honey) and the host (often standardized) were also included. Finally, intrinsic factors were included as well. The branch of biology concerned with population dynamics has produced general rules (such as local-mate-competition or LMC) that must apply to parasitoid rearing. The list of topics and expressions to match was as follows:

- Sex-ratio: `sex_ratio`, `*male_biased`
- Temperature: `degre*`, `°*`, `temper*`, `warm*`, `heat*`
- Humidity: `humid*`, `damp`, `hygro*`
- Pressure: `Pa`, `bar`, `atmos*`
- Diet: `diet*`, `nutri*`, `food`, `forag*`
- Structure: `patch*`, `habit*`, `distan*`, `locat*`, `wind`
- Hosts: `host_quality`, `host_size`
- Dynamics: `LMC`, `competition`, `*ism_rate*`, `life_histor*`, `foundress*`

Certain characters, words or phrases had to be transformed. In particular, phrases that must be interpreted as one token (eg. "sex ratio" should not be counted as "sex" and "ratio"). Very short words and punctuation were also removed. The following steps were applied to each corpus:

1. Remove species abbreviations
2. Replace hyphens with underscores
3. Compound temperatures
4. Remove numeric values
5. Remove parentheses and their contents
6. Remove symbols and apostrophes
7. Remove all text after the word 'References'
8. Remove lone characters
9. Remove very short words
10. Remove 'al'
11. Compound expressions

The corpuses were analyzed in R 4.0.2 with RStudio 1.3 and the text-analysis package Quanteda 2.1.2. The graphs were created in Python 3.9 with Pandas 1.2.1 and Altair 4.2.0. The count data were subjected to separate statistical tests for each corpus. The association of topics was evaluated with a ratio of co-occurrence. In abstracts, where words are fewer but chosen more carefully, the distribution of counts was broken down according to the presence or not of sex-ratio related terms and ANOVA was used to test whether that same variable influenced the distribution. In full texts, chi-square was used to test whether the presence of sex-ratio influenced the presence of other topics. The number of occurrences of each topic after the number of occurrences of sex-ratio was modelled through linear regression.

4.3 Results and discussion

The corpuses were assembled from papers where parasitoids were reared. This is a sufficiently narrow scope, despite the overlap between parasitoid rearing and insect rearing in general. The total counts are shown in Table 4.1 and plotted in Figure 4.2. The per-item counts can be found in the annex. The two corpuses showed similar distributions, with exceptions. Physical properties like pressure and humidity, as well as host quality, were the least often mentioned, totaling together around 5% of hits in each corpus. Environment structure, population dynamics and sex-ratio make up a second group, amounting to about 25% of hits in both corpuses. Diet showed a much higher prevalence in full texts. Diet scored close to temperature in full texts with 30%. In abstracts, it was closer to sex-ratio with 18%. Finally, temperature accounted for 53% of hits in abstracts and 39% in full texts.

One could argue that the evolution of the sex-ratio in a colony is the most important parameter for its survival, yet it did not achieve first place. The overbearing presence of temperature-related words shows that researchers give it a lot of importance. Temperature is indeed essential for the development of insects, governing their development time and lifespan, but this is not specific to parasitoids.

Which parameters occur more frequently together? A correlation plot (Figure 4.3, left) shows no strong relationship between topics, with an absolute maximum of 0.266 occurring between host quality and sex-ratio. A linear regression of each topic against sex-ratio yielded large residuals and P-values. In full texts, the frequency of appearance of topics in pairs can be compared to their total frequency of appearance. This is the Jaccard index, the ratio of the intersection over the union. The result is shown in Figure 4.3 (right). The most frequently associated topics were temperature, diet, environment structure and population dynamics. Sex-ratio was also frequently associated with diet and temperature. To extract actionable information, relationships between each topic and sex-ratio were examined. Sex-ratio was mentioned in 23% of abstracts and 49% of full texts. An ANOVA yielded a P-value of 0.297 for abstracts and 0.420 for full-texts, meaning that the null hypothesis that the presence of sex-ratio has no influence on the distribution of parameters cannot be invalidated by our data. In full articles, chi-square was used to test the relationship between the presence of a topic and the presence of sex-ratio. The test yielded a P-value of 0.339, again indicating that a relationship is not supported by the data.

Table 4.1: Total number of times each parameter was mentioned.

Parameter	Name	Keywords	Abstract	Full texts
Sex-ratio	sexratio	`sex_ratio`, `*male_biased`	319	693
Temperature	temperature	`degre*`, `°*`, `temper*`, `warm*`, `heat*`	1463	2748
Humidity	humidity	`humid*`, `damp`, `hygro*`	53	208
Pressure	pressure	`Pa`, `bar`, `atmos*`	10	43
Diet	diet	`diet*`, `nutri*`, `food`, `forag*`	536	2118
Environment structure	structure	`patch*`, `habit*`, `distan*`, `locat*`, `wind`	167	510
Host quality	hosts	`host_quality`, `host_size`	65	146
Population dynamics	dynamics	`LMC`, `competition`, `*ism_rate*`, `life_histor*`, `foundress*`	181	577

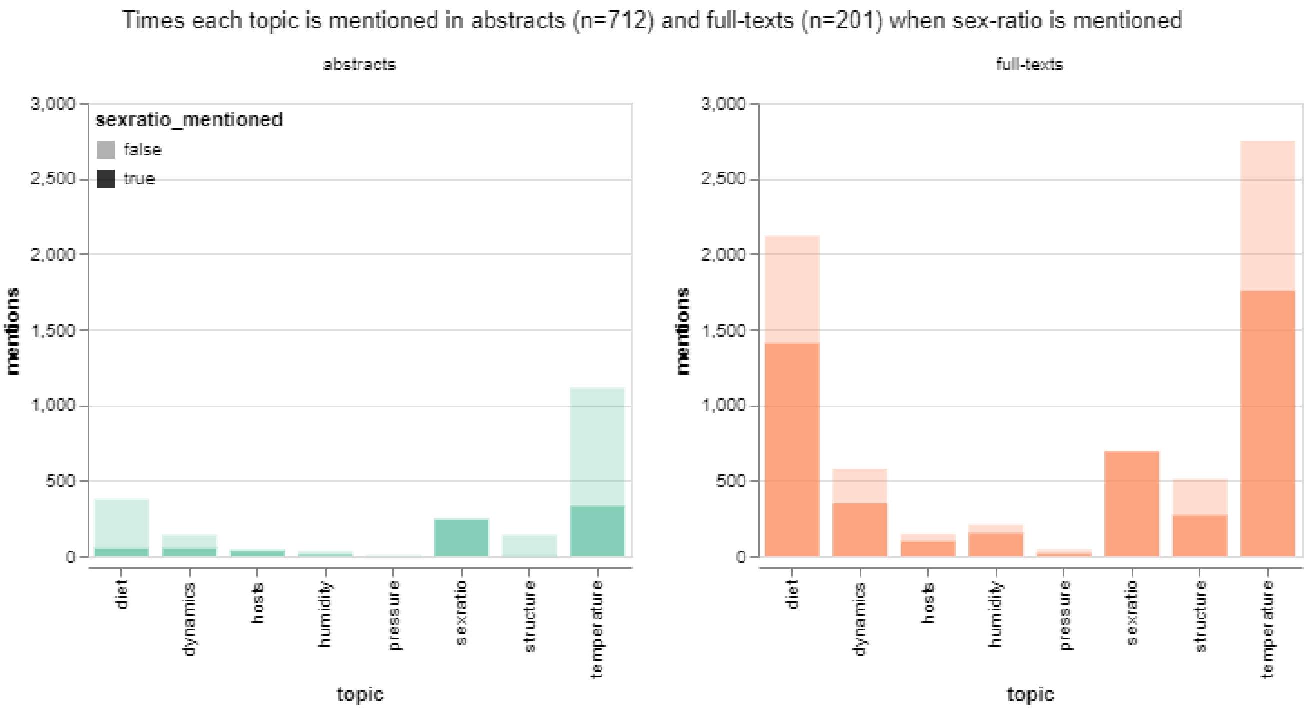

Figure 4.2: Number of hits for each topic in abstracts and full-texts. Hits in items not mentioning sex-ratio are in a lighter shade. Topics have a similar distribution in both corpuses.

