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Refining the implementation of arthropod classical biological control

Klassische biologische Schädlingsbekämpfung: Erfahrungen und neue Entwicklungen für die Praxis

Abstract

The last few decades have been turbulent for the reputation and practice of arthropod classical biological control. Public and scientific support for this pest management approach has risen, fallen and appears to be rising again. Due to reports in the 1980s and 1990s of negative environmental effects caused by classical biological control agents, practitioners have scrambled to develop better risk assessment measures. Since the mid-1990s, several national and international guidelines have been created that assign responsibilities to the players involved in selection and release of exotic beneficial insects. At the same time and on a more technical level, researchers have been working to develop standardised procedures for the risk assessment of proposed classical biological control agents. The lack of well-established protocols (like those that exist for weed biological control) has been a great impediment to the implementation of meaningful risk assessments. Along with other research groups, the biological control scientists at CABI Europe-Switzerland are using current projects to tackle problems associated with estimating agent host specificity and risk assessment. In particular, the arthropod classical biological control team is working on key host range testing issues including methods for selection of non-target species, design and implementation of host specificity experiments, and extrapolation of laboratory results to a field context.

Key words: Host range, risk assessment, leek moth, agent selection

Zusammenfassung

In den letzten zwei Jahrzehnten ist die Anwendung von klassischer biologischer Schädlingsbekämpfung in Verruf geraten. Zurzeit erfährt dieser Ansatz allerdings wieder wachsenden Zuspruch. Durch Berichte über negative Auswirkungen von Nützlingen, eingesetzt in der klassischen biologischen Schädlingsbekämpfung, auf ihre Umwelt, waren Wissenschaftler gezwungen, neue Verfahren zu entwickeln, um eventuelle Risiken besser abschätzen zu können. Seit Mitte der neunziger Jahre wurden daher zahlreiche nationale und internationale Richtlinien entwickelt, um den an der Auswahl und Freilassung von exotischen Nützlingen beteiligten Personen die jeweiligen Pflichten aufzuzeigen. Zur gleichen Zeit begannen Wissenschaftler praxisnahe, standardisierte Verfahren zur Risikoabschätzung von Nützlingen zu entwickeln. Der Mangel an im praktischen Einsatz erprobten Verfahren, wie sie zum Beispiel für die klassische biologische Bekämpfung von Unkräutern bereits existieren, stellt nach wie vor ein großes Hindernis für eine sinnvolle Risikoabschätzung von Nutzarthropoden dar. In Zusammenarbeit mit anderen Forschungsgruppen nutzen die Mitarbeiter von CABI Europe-Switzerland laufende Forschungsprojekte, um Probleme bei der Abschätzung der Wirtsspezifität von Nützlingen und den damit verbundenen Risiken zu lösen. Besonders die Arbeitsgruppe zur klassischen biologischen Bekämpfung von Arthropoden richtet ihr Hauptaugenmerk auf die Entwicklung von Laborverfahren zur Abschätzung der Wirtsspezifität von Nützlingen und deren Aussagekraft im Vergleich mit Freilanduntersuchungen.

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Arthropod Classical Biological Control under Fire

Classical biological control is a process in which exotic natural enemies are introduced to control invasive alien pests. It is often more feasible, more efficient and less environmentally damaging than alternative strategies, such as the use of pesticides or large-scale habitat manipulation. Classical biological control works on the philosophy that the high density of many invasive species is caused by their separation from the natural enemies that controlled them in their native ranges. Therefore, in most cases, the beneficial agents come from the area of origin of the pest and have an evolutionary association with the pest. This pest management approach typically targets weed and arthropod pests, but has even been used against vertebrates and molluscs. This paper, however, will deal solely with classical biological control of arthropods.

