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Spill-over attack by the gall fly, *Urophora stylata*, on congeners of its target weed, *Cirsium vulgare*

Michael Cripps[1]*, Jovesa Navukula[2], Benjamin Kaltenbach[1], Chikako van Koten[1], Seona Casonato[2], Hugh Gourlay[3]

[1]*AgResearch, Lincoln Science Centre, Private Bag 4749, Lincoln, New Zealand*
[2]*Lincoln University, Pest Management and Conservation Department, PO Box 85084, Lincoln, New Zealand*
[3]*Manaaki Whenua Landcare Research, PO Box 69040, Lincoln, New Zealand*

**Corresponding author: mike.cripps@agresearch.co.nz*
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Abstract The gall fly, *Urophora stylata*, was released in New Zealand in 1998 as a biocontrol agent against the thistle weed, *Cirsium vulgare* (Scotch thistle). In the summer of 2018, a survey was conducted to assess the field host range of the biocontrol agent in New Zealand. A random selection of 18 pasture populations under sheep and/or beef production, where *C. vulgare* was present, was surveyed to quantify the attack intensity (gall size relative to seedhead size) on *C. vulgare*, and the attack rate on other thistle weeds within the same population. At each location, seedheads were collected from *C. vulgare* and all other thistle species (Cardueae) present, which included *Cirsium arvense* (Californian thistle), *Cirsium palustre* (marsh thistle), *Carduus nutans* (nodding thistle), and an *Arctium* species (burdock). In addition to *Cirsium vulgare*, the gall fly was recorded on *C. arvense* (six locations) and *C. palustre* (one location). The probability of attack on *C. arvense* was positively correlated with attack intensity on *C. vulgare*, suggesting that attack on *C. arvense* is a 'spill-over effect' occurring where seedheads of *C. vulgare* are in limited supply.

Keywords host range, host specificity, non-target attack

INTRODUCTION

Post-release monitoring is an important part of classical biocontrol programmes. Monitoring is essential to determine the effectiveness of biocontrol agents, and the benefits of biocontrol programmes (Morin et al. 2009; Blossey 2016). It is also important to assess the safety of biocontrol agents to non-target plants including native species and crop plants of economic importance. Systematic surveys designed to assess attack on non-target plants are uncommon globally (Hinz et al. 2019) but New Zealand stands out as an exception, with several thorough assessments of non-target attack on valued native and economic plant species (Fowler et al. 2000; Paynter et al. 2004; Waipara et al. 2009). Results of non-target attack assessments in New Zealand have highlighted some cases of unanticipated attack on native species, and exotic species of minor economic importance (Paynter et al. 2008b; Withers et al. 2008). However, none of these cases of non-target attack were considered to have significant population impacts on the non-target species. Furthermore, these cases of non-target attack would have been predictable with more thorough pre-release host-range testing, since they occurred on closely related plant species (Pemberton 2000; Paynter et al. 2008a; Paynter et al. 2015).

In addition to assessing the safety of introduced biocontrol agents, post-release monitoring can be used to assess if the pre-release host specificity testing matches the realised 'field host range' in the introduced region (Fowler et al. 2012; Schaffner et al. 2020). Prior to introducing a biocontrol agent, laboratory-based pre-release host specificity studies are carried out to define the 'fundamental host range' of the species under consideration (i.e. the group of plant species on which the biocontrol agent can complete development) (Schaffner 2001). After host specificity testing, the fundamental host range of a potential biocontrol insect is often phylogenetically defined, such as development being restricted to a genus, or tribe of plants.

Under field conditions, many plants within the fundamental host range are seldom attacked (Cristofaro et al. 2013). Biocontrol agents are unlikely to encounter some potential host plants due to different phenologies (temporal separation), or different environmental niches (spatial separation), compared to the primary host (Wapshere 1989). While temporal and spatial separation of some potential host plants can limit the realised field host range of biocontrol agents, often the field host range is limited due to the expression of preference hierarchies, where herbivorous insects preferentially utilise host plants that maximise their fitness (or actively avoid suboptimal hosts that result in reduced fitness) (Sheppard et al. 2005). Where utilisation of less preferred host plants occurs under field conditions, it is often a result of 'spill-over attack'. Spill-over attack is defined as transient utilisation of less preferred species, as a result of depletion of the primary host plant(s) (Hinz et al. 2020). In other words, it is attack that is unlikely to occur when the primary resource is plentiful.