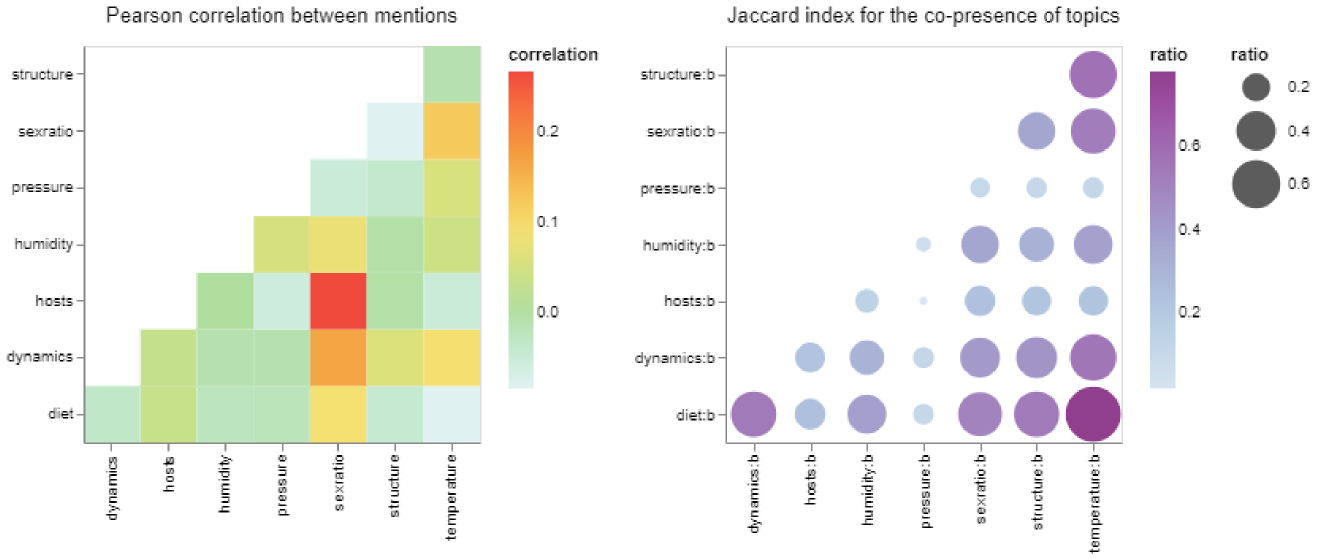

Figure 4.3: Left: Pearson correlation of topics in full texts. Right: Jaccard index for each topic pair in full texts. The Jaccard index is the number of co-occurences over the total number of occurrences.

4.4 Conclusion

No meaningful relationship was found between the number of mentions of sex-ratio and that of other topics. We can still draw a few conclusions with respect to the initial question: what parameters are considered important in parasitoid rearing? We have determined that sometopics are more prevalent than others in scientific articles. Parasitoid-specific topics such as host quality did not achieve particularly high scores. This suggests that the same parameters that are important in general insect rearing are important for parasitoid rearing as well. Aside from their primary role in parasitoid development, parameters may be included for other reasons, such as reproducibility. The lack of a decisive trend in our data can in part be attributed to our methodology. First, as many as 294 papers were excluded from this analysis because they were not in Open Access. This is one of the items that could easily be improved upon. Second, topic occurrence has shown its limits. The list of keywords, as carefully crafted as it might be, is a limiting factor. Ideally, it should be balanced so as not to give a topic more chances simply because it is associated with more general keywords. The probability of each of those words to appear in any given text is intrinsic to the language used, in our case scientific English. In a narrow field like parasitoid rearing, word frequencies do not show what underlying ideas motivated their use. In technical terms, the corpuses have low *keyness* (high keyness is found in political tracts, for instance). Second, whether a topic is mentioned in relation to rearing success or something else cannot be determined. A topic might be mentioned but not investigated at all. Another way of counting would be required to go past this problem, such as natural language processing which extracts meaning from words' positions inside sentences and their relationships.

In reality, as shown by the preliminary round-up of the literature on parasitoids, environmental and population-level parameters can only partially explain the evolution of a population. They vary greatly in nature and, as a product of evolution, the host-parasitoid system should be able to cope with these fluctuations. Successful rearing of parasitoids is a function of several factors and, as outlined by Smart & Mayhew (2009), each species might require its own dedicated study to see which ones are the most important. Researchers concerned with rearing their insects in good conditions should look at temperature and diet, and perform sex-ratio counts whenever practical. The role of environment structure must be studied more closely. Laboratories are limited in the space and materials available, but there may be an optimal way to split a colony between different cages. The best insurance against colony extinction is probably to maintain multiple inbred lines and inject new genetic material regularly, from the field or from another colony.

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Annex: Processed bibliographic items

The list of bibliographic items included in the analysis is available online.

- List of abstracts: https://pastebin.com/raw/qxhy44su
- List of full-texts: https://pastebin.com/raw/WH5JHvbh

5 Population dynamics and sex-ratio in a laboratory-reared colony of *Chelonus insularis*: can the erosion of allelic richness explain population bottlenecks?

Abstract

Hymenopterans are haplodiploid. One sex has two of each chromosome while the other has one. Under arrhenotoky, females are diploid while males are haploid. The laying female can decide of the sex of its progeny by laying an haploid egg from her calyx, or fecundating it first with sperm stored in the spermatheca, making it diploid. In the first case, the offspring will have one chromosome and be male. In the latter, it will have one chromosome from each parent and be female. However, a third case is possible. In most Hymenoptera, sex is determined at a single locus in the genome. Only individuals that are heterozygous at that locus develop into females. If the offspring has two chromosomes with the same allele at the sex-determining locus, it will develop into a diploid male. Whether diploid males are viable or fertile depends on the species, and has only been studied since recently. In laboratory-reared colonies, inbreeding is common, as bringing genetic material from the outside is quite costly. An advantage of inbreeding is that it exposes deleterious alleles quickly. However, if the number of alleles at the sex-determining locus in a population is too low, a large proportion of diploid males should be produced. This requires advanced techniques to establish for certain. As an alternative, a simulation is run to forecast the number of alleles present in subsequent generations from a starting population. The results indicate that while inbreeding does not change the diversity of male genotypes, it does increase the proportion of diploid males to a third of the population after just three generations. Individuals from outbred lines counteract this trend and reduce this proportion to a fifth.

5.1 Introduction

5.1.1 Sex-ratio and the rearing system

During experiments involving insects, researchers typically keep one or several colonies in the laboratory. A continuous supply of insects from the outside is impractical, especially with less common species. Such a solution would put the experiments at the mercy of supply-chain effects. It is much easier to have a colony on-premise from which insects are available on-demand. The burden is that of maintaining the colony. The ensemble of procedures by which parasitoids are reared for experiments can be considered a system. It has certain parameters and a certain boundary. Concretely, the boundary is the cages in which the insects are kept between experiment runs. In this closed loop, the parameters can be summarized by one notion, lineage.

