**INTERACTIONS BETWEEN *PIERIS OLERACEA* AND *PIERIS RAPAE* (LEPIDOPTERA: PIERIDAE) BUTTERFLIES, AND THE BIOLOGICAL CONTROL AGENTS *COTESIA GLOMERATA* AND *COTESIA RUBECULA* (HYMENOPTERA: BRACONIDAE).**

A Thesis Presented

by

MEGAN V. HERLIHY

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## DEDICATION

For Rhianna

# ACKNOWLEDGEMENTS

# ABSTRACT

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*Pieris oleracea,* formerly *Pieries napi*, was once a widespread pierid butterfly in New England until the introduction of a biological control agent, *Cotesia glomerata*. It has been suggested that *C. glomerata* is responsible for the range reduction of *P. oleracea*. There are been several introductions of a second more specialized biological control agent, *Cotesia rubecula*, to the United States since the 1960's. My first goal was to determine the current distribution and status of *P. rapae* parasitoids and the effectiveness of *C. rubecula* as a biological control agent since its release. The findings of a survey I conducted of the parasitoid community of *P. rapae* indicate that *C. rubecula* now occurs as far west as North Dakota and has become the dominant parasitoid of *P. rapae* in the northeastern and north central United States and adjacent parts of southeastern Canada, where it has displaced *C. glomerata*, the previously dominant parasitoid.

Survival of artificially established cohorts of *P. rapae* larvae was assessed in a collard patch on an organic vegetable farm in western Massachusetts. There was a significant drop in larval survival between the 4$^{th}$ and 5$^{th}$ instar due to parasitism by *C.*

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*rubecula*. This was change from survival curves of *P. rapae* from a 1985-1986 study, in which there was a significant drop in survival between the 5<sup>th</sup> instar and pupal stage due to *C. glomerata*.

The final goal of my thesis work is to try to understand why *P. oleracea* was able to survive at the focal study site in Lenox, MA despite parasitoid pressure and range reduction elsewhere in New England. In olfactometer tests, there was no difference in attractiveness of naïve *C. glomerata* females to volatiles of either *Cardamine pratensis* (cuckooflower) foliage, the host plant of *P. oleracea* or *Brassica olercea* (collard) foliage ($P = 0.51$). In order to determine if overtopping by other vegetation may provide an enemy free space for *P. oleracea* by affecting detection by *C. glomerata,* cage experiments were conducted. Overtopping vegetation had a significant effect on parasitism by *C. glomerata* ($F = 12.8$, df $= 3$, $P < 0.001$), and may be the reason *P. oleracea* has been able to thrive at the Lenox, MA site.

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**CHAPTER 1**

**DISTRIBUTION OF *COTESIA RUBECULA* (HYMENOPTERA: BRACONIDAE) AND ITS DISPLACEMENT OF *COTESIA GLOMERATA* IN EASTERN NORTH AMERICA**

**<u>Introduction</u>**

The parasitoid *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) was introduced to the United States as a biological control agent against the invasive vegetable pest *Pieris rapae* (L.) (Lepidoptera: Pieridae) in 1884 near Washington, District of Columbia (Clausen 1978). *Cotesia glomerata* is a gregarious endoparasitoid of several species of pierid butterflies. Although *C. glomerata* established, it was unable to reduce damage from *P. rapae* larval feeding to a level acceptable to vegetable growers. *Cotesia glomerata* kills *P. rapae* larvae at the end of the fifth instar, after virtually all larval feeding has occurred, so that per larvae parasitism by *C. glomerata* does little to reduce within generation impacts of *P. rapae* larvae. In fact, larvae parasitized by *C. glomerata* consume significantly more food during their development than unparasitized ones (Rahman 1970). Thus any pest control benefit from *C. glomerata* is limited to intergenerational reduction in *P. rapae* density, which has not been sufficient to reduce *P. rapae* to non-pest status in the United States. Also, *Cotesia glomerata* is not host specific, and has non-target impacts on native pierid butterflies, including *Pieris oleracea* Harris (formerly *Pieris napi oleracea*) (Benson et al. 2003).

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*Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) is a solitary host specific endoparasitoid of *P. rapae* that attacks first and second instars. *Cotesia rubecula* not only attacks *P. rapae* at a high rate (e.g., Van Driesche 2008), but also reduces feeding damage on a per larva basis (Le Masurier and Waage 1993). *Cotesia rubecula* is successful at reducing feeding damage because it kills *P. rapae* in the fourth instar before most larval feeding occurs which greatly reduces total per larval feeding, since 85% of total feeding by a P. rapae larvae occurs in this instar (Parker and Pinnell 1973). Also, because *C. rubecula* is host specific, it rarely attacks native pierids in the field (Van Driesche et. al. 2004).

There have been several introductions (accidental or deliberate) of *C. rubecula* into North America since the 1960s. A population of *C. rubecula* that was not deliberately introduced was detected on Vancouver Island, British Columbia in 1963 (Wilkinson 1966). By the 1980s, this strain had spread as far south as Oregon and displaced *C. glomerata* there, but did not do so below latitude 44°35' (Biever 1992). The Vancouver strain of C. rubecula was later released in Ontario, Missouri, New Jersey, and South Carolina in the 1960s (Puttler et al. 1970; Williamson 1971, 1972). The Vancouver strain of *C. rubecula* established in Ontario (Corrigan 1982), but failed to establish in more southern areas, including Missouri (Parker and Pinnell 1972).

It was suggested that this strain failed to establish in more southern areas because its diapause requirements were not met (Nealis 1985), although lack of tolerance to colder winters was also considered a possible explanation. To overcome thisfailure to obtain establishment, a strain of *C. rubecula* from the former Yugoslavia was introduced in the 1980s to Ontario, Missouri, and Virginia (McDonald and Kok 1992). In 1988, the

Yugoslavian strain of *C. rubecula* was recovered in Virginia, but it did not persist. This may have been due to either its diapause requirements not being met, or perhaps the negative effects of hyperparasitism (McDonald and Kok 1992; Gaines and Kok 1999). In a third attempt to find a well adapted population, *C. rubecula* was collected in Shenyang, China in 1988, and this strain was released in 17 locations in southern New England (Van Driesche and Nunn 2002), where it established and spread. In the early 1990s, individuals from both the former Yugoslavian and Chinese populations were released in Minnesota and *C. rubecula* recoveries were made beginning in 2000 (Wold-Burkness et al. 2005; Lee and Heimpel 2005).

Before the release of *C. rubecula* in New England, the dominant parasitoid of *P. rapae* was *C. glomerata* (Van Driesche and Bellows 1988). By 2002, *C. rubecula* was widely distributed in southern New England, and had become the dominant parasitoid of *P. rapae* (Van Driesche and Nunn 2002). In western Massachusetts, Ontario, and the western United States, *C. rubecula* has outcompeted and displaced *C. glomerata* (Corrigan 1982; Biever 1992; Van Driesche 2008).

The purpose of this study was to assess the current geographical distribution of *C. rubecula* and *C. glomerata* in the northeastern and north central parts of the United States and adjacent parts of Canada in order to determine if *C. rubecula* has displaced *C. glomerata* at this scale as it has done locally in New England. We hypothesized there would be a southern limit to the spread of *C. rubecula* due to an incompatibility between local seasonal day length patterns and diapause cue sensitivity of the parasitoid, as suggested by Nealis (1985). We also hypothesized that *C. rubecula* would displace *C.*

*glomerata* over some larger spatial scale given that it has done so in New England, Ontario, Washington, and Oregon (Corrigan 1982; Biever 1992; Van Driesche 2008).

