Figure 1. A parasitized *Popillia japonica* collected from a trap after the release of *I. aldrichi* in Port Coquitlam, British Columbia.

Istocheta aldrichi (Mesnil) makes its biological control debut in British Columbia, Canada

by Victoria Makovetski[1,2] and Paul K. Abram[2]

[1]*University of Victoria, Department of Biology, Victoria, BC, Canada*
[2]*Agriculture and Agri-Food Canada, Agassiz Research and Development Centre, Agassiz, BC, Canada*
E-mails: vi2001ka@gmail.com, paul.abram@agr.gc.ca

Introduction

Western North America was free of the invasive Japanese beetle, *Popillia japonica*, for 100 years after the species was first accidentally introduced to the eastern part of the continent. Now there is a coordinated effort to stamp out new populations of this agricultural and ornamental pest in British Columbia. In 2023, the first experimental releases of a tachinid fly in British Columbia (BC) were done in support of this goal.

The effort to eradicate *P. japonica* from BC began in 2017, when the beetle was discovered in a localized area of downtown Vancouver. The eradication program has since grown to involve cooperation between municipalities, the provincial government, and the federal government (the Canadian Food Inspection Agency, CFIA). The eradication strategy involves an extensive trapping network for surveillance (5191 traps in 2023!), soil movement restrictions, and the widespread application of a larvicide to turf grass in areas where the beetle is detected. Thus far, this initiative has greatly reduced the population of this pest within Vancouver. However, *P. japonica* has spread into two neighboring cities, where smaller populations still persist. Two states in western United States, Washington and Oregon, are also attempting to eradicate recently detected populations of this pest. We became interested in whether biological control could be added to existing eradication strategies to further reduce beetle populations.

The first explorations into biological control options for *P. japonica* started more than a century ago, soon after it was first detected in North America in New Jersey in 1916. A team from the United States Department of Agriculture surveyed *P. japonica* in its native country of Japan and found various natural enemies attacking the beetle (Clausen et al. 1927). Among these, one species – the tachinid fly *Istocheta aldrichi* (Mesnil) – stood out for its impressive ability to parasitize *P. japonica*, particularly when the beetle populations were relatively low.

Like its host, *I. aldrichi* has a single generation per year, with adult flies typically emerging in late June in Japan (Clausen et al. 1927, Clausen et al. 1933). Mated females lay conspicuous, milky-white eggs on the beetle's pronotum. Female beetles are more often parasitized than males (Clausen et al. 1927, Legault et al. 2023). After the egg is laid, the fly larva enters the host's body within 24 hours by burrowing downwards through both the underside of the egg and the beetle's pronotum. Once inside, it feeds on the internal tissues and molts twice to become a mature third instar larva. The beetle falls to the ground soon after it is parasitized, buries itself in the soil, and dies within approximately five days. The tachinid larva completes development and enters the pupal stage. The puparium enters a diapause that lasts for eight months. In Japan, the adult fly emerges in late June, just before its host emerges. *Istocheta aldrichi* appears to be specialized on *P. japonica*, as it has not been recorded parasitizing any other insect species in Asia or North America (Fleming 1968, Arnaud 1978).

Following the field investigations of 1921–1923 (Clausen et al. 1927), *I. aldrichi* was released on multiple occasions in several places in eastern United States where *P. japonica* populations were very high. The fly became established, though recorded parasitism rates after releases were relatively low (<10%; Fleming 1968, Cappaert & Smitley 2002). Over the course of the past century, *P. japonica* has extended its range into neighboring states and southeastern Canada. Interestingly, *I. aldrichi* has accompanied *P. japonica* populations northward, including Ontario (O'Hara 2014) and Quebec (Gagnon et al. 2019), and to a lesser extent southward, as the beetle's range expanded. In sampled sites within Quebec, parasitism rates in *P. japonica* by *I. aldrichi* ranged from 3.9% to 27.3% (Gagnon et al. 2023), suggesting that the tachinid may be providing some level of helpful biological control.