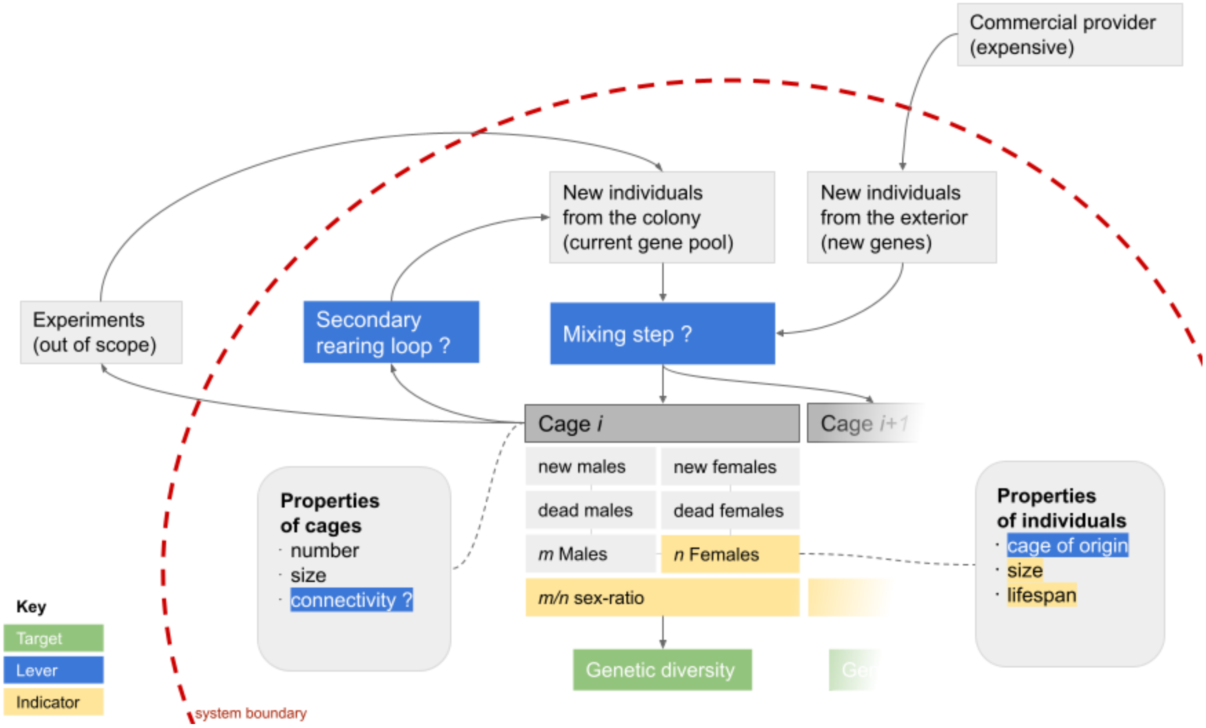


Figure 5.1: Production system for parasitoids in captivity. When conducting specificity experiments, the rearing system (red) takes two inputs, one of which flows from its own single output through the experiment runs (left). Individuals are assigned a cage during the mixing step. This ‘algorithm’ should not be random but rather optimized to maintain genetic diversity (green). This can be achieved by considering the origin of individuals (generation, ancestry, experiment type) and by ensuring optimal conditions in the cages (notably, an appropriate number of females). Individuals’ health can be monitored by looking at their size and lifespan. With gene inflow from commercial providers too expensive in both time and money, a solution to investigate would be a secondary rearing loop which would bypass the experiment runs and counterbalance the selection pressure that they inevitably exert. Furthermore, cages could be connected to simulate a patchy environment in which mate-finding strategies are more likely to take place.

In parasitoid studies, both parasitoid and host colonies must be kept healthy and stable at the same time. This is not too difficult in itself, but the sensitivity of this equilibrium becomes apparent when problems arise, some of which are specific. One concern is the quality of egg masses. They should be large, but not too large, and fertile. Unless a procedure has been put in place, diagnosing the reason for eggs not hatching can be difficult. Another concern is larval mortality due to factors other than parasitoidism. Cannibalism and viruses can decimate a group of hosts in a few hours. Viruses are particularly difficult to contain. Problems at any of these points reveal the bottlenecks in the system. The number of insects available for experiments may fall to zero for several days, or be too large, meaning resources are being wasted. Their quality may also put experimental results in question.

Sex-ratio is the ratio of males to females in a population. For biological control, the sex-ratio in a parasitoid population is of interest because only females lay eggs. Sex-ratio is not a constant but varies in time. It is known to fluctuate in arrhenotokous species, which most Hymenopterans are (Huffaker & Messenger 1976, p149). In these species, the female decides on the sex of the egg that is laid. Understanding this decision is essential if population forecasts are to be made. However, the laying female is not solely responsible for the sex-ratio of its offspring. A distinction is made between primary sex-ratio, the one assigned to the embryo, and secondary sex-ratio, which is based on the sex of the emerging insects. Sex-ratio is known to be male-biased in the laboratory (Wajnberg et al. 2007, p254 and figure p255; Heimpel & Lundgren 2000), though it is unclear if endogenic or exogenic factors are at work. Among the information available to the female at oviposition, sex-ratio inside a cage may have an influence on the primary sex-ratio of offspring. According to local mate competition (LMC) theory described below, females should lay more male eggs as the number of females in the cage increases. In practice, different species apply this rule differently.

Causes for sex-ratio fluctuations have been searched for in theory as well as empirical observations. Different models have been proposed to explain primary sex-ratio, as chosen by the laying female. These explanations often involve an ideal ratio, one that would return maximum fitness, that the female will try to approach. Their adaptability to the case of Hymenopteran parasitoids is not always straightforward. Fisher, in 1930, posited that equal cost and equal fitness gain means that the ideal ratio to lay is 1:1, with a stabilizing effect (Wajnberg et al. 2007, p256). However, under arrhenotokous haplodiploidy, female eggs are more expensive and do not provide the same fitness gain. Therefore, the ratio should be skewed towards males (the least costly) (Wajnberg et al. 2007, p254). In practice, fisherian theory concerns investment at the population level, not at the individual decision level. There is little evidence of these assumptions holding for parasitoids (Wajnberg et al. 2007, p257). In 1967, Hamilton added the number of males and females to this model. The optimal ratio would be $\frac{n-1}{2n}$ with n the number of females. This is known as local mate competition (LMC). Assuming one female contributing to offspring per patch, the evolutionary stable strategy is to lay only as many males as needed to mate with the females. This reduces competition between brothers for mates and increases mother-offspring relatedness (Wajnberg et al. 2007, p267). In practice, host quality may be a bigger driver than the number of peers, and push the sex-ratio towards males, as female eggs are reserved for higher quality hosts. Hassell et al. (1983) tried to model sex-ratio as function of the ratio of females to hosts. They found that the portion of females in the offspring decreased as the number of laying females increased. A unifying hypothesis between these two explanatory factors is that the number of competing females is estimated not by the number of females currently present in the patch, but rather by the presence of their eggs. Shuker & West (2004) used a normal strain as well as red-eyed mutants in thee settings: the normal female alone, with red-eyed mutants who could not oviposit, and with ones who could. In both latter settings, a more male-biased sex-ratio was observed. The effect was stronger when the mutant females could oviposit. A split design allowed them to conclude that direct cues (presence of females) account for 8.6% of the effect and indirect cues (presence of eggs) account for 31.5%.

In 1973, Trivers & Willard argued that when each sex provides different fitness returns, the female should adapt its strategy in accordance with the available resources (Wajnberg et al. 2007, p257). Charnov et al., in 1981, took this argument into account and adapted the model to solitary parasitoids. Mothers with more resources should invest on the most expensive sex, because it will benefit more from investment. Conversely, a mother with few resources “should produce the sex whose fitness is less sensitive to investment”. Resource can be understood as host quality. This implies that female fitness is more affected by host quality than male fitness is. Therefore, King concluded in 1993, it makes evolutionary sense to reserve higher quality hosts for female eggs, or to reserve female eggs for the highest quality hosts. If laying female eggs in higher quality hosts provides greater fitness gain to the mother, a slight male bias should be expected. This has only been shown empirically in a few species (Wajnberg et al. 2007, p259). In experiments, host quality is often equated to host size, an absolute notion. In reality, host quality appreciation has been shown to be relative to other hosts in the patch (see below). Murdoch et al. added host quality effects in a discrete-time model. They found that an increase in the density of laying females would lead them to parasitoidize younger, smaller hosts at time t with male eggs, leaving fewer older, bigger hosts at time $t+1$ to be parasitoidized with more costly female eggs. This could result in either a stabilizing or destabilizing effect on sex-ratio.