## <u>Materials and Methods</u>

Samples of *P. rapae* and their *Cotesia* parasitoids were collected from May to late Sept 2011 in fourteen states and two Canadian provinces, from New England to North Dakota, southward to North Carolina and northward to New Brunswick and Quebec. Samples were collected from various types of cole crops at organic vegetable farms or private gardens. All *P. rapae* larvae from first to fifth instars, as well as pupae, and cocoons of both species of *Cotesia* parasitoids (emerged or not) were collected. Collectors were provided with pictures and descriptions of these life stages. Up to one hour was spent examining crop plants, collecting all of the above life stages until 30 or more "individuals" (one *C. glomerata* cocoon mass was considered one individual, as it came from one host larva) had been collected. Actual sample numbers per site ranged from 5-103 individuals, depending on local *P. rapae* density. First and second instar *P. rapae* larvae may be underrepresented in the survey samples due to their small size. Insects in samples were counted by species and life stage, and all *P. rapae* larvae were dissected to determine the level of parasitism by each parasitoid species. The only parasitoids observed in dissection were *C. glomerata* and *C. rubecula*. All dissections were done by the senior author. The immature stages, including eggs, of these two parasitoids can be readily separated in dissection by several characteristics. Visible mandibles and an anal hook are present in first instars of *C. rubecula*, but not in those of *C. glomerata* (Van Driesche 2008). Also, the number of parasitoid larvae per host is

diagnostic (*C. rubecula* is solitary; *C. glomerata* is gregarious). No parasitoid eggs were seen in this survey although they also can be distinguished to species, by size, shape, and number (Van Driesche and Nunn 2002).

In total, 32 samples of *P. rapae* larvae or pupae and parasitoid cocoons were examined, comprising 1571 individuals. Sample percent parasitism rates for each species were calculated at each location and mapped to look for geographical patterns. Average parasitism rates per species across all sites with any parasitism were also calculated. The percentages were arcsine transformed to better meet the assumption of normality, and then compared with a t test. Hyperparasitism was not examined in this study.

### Results

Summed across all 32 samples collected in the survey, 1571 individuals, were obtained and examined (Table 1). From that pool of samples, the only parasitoids recovered were *C. rubecula* and *C. glomerata*. *Cotesia rubecula* was present at 22 of the 32 sample sites (Table 1) and parasitized $20.6 \pm 0.02\%$ (95% CI) of the 1571 individuals examined. *Cotesia glomerata* was present at 12 sites and parasitized $7.3 \pm 0.01\%$ (95% CI) of the 1571 indviduals. When parasitism was calculated based only on sites where a given parasitoid actually occurred, we found an average parasitism rate of $47 \pm 0.03$ % (95% CI, $n = 1041$) for *C. rubecula* and $25 \pm 0.03$ % (95% CI, $n = 641$) for *C. glomerata* (t-value: 2.748, df: 31, $P = 0.0049$).

Spatially, *C. rubecula* and *C. glomerata* were largely exclusive in the distribution of their recoveries (Fig. 1). Only at 4 out of the 32 sites sampled was parasitism by both

*C. rubecula* and *C. glomerata* detected. Three of these four sites (exclusive of the Charlestown, Rhode Island site) were on the border of what appears to be a latitudinal point of separation of the regions that each parasitoid now occupies. *Cotesia rubecula* recoveries were highly concentrated in the north, while *C. glomerata* was dominant farther south. *Cotesia rubecula* was not found below latitude N 38° 48', and is the only parasitoid found in our samples above latitude N 40° 2'. Within the area surveyed, no westward limit was detected for the distribution of *C. rubecula* (i.e., it was present in the most western of our sample locations [North Dakota]).

## Discussion

The *Cotesia* spp. distribution patterns observed in our survey (Fig. 1) are not explained by the history of these species. *Cotesia glomerata*, now largely absent in the northern portion of our survey area, was once widely present there (Fig. 2) and likely still occurs there at very low levels (e.g., in Massachusetts [Van Driesche 2008]). Similarly, the absence of *C. rubecula* in the southern portion of our survey area is not due to failure to release the parasitoid there, since releases were made in both Virginia and Missouri (Fig. 2). The absence of *C. rubecula* in the southern portion of our survey area is consistent with previous studies on the diapause needs of this species (Nealis 1985). Diapause in *C. rubecula* is induced by short day length. Cool temperatures during diapause are believed to preserve the insect's fat supply and coordinate post-diapause development (Nealis 1985). Nealis (1985) further suggested that the mechanism for poor establishment of some populations of *C. rubecula* in southern locations was the

premature induction of diapause, caused by locally reaching a critical short daylength, before seasonal temperatures had declined adequately. Temperatures above 15 °C on average have been hypothesized to be lethal to diapausing prepupae of *C. rubecula* (Nealis 1985). Another potential explanation for the failure of *C. rubecula* to establish in some areas of the United States is the effect on *C. rubecula* densities of high rates of mortality to its immature stages due to hyperparasitism, as observed in Virginia (McDonald and Kok 1992; Gaines and Kok 1998); however, there is no evidence in the literature that hyperparasitism rates in southern states are higher than in other areas. We did not examine hyperparasitism rates in our survey.

The absence of *C. glomerata* in samples from the northern portion of our survey area, where it was formerly widespread, is likely related to competitive displacement by *C. rubecula*. The phenomenon of parasitoid displacement has been well documented in other systems (e.g., DeBach and Sundby 1963; Le Brun et al. 2009). Our study suggests that such displacement of *C. glomerata* by *C. rubecula* has occurred at this larger spatial scale, as was previously observed for these species at the state/province level in Massachusetts (Van Driesche 2008), Quebec (Godin and Boivin 1998), and Oregon and Washington (Biever 1992). *Cotesia rubecula* is now widespread in the northeastern and north central United States and parts of southeastern Canada. The lack of such displacement in Europe, where both *Cotesia* species coexist, is likely due to the presence of the specific host of *C. glomerata*, *Pieris brassicae* L.,which is not attacked by *C. rubecula*.

The observed increase in prevalence and dominance of *C. rubecula* provides benefits both by increasing the level of control of the imported cabbageworm (*P. rapae*)

and lessening the damage to non-target native pierids from *C. glomerata*. The displacement of *C. glomerata* in the northern United States by the more host-specific *C. rubecula* should allow some native pierids such as *P. oleracea* (Benson et al. 2003; Van Driesche et al. 2004) and *Pontia protodice* Boisduval and Leconte (Dave Wagner, University of Connecticut, pers. comm.), whose ranges collapsed in some regions due to attack by *C. glomerata*, to recolonize areas from which they were extirpated, providing a benefit to protection of native biodiversity.

Also, vegetable producers will benefit from this change in parasitoid species. *Cotesia rubecula*, which is now the dominant parasitoid of *P. rapae* in the northern part of our survey area, causes high levels of mortality to *P. rapae* ($47 \pm 0.03\%$). and kills individual larvae before most of their feeding occurs. Although we cannot say with certainty which strain of *C. rubecula* is now found at particular sites, the introduction of *C. rubecula* in North America appears to be at least a partially successful biological control program that has met its objectives.

**Table 1.1**

Rates of parasitism in 2011 by *Cotesia* wasps (Hymentoptera: Braconidae) of *Pieris rapae* from organic vegetable farms in the eastern United States and Canada.