By experimentally introducing the fly to Vancouver, BC for the first time in 2023, we explored the potential for incorporating this biological control agent into a comprehensive eradication strategy while pest populations were low. This is not typical; biological control releases are typically done when pest populations have already spread extensively and have reached high densities. This provided us with a unique opportunity to study what happens when a biological control agent is released pro-actively into a not-well-established, low-density pest population.

Building support for conducting experimental releases

Moving biological control agents from Ontario to BC is not regulated by the federal or provincial government, but we nonetheless wanted to practise due diligence before doing the first releases of *I. aldrichi* in BC. We developed a detailed proposal outlining the background scientific information about the pest issue, goals of the releases, the biology and host specificity of *I. aldrichi*, and plans for post-release monitoring. This document was reviewed by a variety of pest management, entomology, and conservation experts from multiple agencies, coordinated by the British Columbia Plant Protection Advisory Council (BCPPAC). These reviews supported the releases. Similarly, the municipalities of Vancouver, Burnaby, and Port Coquitlam, where *P. japonica* populations were known to be present, were consulted and they supported the use of public parks to conduct these experimental releases. We had the 'green light' to proceed!

Collecting parasitized beetles and rearing out *Istocheta*

In the meantime, we needed to develop practical ways of collecting parasitized beetles and rearing out the tachinid. We collected more than 10,000 parasitized beetles from areas of greater Montreal and Ottawa in 2022, in collaboration with colleagues from Agriculture and Agri-Food Canada (the Ottawa and Saint-Jean-sur Richelieu Research and Development Centres) and the Université de Montréal. The beetles were collected using a combination of commercially available traps and semiochemical attractants, and by hand-picking beetles off their host plants. Then it was time to sort out the parasitized beetles – this involved going through many trays of beetles by hand and picking out the ones with clearly visible *I. aldrichi* eggs on their pronota. These were placed in groups on top of soil with some plant material for the beetles to feed on as they succumbed to parasitism and buried themselves in the soil. The beetle 'cadavers' containing parasitoid puparia were then shipped to our research centre in Agassiz, BC and dissected. About 56% of the cadavers, on average, contained *I. aldrichi* puparia, suggesting that some proportion of parasitism fails after eggs are laid on beetles.

Figure 2. A. Agriculture and Agri-Food Canada technicians Yonathan Uriel [left] and Jason Theissen [right] digging "crypt" in Agassiz, BC for burying overwintering *Istocheta aldrichi* puparia. **B.** Balls of *I. aldrichi* in "crypt".

Getting parasitoid puparia through the winter

Next, we needed to figure out how to successfully overwinter the parasitized puparia. We wanted to know whether the mild winter climate of south coastal BC (relative to Ontario and Quebec) would be cold enough to allow the flies to complete their diapause. We also wanted to see what the best conditions were to keep them in after overwintering, and when they would emerge in the spring in BC if kept outdoors.

We buried half of the puparia in a shallow "crypt" outdoors at our research centre in Agassiz, BC (Fig. 2), and kept half of them in a refrigerated chamber indoors at 5°C (Fig. 3; these were dissected incrementally). We then experimented with different temperature treatments during the spring, including keeping them in the soil outdoors or warming them up gradually in incubators indoors. No matter what the overwintering or spring temperature treatments, about half the puparia survived and produced adult flies. The timing of fly emergence depended strongly on the spring temperature treatment – but most interestingly, the flies that were kept at BC soil temperatures (whether indoors or outdoors) emerged just a few days before the first *P. japonica* was caught by the CFIA in BC in 2023.

These results were encouraging as they indicated to us that if *I. aldrichi* were to establish in BC, it would be able to successfully overwinter and emerge at the right time to parasitize *P. japonica*.