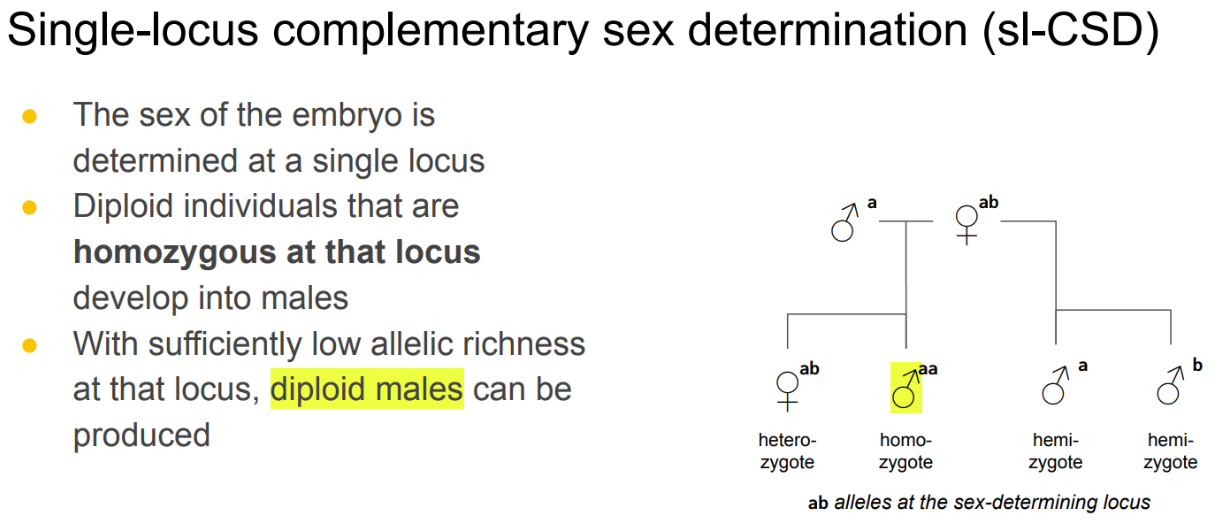
The theories seen so far predict at best a small male bias in parasitoid populations reared in the laboratory, but fluctuations were observed, sometimes massive. Among environmental parameters having a possible influence on primary and secondary sex-ratio, laboratory climate springs to mind. Climate could affect the individuals’ perceived lifespan (Jervis & Kidd 1996, p84-85) which could guide sex allocation. For instance, if the environment becomes unfavorable (storm), producing a large number of males, some of which will escape, returns greater fitness than producing a handful of females, which can instead be kept for when the conditions are more amenable. Schneider (2009, p294) argues that everything needs to be adapted to, in both genotype and behavior, including materials, and that individuals adapt as soon as the first generation. For instance, courtship typically happens on plants in the field but happens just as well on synthetic materials in the laboratory. Host egg quality has already been mentioned as an important factor in models. In a 1913 experiment by Chewryreuv, reported on by Wajnberg et al. (2007, p360), female parasitoids assign female eggs to the highest quality hosts and male eggs to the others. This is done regardless of their absolute quality. The eggs are evaluated against the other eggs in that same egg mass. This is actually the best strategy (the bayesian optimal), as the female has no knowledge of host quality in the other patches. The distribution of host quality would therefore be the main predictor of sex allocation. This means that the sex allocation process can be manipulated by providing a certain combination of relative qualities. This has been tried with larval parasitoids (Wajnberg et al. 2007, p15). Mixing big and small hosts together or over time lead to a greater proportion of females in the offspring (Chow & Heinz 2005).

For *Chelonus insularis*, all eggs are the same size, but other cues must indicate quality, and eggs could be evaluated relative to each other too. These factors are not well known. If the goal is to obtain a female offspring with only a few males, could ideal eggs be produced on demand? In the 1990s, artificial eggs were investigated for rearing parasitoids, and especially *Trichogramma* (Cônsoli & Parra 1999a and b). Cônsoli & Grenier (2009) reviewed this period in scientific publishing. The host egg is both a shelter and a food resource for the parasitoid larva. The challenges are therefore diet composition and packaging. The diet inside the artificial egg must suit the laying female and the egg shell must be thin enough to allow chemical cues through, without risk of desiccation. It must also support the different developmental stages of the parasitoid. Interestingly, there must also be the right quantity of diet, as the larva only enters pupal stage once it is depleted. Larvae that continue to feed after a certain age have more malformations. Rearing on artificial hosts is two orders of magnitude cheaper than on natural hosts, even taking into account the rearing of silkworms as a substrate for the artificial diet. Artificial rearing was successfully implemented at scale at the Guangdong Entomological Institute and Wuhan University, with several millions *Trichogramma* wasps produced per day. However, the authors report that attempts with other species were not successful. It is possible that these species expect slightly older eggs, where some of the base nutrients have already been transformed. As to the absence of artificially-reared *Trichogramma* on the market, the simple lack of automated means of production outside of China would be the main factor. Artificially reared parasitoids have generally good performance. Some showed lower preference for their natural host, but this difference disappeared after being reared on their natural host for one generation (Gandolfi et al. 2003).

There are certainly interactions between ovipositing females when there are several in a patch. Each female leaves chemical cues on the egg it has parasitoidized examined. Outside of superparasitoidism, these cues must also inform the female's strategy. Females of certain species lay male eggs, and switch to female eggs when they encounter already-parasitoidized eggs. This is a sign that males have already been laid, and thus that females that are laid will find mates (Jervis & Kidd 2007, p37-38). Once inside the host, mortality could be different for parasitoid males and females (Godfray 1994, p174). For any given combination of host and parasitoid, this requires systematic dissections to determine. Finally, certain factors apply on genome expression. Supernumerary chromosomes, if present in a diploid individual, destroy half the genotype, leaving a male (Wajnberg et al. 2007, p277). *Wolbachia*, a genus of symbiotic bacteria (Jervis & Kidd 2007, p88; Wajnberg et al. 2007, p277) affects secondary sex-ratio by changing the sex of their host during development. *Wolbachia* can be the cause of thelytoky in certain species (Leach et al. 2009). This has been shown in the parasitoid *Trichogramma dendrolimi* (Zhou et al. 2021). Thelytokous species produce diploid females without fecundation. The Ichneumonidae *Venturia canescens* exists simultaneously in arrhenotokous and thelytokous forms. Inducing thelytoky remains to be attempted. *Wolbachia* can also inhibit a parasitoid's preference for the host they developed on (Abroon et al. 2019). Luck et al. (1999) provide a review of genetic and epigenetic sex-determination factors.

5.1.2 Are genetic effects at the source of fluctuating sex-ratios?

Sex-ratio might also be influenced by factors specific to a genetic line. Laboratory rearing introduces biases that are either not present in nature, or present but compensated by natural selection. With regard to parasitoids specifically, inbreeding is one such area where field and laboratory rearing can result in different outcomes. Sib-mating is common in parasitoids. For *C. insularis*, adult males emerge a couple of days before females, and wait around until they hatch as well. They then mate between siblings. As an arrhenotokous species, females are diploid but need insemination to be able to lay female eggs. Sib-mating guarantees that males are available right after hatching. Under haplodiploidy, deleterious alleles are purged from the population within one generation. Haploid individuals (here males) only have that one deleterious allele and do not propagate it. As a result, inbreeding depression is avoided (Heimpel & Mills 2017, p192). Fitness does not decrease over time. In the laboratory, the effects of inbreeding include a more male-biased sex-ratio and lower genetic diversity (Bueno et al. 2017). But what could be the cause?



Single-locus complementary sex determination (sl-CSD)

- The sex of the embryo is determined at a single locus
- Diploid individuals that are **homozygous at that locus** develop into males
- With sufficiently low allelic richness at that locus, diploid males can be produced

Figure 5.2: Emergence of diploid males in arrhenotokous Hymenoptera. Eggs that are hemizygous or homozygous at the sex-determining locus develop into males. After Cowan & Stalhut (2003) and de Boer et al. (2007).

In most Hymenopterans, the sex of the emerging individual is determined by zygosity at a single locus. This mechanism was discovered in 1943 and named sl-CSD, for single-locus complementary sex determination. Individuals that are heterozygous at this locus become female, and the ones that are homozygous, or hemizygous as are haploid individuals, become male (Heimpel et al. 1999). This means that a fertilized, homozygous egg can develop into a diploid male individual. sl-CSD has long been suspected of causing sex-ratio fluctuations (Huffaker & Messenger 1976, p58) (Wajnberg et al. 2007, p275). Cowan et al. (2004) note that male diploidy has not been extensively studied in Hymenopteran parasitoids. Harpur et al. (2013) found that out of 83 species, 24 were confirmed to be governed by sl-CSD while 58 were only assumed to be (1 was confirmed ml-CSD). The role of diploid males in nature may be different from that predicted. Depending on the species, diploid males may or may not be viable, fertile, or otherwise useful at the population level. The reasons may be genetic, strategic or practical. For instance, the male may die prematurely or not be able to produce gametes. It may be excluded from the colony (certain social wasps kill diploid males upon emergence) or remain undetected. Finally, in some species, diploid males are too big to be able to mount females. Under sl-CSD, females mating with diploid males cannot produce daughters unless the male carries a third allele.