| State/Province[2] | Date | Coordinates | % parasitized $\bar{x}$, 95% CI, (n) | | Crop[1] |
|---|---|---|---|---|---|
| | | | *C. rubecula* | *C. glomerata* | |
| Newark, DE | 15 Jun | N 39° 41'; W 75° 44' | 70.7 ± 0.14 (41) | 19.5 ± 0.12 (41) | B, Cau. |
| Champaign, IL | 15 Jun | N 40° 4.5'; W 88° 12' | 0.9 ± 0.02 (103) | 5.8 ± 0.05 (103) | C, Cau., K |
| Lexington, KY | 20 Jul | N 38°7'; W 84° 30' | 0 ± 0 (65) | 5 ± 0.05(65) | Ko |
| Midway, KY | 7 Sept | N 38° 11'; W 84° 42' | 0 ± 0 (32) | 0 ± 0 (32) | C |
| Westhampton, MA | 12 Aug | N 42° 57'; W 72° 46' | 41 ± 0.15 (41) | 0 ± 0 (41) | C |
| Northampton, MA | 24 Aug | N 42° 19'; W 72° 38' | 53 ± 0.16 (38) | 0 ± 0 (38) | B, C, K |
| Ashfield, MA | 18 Aug | N 42° 18'; W 72° 45' | 5.9 ± 0.11 (17) | 0 ± 0 (17) | Misc. |
| Westhampton, MA | 19 Aug | N 42° 31'; W 72° 47' | 0 ± 0 (31) | 0 ± 0 (31) | Misc. |
| Upper Marlboro, MD | 8 Jun | N 38° 49'; W 76° 45' | 0 ± 0 (67) | 52 ± 0.12 (67) | K |
| East Lansing, MI | 13 Jul | N 42° 42'; W 84° 29' | 70.6 ± 0.13 (51) | 0 ± 0 (51) | C |
| East Lansing, MI | 13 Jul | N 42° 42'; W 84° 29' | 13.6 ± 0.07 (103) | 0.9 ± 0.02 (103) | C |
| St. Paul, MN | 11 Jul | N 44° 56'; W 93° 5' | 14 ± 0.10 (50) | 0 ± 0 (50) | B, C, K, |
| St. Paul, MN | 31 Aug | N 44° 56'; W 93° 5' | 60 ± 0.30 (10) | 0 ± 0 (10) | B, C |
| Northampton, NB | 27 Jun | N 46° 3'; W 67° 33' | 88.9 ± 0.21 (9) | 0 ± 0 (9) | B |
| Pittsboro, NC | 2 Jun | N 35° 42'; W 79° 17' | 0 ± 0 (36) | 19.4 ± 0.13 (36) | C, Co, K |
| Chapel Hill, NC | 2 Jun | N 35° 51'; W 79° 12' | 0 ± 0(29) | 10.3 ± 0.11 (29) | C, Co, K |
| Harwood, ND | 14 Sept | N 47° 25'; W 96° 50' | 8.6 ± 0.07 (70) | 0 ± 0 (70) | B, C |
| Geneva, NY | 10 Aug | N 42° 52'; W 77° 50' | 0 ± 0 (103) | 0 ± 0 (103) | C |
| Fairville, NY | 29 Sept | N 43° 7'; W 77° 4' | 13 ± 0.09 (55) | 0 ± 0 (55) | Cau. |
| Fairville, NY | 29 Sept | N 43° 7'; W 77° 4' | 44 ± 0.12 (71) | 0 ± 0 (71) | Misc. |
| Terre Hill, PA | 20 May | N 40° 9'; W 76° 3' | 4.1 ± (97) | 0 ± 0 (97) | Misc. |
| Hustontown, PA | 31 Aug | N 40° 2'; | 0 ± 0 (44) | 2.3 ± 0.04 (44) | Br |

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| Location | Date | Coordinates | | | Crop[1] |
|---|---|---|---|---|---|
| | | W 78° 1' | | | |
| Montreal, QC | 14 Sept | N 45° 30'; W 73° 36' | 59 ± (66) | 0 ± 0 (66) | Misc. |
| Charlestown, RI | 2 Aug | N 41° 21'; W 71° 42' | 60± 0.18 (30) | 3 ± 0.04 (30) | Co, K |
| South Kingston, RI | 2 Aug | N 41° 28'; W 71° 31' | 41.9 ± 0 (31) | 0 ± 0 (31) | C, Co |
| Birdsnest, VA | 15 May | N 37° 25'; W 75° 51' | 0 ± 0 (32) | 3.1 ± 0.06 (32) | C, Co |
| Birdsnest, VA | 30 May | N 37° 13'; W 75° 59' | 0 ± 0 (40) | 42.5 ± 0.15 (40) | C, Co |
| Blacksburg, VA | 15 Jun | N 37° 13'; W 80° 24' | 0 ± 0 (51) | 62.7 ± 0.13 (51) | CC, K, RC |
| So. Burlington, VT | 2 Aug | N 44° 38'; W 72° 52' | 100 ± 0 (18) | 0 ± 0 (18) | C, K |
| Burlington, VT | 2 Aug | N 44° 26'; W 73° 9' | 100 ± 0 (5) | 0 ± 0 (5) | C, K |
| Cambridge, VT | 8 Aug | N 44° 28'; W 73° 13' | 100 ± 0 (41) | 0 ± 0 (41) | C, Co. |
| Madison, WI | 3 Aug | N 43° 4'; W 89° 24' | 5.3 ± 0.05 (94) | 0 ± (94) | Misc. |
| Total | | | 20.6 ± 0.02 (1571) | 7.3 ± 0.01 (1571) | |

[1]Crop key. B-broccoli, Br-brussel sprouts, C-cabbage, CC-Chinese cabbage, Cau-cauliflower, Co-collards, K- kale, Ko- kohlrabi. RC-red cabbage, Misc.-miscellaneous cole crops.