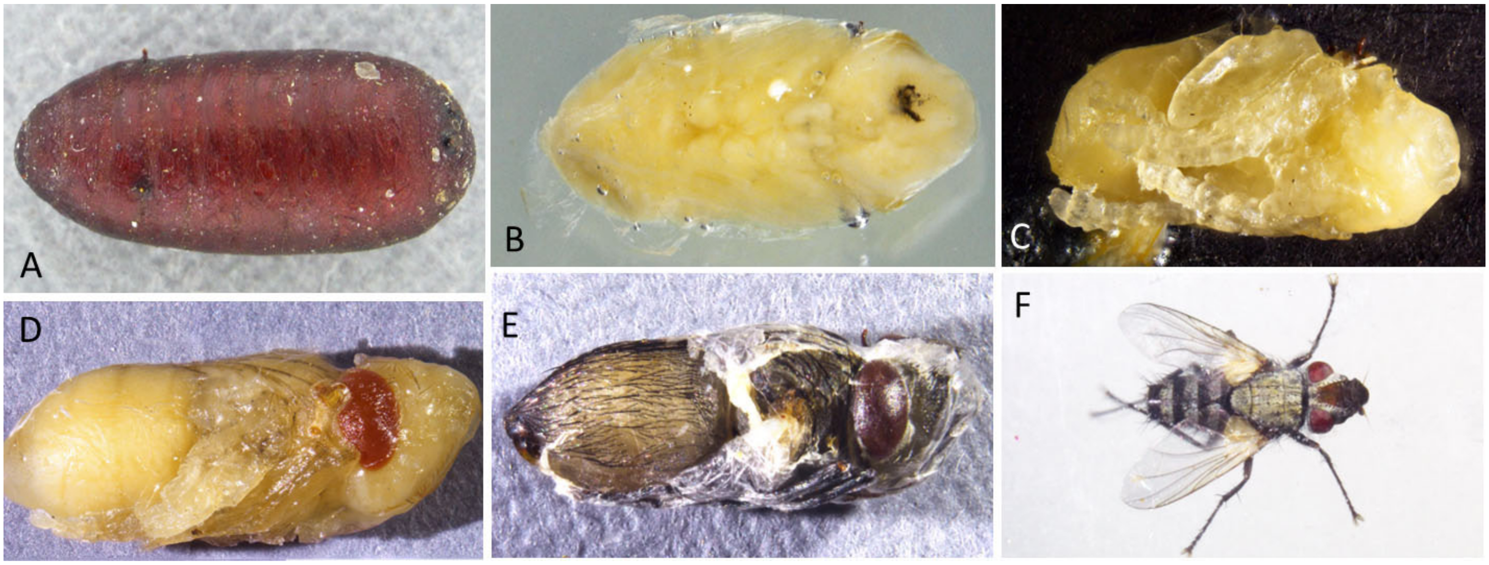

Figure 3. The development of *Istocheta aldrichi* during diapause. **A.** Puparium. **B.** Pre-pupa, segments beginning to differentiate. **C.** Dissected-out pre-pupa, segments continue to differentiate. **D.** Dissected-out pre-pupa, segments are distinguishable, eyes and hairs beginning to develop. **E.** Dissected-out adult fly nearing the end of its development, emergence soon to occur. **F.** The emerged adult fly.

Keeping the adult tachinids alive between emergence and release

As the first adult tachinids emerged, we had to find out what to feed them. First, we offered them sucrose, honey, bee pollen, and royal jelly (separately) to see which diet would keep them alive the longest. Each food source was mixed with water, making a concentration mimicking that of flowers and other natural nutrition sources. We recorded the lifespan of flies in days for each trial. Two treatments stood out as resulting in the longest-lived flies: those that were fed honey, and those that were fed pollen. These findings guided us in designing the next set of tests.

We next presented honey and pollen separately, as well as a combination of honey and pollen to see if the combined effect extended lifespan beyond that of honey or pollen alone. We also compared the lifespans of males and females. We did these tests at both warmer and cooler temperatures, expecting cooler temperatures to extend the lifespan of the flies (Fig 4).