The effect on sex-ratio is even more male bias. If the two parents share an allele, the female is said to be match-mated. If the haploid male brings an allele not possessed by the female, this is unmatched mating. At oviposition, the female can allocate eggs to be male or female by using the semen stored in the spermatheca after mating, thereby adapting its own fitness. For match-mated females, fertilized eggs will produce diploid sons where daughters were intended. The effect of diploidy under these circumstances is not straightforward. Diploid males are homozygous, and can only bring one allele. Yet, the distribution of their offspring does not match that of haploid males. Females mated with diploid males produce fewer daughters (Harpur et al. 2013). This depends on the species and the causes are not clear. One possible explanation is a preference of the female for laying haploid eggs. In *Cotesia vestalis*, diploid males display normal mating behavior and father infertile triploid daughters (de Boer et al. 2007), and the same has been recorded in *Polistes dominulus* (Liebert et al. 2005). In *Cotesia glomerata*, diploid males father fertile diploid daughters (Elias et al. 2009). Wajnberg et al. (2007, p272) report that sex-ratio changes have been attributed to lineage in several species of parasitoids. Sex-ratio is also correlated with other factors such as realized fecundity, making the effect of genetic variation hard to isolate.

With regard to parasitoids reared for experiment, genetic quality is of concern and specific effects may come into play. At colony establishment, the population is a sample from the actual population in the field, and might only have a fraction of the alleles (Singh & Moore 1985, p9; Schneider 2009, p292). In females, inbreeding favors homozygosity and recessive traits may become common (Singh & Moore 1985, p11). Under sl-CSD, homozygosity leads to the creation of diploid males, a dead-end with regard to fitness. In *Venturia canescens*, inbred females had lower egg-load at emergence and laid more males (Vayssade et al. 2014). Depression and allele loss (Heimpel & Mills 2017, p192-195) as well as genetic drift (Schneider 2009, p87) are parameters to watch should the colony persist in time. Given the preponderance of genetic diversity in matters sex-ratio, what effect does the experimental protocol have, and can it be improved?

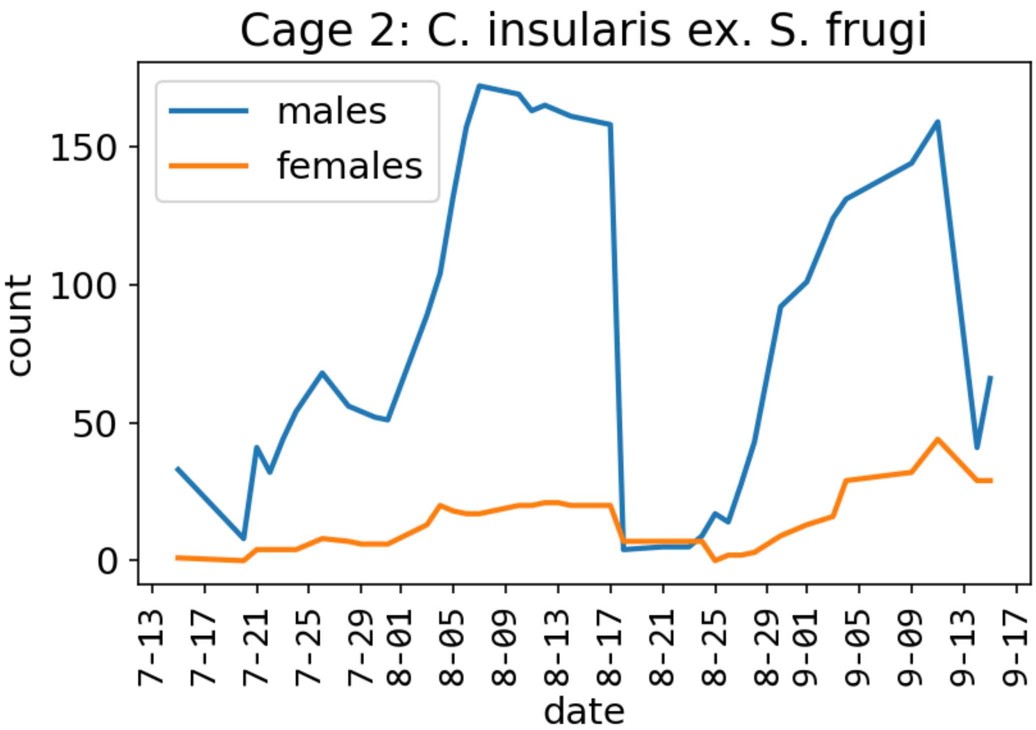


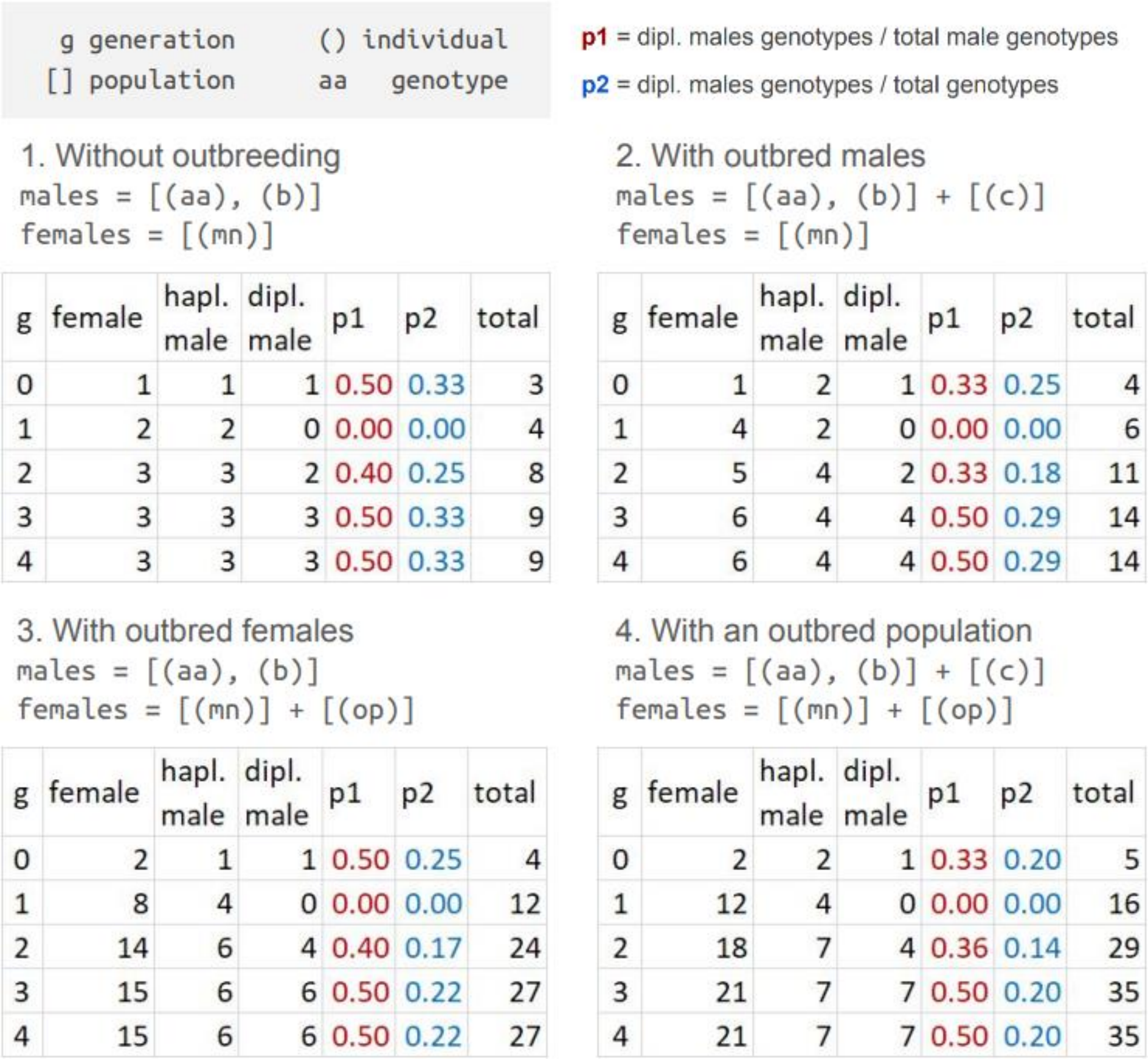
Figure 5.3: Number of males and females in cage n°2 during oviposition tests with *Chelonus insularis*, *Spodoptera frugiperda* and *Spodoptera exigua*. The total number of individuals increased but the number of females stayed relatively low.