Since the historic first releases of the vedalia beetle (*Rodolia cardinalis*) against cottony cushion scale (*Icerya purchase*) in Californian citrus orchards in 1888, this method of arthropod pest management has had widespread use. Over the last 120 years, more than 2000 species of entomophagous agents have been used in classical biological control. Against the backdrop of severe environmental and human poisoning from synthetic insecticides in the 1960s and 1970s, biological control was considered an environmentally friendly and "green" technology. Because there was no evidence of negative effects on wildlife, little thought was given to risk assessment or risk mitigation.

However, the scientific and public image of biological control began to change in the 1980 s with the publication of reports indicating that certain introduced agents were having negative impacts on native non-target species (HOWARTH, 1983, 1991). In retrospect, it is interesting that this should have come as a surprise, given that the potential for non-target impacts had long been recognised in weed biological control. Phytophagous classical biological control agents were already regularly undergoing risk assessments that were intended to minimise non-target effects. The key difference was that native and economically important plants were highly valued whereas people were less sympathetic towards poorly understood non-target arthropods. It was primarily due to a growing interest in biodiversity that people became more concerned about non-target arthropods.

The growing criticisms of classical biological control led practitioners to re-evaluate their agent selection process. By the mid-1990s, national and international policies began to be developed and adopted by numerous countries in an attempt to ensure responsible practice. One notable document was the Code of Conduct for the Import and Release of Exotic Biological Control Agents (International Standards for Phytosanitary Measures No. 3). This guideline helped to clarify the responsibilities of parties involved in the shipping, receiving and releasing of biological control agents. However, like similar documents that were produced at the time, it indicated what information was required, but did not specify effective methodologies to obtain that information. For instance, there were no explanations available on *how* to select agents in a responsible and standardised way. This lack of robust methodologies to minimise risks associated with agent introductions has become one of the biggest topics in arthropod classical biological control. Some of the key issues for which protocols are currently being developed include: (1) selecting non-targets for testing, (2) running host specificity experiments, (3) interpreting laboratory results and extrapolate them to field situations and (4) conducting risk-benefit analyses.

Restoring Arthropod Classical Biological Control as a Green Alternative

With its long history of involvement in weed and arthropod classical biological control, CABI has continuously employed practitioners over the turbulent decades which saw the rise, fall and recent renewal of public faith in biological control. In particular, researchers at CABI Europe-Switzerland have had first-hand experience with these issues and are currently working to develop needed methodologies for the selection of safe and effective agents. One of the most significant and recent contributions, in close collaboration with Agroscope, FAL Reckenholz, Switzerland, has been the production of a book discussing methodologies for risk assessment (BIGLER et al., 2006). This book, written by a large team of internationally recognised biological control experts, describes emerging methods for things such as determination of agent specificity, molecular-based species identification and evaluation of post-release establishment, dispersal and impact.

Along with other research institutions in Europe, CABI has also been engaged in shaping European policy on the regulation of exotic agents. Europe lags far behind Australia, New Zealand and North America in terms of implementing regulatory procedures for the import and release of classical biological control agents. For example, by 2000, very few European countries were regulating the release of beneficial arthropods. It has therefore been the Europe Union's priority to formulate a regulatory system that will be readily approved of and adopted by all member countries. Through an EU-funded policy support action called 'Regulation of Biological Control Agents' (REBECA), two reports were compiled comparing regulatory frameworks among European countries (LOOMANS, 2007) and those in Australia, New Zealand, Canada and the USA (HUNT et al., 2008). This project identified strengths and weaknesses in the different systems and offered suggestions for a European system. Furthermore the working group prepared specific guidelines on how to conduct environmental risk assessments and submit the resulting information to national authorities for permission to release new agents (http://www.rebeca-net.de/).