[2]State/Province abbreviations key. DE-Delaware, IL-Illinois, KY-Kentucky, MA-Massachusetts, MD-Maryland, MI-Michigan, MN-Minnesota, NB-New Brunswick, NC-North Carolina, ND-North Dakota, NY-New York, PA-Pennsylvania, QC- Québec, RI-Rhode Island, VA-Virginia, VT-Vermont, WI-Wisconsin.
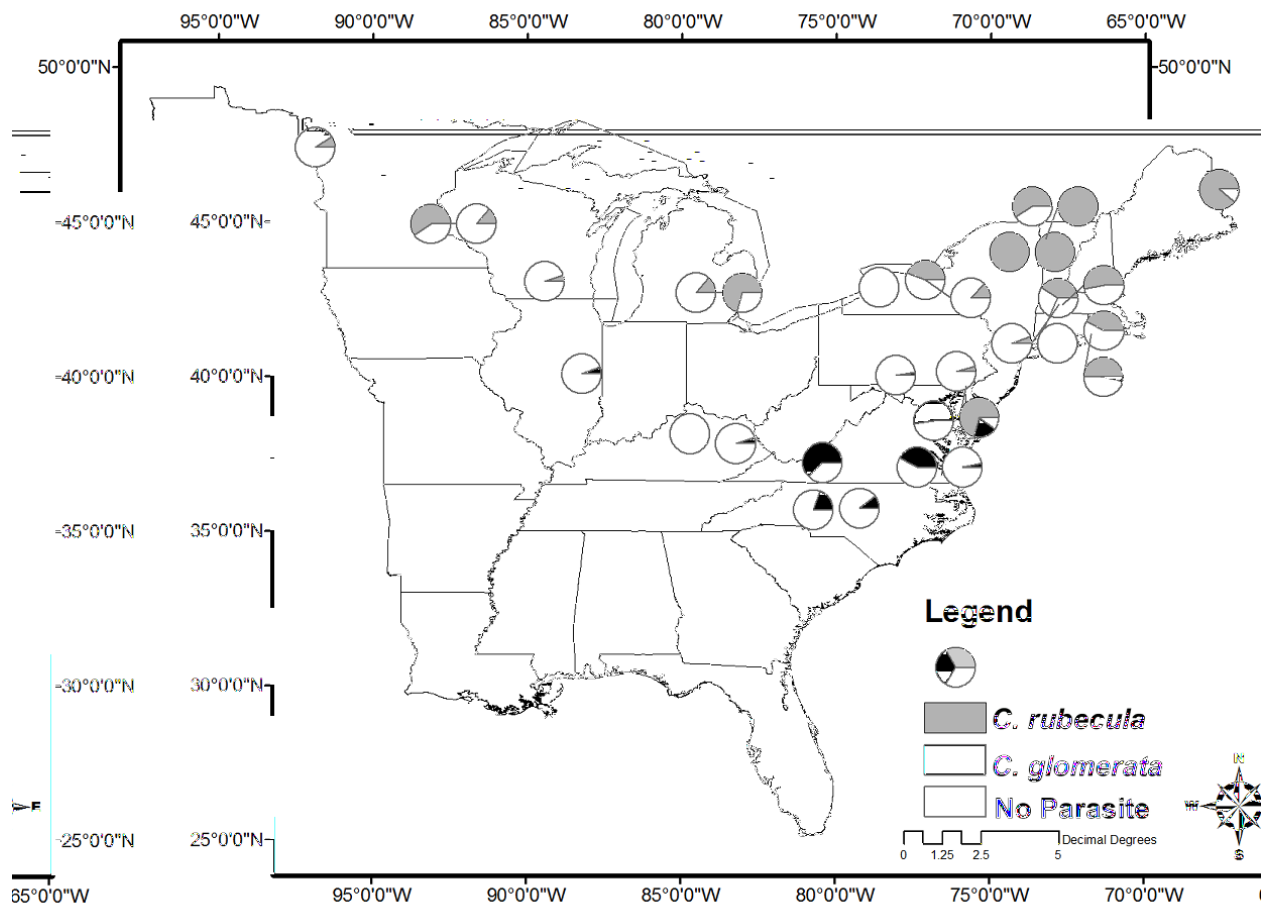
**Figure 1.1** Observed pattern of *Cotesia* parasitism of *Pieris rapae* in parts of the eastern United States and southeastern Canada in 2011. Parasitism by *Cotesia rubecula* is shown in gray and *Cotesia glomerata* in black. The percentage of unparasitized larvae is shown in white.
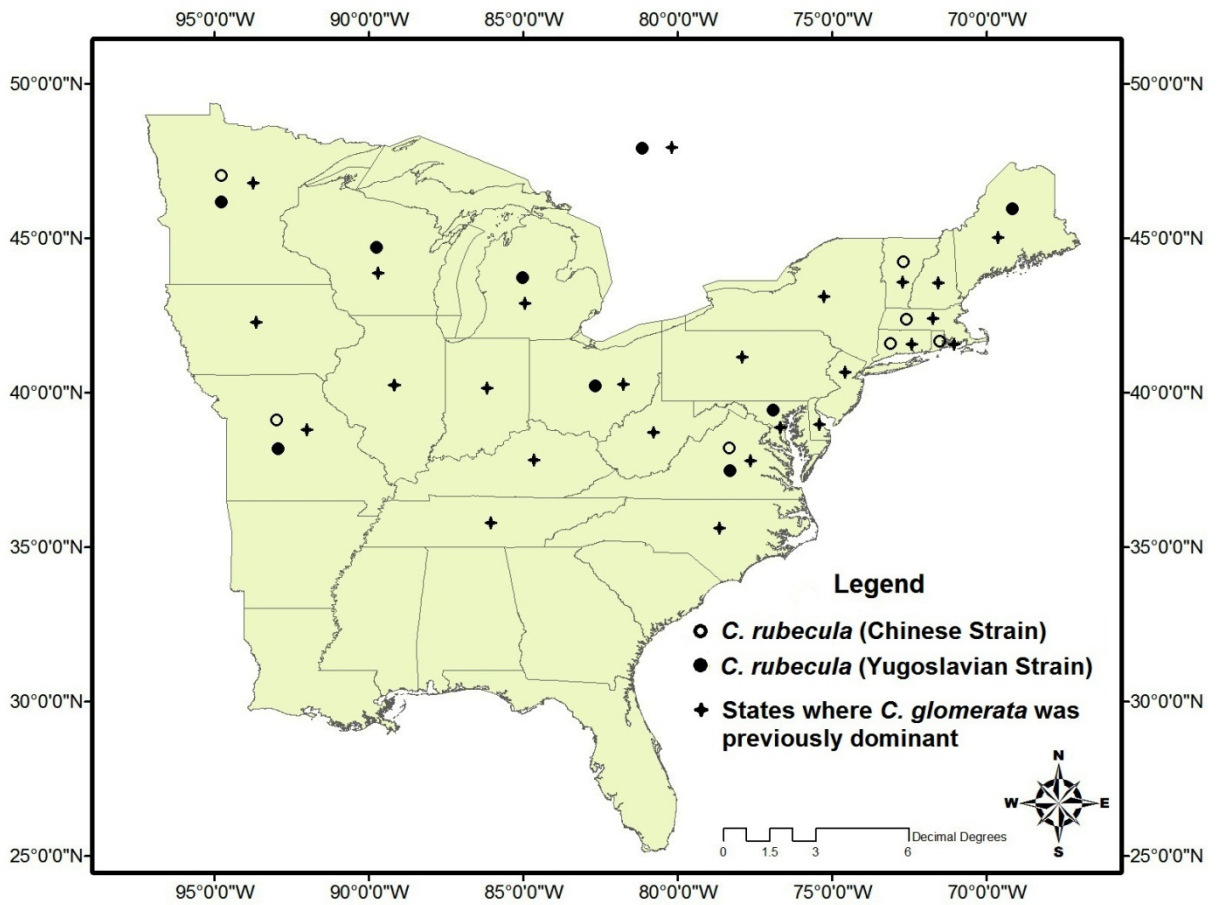
**Figure 1.2** USA States and Canadian Provinces where the *Pieris rapae* parasitoids *Cotesia glomerata* was previously dominant (crosses), and where releases of the Chinese strain of *Cotesia rubecula* (open circles) or the Yugoslavian strain (black circles) were made.

# CHAPTER 2

# EFFECT OF *COTESIA RUBECULA* (HYMENOPTERA: BRACONIDAE) ON SURVIVAL OF LARVAL COHORTS OF *PIERIS RAPAE* (LEPIDOPTERA: PIERIDAE) ON COLLARDS: EVALUATION OF THE IMPACT OF AN INTRODUCED BIOLOGICAL CONTROL AGENT

## <u>Introduction</u>

*Pieris rapae* (L.) (Lepidoptera: Pieridae) is an invasive agricultural and garden pest of several varieties of *Brassica* crops in North America, Australia, and New Zealand (Jones et al. 1980). In the United States, two biological control agents attacking larvae have been released against *P. rapae* larvae during different historical periods, in an attempt to reduce feeding damage to levels acceptable to growers.

The gregarious, generalist parasitoid *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) was introduced to the United States in 1884 near Washington, D.C. (Clausen 1978). *Cotesia glomerata* parasitizes first and second instars of *P. rapae*, and kills *P. rapae* larvae at the end of the fifth instar, after most larval feeding has occurred (Parker and Pinnell 1973). Larvae parasitized by *C. glomerata* consume significantly more food than unparasitized larvae during their development (Rahman 1970).

The second *P. rapae* larval parasitoid introduced to North America was *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae), a solitary, host-specific parasitoid. Introductions in the United States were made from 1960 to 1992 (Puttler et al. 1970;

Williamson 1971, 1972; Parker and Pinnell 1972; McDonald and Kok 1992; Wold-Burkness et al. 2005; Lee and Heimpel 2005). In 1988, releases of a Chinese strain of this species were made at 17 different locations in New England (Van Driesche and Nunn 2002). Like *C. glomerata*, *C. rubecula* attacks only first and second instars of *P. rapae*, but it kills larvae as fourth instars (Le Masurier and Waage 1993), before most of the larval feeding damage has occurred. *Cotesia rubecula* attacks *P. rapae* at a higher rate than *C. glomerata* and has replaced *C. glomerata* in the north and north central United states above latitude N 38° 48' (Herlihy et al. 2012). Such displacement, while not the topic of this study, likely results from some mix of intrinsic superiority of *C. rubecula* larvae (which are mandibulate and hence attack and kill the non-mandibulate *C. glomerata* larvae in cases of co-parasitism) (Laing and Corrigan 1987).

Here, we estimated the survival of cohorts of larvae of *P. rapae* on collard crops in an organic vegetable farm in 2011 in western Massachusetts, 23 years after the release of *C. rubecula,* and compare current survival rates to similar data from experiments done in 1985 and 1986 (Van Driesche 1988), when *C. glomerata* was the only important larval parasitoid of *P. rapae*. We predicted that *C. rubecula* would be the dominant parasitoid because it has largely displaced *C. glomerata* in the study region (Van Driesche 2008; Herlihy et al. 2012.) and that the survival of *P. rapae* larvae would decrease sharply from the fourth to fifth instar due to parasitism by *C. rubecula*.

**Materials and Methods**

**Study location**

In June of 2011, 130 collard transplants were established at an organic vegetable farm (Intervale Farm in Westhampton, Massachusetts, 42°17'41" N; 72°46'19" W) to form an experimental plot where the interaction of *C. rubecula* and *P. rapae* larvae could be studied. Seedlings were planted in 6 rows of about 22 plants each, at 50 cm intervals. The land used for the patch had not been planted previously with cole crops, and was used the preceding year for corn production. During the 2011 season, the area around the patch was planted with various cole crops, corn, and tomato plants, since it was part of an actively functioning organic vegetable farm. Neither the study patch nor the surrounding area was treated with pesticides during 2011.