The purpose of the present study was to assess the field host range of the gall fly, *Urophora stylata* (F.), a biocontrol agent introduced to New Zealand for control of the thistle weed, *Cirsium vulgare* (Savi) Tenore (Scotch thistle). The

fly is native to the Palearctic region, where it is commonly reported to attack the seedheads of *C. vulgare*, its primary host plant (Redfern 1968). The fly oviposits eggs between the bracts of developing seedheads, or sometimes inserts eggs in the tips of florets. The larvae hatch as second instars and move down the floret tubes, and then feed on the developing seeds. Multiple larvae often develop in a single seedhead, creating a hardened multi-chambered gall within the seedhead. By mid-summer the next generation of adults emerge, completing a partial second generation per year (Redfern 1968).

Surveys in central Europe have recorded *U. stylata* from *Cirsium* and *Carduus* species (Zwölfer 1965; Korneyev & White 2000). However, different biotypes of *U. stylata* have been reported from its native range, including a biotype from the eastern Mediterranean region considered to be oligophagous, developing on a wide range of Cardueae host plants (Knio et al. 2002).

Pre-release host specificity testing carried out in the native range, evaluated oviposition of the fly on 44 test plant species (including 10 non-Cardueae species). Oviposition by the fly was reported on three Cardueae test plants (*Arctium tomentosum* Miller, *Carduus acanthoides* L., and *Onopordum acanthium* L.), but development was completed only on the primary host plant, *C. vulgare* (Zwölfer, unpublished)[1]. The host specificity testing was carried out at CABI Switzerland (Delémont) exclusively using the Swiss Jura biotype of the fly, which was considered to be highly specific to *C. vulgare* (Zwölfer, unpublished)[1].

Additional pre-release host specificity testing was carried out in New Zealand and Australia, primarily to ensure safety to native plant species. In New Zealand, the closest native relatives to Cardueae belong to the Cichorieae tribe and include *Sonchus* species. While *Sonchus* species are very distantly related to *Cirsium vulgare* (Funk et al. 2009), and unlikely to be attacked, it was considered prudent to test these species since some are culturally valued as traditional food plants (known to Māori as pūhā), and classified as threatened, or nationally vulnerable species (de Lange 2020). As expected, no attack was detected on native *Sonchus* species (Table 1). Of three thistle weeds (*Cirsium arvense* (L.) Scop., *Carduus nutans* L. and *Silybum marianum* (L.) Gaertn.) tested in addition to *Cirsium vulgare*, attack was detected only on *Cirsium arvense* (Table 1). In Australia, there are two Cardueae species recorded as native, *Rhaponticum australe* (Gaudich.) Soskov and *Saussurea lyrata* (Bunge) Franch., although their native status is doubtful (Hidalgo et al. 2006; Susanna & Garcia-Jacas 2007). No attack was reported on these Cardueae species, or an additional 10 species from nine different Asteraceae tribes (Harris et al, unpublished)[2].

In New Zealand, *U. stylata* was released in 1998 via a shipment from Australia, followed by a supplemental release sourced from the USA in 1999 (Winston et al. 2014). The original sources of *U. stylata* released in Australia and North America were collected from populations of the fly from its native range in France, Germany, and Switzerland. Thus, it is likely that the fundamental host range of *U. stylata* released in New Zealand corresponds with host records from surveys in central Europe, and that the fly is restricted to *Carduus* and *Cirsium* species. *Urophora stylata* is the only species deliberately released for biocontrol of *Cirsium vulgare* in New Zealand, although the weevils, *Rhinocyllus conicus* (F.) and *Trichosirocalus horridus* (Panzer), have been reported to attack the weed (Zwölfer & Harris 1984; Groenteman et al. 2008). *Rhinocyllus conicus* was released along with *Urophora solstitialis* (L.) to control thistle weeds in New Zealand. Both are seedhead-feeding biocontrol agents but diverge in their host range. *Urophora solstitialis* has not been recorded on any host plants other than *Carduus nutans* in New Zealand, although closely related *Carduus* species are within the its host range (Woodburn 1993; Korneyev & White 2000). *Rhinocyllus conicus* is known to have an oligophagous host range attacking many plants in the subtribe Carduinae (true thistles) (Zwölfer 1965; Zwölfer & Harris 1984).