Besides the publication of guidance documents, CABI is heavily involved in several on-going weed and arthropod classical biological control programmes. The agricultural pest research group focuses primarily on Palaearctic crop pests that are invasive aliens in North America. Recent projects include pest species such as cabbage seedpod weevil (*Ceutorhynchus obstrictus*), cabbage root maggot (*Delia radicum*), swede midge (*Contarinia nasturtii*), leek moth (*Acrolepiopsis assectella*), tarnished plant bugs (*Lygus* spp.) and cherry bark tortrix (*Enarmonia formosana*). One exception to this list is the western corn rootworm (*Diabrotica virgifera virgifera*) which is a new world pest that is highly invasive in many parts of Europe. However, in all cases, the group conducts research to identify and evaluate candidate classical biological control agents for these various pests. This work generally begins with the initial exploration for and cataloguing of natural enemies, particularly parasitoids since these are considered to pose fewer risks to non-target species. Once one or a small number of "most promising" natural enemies are identified, studies are conducted to fill any important knowledge gaps concerning their biology and ecology.

Currently, issues regarding agent risk assessment are of particular interest and the research focuses largely on exploring different methods for measuring host range. One of the first hurdles to determining host specificity for entomophagous agents is deciding which non-target species to include in tests. This test list is an important part of the process as it could have a significant impact on the host range data obtained. In contrast to the assessment of phytophagous agents, for which a large proportion of the non-target species may be tested, the selection of non-targets for assessing entomophagous agents is complicated by several factors: (1) arthropod species far outnumber plant species in most ecosystems, (2) arthropod phylogenies are often poorly understood, (3) entomophagous agents respond to two trophic levels (the host and its host plant), (4) disjunct host ranges appear to be more common among entomophagous than phytophagous agents and (5) it is often much more difficult to obtain and rear large numbers of non-target arthropod species than plant species. In order to develop schemes that simplify the process of non-target selection, CABI is working alongside other research groups to determine which types of non-target characteristics (e.g. phylogenetic affinity to target or ecological similarity to target) are better predictors of suitability for the agent.

Once a non-target test list is generated and validated, it is vital to design appropriate tests for the suitability of non-targets for the agent. This next step can be even more daunting than preparing the test list because a number of features of the experimental set-up can influence the behaviour of the test insects. Very basic, but important, decisions include whether to run trials in large or small arenas, to include the bottom trophic level (plants) or not, to run trials over short or long time intervals, and to run choice or no-choice tests. On a finer scale, details such as parasitoid and host densities, temperature, and previous experience and physiological state of parasitoids may also influence foraging decisions and lead to interactions that are unlikely to occur in nature. At this point in time, there is no universal set of host range tests that suits all entomophagous agents. The idiosyncratic nature of parasitoid life histories means that a degree of customisation will almost inevitably be required. Nonetheless, foraging and life history theory can help to describe general patterns in behaviour and stimulus response as well as to identify those taxonomic groups to which the generalisations hold true. By conducting comparative studies on host acceptance under varying experimental conditions, CABI is hoping to help clarify which types of factors can influence host range test results and, ultimately, risk assessment conclusions.

Finally, when data are obtained from laboratory-based host specificity trials, they must be interpreted in such a way that makes sense under natural conditions. It occurs commonly that parasitoids will attack non-target hosts under laboratory conditions that they do not attack in nature. This is particularly true in no-choice tests where the agent is confined for long periods with the host. So what does it mean if the proposed agent parasitised a non-target species in 4 of 100 trials? Will there be 4% parasitism of that non-target in the field? Almost certainly not; however, this extrapolation from laboratory to field is still in its infancy and needs further development. Through various projects, organisations like CABI have begun to tackle these kinds of questions by obtaining and comparing field and laboratory data. Thus far, it appears that laboratory host range tests will typically, and sometimes grossly, overestimate an agent's host range.

A Recent Success in the Battle against Leek Moth

Although not yet complete, the classical biological control programme against leek moth can, in a sense, already be considered a success story because a classical biological control agent was made available in only five and a half years (from exploration for potential agents until permission to release an agent). This was accomplished despite heavy data requirements needed to obtain a release permit for Canada, where leek moth is invasive. Below is a description of the work conducted to prepare a classical biological control agent for release.