5.2 Methods

We are wondering if inbreeding is a possible factor in the presence of diploid males. Another way to turn the question is: does outbreeding increase allelic richness? According to Wajnberg et al. (2007, p276), outbreeding can divide the sex-ratio by 2. The source of new alleles can be another colony or the field (Schneider 2009). A very simple computer simulation was written. The program takes the genotypes of a starting population and gives the number of genotypes available after n generations. This is a basic approach. A real genomics simulation would require knowledge of certain essential ecological parameters related to mating and sex-allocation strategy. These parameters are different in each species and unknown in *Chelonus insularis*. The program does not simulate the number of individuals. Instead, starting with a given population of genotypes, it outputs all possible genotypes at each generation. Genotypes are denoted using lowercase letters, as the exact alleles and their dominance are out of scope. The program was run for 4 generations, assuming diploid males can survive but not reproduce. There were four scenarios: (1) without outbreeding (2) when crossing with outbred males (3) when crossing with outbred females (4) when crossing with outbred males and females. For each generation, the proportion of diploid male genotypes available in all male genotypes (p1), as well as the proportion of diploid males genotypes available in all possible genotypes (p2), were calculated.

5.3 Results

The base population contained a single female genotype, a haploid male genotype and a diploid genotype. Without outbreeding, after 3 generations, the offspring had 3 available female genotypes, 3 haploid males genotypes and 3 diploid male genotypes. The total number of available genotypes was therefore 9. The proportion of available diploid male genotypes in all male genotypes was 50%. The proportion in all possible genotypes was 33%. With an additional haploid male genotypes, corresponding to an outbred males scenario, the total number of available genotypes was 14 after 3 generations. There ware 6 females genotypes and 8 male genotypes available. The proportion p1 was 50% while p2 decreased to 29%. With an outbred female, two additional alleles enter the genetic pool. The total number of available genotypes was 27. There were more female genotypes than male genotypes available, at 15 versus 12. Diploid male genotypes still represented 50% of all male genotypes. However, they only represented 22% of all possible genotypes. When a female genotype and a haploid male genotype were added to the genetic pool, the total number of available genotypes was 35. There were 21 female genotypes and 14 male genotypes available. Proportion p1 remained 50% but p2 decreased to 20%.



```
g generation      () individual
[] population     aa    genotype
```

p1 = dipl. males genotypes / total male genotypes

p2 = dipl. males genotypes / total genotypes

1. Without outbreeding

```
males = [(aa), (b)]
females = [(mn)]
```

g	female	hapl. male	dipl. male	p1	p2	total
0	1	1	1	0.50	0.33	3
1	2	2	0	0.00	0.00	4
2	3	3	2	0.40	0.25	8
3	3	3	3	0.50	0.33	9
4	3	3	3	0.50	0.33	9

2. With outbred males

```
males = [(aa), (b)] + [(c)]
females = [(mn)]
```

g	female	hapl. male	dipl. male	p1	p2	total
0	1	2	1	0.33	0.25	4
1	4	2	0	0.00	0.00	6
2	5	4	2	0.33	0.18	11
3	6	4	4	0.50	0.29	14
4	6	4	4	0.50	0.29	14

3. With outbred females

```
males = [(aa), (b)]
females = [(mn)] + [(op)]
```

g	female	hapl. male	dipl. male	p1	p2	total
0	2	1	1	0.50	0.25	4
1	8	4	0	0.00	0.00	12
2	14	6	4	0.40	0.17	24
3	15	6	6	0.50	0.22	27
4	15	6	6	0.50	0.22	27

4. With an outbred population

```
males = [(aa), (b)] + [(c)]
females = [(mn)] + [(op)]
```

g	female	hapl. male	dipl. male	p1	p2	total
0	2	2	1	0.33	0.20	5
1	12	4	0	0.00	0.00	16
2	18	7	4	0.36	0.14	29
3	21	7	7	0.50	0.20	35
4	21	7	7	0.50	0.20	35

Figure 5.4: Results of the simulation of available alleles after 4 generations under arrhenotokous haplodiploidy, when diploid males cannot reproduce. With outbreeding, diploid male genotypes represent a lower portion of all available genotypes.

5.4 Discussion

Our simulation showed that comparatively fewer diploid male genotypes are available with outbreeding. The benefit is stronger the more genotypes are brought in from the outside. With up to two male and two female genotypes in the starting population, available genotypes stabilize after the third generation. In general, under arrhenotokous haplodiploidy, males cannot have sons but only grandsons. However, with sl-CSD, haploid males can have diploid sons. In our simulation, diploid males were infertile. Females had no common alleles with males in the starting population used. If that were the case, the available genotypes would stabilize after only 2 generations, with 1 female, 2 haploid male and 2 diploid male genotypes. The proportions would be 50% for p1 and 40% for p2.

Outbreeding is clearly key to maintaining a low number of diploid males. How can experimenters improve colony security? In most cases, wild specimens cannot be continuously collected, for reasons of cost. There are two lines of reasoning. In the first, wild specimens are sampled only once and from one location, and make up a somewhat temporary colony. In this case, simply shuffling individuals among cages could reduce inbreeding depression. A mixing step should be put in place so that only a portion of individuals originating from a cage are returned to it (Figure 5.5). Crossing among cages was tried towards the end of the experiments, but the colony was not monitored for long enough afterwards to see any benefits. The second option is to periodically introduce one (or more) female individuals from the wild or from a colony reared from another location or containing different alleles. In fact, Cook (1993) notes that inbred lines can serve as reservoirs of good alleles at the sex-determination locus, to be called upon to avoid diploid males. In commercial rearing systems, inbreeding is remediated with periodic outbreeding (Huffaker & Messenger 1976, p11). Allelic richness is also associated with the general quality of insect agents. Final genetic diversity is more associated with population history and habitat than with male diploidy (Collet et al. 2016).

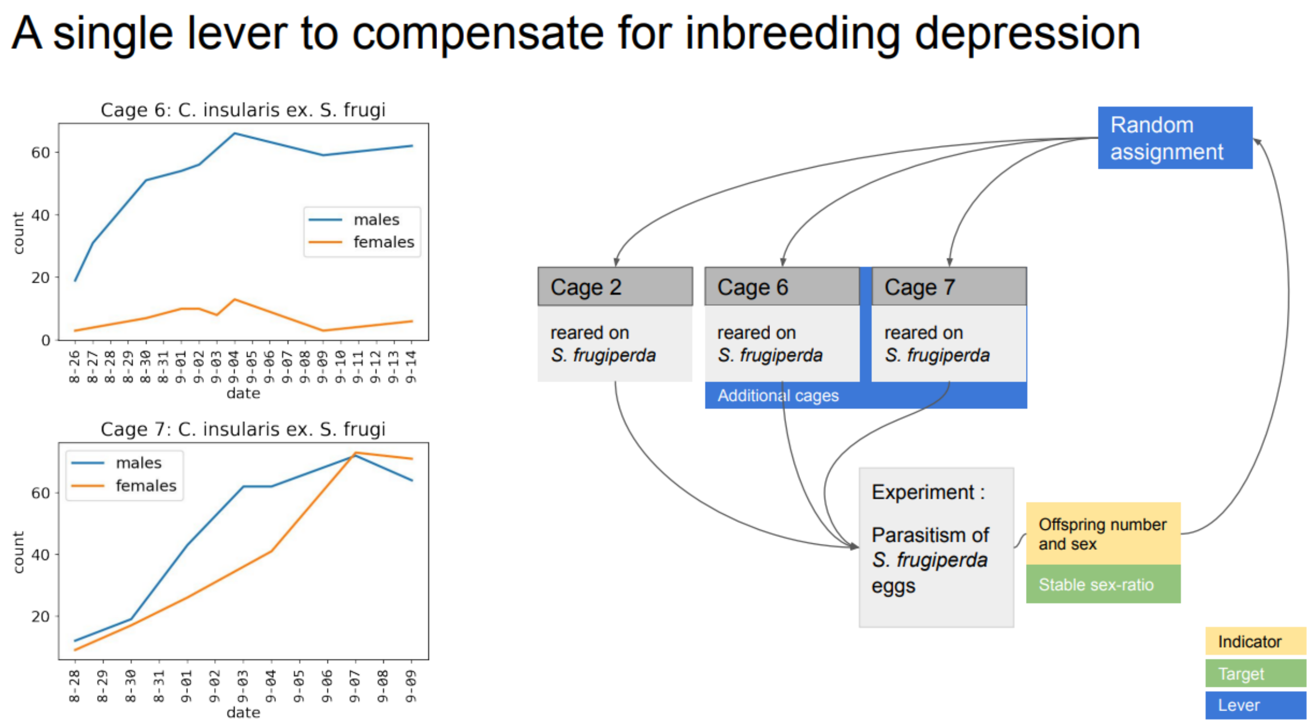

A single lever to compensate for inbreeding depression

Figure 5.5: A mixing step allocating individuals to cage according to their genetic heritage would reduce the proportion of available diploid male genotypes, and therefore risk. The male and female populations in two cages of *C. insularis* raised on *S. frugiperda* are shown. Individuals in Cage n˚7 had greater allelic richness thanks to outbreeding, but the cage was not monitored for long enough to confirm the effect on sex-ratio.