Table 1 Summary results of multi-choice tests carried out with *Urophora stylata* at Lincoln, New Zealand, summer 1997–1998. This table is reproduced from an unpublished report (Harris et al, unpublished)[2] with permission from Manaaki Whenua Landcare Research.

Test plant species	Common name	No. plants	No. seed-heads	Seedheads attacked	No. larvae
Cirsium vulgare (Savi) Tenore	Scotch thistle	14	42	27	83
Cirsium arvense (L.) Scop.	Californian thistle	4	76	2	3
Carduus nutans L.	nodding thistle	6	24	0	0
Silybum marianum (L.) Gaertn.	variegated thistle	6	226	0	0
Sonchus novae-zelandiae (Hook.f.) Garn.-Jones [= *Kirkianella novae-zelandiae* (Hook.f.) Allan]	dryland sow thistle	5	7	0	0
Sonchus kirkii Hamlin	New Zealand sow thistle	6	43	0	0
Picris sp.		3	16	0	0

[1] Zwölfer H 1972. Investigations on *Urophora stylata* Fabr., a possible agent for the biological control of *Cirsium vulgare* in Canada. Weed projects for Canada. [Commonwealth Institute of Biological Control Progress Report 29] 20 p.

[2] Harris RJ, Rose EAF, Gourlay AH 1998. Introduction of *Urophora stylata* for biological control of Scotch thistle *Cirsium vulgare*: An importation impact assessment. [Landcare Research Contract Report: LC9798/096] 22 p.

There are no native thistle species (Cardueae) in New Zealand (Webb et al. 1988) so the only non-target species of concern were exotic crop species of economic importance. Paynter et al. (2004) reported no non-target attack by introduced thistle biocontrol agents; and specifically, no attack on commercial production of artichoke (*Cynara scolymus* L.) by the seedhead gall fly, *U. stylata*. While Paynter et al. (2004) assessed potential non-target attack by *U. stylata* in New Zealand, it remained uncertain to what extent *U. stylata* might attack related thistle weeds. Thus, as part of a recent post-release assessment to determine the impact of *U. stylata* on seed production of *Cirsium vulgare* (Cripps et al. 2020), attack on co-occurring thistle species within the same population as the target weed was assessed. The field hosts reported here are considered in relation to the pre-release host specificity testing and the likely fundamental host range of *U. stylata* released in New Zealand.

MATERIALS AND METHODS

New Zealand host-specificity testing (summer 1997–1998)

Pre-release host specificity testing of *Urophora stylata* was carried out in a quarantine facility under controlled environment conditions (16 h light at 22°C and 60% RH, alternating with 8 hr dark at 13°C and 70% RH). Each of seven replicate cages contained two *Cirsium vulgare* plants and a single plant of up to five of other test species (Table 1). Four to six females and four or five males of *U. stylata* were placed in each cage for four days. The test plants were maintained until the end of their flowering period, and then all seedheads were dissected and inspected for the presence of *U. stylata* larvae.