The leek moth is a pest of *Allium* crops (leek, onion, garlic, chives). The larvae mine the stems, leaves and flower heads, but can even attack the bulb if all green material is consumed. It is a Palaearctic species common throughout Europe, but was identified in Canada in 1993. The first records were from private gardens in the capitol city of Ottawa (southeastern Canada). The pest did not become a significant problem until 2000, when organic farmers in the area began noticing damage to their *Allium* crops. At this time, monitoring of the pest was initiated. Leek moth showed only minimal dispersal for several years but has more recently appeared in south-central Quebec and southern Ontario towards Toronto. Because leek moth was not known to exist in the USA, it was considered to be a quarantine pest in that country. The result is that trade restrictions have been placed on all fresh *Allium* products imported into the USA from Canada. Only material that has been certified as "clean" is permitted to cross the border into the USA. As this is a nationwide restriction, it applies not only in the east where leek moth is present, but all the way to the Pacific coast, 2500 km away. Thus, leek moth is having a double-impact on *Allium* growers: (1) direct losses in yield and (2) lost sales through delays getting produce certified before it can be shipped to the USA. While the *Allium* industry is not Canada's largest, it is significant for many farmers, with the export value of fresh *Allium* produce exceeding CAN $40 million in 2006. Coincidentally, the region of Canada in which leek moth occurs is also the source of approximately 85% of the material exported to the USA.

Field surveys in the affected areas of Canada showed high infestation rates and no evidence of suppression by indigenous or exotic natural enemies. Classical biological control was considered to be an important part of an integrated pest management approach for dealing with leek moth in Canada. Field surveys were initiated in central Europe to obtain, identify and rear parasitoids of leek moth. At the same time, a life table study revealed that natural enemies contributed most to leek moth mortality in the pupal stage (Jenner et al., 2010). Of the parasitoid species identified, *Diadromus pulchellus* (Ichneumonidae) was considered the most promising candidate. Not only was this pupal parasitoid believed to have the narrowest host range, it was the species most often reared from leek moth, it was the only one attacking leek moth in all three host generations and it is a close relative of *Diadromus collaris*, which has been used in several classical biological control campaigns against diamondback moth (*Plutella xylostella*).

Once *D. pulchellus* was chosen for further evaluation, studies were conducted to estimate the likely efficacy and risk of the proposed agent if it were released in Canada. Experiments on the cold hardiness of *D. pulchellus* demonstrated that this agent should survive winters in the targeted release areas of Ontario and Quebec. In addition, field trials conducted in Europe showed that mass-released parasitoids can significantly reduce leek moth survival in infested leek plots. The final, and arguably most important, set of experiments were carried out to investigate the agent's host specificity. In the process of conducting this study, a secondary question was investigated: Could changes in parasitoid state or environmental conditions alter host acceptance behaviour to the extent that it would affect host range expression? Preliminary experiments had shown that variation in parasitoid age (3-day-old versus 10-day-old) caused differences in the "motivation to oviposit" (i.e. parasitism rate) in the target host, leek moth. Parasitoids of these two ages were thus used in the host range trials to test for an age effect on acceptance of non-target hosts.

The non-target test list was compiled with the assistance of a Canadian taxonomist who specialises in microlepidoptera. Initially, the list consisted of 24 non-target species, each of which met one or more of the following criteria: (1) phylogenetic affinity to target pest, (2) ecological similarity to target, (3), safeguard species (i.e., beneficial or rare), (4) morphological similarity to target and (5) known host of another *Diadromus* sp. parasitoid. As host range trials progressed, it was possible to omit certain non-target test species belonging to taxonomic or ecological groups that were previously represented by another non-target and that were highly unlikely to be suitable hosts based on earlier results. Certain species were not tested due to difficulty in obtaining sufficient live specimens to test or start a culture. The final test list consisted of 12 non-target species of varying phylogenetic and ecological relatedness to leek moth.