**Plants**

Before each of four experiments, groups of 70 large collard plants (ca 30 cm tall with 7-10 leaves) in 15 cm dia pots were purchased from Harvest Farm in Whately, Massachusetts, where they had been grown in greenhouses without applications of pesticides. After purchase, the plants were held for about 1 week in the laboratory and watered daily until used in the field experiment. Before starting an experiment, we took plants to the field plot to acclimatize them to outdoor conditions. Potted plants were placed within the existing rows between soil-grown collard plants at the research site; there were 15 potted plants in each of the plot's four rows (which were used for the main treatments) and 10 potted plants in a fifth row (used for plants with larvae needed to determine the accuracy of visual estimation of parasitism rates). Pots were set into evenly spaced shallow holes dug in the ground to help conserve moisture and prevent

pots from blowing over. The plants were allowed two days to adjust to being in full sun, and watered every other day during the experiments.

## Insect rearing and handling

*Pieris rapae* larvae used in the experiment came from a laboratory colony, initiated with butterflies collected at local sites near Amherst, MA, in 2010 and reared in the laboratory on collards. Butterflies were provided with sugar solution ("Instant Hummingbird Nectar," Perky Pet Woodstream Corp. Lititz, PA) and were allowed to oviposit on collard seedlings. After egg hatch, larvae were reared on seedling collard plants until they were just beyond the neonate stage (e.g., had taken their first meal and turned from yellow to green due to food in their guts). Young first instars were then taken, along with plants, to the field where larvae were placed on the experimental plants at the field site using a paintbrush dipped in nectar water (sugar induced plant acceptance by neonates). Five first instars were placed on each potted plant.

## Experimental design

We tested the effects of four levels of natural enemy exclusion on survival of our cohorts of *P. rapae* larvae: (1) no exclusion (uncaged plants without Tanglefoot [Contech Enterprises Inc. Victoria, BC, Canada] on pots), (2) full exclusion (plants inside net sleeves and with Tanglefoot on pots), and (3, 4) two levels of partial exclusion, being either with sleeves and no Tanglefoot, or the reverse. Treatments were placed within their own rows, with all 15 plants arranged in a line along one row, but immediately next to

(<0.5 m) a similar row of plants for treatments two, three, and four.  The maximum distance between pots of different treatments was therefore 1.5 m.

In two of the four treatments, Tanglefoot was applied to pots to prevent walking predators from moving up the pots from the soil onto the plants. Tanglefoot was painted in a band around the outsides of each pot in this treatment at the beginning of the experiment, but was not renewed during the experiment. For two of the four treatments, organdy sleeves were placed over the entire plant to exclude flying parasitoids and predators. Sleeves were held slightly away from the foliage of the potted plants by four stakes driven into the ground around each pot; sleeves were fitted over the stakes, dropping to the mid-pot level (about 5 cm above soil level). The bottom edges of the sleeves were left loose and were not secured to pots in order to facilitate sleeve removal, which was needed for frequent counting of larvae and watering of plants.

During insect counts, parasitism was estimated visually as soon as it could be detected, but live, putatively parasitized larvae were left on plants for continued observation. To determine the relationship between these visual estimates of parasitism and the real rates of parasitism (as would have been seen in dissection if larvae were removed and killed), in the fifth row we placed ten additional potted plants without organdy bags or Tanglefoot. Larvae in this group were placed on plants at the same time and in the same manner as for the cohorts of the main experiment. When larvae in the cohorts reached the fourth instar, we assessed parasitism in the field in the same manner as the larvae in the cohorts of the main experiment, and then we validated our field classification by dissecting these larvae in the laboratory.  The comparison of parasitism rates of these larvae via field visual assessments and their subsequent dissection  in the

laboratory allowed us to measure the accuracy of our visual assessments of parasitism in the main experiment.

To assess seasonal changes in larval survival or parasitism, the experiment was repeated four times (July, August, September and October). The same plot was used for all four runs of the experiment, but in each run, treatments were assigned to different rows.

**Data collection**

Within each treatment in each of the four runs, we counted the number of surviving *P. rapae* larvae at a series of time points over their development from first instars to pupae. Larval survival was checked visually in the field by examining plants leaf by leaf and recording all living larvae, by instar, that were present, as well as any parasitoid cocoons that had been spun. For treatments in which plants were covered with organdy sleeves, these were lifted up to examine plants and then replaced. If any eggs of wild *P. rapae* butterflies were found to have been laid on our experimental plants (as might happen in the unsleeved treatments), they were removed by hand. During the first two runs (7-20 July and 4-16 August), plants were examined every second day, providing six observations in each run. During the last two runs (3-24 September and 27 September to 17 October), the plants were initially examined every second day, but it became clear that the larvae were developing much more slowly than in earlier runs due to lower seasonal temperatures. Therefore, after the first two observations, the period between observations was changed to every third day, providing seven observations in run three and eight observations in run four.

When the surviving larvae of a cohort reached the fourth instar, each larva was assessed visually in the field and classified as parasitized or not, by either *C. rubecula* or *C. glomerata*. (Parasitism by this stage being visible due to a change in body color and parasitoid species being recognized by larval size and appearance, changes which were familiar to us from rearing both parasitoids in the laboratory).

Larvae on the 10 additional plants (not part of the experimental cohorts) without organdy or Tanglefoot were examined for parasitism visually in the field when they were fourth instars in the same manner as experimental larvae and then were brought back to the laboratory for dissection to determine the accuracy of the visual inspection method.

**Life table construction**

Life tables were constructed for the cohort of each treatment group within each monthly run of the experiment, but only four life tables (one per treatment, pooled over months) are presented. We used the marginal rate equation $m_B = d_B/(1-m_A)$, where $m_B$ is the marginal attack rate of the parasitoid, $d_B$ is the death rate from parasitism, and $m_A$ is the marginal attack rate for predation (here, disappearance), to calculate the underlying rates of parasitoid attack from the observed rates of parasitism and disappearance for fourth instars in life tables (the only stage with two observed mortality factors) (Elkinton et al. 1992).

**Statistical analyses**

All statistical analyses were conducted using the program R (version 2.13.1). All parasitism and survival proportion data were arcsine transformed in order to meet the

assumption of normality. Rates of survival for selected life stage and rates of parasitism were compared across sample dates. Rates of parasitism were compared using a t-test for treatments with and without sleeves. Survival data were analyzed with an ANOVA followed by Tukey HSD tests.

## Results

### Survival of *P. rapae* cohorts

Analysis (ANOVA) of data from the four monthly runs showed that month of exposure had no significant effect on survival to fifth instar (F = 0.17, df = 3, $P$ = 0.92), or survival to pupae (F = 0.05, df = 3, $P$ = 0.83). Therefore, in further analyses, data were pooled across months to explore differences among the four levels of natural enemy exclusion.

Differences in the survival of experimental cohorts to the pupal stage with sleeves (presence vs. absence) and Tanglefoot (presence vs. absence) as factors was highly significant (ANOVA, F = 63.2, df = 3, $P$ < 0.0001). Differences between treatment means (levels of exclusion) were then examined using a Tukey HSD test in order to determine the treatments causing significance (Table 1). Survival of cohorts to the pupal stage with and without Tanglefoot did not differ significantly (for sleeved: t = 0.21, df = 6 $P$ = 0.84 or for exposed (not sleeved): t = 2.43, df = 6, $P$ = 0.051 groups), although the latter was very close to significance. Survival to the pupal stage in cohorts without organdy sleeves averaged 3.0 ± 2.9% with Tanglefoot and 3.7 ± 2.7% without Tanglefoot

(pooled over all experimental runs). Survival to the pupal stage for cohorts on sleeved

pots averaged 36.3 ± 6.6% with tanglefoot and 27.3 ± 3.4% without Tanglefoot (Table 1).