Collection of seedheads

The populations selected for this study were part of a nationwide survey to assess the impact of *U. stylata* on seed production of *Cirsium vulgare* in New Zealand pastures. Details of the selected populations are given in Cripps et al. (2020) and briefly summarised here. Twenty locations were randomly selected from the 34,167 farms designated as sheep, beef, or sheep + beef properties in the 2015 AgriBase dataset (Assure Quality, New Zealand), and stratified to ensure an equal number of populations in both the North and South Islands of the country (10 in the South Island, and 10 in the North Island). For the current study, the seedheads for assessing *U. stylata* attack on co-occurring thistle species were taken from 18 locations, i.e. the first two surveyed locations by Cripps et al. (2020) (Lincoln and Oxford, Canterbury) were excluded. Only pasture land designated as sheep and/or beef production was included since this is where the weed is most problematic (Kelly & Popay 1985; Bourdôt & Kelly 1986). The randomly selected locations were assessed in consultation with the relevant Beef+Lamb NZ farm extension manager for each region, and the nearest suitable location known to have *C. vulgare* present was selected for surveying. Of the randomly selected 18 *C. vulgare* populations, 15 had additional thistle species present (Table 2).

To assess potential attack on other thistle species, seedhead collections were carried out for all thistle species (Cardueae) present at the surveyed locations and included *Cirsium arvense, Cirsium palustre* (L.) Scop.*, Carduus nutans*, and an *Arctium* species (Table 2). These Cardueae species have similar phenologies to *Cirsium vulgare*, producing flower buds from spring to summer when the fly is active (November to February) (Webb et al. 1988; Cripps et al. 2020). A maximum of ten plants (or shoots in the case of *C. arvense*) of each species were haphazardly selected and three post-flowering seedheads were collected (maximum of 30 seedheads per thistle species for each surveyed location). These seedhead samples were kept in ventilated containers in a laboratory at AgResearch (Lincoln), maintained at constant temperature (20 °C) and exposed to indirect natural ambient light. The containers were periodically inspected for the presence of adult biocontrol agents (*U. stylata, U. solstitialis*, and *R. conicus*) from 25 May 2018, when the first adult emergence was observed, until 23 January 2019 after which no more emergence of adult biocontrol agents was observed. At each inspection time, all adult biocontrol agents were counted, and removed from the containers.

Supplemental to this systematic survey, an opportunistic collection of thistle seedheads from three locations in the Gisborne region was carried out in December 2019. At each of these locations, *Cirsium vulgare* was present along with at least one other thistle species, including *Cirsium arvense*, *Carduus tenuiflorus* W. Curtis, and *Silybum marianum*. As described above, three seedheads from ten individual plants of each thistle species present were collected, maintained in boxes in the laboratory, and inspected for emergence of adult biocontrol agents (Table 3).

Attack intensity on the primary host

The mean attack intensity for each *Cirsium vulgare* population was determined from seedhead dissections. At each population, a maximum of 30 individual plants were haphazardly selected for collection of seedheads for later dissection. At populations with less than 30 plants, all plants present in the population were surveyed. From each plant, up to three seedheads were collected for subsequent dissection in the laboratory (Table 2). Only seedheads visually assessed to be in the post-flowering growth stage were collected. For plants with less than three seedheads in the post-flowering stage, all seedheads in this growth stage were collected. The seedheads were refrigerated at 4 °C until they were dissected in the laboratory. Each seedhead was cut in half, and the receptacle diameter (mm) was measured. If a gall was present, the gall diameter was measured (mm), and the percentage of each seedhead that was occupied by a gall (% galled = gall diameter/seedhead diameter x 100) was calculated and used as the measure of 'attack intensity'. The percentage of a seedhead occupied by a gall is a good measure of attack intensity since gall size is directly related to the number of larvae developing within the gall (Harris & Wilkinson 1984).

Statistical analyses

Presence of *U. stylata* attack on *Cirsium arvense* per

Table 2 Locations of thistle species populations surveyed for attack by seedhead-feeding biocontrol agents present in New Zealand. At each location, three seedheads were collected from 10 plants of each species (total of 30 seedheads per thistle species for each population), except for *Cirsium vulgare* at Ararimu where only three plants were present and six seedheads collected in total, and for the *Arctium* sp. at Rotorua where only one plant was present from which 30 seedheads were collected.