These non-targets were offered to both 3- and 10-day-old parasitoids in a series of no-choice and choice oviposition trials. Only the three non-targets most closely related to leek moth (*Acrolepiopsis incertella*, *Plutella porrectella*, and *Plutella xylostella*) were suitable for *D. pulchellus* development. To assess whether attack of these non-targets was an artefact of the no-choice experimental design, several choice tests were run in which leek moth and *P. xylostella* were presented simultaneously to *D. pulchellus*. Although there was a clear preference for leek moth, the non-target was still occasionally attacked. This was a striking result given that a comprehensive literature search and discussions with European ichneumonid specialists had failed to identify any field records of *D. pulchellus* from hosts other than leek moth in its native range. If *D. pulchellus* commonly attacked *P. xylostella* in its native range, then this host association should have been reported among the immense number of European field studies on this major crop pest. As a final test of the association between *D. pulchellus* and *P. xylostella*, parasitoids were mass-released into a field site where *P. xylostella*-infested cabbage plants were planted among rows of leeks infested with leek moth. Parasitism of the target and non-target species was assessed by recollecting and rearing the pupae that had been exposed in the field. There was 62% parasitism of leek moth pupae collected from leeks. In stark contrast, none of the recollected *P. xylostella* pupae showed signs of parasitism by *D. pulchellus*. Moreover, several leek moth pupae had been artificially attached to the cabbage plants as an additional test of *D. pulchellus* foraging behaviour. None of these leek moth pupae was parasitised, suggesting that the parasitoids spent very little or no time foraging on the cabbage plants, despite their close proximity to the leeks. The outcome of this field trial indicates that although *D. pulchellus* can develop in hosts that are closely related to leek moth, it is quite unlikely to encounter them under natural conditions due to its foraging strategy, which relies on various habitat- and host-specific stimuli.

In the laboratory trials, younger *D. pulchellus* females had previously demonstrated greater motivation to oviposit in leek moth and were, therefore, predicted to express a broader host range than older females. During non-target testing, parasitoid age had a minor effect on host range expression that was contrary to expectations. Older females more readily attacked one of the non-target species in no-choice tests and inflicted higher mortality in one of the choice tests with *P. xylostella*. Ultimately however, young and old parasitoids still successfully parasitised the same three non-target species. There was an interaction between the effects of parasitoid condition and experimental design on responsiveness to low-ranked hosts: increasing non-target density in choice tests significantly altered attack rates by 10-day-old, but not by 3-day-old, parasitoids. These findings indicate that experimental design and the condition of the agent can influence test results. However, as the effects were quite minor, adequate replication of specificity trials is probably the most effective way to overcome misleading results.

The combined results from the studies described above create a positive case for the use of *D. pulchellus* as a classical biological control agent against leek moth. A petition for the import and release of *D. pulchellus* into Canada was therefore drafted and submitted for evaluation. This petition contained a summary of all information considered relevant for deciding whether to permit or reject the introduction of an exotic biological control agent into Canada. The dossier was submitted to the Import Office of the Canadian Food Inspection Agency (CFIA). A Biological Control Review Committee was then organised to provide feedback on the petition. The petition review committee typically consists of taxonomists in entomology and botany, ecologists, scientists and/or specialists within the federal and provincial governments and Canadian universities. The conclusions of this peer-review were transferred to regulatory entomologists within CFIA, who then made a recommendation to the Director of the Plant Health Division of CFIA (CFIA-PHD). Based on this information, the Director of CFIA-PHD informed the petitioners that authorisation was granted for the import and release of *D. pulchellus*.

Although releases of *D. pulchellus* in Canada have not actually started yet, this programme has been a success in terms of outputs over time. It has demonstrated how classical biological control agents can be identified and evaluated over a relatively short period of time. The ease with which assessments of candidate agents can be implemented depends largely on the availability of efficient methodologies. The efforts over the last 15 years to develop harmonised host range testing protocols have contributed significantly, and continue to contribute, to the efficient operation of arthropod classical biological control programmes. Thus, despite a reduction in the rate of agent introductions during recent decades, there is significant progress being made. Projects like the leek moth example are perfect opportunities to test emerging methodologies to make agent selection more scientific and risk averse.

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