We found that the tachinids lived the longest when fed just honey when they were kept in cooler conditions. We also found that males and females lived for about the same amount of time. Contrary to our expectations, the combination treatment of honey and pollen did not lead to a longer lifespan compared to each treatment individually. Flies that were fed honey alone, and kept in cooler conditions, lived (on average) for 18.3 days (the longest-lived fly was a female that made it to 43 days).

Based on the results of these trials, flies that emerged for the purpose of releases were held in cooler incubators and fed honey. The flies that were allocated for the field releases were counted as they emerged and kept together in a large net-mesh cage. As the first scheduled release date


Figure 4. For feeding trials, adult *I. aldrichi* were kept in mesh lid containers with a moistened Kimwipe and 2 cotton vial plugs: one for water and one for food treatment. The fly pictured is being fed with liquid honey.

approached, there was concern about the mating status of the female flies, as tachinid flies can be notoriously difficult to rear (Zhang et al. 2003, Plowes et al. 2012, Dindo & Grenier 2014). We monitored the flies closely for some time but did not see any mating activity. However, one day before the first release, mating among the caged *I. aldrichi* was finally observed. This observation raised our confidence in their ability to produce fertilized eggs and parasitize the beetles once released.

Identifying when and where to perform releases

Now that we had viable adult flies, we needed to know where best to release them. The incredible surveillance network of *P. japonica* traps set up by the CFIA, and the fact that the traps were checked frequently and the results posted to a real-time GIS application that we had access to, proved invaluable. We closely coordinated with the very helpful team at CFIA on a weekly basis to identify areas where they were catching beetles, so we could allocate our released flies to those areas. In the end, the vast majority of *P. japonica* were caught in Port Coquitlam in 2023, so most of the flies we released (i.e., 774 of a total of 801) were released there in a public park.

On the first day of releases, all the folks involved in coordinating the releases got together for a release and "ribbon-cutting" (Fig. 5). It was, in a way, a strange biological control release: despite *P. japonica* having recently been detected in CFIA's traps in the vicinity of the park where we were doing releases, the beetle populations were at such low densities that it was impossible for human observers to find them on vegetation in the area. So, we were releasing flies into a park where we couldn't see any of their hosts – not a typical situation for biological control researchers or practitioners! It was hard to know if we would see any results of these releases – we just had to wait and see what turned up in the traps.