The question of sex allocation remains the central one. The driver for sex allocation in ovipositing females is not sex-ratio but foundress number. West and Sheldon (2002) found that sex-determination mechanisms were not sufficient to explain sex allocation. When fitness returns for each sex change with the environment, parents should adapt by changing the sex of their offspring. In a meta-analysis, the authors compared parasitoid wasp populations to birds. They found that sex-allocation was indeed the result of adaptation to environmental conditions across these taxa. When conditions were difficult to predict, the effect was stronger. The authors add that in this case, large quantities of the least profitable sex could be produced. The dependence on environmental predictability was illustrated by differences between types of parasitoids. Idiobionts kill or paralyze hosts at oviposition, while koinobionts let them live and grow. Koinobionts showed a lower effect of the adaptation to environmental conditions, in this case host size. This makes sense because for idiobionts, host size does not change after oviposition. All the information about host quality is known at that time. For koinobionts, there is uncertainty as to host quality over the course of development. As a result, sex allocation is less dependent on this factor. Which factors it depends on remains to be discovered for each species.

5.5 Conclusion

The stakes of mass insect rearing have long been established (Chambers 1977). The present work has shown that even for a few common experiments, colony size, sex-ratio and genetic diversity are difficult to control and there is considerable risk. In the recent decades, more techniques have become available, especially in relation to quality control (encouraged by the advent of edible insect products), and costs have decreased. The FAO has published a manual on industrial-scale insect rearing (Cáceres et al. 2012). Whether a given laboratory must resort to mass-scale techniques can be evaluated on a case-by-case basis. A selection can be unintentionally performed by rearing under laboratory conditions (Singh p59). At the very least, the production system should be the result of a design phase, taking into account all the goals and risks. However, the case has been made that experiments should reach for as high a genetic diversity as possible within their constraints.

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Annex: Simulation source code

The simulation was written and run in Python.

```
"""
Effect of outbreeding on genotype richness under arrhenotokous haploidiploidy.

[] population
() individual

Example uses:

male = ('♂ a')
female = ('♀ ac')

has_diploid_males(('♂ aa', '♀ ac', '♂ a', '♂ c'), True)

males = [('aa'), ('b')] + [('c')]
females = [('mn')]

run(*parse(males, females), True, False)
"""

def f(sex, alleles):
    return (f'{sex} {alleles}')

def offspring(male, female, sex_locus):
    def a(i):
        return i.split(' ')[-1]
    def s(alleles):
        sex = '♂' if alleles[0]==alleles[1] and sex_locus else '♀'
        return f(sex, ''.join(sorted(alleles)))
    male, female = (a(e) for e in (male, female))
    x = itertools.product(male, female)
    d = (s(i) for i in x)
    h = (f('♂', i) for i in female)
    return tuple(d) + tuple(h)

def has_diploid_males(offspring, count):
    d = (e[0]=='♂' and e[2]==e[3] for e in offspring if len(e)==4)
    return len([e for e in d if e]) if count else any(d)
```

```
def all_offspring(males, females, sex_locus, fertile_diploids):
    def o(male, female):
        return offspring(male, female, sex_locus)
    if not fertile_diploids:
        males = (e for e in males if len(e)<4)
    i = itertools.product(males, females)
    a = (o(male, female) for male, female in i)
    x = functools.reduce(lambda x, y: {*x} | {*y}, a, {})
    return tuple(x)

def sex_split(population):
    males = [e for e in population if '♂' in e]
    females = [e for e in population if '♀' in e]
    return males, females

def summary(population):
    m, f = (len(e) for e in sex_split(population))
    w = len(population)
    d = has_diploid_males(population, True)
    return f'genotypes: {m} male, {f} female, {d} diploid male, {w} total'

def run(males, females, sex_locus, fertile_diploids, generations=5):
    """
        Parameters:
        sex_locus: whether the alleles target the sex-determination locus
        fertile_diploids: whether homozygous diploid males can reproduce
    """
    print(f'Generation 0:', end=' ')
    population = tuple(males) + tuple(females)
    print(summary(population))
    print(population, end='\n\n')
    for generation in range(1, generations):
        print(f'Generation {generation}:', end=' ')
        males, females = sex_split(population)
        population = all_offspring(males, females, sex_locus, fertile_diploids) # !
        print(summary(population))
        print(population, end='\n\n')

def parse(males, females):
    males = (f('♂', i) for i in males)
    females = (f('♀', i) for i in females)
    return males, females
```

6 General discussion and conclusion

Over the course of five investigations, all the aspects of biological control have been explored, from matching agent and pest to evaluating them to rearing them. The systematic approach found 85 Hymenopteran parasitoids of the Fall Armyworm. For comparison, CABI's Invasive Species Compendium lists 36. Natural parasitoids were present in all climates invaded by the pest. Field performance information was difficult to obtain, as each study had specificities that could not be accounted for in a purely systematic way. Per-species reviews may be required to obtain more reliable numbers. Host-parasitoid systems were shown to be much more complex than simple consumer-resource systems. This is due to the many decisions that a parasitoid female must make to optimize fitness returns, which depend on spatial factors. Host density and environment patchiness are the two parameters considered essential. However, they are difficult to quantify. Spatial distance cannot fully define the constraints felt by a species, as local conditions (eg. climate, predators) also restrict movement and accretion, adding a layer of complexity. As a result, forecasts may remain difficult for a long time and limited field releases will remain necessary to assess agent propagation.

C. insularis's biology and distribution showed that it could be a good choice for a biological control agent against *S. frugiperda*, being on of its main parasitoids in the pest's native range. The host specificity and preference experiments produced straightforward results. *C. insularis* prefers *S. frugiperda* eggs but will also attack *S. exigua* eggs. Parasitoidism was successful on this alternative host, leaving open the question of adaptation. Should a released population of *C. insularis* use this alternative host for more than one generation, what is the probability that it switches to it as its main host? Certain release strategies might still be possible, provided the agent does not persist. This will require further study. *C. insularis* showed no discernable differences in performance with regard to egg age. However, egg age and superparasitism directly relate to egg quality. Of species, age, texture and chemicals, exactly which qualities elicit which behavior is still unknown.

Explaining sex-ratio fluctuations proved an interesting and challenging question. The topic is central to agent quality, yet poorly understood across species. The systematic search for preponderant rearing parameters highlighted temperature and diet. Although no relationship was found with sex-ratio, the sheer number of mentions compared to the other parameters indicate that researchers give them great importance. This might be related to scientific credibility. The influence of temperature on insect development time is well known. Given the importance of biochemical signals in insect behavior, diet is also justified as a determining factor for the outcome of certain experiments. Therefore, researchers might want to include them at minima, should reproduction of their results be attempted. The other parameters chosen for the analysis may have been too specific or complex to apply to experiments in general. On the other hand, the role of allelic richness in realized sex-ratio was made clear by the simulation. Inbreeding favors homozygosity which can generate diploid males. Outbreeding was shown to be sufficient to reduce the proportion of possible diploid male genotypes from 33% to 20%. The present work gives good hope that *C. insularis* can make a good biological control agent against *S. frugiperda*. Taken together, the results obtained in each part amount to a broad overview of the situation with regard to performance as well as which questions are left to answer.