Because *C. rubecula* kills its hosts in the fourth instar, we also compared rates of

survival to the fifth instar for treatments in all four runs. Differences in the survival of

experimental cohorts to the pupal stage with sleeves (presence vs. absence) and tangle

foot (presence vs. absence) as factors was highly significant (F = 53.4, df = 3, *P* <

0.0001). Survival to the fifth instar in cohorts without organdy sleeves averaged 5.3 ±

2.9% with Tanglefoot and 5.0 ± 1.3% without Tanglefoot (pooled over all experimental

runs). Survival to the fifth instar for cohorts on sleeved pots averaged 43.0 ± 8.7% with

Tanglefoot and 33.4 ± 5.2% without Tanglefoot (Table 1).

The survival of larvae in cohorts (Fig. 1) in all treatments decreased at a steady

rate after an initial sharp drop between the first and second instar, which was likely due to

a combination of predation and failure of young larvae to establish and feed. There was

another sharp drop in the number of larvae (considering visually parasitized larvae as

"dead" for this comparison) in the unsleeved treatments during the fourth instar, due to

the effect of parasitism by *C. rubecula*. The fourth instar was the first point in which

parasitism could be scored visually and was the instar in which larvae parasitized by *C.

rubecula* died.


### Parasitism of *P. rapae* by *C. rubecula*

Comparison of visually determined rates of parasitism to rates seen in larval

dissection for  48 "extra" fourth instars placed in the experimental plot for this purpose

(and on the same dates as the cohort larvae in the main experiment) revealed that our

estimates of the rates of parasitism by visual determination was 94% accurate. For these larvae, rates of parasitism based on visual inspection (65%) versus dissection (71%) (of the same larvae) were not significantly different based on an unpaired t-test (t = 0.81, df = 4, $P$ = 0.47). The few cases of error in assessing parasitism visually, were, in all cases, instances where a larva that appeared unparasitized in the field, was found to be parasitized upon dissection. There were no cases of larvae being classified as parasitized in the field by visual inspection that upon dissection were found not to be parasitized. Consequently, our reported parasitism rates are thus likely to be somewhat conservative.

A two-factor (month, treatment) factorial ANOVA found that there was no effect of month on parasitism rates (F = 0.0805, df = 3, $P$ = 0.97) and therefore data on parasitism were pooled by month to examine the effects of treatments on rates of parasitism. In both treatments lacking organdy sleeves (two such treatments per monthly experiment), averaged over experimental runs in the four months, the rate of parasitism by *C. rubecula* ) was high (62.4 ± 8.6 %) (Fig. 2), in contrast to treatments protected by organdy sleeves, in which very little parasitism occurred (4.2 ± 4.0 %) (data pooled over the four runs), with "sleeves" (presence vs absence) being a significant effect on rates of parasitism (ANOVA: F = 66.73, df = 3, $P$ < 0.001). A Tukey HSD test was used to determine which treatment types were significant. As with the survival data, the only significant difference was between sleeved and exposed plants, with no effect of the presence or absence of Tanglefoot on pots (Table1).

**Cohort life tables and marginal rates of parasitoid attack**

Life tables for all treatments (4) by all runs (4) were constructed (16 tables) and examined.  However, since run date (month of experiment) had no significant effect on any survival or parasitism parameters examined, for presentation, tables were collapsed across run dates within treatments and the resultant four summary life tables, one per treatment, are presented (Table 2).

In a three-way ANOVA of the effect of treatment, number of early instar larvae (1-4) and run date on survival, we found no significant three-way interaction (F = 0.75, df = 27, $P$ = 0.65), but there was a significant effect of run date on survival during the early instar stages in all treatment types (F = 2.8, df = 3, $P$ = 0.045) . Specifically, there was a significant difference between survival of all instars in all treatment types between the July and October experimental runs, but not between survival of instars in all treatment types of any other months based on a Tukey HSD test.

Marginal rate analysis (Table 2) revealed that parasitoid attack rates over both unsleeved treatment types (with and without Tanglefoot) were 13% higher than the apparent parasitism by *C. rubecula* as directly observed in field sampling.

## Discussion

Because it is often not feasible to continuously monitor a biological control agent each year for many years after it has been released, an alternative is to measure the effects of the biological control agent after a lapse of some years, after it has had an opportunity to fully establish and increase its population. Monitoring cohorts of individuals with known and standardized histories can provide especially useful data. In

this case, use of cohorts of *P. rapae* larvae on potted collard plants placed on an organic farm, with and without natural enemy exclusion, allowed us to quantify losses by instar, giving a more precise measure of the survival patterns of *P. rapae* life stages in the field over time. Monitoring cohorts allowed us to determine that there is a relatively large drop in survival (77.5%) between the fourth and fifth instars due to parasitism by *C. rubecula* (Table 1). This is important for growers because most damage to crops due to *P. rapae* feeding is done in the fifth instar. Consequently, lowering the number of fifth instars by this amount should greatly reduce feeding damage of *P. rapae* on *Brassica* crops.

While tanglefoot had no significant effect on survival of cohorts, sleeves were successful at preventing parasitism: survival of cohorts was much higher for cohorts with sleeves (31.8 ± 6.9%) than without (3.3 ± 2.7%). This suggests that parasitism rather than predation by ground predators was responsible for most *P. rapae* mortality in late instars (fifths), while ground predation is responsible for most *P. rapae* mortality in early instars (one through three). However, since this study was designed to estimate the effects of parasitism, our insights into predation are limited.

Our estimates of parasitism were likely underestimates for two reasons. First, parasitism in the field measured visually was determined to capture only 94% of the true rate, missing some parasitized larvae. Secondly, parasitism rates in the cohort data were influenced by the problem of simultaneous mortality, since some larvae disappeared steadily in all exposed treatments, likely due to predation. As a correction for this issue, we calculated the underlying marginal rates of attack by parasitoids, which revealed that the underlying parasitoid attack rates was 13 percentage points higher than the observed value (72.5% [marg. rate] Vs. 59.5% [observed rate]) (Table 2) (Elkinton et al. 1992).

Our results are consistent with a study conducted in New Zealand that assessed the rates of parasitism of *P. rapae* by both *C. rubecula* and *C. glomerata* (Cameron and Walker, 2002). It is clear that *C. rubecula* is now the dominant parasitoid of *P. rapae* in western Massachusetts and has displaced *C. glomerata there* (Herlihy et al. 2012), as it did at many of the New Zealand sites studied (Cameron and Walker 2002). As in Cameron and Walker (2002), in Massachusetts *C. rubecula* attacked *P. rapae* at a higher rate (in 2011 in our study) than did *C. glomerata* in a previous study (Van Driesche 1988 [done in 1985 and1986]), in the same area before the introduction of *C. rubecula*. As expected, in comparison to the 1985, 1986 study (Van Driesche 1988), the survival pattern of *P. rapae* has changed significantly since the introduction of *C. rubecula*. In the 1985-1986 study, survival of *P. rapae* declined steadily throughout all life stages in the first generation each year. During the second, third, and fourth generations, *C. glomerata* (the dominant parasitoid) caused approximately a 50% decrease in survival between the fifth instar and pupal stage. Since the displacement of *C. glomerata* by *C. rubecula*, the decrease in survival of *P. rapae* (77.5%) is now both steeper and earlier, occurring between the fourth and fifth instars. This is an important result for organic vegetable growers because earlier mortality of *P. rapae* caterpillars likely translates into a less damage from larval feeding.

**Table 2.1**

Number of *P. rapae* larvae reaching designated life stage in cohorts by level of exclusion, pooled over months. Percentages of *P. rapae* larvae parasitized by *C. rubecula* observed during the fourth instar by each level of exclusion pooled over months, including the marginal rate of attack.

| | No.[1] of larvae surviving to | | % Parasitism | |
| --- | --- | --- | --- | --- |
| | 5$^{th}$ instar | Pupae | Apparent rate | Marginal rate |
| S (-), TF (-)[2] | 15 a[3] | 11 a | 59 aA | 72 aA |
| S (-), TF (+) | 16 a | 9 a | 60 aA | 73 aA |
| S (+), TF (-) | 102 b | 82 b | 4 bB | 5 bB |
| S (+), TF (+) | 136 b | 109 b | 1 bB | 1 bB |

[1] Number of 300 original first instars surviving to stage.
[2] Treatments: S = sleeved, TF = tangle foot; - is without this type of exclusion; + is with the type of exclusion
[3] Differing lower case letters denoting statistically significant differences within columns (Tukey HSD test). Differing upper case letters denoting statistically significant differences within rows (Tukey HSD test).