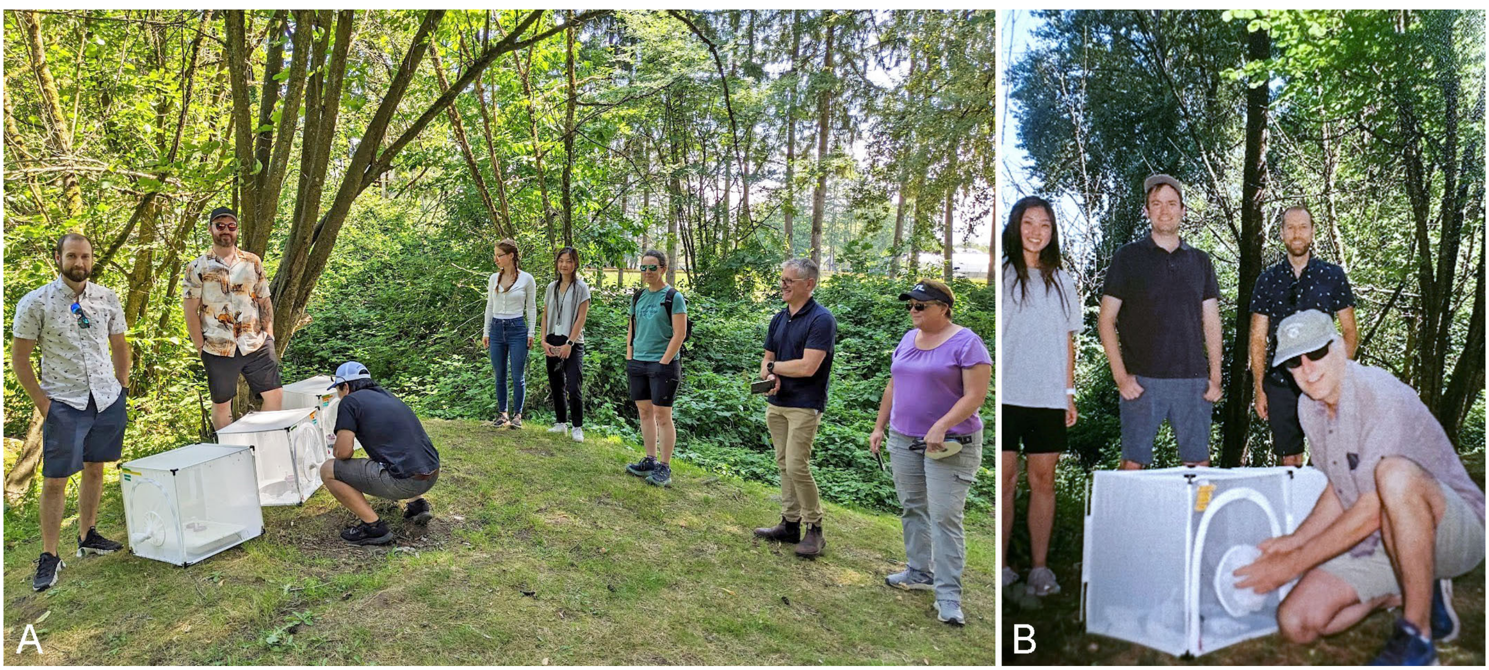

Figure 5. A. Preparing for the experimental releases of *Istocheta aldrichi* in Port Coquitlam, British Columbia, in 2023. Representatives of the city of Port Coquitlam, Canadian Food Inspection Agency, Agriculture and Agri-Food Canada, the Université de Montréal, and the province of British Columbia are pictured. **B.** Releasing the flies!

Monitoring for parasitism after releases

After a few days passed, more *P. japonica* were caught and reported in traps. We checked the database, sometimes 3–4 times a day, in high anticipation of signs of *I. aldrichi* presence. Amazingly, only 12 days after our first releases, a parasitized *P. japonica* was caught! After this first parasitism, more and more beetles were caught with the distinctive white tachinid eggs on their pronota. A total of 24 *P. japonica* were observed carrying one or more eggs (Fig. 1). These parasitized beetles were captured across seven different traps, all of them close to our release site. Because no parasitized beetles had ever been trapped in previous years or in locations farther away from our 2023 release sites, we were confident that these beetles were parasitized by our released flies. Remarkably, the parasitized beetles were found, on average, approximately 337 meters away from the release site, with the farthest capture occurring at an impressive distance of 470 meters. This meant that not only could the flies identify and parasitize *P. japonica*, but they could find them at very low densities and over some distance, and survive in BC's coastal climate.

Moving forward

We are eagerly anticipating the 2024 field season to see what another year of *I. aldrichi* experimental releases might bring: we have about another 5,000 fly puparia overwintering at the time of writing. We will be particularly interested to see whether the first *P. japonica* caught by traps in 2024, before we do any more *I. aldrichi* releases, show any evidence of parasitism – this would mean that parasitism took place because of our releases the previous field season and resulted in successful overwintering and emergence in the field.

Over the longer term, the role of *I. aldrichi* in providing biological control of *P. japonica* in BC will depend on how the eradication effort goes. If *P. japonica* is eradicated from BC, the fly will have helped to take out a few additional beetles that were not killed by other control measures along the way. If the beetle is not eradicated, then it is possible that our releases could result in the establishment of an *I. aldrichi* population that could spread geographically along with that of its host, hopefully "taking the edge off" the economic and environmental damage the beetle is expected to cause. We are developing mathematical modeling approaches to better understand the true level of Japanese beetle biological control provided by *I. aldrichi* so we can better track the impact of this parasitoid in the future, in BC and elsewhere.

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