			No. adults emerged		
Collection date	Location	Thistle species	*Urophora stylata*	*Urophora soltitialis*	*Rhinocyllus conicus*
22/02/2018	Waimate	*Carduus nutans*	0	390	13
		Cirsium arvense	0	0	0
		Cirsium vulgare	29	0	0
23/02/2018	Portobello	*Cirsium arvense*	0	0	0
		Cirsium vulgare	0	0	0
27/02/2018	Dobson Moana	*Cirsium vulgare*	0	0	0
27/02/2018	Kumara	*Cirsium vulgare*	0	0	0
		Cirsium palustre	0	0	0
28/02/2018	Mt Somers[1]	*Carduus nutans*	0	81	3
28/02/2018	Methven	*Cirsium arvense*	0	0	0
		Cirsium vulgare	58	0	0
2/03/2018	Dipton	*Cirsium arvense*	0	0	0
		Cirsium vulgare	0	0	0
2/03/2018	Happy Valley	*Cirsium arvense*	0	0	0
		Cirsium vulgare	0	0	0
		Cirsium palustre	0	0	0
5/03/2018	Portland	*Cirsium vulgare*	64	0	4
5/03/2018	Tangiteroria	*Cirsium vulgare*	41	0	5
6/03/2018	Ararimu	*Cirsium arvense*	8	0	0
6/03/2018	Maramarua	*Cirsium vulgare*	26	0	0
		Cirsium arvense	17	0	1
		Cirsium vulgare	60	0	1
7/03/2018	Rotorua	*Arctium* sp.	0	0	0
		Cirsium arvense	1	0	0
		Cirsium vulgare	128	0	0
8/03/2018	Moawhango	*Cirsium arvense*	3	0	0
		Cirsium vulgare	69	0	2
8/03/2018	Taihape	*Cirsium arvense*	9	0	0
		Cirsium vulgare	39	0	0
8/03/2018	Fordell[2]	*Cirsium arvense*	36	0	0
9/03/2018	Hastwell	*Cirsium arvense*	0	0	0
		Cirsium vulgare	6	0	0
		Cirsium palustre	1	0	0
9/03/2018	Rangitumau	*Cirsium arvense*	0	0	0
		Cirsium vulgare	5	0	0

[1]At the Mt Somers population, *Cirsium vulgare* was present, and assessed for larval attack intensity by *Urophora stylata* (see Cripps et al. 2020), but extra seedheads to assess adult biocontrol agent emergence were not collected.
[2]At the Fordell population, 15 *Cirsium vulgare* plants were present from which all available seedheads in the post-flowering stage were collected for dissection to assess the larval attack intensity by *Urophora stylata* (see Cripps et al. 2020).

Table 3. Supplemental thistle populations surveyed for attack by seedhead-feeding biocontrol agents present in New Zealand.

			No. adults emerged		
Collection date	Population	Thistle species	*Urophora stylata*	*Urophora solstitialis*	*Rhinocyllus conicus*
4/12/2019	Ngatapu	*Cirsium vulgare*	39	0	1
		Carduus tenuiflorus	0	0	3
5/12/2019	Tangihanga Station	*Cirsium vulgare*	1	0	0
		Cirsium arvense	0	0	0
		Carduus tenuiflorus	0	0	0
		Silybum marianum	0	0	0
6/12/2019	Whatatutu	*Cirsium vulgare*	21	0	1
		Silybum marianum	0	0	0

population was expressed as a binary response (yes or no) and the relationship of this response with attack intensity on *Cirsium vulgare* in the corresponding populations was analysed using a generalised linear model (GLM) with a binomial distribution through a logit link function. The GLM consisted of the attack intensity covariate only, and the analysis estimated the relationship between the probability of attack on *C. arvense* and attack intensity on *C. vulgare*. The number of *U. stylata* per seedhead of *C. arvense* was calculated for each population (rate of attack) and the relationship between this rate and attack intensity on *C. vulgare* was analysed using polynomial regression. A quadratic equation modelled the observed nonlinear pattern and restricted the rate of attack to be equal or greater than zero. Statistical analyses were performed with Minitab version 16.