**Table 2.2**

Life tables for cohorts of *P. rapae* larvae on potted collards deployed in a collard patches at an organic vegetable farm, Intervale Farm, in Westhampton, MA, across four months (July, August, September, October) in 2011, with four levels of natural enemy exclusion (+/-tanglefoot on pots crossed with +/-sleeves over plants), numbers entering and dying in each life stage, pooled over months by treatment.

**Tangle foot No Sleeve**

| | | Stage | | Factor | | Apparent mortality | |
| | | | | | | Stage | Factor |
| Stage | Factor | lx | dx | dx | Marginal attack rate | qx | qx |
|---|---|---|---|---|---|---|---|
| L1 | | 300 | 147 | | | 49 | |
| | Disappeared | | | 147 | | | 49 |
| L2 | | 153 | 43 | | | 28 | |
| | Disappeared | | | 43 | | | 28 |
| L3 | | 110 | 37 | | | 34 | |
| | Disappeared | | | 37 | | | 34 |
| L4 | | 73 | 57 | | | 78 | |
| | Disappeared | | | 13 | | | 18 |
| | Parasitized by *C. rubecula* | | | 44 | 73 | | 60 |
| L5 | | 16 | 7 | | | 44 | |
| | Disappeared | | | 7 | | | 44 |
| P | | 9 | | | | | |
| | Not observed | | | | | | |

**No Tangle foot No Sleeve**

| | | Stage | | Factor | | Apparent mortality | |
| | | | | | | Stage | Factor |
| Stage | Factor | lx | dx | dx | Marginal attack rate | qx | qx |
|---|---|---|---|---|---|---|---|
| L1 | | 300 | 144 | | | 48 | |
| | Disappeared | | | 144 | | | 48 |
| L2 | | 156 | 28 | | | 18 | |
| | Disappeared | | | 28 | | | 18 |
| L3 | | 128 | 62 | | | 48 | |
| | Disappeared | | | 62 | | | 48 |
| L4 | | 66 | 51 | | | 77 | |
| | Disappeared | | | 12 | | | 18 |
| | Parasitized by *C. rubecula* | | | 39 | 72 | | 59 |
| L5 | | 15 | 4 | | | 27 | |
| | Disappeared | | | 4 | | | 27 |
| P | | 11 | | | | | |
| | Not observed | | | | | | |

**No Tangle foot with Sleeve**

| Stage | Factor | Stage lx | dx | Factor dx | Marginal attack rate | Apparent mortality Stage qx | Factor qx |
|---|---|---|---|---|---|---|---|
| L1 | | 300 | 74 | | | 25 | |
| | Disappeared | | | 74 | | | 25 |
| L2 | | 226 | 36 | | | 16 | |
| | Disappeared | | | 36 | | | 16 |
| L3 | | 190 | 51 | | | 27 | |
| | Disappeared | | | 51 | | | 27 |
| L4 | | 139 | 37 | | | 27 | |
| | Disappeared | | | 31 | | | 23 |
| | Parasitized by *C. rubecula* | | | 6 | 5 | | 4 |
| L5 | | 102 | 20 | | | 20 | |
| | Disappeared | | | 20 | | | 20 |
| P | | 82 | | | | | |
| | Not observed | | | | | | |

**Tangle foot with Sleeve**

| Stage | Factor | Stage lx | dx | Factor dx | Marginal attack rate | Apparent mortality Stage qx | Factor qx |
|---|---|---|---|---|---|---|---|
| L1 | | 300 | 64 | | | 21 | |
| | Disappeared | | | 64 | | | 21 |
| L2 | | 236 | 30 | | | 13 | |
| | Disappeared | | | 30 | | | 13 |
| L3 | | 206 | 47 | | | 23 | |
| | Disappeared | | | 47 | | | 23 |
| L4 | | 159 | 23 | | | 14 | |
| | Disappeared | | | 21 | | | 13 |
| | Parasitized by *C. rubecula* | | | 2 | 1 | | 1 |
| L5 | | 136 | 27 | | | 20 | |
| | Disappeared | | | 27 | | | 20 |
| P | | 109 | | | | | |
| | Not observed | | | | | | |

**Figure 2.1** Survivorship curves of cohorts of *P. rapae* larvae (from first instar to pupa) on potted collards in four successive months in 2011 on an organic vegetable farm in Westhampton, Massachusetts, USA. All cohorts began with 75 larvae on day zero. *Healthy is defined as larvae that are alive, present, and not visibly parasitized (early stage parasitism is not detectable in living larvae).

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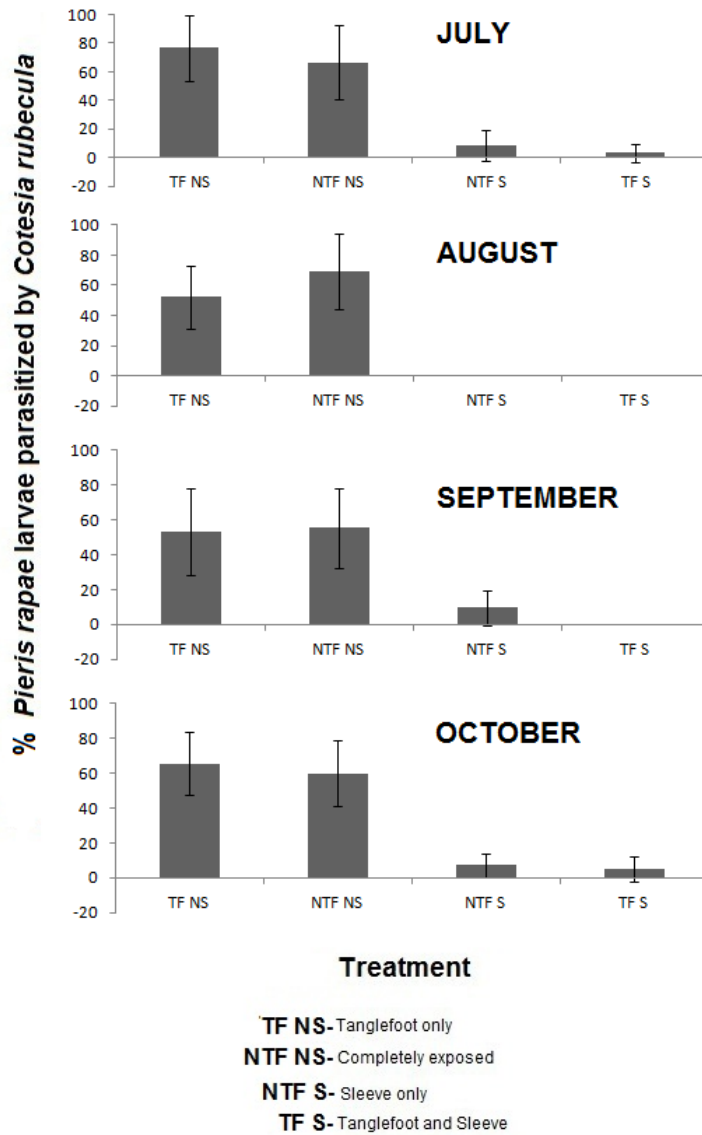
**Figure 2.2** Percentages of *P. rapae* larvae in experimental cohorts on potted collards that were parasitized by *C. rubecula* during their larval life. Parasitism (± 95 % C.I.) is shown for four levels of natural enemy exclusion (with and without sleeve, and/or tanglefoot on pots), in four successive months in 2011 at an organic vegetable farm in Westhampton, Massachusetts.