Given their central place in the endeavor, a retrospective look on the oviposition experiments conducted at CABI is apropos. More specifically on the rearing part. Some challenges were encountered. Two of the four *Spodoptera* species selected for oviposition were infertile or sick. Cannibalism and fungi taking advantage of humidity in the rearing boxes caused concern in the beginning. Finding a time to collect parasitoid cocoon after they were well established but before they hatched was not evident. Manipulation always incur some danger on parasitoids adults. Some adults were possibly slightly injured during sexing or when transferred from cages to experiment boxes. Still, rearing was very successful overall. No obvious pathogens were present in the parasitoids. Failed parasitoid pupae were only a handful, covered in an orange organism, probably a fungus. Though it was not identified, it seemed to develop after death, and not cause it.

Commercial rearing systems can provide indications of the best rearing practices (Schneider 2009). To a certain extent, parasitoid rearing is host rearing. *S. frugiperda* is already reared at an industrial scale and in certain university laboratories. The process would need to be adapted to permit *C. insularis* individuals to hatch and be collected. This is where the bulk of the work lies. Genetic diversity has been discussed but is just one component of risk mitigation. For rearing facilities even more than for experiments, the idea is to avoid possibly catastrophic difficulties. For this, they use what-if scenarios, exit strategies, emergency procedures. Rearing can have different orientations according to its end goal. Processes can optimize for purpose (in the field), quantity, qualities, age distribution or price. Quality is certainly a goal whatever the orientation. The International Organisation for Biological Control (IOBC, headquartered in Switzerland) provides guidelines for quality assurance (Schneider 2009, p290), as do the Association of Natural Biocontrol Producers (ANBP, United States) and the International Biocontrol Manufacturers Association (IBMA, Belgique). Quality control covers three scopes (Schneider 2009, p298 fig9). Production control is the monitoring of operations, procedures and equipment. Process control is concerned with process quality and the monitoring of unfinished products (eg. larval weight). Product control encompasses all measures of the final product's quality (eg. sex-ratio). The use of industry standards such as Total Quality Control is recommended, since these methods are abstract and can easily be adapted to parasitoid production. An acceptable sample size can be obtained via a formula (Schneider 2009, p297). Quality assurance is not complete without a set of procedures to remediate performance defects (Schneider 2009, p300 fig11). The produced parasitoids must be efficient (resources are spent on field fitness) and effective (maximum host-locating and parasitoidizing abilities). A performance indicator for efficiency could be size (Godfray 1994, p262). Effectiveness could be measured by the capacity to resist encapsulation. The produced parasitoids must also be genetically diverse and have a long lifespan. In a rearing system dedicated to *Trichogramma maidis*, both a stock colony and a production colony were maintained (Schneider 2009, p294 fig8). Individuals were taken from the stock colony's greenhouse, where conditions were fluctuating, and reared in a laboratory on factitious eggs for 6 generations, with constant conditions. The final generation was then stored in diapause until release.

At laboratory scales, rearing is considerably simplified. Production is not subjected to any standards or contracts, or to market dynamics (quantities, price). Quality control and working conditions can be relaxed. The number of individual targeted is much lower. The system should require very little real estate and little maintenance. Still, the experimenters should be prepared against incidents using procedures. Some observations can be made if *C. insularis* was to be reared for experiments again. Although stewardship of a colony proved exciting and extremely interesting, the number of replicates in experiments was ultimately limited by the capacity to process vials. With changes to the protocols and the right tools, production could be facilitated and scaled up.

The system can be designed with the idea of insuring against catastrophic incidents (eg. a pathogen). Evidently, a colony should be started with a big, healthy sample (Schneider 2009, p292). The insect could be fed a diet containing antipathogens. Parasitoid wasps such as *C. insularis* are fed honey, which can become unwieldy. Feeding devices can be used to keep cages clean and easy to refurbish. Wasps can eat and drink from drops or from soaked cellulose. Big expanses of honey and water are deadly because wasps get stuck in them. Any container exposing only a few drops at a time would be appropriate for honey and water. Such a feeding tower device can be made with a weighted and perforated cap resting over the liquid, which would sink only a millimeter, leaving the liquid accessible. During the experiments, honey sticks were used to disperse sources of honey inside the cages. They consisted of a plastic tab with honey smeared at one end. Wet cellulose plugs are the equivalent for water dispensal. They were placed in upside-down bottle caps. Both made refurbishing more straightforward. The distribution of individuals in different cages should be made with the aim of obtaining the best mixing of genes while under the constraints of the laboratory setting and while insuring against catastrophic events. At the very least, the sex and origin of each individual should be determined before it is assigned a cage. To gain leverage on the mixing strategy, individual tracking could be put in place. Females, which carry more alleles, and males could be assigned an identifier. This way, allele flow among cages is known at all times and adjustments can be made. Genetic diversity should be periodically reinforced through cross- or out-breeding. A PCR test should be performed periodically on sampled individuals. This will reveal symbionts such as pathogens and bacteria (eg. *Wolbachia*) that could have an influence on quality. Periodically, the genotype of individuals should be established and compared to the previous records to quantify genetic depression. Additionally, known fitness factors for the insect in question (eg. larval mortality) should be monitored. Freezing preserves DNA and can be used for sample storage. Host larvae should be reared in individual vials or in heavily furnished boxes. The processing of vials could be made quicker with a cell tray. Diet and furnishings can be deposited on the tray in advance and then the vials and larvae simply transferred. On the topic of diet, its preparation and apportion could be partly automated. During manual preparation, certain ingredients were difficult to work with. A small quantity might sometimes be left at the bottom of the pot. For these reasons, glassware should be used as well as a chemist's blender, which will be easier to clean. Although no larva went hungry during the experiments, cutting diet blocks of the exact right size was not evident. A block that is tool small will dry out much quicker, as its water content is tied to its dimension cubed (volume) while its evaporation rate is tied to its dimension squared (the number of exposed faces times the area of one face). A block that is too big can bring too much humidity depending on vial size and is simply wasteful. Therefore, a tool should be used to cut blocks of diet to the right size. Diet and processing time can be saved if parasitoidism is detected early. For *C. insularis* reared on *S. frugiperda*, the difference in size between parasitoidized and non-parasitoidized larvae can vary. Weighting and measuring larvae daily would provide sufficient data to find a soft threshold. If the weight of the diet is known, a whole tray can be weighted and the average weight gain of larvae obtained. With many trays, the number of parasitoidized individuals at the end of rearing allows deriving the average weight of one parasitoidized larva over time. Cleaning should also be automated as much as possible. The cleaning system should be inside the quarantine for reasons of logistics. Steam should be used as cleaning media. Steam is cheaper than alcohol and can be dispensed at high pressure in one step. In fact, industrial facilities use steam for cleaning.

The improvements proposed here have a reasonable cost and their benefit to experimenters are clear. More advanced techniques such as artificial eggs and secondary hosts were left out of these recommendations. Artificial eggs have been described above. Secondary host species are useful for rearing at a lower cost, though possible specialization of the parasitoid is difficult to estimate in this case. In the laboratory, parasitoid eggs can even be manually moved to secondary hosts (Heimpel & Mills 2017, p125) so as not influence host choice. Such advanced methods, given their cost, should be reserved for solving specific problems.

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Annex

This thesis and all original content can be consulted and downloaded at

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Acknowledgements

The present work concludes three years at HAFL. This small contribution to biological control research was also rewarding. My time at CABI remains in my memory as the most enjoyable learning experience I've ever had. In addition to previously unsuspected scientific knowledge, a few general lessons were learned. Diving into theory while the experiments are running is really motivating. So many questions arise while working that it's really worth preparing a number of small experiments to run on the side, while everything is already set up. I would like to thank Lindsey for sharing this opportunity with the Master's students, for her support and for her patience. I would to thank Marc for sharing his knowledge, setting up the experiments and ensuring that they ran smoothly. The CABI staff's openness and helpfulness also deserves mentioning. I am grateful to Phillip for introducing me to the staff, showing me around the premises and organizing great barbecues, Nataly for teaching me how to rear insects, Lukas for support in statistics, Baptiste for showing me how to use the lab and directing me to the right resources and materials, and finally everyone else for being so friendly. I hope further evaluations of *C. insularis*, at CABI or somewhere else, can use these results profitably.