RESULTS

Urophora stylata was detected in 13 of the 18 randomly selected *Cirsium vulgare* populations. Thistle species other than *C. vulgare* were present at 15 of the 18 locations. At locations where there was no attack by *U. stylata* on *C. vulgare*, there was no attack recorded on any other thistle species. *Cirsium arvense* was present at 13 locations, of which 10 had *U. stylata* detected. Attack by *U. stylata* on *C. arvense* was recorded at six of the 10 locations where both were present (Table 2).

The probability of attack on *C. arvense* was positively correlated with attack intensity (i.e. mean percentage occupation of seedheads by *U. stylata* galls) on the primary host, *C. vulgare* (χ^2=8.24, df=1, P=0.004) (Fig. 1). The maximum attack intensity where no spill-over attack occurred on *C. arvense* was 18%; and the minimum attack intensity where spill-over occurred was 42%. The inflection point in the logistic model is at 29% (95% CI=28%, 30%) attack intensity on *C. vulgare*. Beyond this attack intensity, the probability of spill-over attack occurring on *C. arvense* is greater than the probability of it not occurring. At an attack intensity of 50% or more on the primary host plant, spill-over attack on *C. arvense* is predicted to be a near certainty (Fig. 1). As the intensity of attack on *C. vulgare* increased, the rate of attack (*U. stylata* per seedhead) on *C. arvense* also increased ($F_{2,11}$=6.34, P=0.015) (Fig. 2). The mean attack rates on *C. arvense* ranged from 0.1 to 1.2 *U. stylata* per seedhead.

The only other thistle species attacked by *U. stylata* in this study was *Cirsium palustre*. One specimen of *U. stylata* emerged from the *C. palustre* seedheads collected from the Hastwell field site (Table 2). At this location there was relatively low attack intensity on *C. vulgare* (mean of 18%), and no attack on *C. arvense* detected, despite its presence there. From the supplemental surveys carried out in the Gisborne region, no attack by *U. stylata* was recorded on other thistle species (*Cirsium arvense*, *Cardus tenuiflorus*, or *Silybum marianum*), despite attack recorded on *Cirsium vulgare* at these locations (Table 3).

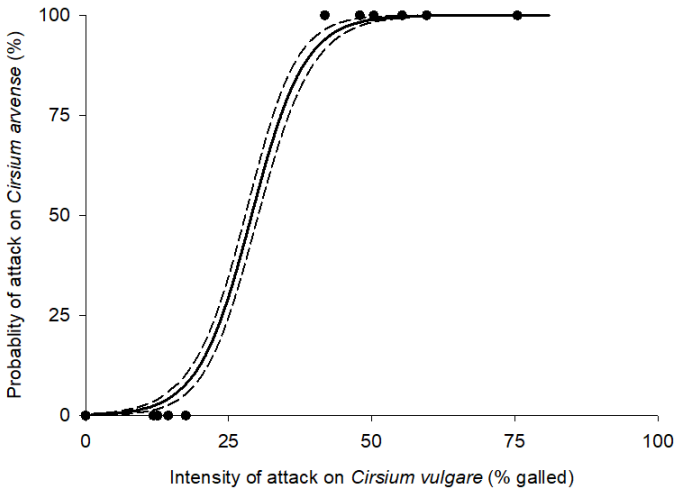

Figure 1 Probability of attack on *Cirsium arvense* in relation to the mean attack intensity on the primary host plant, *Cirsium vulgare*. The attack intensity values are from 13 randomly selected populations of *C. vulgare* where *C. arvense* was also present. The relationship (solid line) is described by the logistic equation: Probability (%) = 100 / (1 + exp (6.230 – 0.215 × attack intensity)). Dashed lines depict the 95% confidence bands.

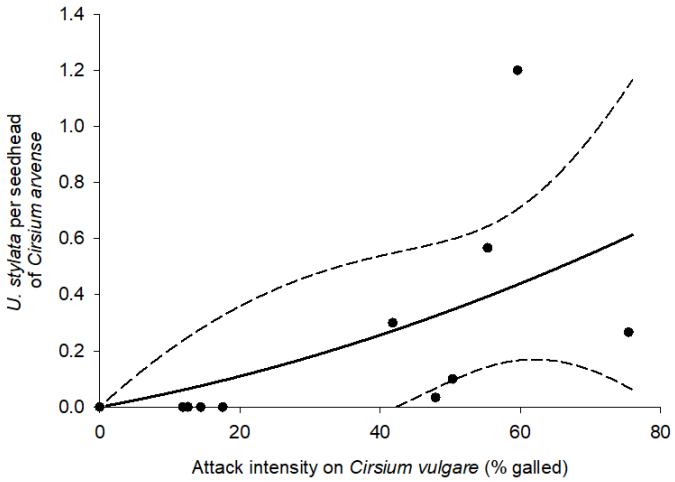

Figure 2 Mean number of *Urophora stylata* emerged from seedheads of *Cirsium arvense* in relation to the mean attack intensity on the primary host plant, *Cirsium vulgare*. The mean values are calculated from 13 randomly selected populations of *C. vulgare* where *C. arvense* was also present. The relationship (solid line) is described by the quadratic equation: *U. stylata* per seedhead = 4.56×10^{-3} (Attack intensity) + 4.6×10^{-5} (Attack intensity)^2. Dashed lines depict the 95% confidence bands.

DISCUSSION

This study has documented that *Cirsium arvense* and *Cirsium palustre* are field host plants of the biocontrol agent, *Urophora stylata*, released in New Zealand for control of the weed, *Cirsium vulgare*. Attack on these congeneric hosts was predictable based on native range records and pre-release host specificity testing in New Zealand but the extent of host utilisation under field conditions was unknown. At least in the case of *C. arvense*, the data from this survey suggest that utilisation of this host plant is limited to spill-over attack (*sensu* Hinz et al. 2019). This situation contrasts with full utilisation, where a biocontrol agent can sustain its population on an alternative host plant, even in the absence of its primary host. In this study we did not survey *C. arvense* in the absence of *C. vulgare*, and therefore cannot say for certain if *U. stylata* can fully utilise this or other congeneric hosts. While we did not specifically seek out populations of *C. arvense* occurring in the absence of *C. vulgare*, such populations in New Zealand would be difficult to find, since the co-occurrence of the two species is common (Bourdôt & Kelly 1986; Klinkhamer & de Jong 1993).

The pattern of attack on *Cirsium arvense* is consistent with spill-over at all 13 locations surveyed in this study where both *C. arvense* and *C. vulgare* were present. That is, attack on *C. arvense* occurred only at locations where attack intensity on the primary host was high, and never occurred where attack intensity on the primary host was low. At an attack intensity of 50% on the primary host, the predicted probability of spill-over attack on *C. arvense* is nearly 100%. While this degree of attack intensity is unlikely to directly cause spill-over attack, it is probably a good indicator of a point where the preferred resource is in limited supply. Previous studies have reported that the seedhead resource tends to be underutilised by *U. stylata* (Harris & Wilkinson 1984; Cripps et al. 2020), and that intraspecific competition among developing larvae is negligible (Michaelis 1986). How *U. styalta* avoids competition within the spatially constrained niche of the seedhead is unclear, but there is no evidence that females avoid ovipositing into already occupied seedheads. In fact, females will readily oviposit into seedheads that already contain eggs from other females (Michaelis 1986). Cripps et al. (2020) hypothesised a critical 'close-off time' during gall formation that might exclude subsequent larvae from entering the receptacle area of the seedhead, and thereby avoid competition.

The critical close-off time hypothesis may explain the underutilisation of seedheads but does not provide an explanation for the spill-over effect, or more precisely, what is causing the fly to use an alternative host that is likely suboptimal. It is possible that the spill-over effect is caused by territorial conflicts between males. Males of *U. stylata* (and other tephritid species) are known to establish territorial breeding grounds that they defend against conspecific rival males (Headrick & Goeden 1994; Daniels 2004). Harris (1989) observed a volatile substance secreted by some male *Urophora* biocontrol agents, including *U. stylata* and its sister species, *Urophora cardui* (L.), and speculated that it might be important for locating mates. The behaviour of *U. stylata* has not been thoroughly studied, but for *U. cardui* the marking pheromone was found to attract both males and females. Females use the pheromone to find mates, and males use it to find safe spaces (i.e. where a male is present, predators are likely absent) (Frenzel et al. 1990; Frenzel & Brandl 1991; Daniels 2004). At high population densities of *U. stylata* (or at least high relative to the number of *C. vulgare* plants), the frequency of male territorial conflicts may increase, forcing some males to establish territories on suboptimal host plants, such as *C. arvense*.

The rate of attack (*U. stylata* per seedhead) on *Cirsium arvense* also increased with increasing attack intensity on *C. vulgare*, indicating that it is not just the probability of spill-over attack that increases, but also the degree of spill-over attack. Overall, the attack rates on *C. arvense* were low, with average attack rates typically much less than one per seedhead. However, the rate of attack on *C. arvense* was high at the Fordell field site, where only 15 *C. vulgare* plants were present. At this location the rate of attack on *C. arvense* was 1.2 *U. stylata* per seedhead. This was somewhat surprising since typically when *U. stylata* attacks *C. arvense* there is only one larva per seedhead (M. Cripps, personal observation). The attack rate on *C. arvense* at the Fordell site indicates that there are occasional cases of more than one larva per *C. arvense* seedhead, likely where the primary host is very limited.

While the pattern of attack on *Cirsium arvense* is indicative of spill-over, the data are too limited to determine if the attack on *C. palustre* is a spill-over effect. From this survey, one specimen of *U. stylata* was reared from *C. palustre*, but it is worth noting that this occurred at the only field site where both *U. stylta* and *C. palustre* were present. Attack intensity on the primary host was low at this location (18%), and no attack was recorded on *C. arvense*. Thus, it is possible that if *C. palustre* was present at a site with high attack intensity on

C. vulgare there could be greater utilisation of this species.

Attack on *Cirsium arvense* and *C. palustre* was predictable, based on historical field host records from the native range, rather than the original host specificity testing carried out in Europe. The pre-release host specificity testing was carried out with one biotype of *U. stylata* (the Swiss Jura biotype). This biotype was considered to be monophagous, only attacking *C. vulgare* (Zwölfer, unpublished)[1]. Indeed, both *C. arvense* and *C. palustre* were included in the host specificity testing and were not accepted for oviposition. However, the biotype(s) released in North America, and subsequently New Zealand, were collected from populations of the fly in France, Germany, and Switzerland (Harris & Wilkinson 1984; Winston et al. 2014), and match the somewhat broader field host range of *U. stylata* known from central Europe.

The disconnection between the host range of the biotype tested and the biotype released is an obvious inadequacy of the specificity testing. However, it is important to recognise that in the early 1970s, the main purpose of host specificity testing was to determine the safety to non-target plants of economic significance (Fowler et al. 2004; Hinz et al. 2014), in this case artichoke and safflower (*Carthamus tinctorius* L.) (Zwölfer, unpublished)[1]. In New Zealand and Australia, where the fly was released much later (1990s), additional host specificity testing ensured safety to native plant species (Harris et al, unpublished)[2]. The specificity testing in New Zealand recorded *Cirsium arvense* as a host, which was not considered problematic, since there are no native thistle species, and the bulk of introduced thistles are either current or potential weeds of economic significance (Cripps et al. 2013). However, the host specificity testing for *U. stylata* released in North America relied exclusively on the European study (Zwölfer, unpublished)[1] that was carried out at a time when testing the safety of biocontrol agents on native species was not common practice (Fowler et al. 2004; Hinz et al. 2014). In North America, where there are numerous native *Cirsium* species (some of which are classified as rare or endangered (Eckberg et al. 2017)), this inadequacy in the original host testing could be problematic. To date, we are unaware of any records of non-target attack by *U. stylata* on native *Cirsium* species in North America, although it is uncertain to what extent, if any, this has been investigated. Possible non-target spill-over attack by *U. stylata* on native *Cirsium* species in North America, particularly at sites with *C. vulgare* present, would be worthwhile to investigate.

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