**CHAPTER 3**

**ABILITY OF *COTESIA GLOMERATA* [HYMENOPTERA: BRACONIDAE] TO DETECT AND PARASITIZE *PIERIS OLERACEA* (FORMERLY *PIERIS NAPI*) [LEPIDOPTERA: PIERIDAE] ON THE NOVEL HOST PLANT *CARDAMINE PRATENSIS***

**<u>Introduction</u>**

*Pieris oleracea* Harris. (Lepidoptera: Pieridae), formerly *Pieris napi*, was widespread in New England before the introduction in 1884 of a biological control agent, the braconid wasp *Cotesia glomerata* (L.), the butterfly once being found throughout Massachusetts (Scudder 1889). *Cotesia glomerata* is a gregarious parasitoid wasp that readily attacks several pierine butterflies. In both field and laboratory choice tests, *C. glomerata* preferred to parasitize *P. oleracea* over the target of this biological control agent, the invasive butterfly *Pieris rapae* (L.) (Lepidoptera: Pieridae), but will readily parasitize *P. rapae* if *P. oleracea* is not present (Van Driesche et al. 2003). *Cotesia glomerata* was introduced to the United States in the 1880s in order to control the invasive agricultural pest *P. rapae,* but failed to reduce this butterfly to non-damaging levels and the parasitoid is thought to have had important non-target impacts on some native pierines, including a significant range reduction of *P. oleracea* in New England (Benson et al. 2003). In a 2001 study, in Berkshire County, Massachusetts, *C. glomerata* caused 100% mortality of a cohort of *P. oleracea* larvae exposed on hedge mustard

(*Sisymbrium officinale* [ L.]) in an open meadow (Van Driesche et al. 2004). In New England, *P. oleracea* is now known to inhabit northern Vermont where it is largely univoltine (and perhaps the rest of northern New England as well), but in Massachusetts this formerly widespread species is now found only a few areas along the Housatonic river and in Savoy State Forest in Berkshire County, Massachusetts, where it has up to four generations per year in the best studied site. The largest western Massachusetts population, in Lenox, was first documented in about 2008, when reports from amateur butterfly collectors indicated the existence of a single, but large, population of *P. oleracea*. Investigation showed that larvae of the population were feeding on a large population of the invasive form of the crucifer *Cardamine praetensis* (L.), known as cuckoo flower.

Why this population of *P. oleracea* survived is of general interest in understanding the interactions of this butterfly with the changes in its environment due to various exotic plant and parasitoid invasions. Reproductive success of summer generations of *P. oleracea* in open habitats in Massachusetts despite parasitoid pressure from *C. glomerata* seems to be related to its switching host plants from various meadow crucifers to stands of cuckoo flower (*C. pratensis*), also in meadows. It is unclear what aspects of the Lenox cuckoo flower population allowed for the development of a high density *P. oleracea* population. Four explanations are possible, three of which have to do with cuckoo flower potentially offering the butterfly escape into enemy free space (Jeffries and Lawton 1984) from *C. glomerata*, while the fourth relates to the size and seasonal duration of cuckoo flower as a larval food resource.

The first three explanations, based on the enemy free space concept, are (1) that the wasp (*C. glomerata*) is unable to locate and parasitize *P. oleracea* larvae because it is unable to detect or respond to volatiles emitted by *P. oleracea*'s new host plant, *C. pratensis,* when it was fed on by *P. oleracea* larvae; this seems to be a plausible explanation because it has been shown that North American cuckoo flower has a different glucosinolate profile than both European cuckooflower and brassica olercea crops (Agerbirk 2010, unpub. data Agerbirk and Chew) (2) alternatively, the complex plant architecture of *C. pratensis* (with pinnate leaves) may physically impede the wasps' ability to search this plant efficiently, allowing larvae to escape; or (3) larval escape from parasitism may be caused by the vegetational complexity of the habitat during the summer and fall, when other meadow plants overtop (by ca 0.6 m) cuckoo flower plants, which occur in summer as rosettes at the soil level; overtopping vegetation may either physically misdirect foraging wasps or prevent them from using odor plumes from plant volatiles effectively to locate hosts detected by the parasitoid.

As an alternative to enemy free space (by whatever mechanism), the success of the Lenox *P. oleracea* population may be caused by the fact that the cuckoo flower population at the meadow field site where *P. oleracea* has survived is a very large, seasonally stable food resource. The plant is an invasive species and currently maintains a very dense population at the site over several ha. During nearly the entire growing season (April to November), cuckoo flower is present either as basal rosettes (year round) or flowering plants (spring only), which are both abundant and highly suitable for larval nutrition as show by successful use of the plant in laboratory rearings (pers. obs., M.H. )

More broadly, it has also been suggested that the historical reduction of *P. oleracea* may have been due to a range reduction of its spring, forest host plant, two-leaved toothwort (*Cardamine diphylla* [Michx.] A.W. Wood), with possible further affects from the invasion of the region by garlic mustard (*Alliaria petiolata* [M. Bieb.]) (Keeler et al. 2006), which acts as an egg trap for *P. oleracea* (Keeler and Chew 2008). However, neither of those events provides an explanation for the survival and expansion of the *P. oleracea* population at our study site. Indeed a dense population of the butterfly exists there despite the presence of garlic mustard at the site.

The evolution of this community continued when *Cotesia rubecula* (Marshall), a second and more effective biological control agent of *P. rapae*, was introduced. Various attempts to establish this species were made into the United States from 1960 to 1992 (Puttler et al. 1970; Williamson 1971, 1972; Parker and Pinnell 1972; McDonald and Kok 1992; Wold-Burkness et al. 2005; Lee and Heimpel 2005), with limited success, until the1988 release of a Chinese strain of this species in New England (Van Driesche and Nunn 2002), which was highly successful.

*Cotesia rubecula* is a nearly monophagous parasitoid that has rarely been observed to attack any species other than *P. rapae* in the field (Van Driesche 2008). Critical for our study is the fact that since its release, *C. rubecula* has largely displaced *C. glomerata* as a parasitoid of *P. rapae,* both in southern New England (Van Driesche 2008), Ontario (Corrigan 1982), Washington and Oregon (Biever 1992), and, more broadly, in eastern North America north of latitude N 40° from New Brunswick to North Dakota (Herlihy et al. 2012). This widespread reduction of the population density of *C. glomerata* throughout southern New England by *C. rubecula* may allow *P. oleracea* to

reoccupy more of its historical native range (Van Driesche et al. 2004). Recent trap host exposures of *P. oleracea* larvae at the Lenox site, documented that currently there is a near zero level of parasitism by *C. glomerata* at the site (unpub. data, Wagner, Van Driesche and Herlihy).

With this background as context, the specific objectives of our study were to explore the above-mentioned mechanisms proposed to explain how *P. oleraceae* survived at this site in the pre-2000 time period, when levels of parasitism by *C. glomerata* at the study site would have been predicted fto be high, at least on other crucifers such as hedge mustard (as in Van Driesche et al. 2004). We wished to determine which attributes of cuckoo flower were protective of the butterfly population when *C. glomerata* was still abundant in Massachusetts. This information is relevant both for insect conservation and study of the non-target effects of biological control agents.

## Materials and Methods

### Sources of Plants and Insects For Experiments

Cuckoo flower (*C. pratensis*) plants used in our experiments were dug at the study site in Lenox, MA (42° 23' 37" N; 73° 14' 33" W) and placed in 10 cm dia pots prefilled with Pro-Mix BX mycorise growing mix. Plants were kept in a greenhouse and watered every two days until used for experimentation.

Collards (*Brassica oleracea* L. var. Blue max hybrid) for experiments were purchased as four-week-old plants from Harvest Farm in Whately, MA. They were

transplanted into 10 cm dia pots prefilled with Pro-Mix BX mycorise growing mix. The plants were kept in a greenhouse and watered every two days until used.

*Cotesia glomerata* wasps used in experiments were obtained from our laboratory colony maintained, originally started with cocoons collected in Massachusetts and supplemented with material from Virginia, and Illinois. *Cotesia glomerata* cocoon masses were held at about 3 °C until needed for experiments. Cocoons were then put into cages with honey and sugar solution ("Instant Hummingbird Nectar," Perky Pet Woodstream Corp. Lititz, PA), at 20°C, natural light, and 50-65% R. H. and left for adult emergence. Once adults had emerged, wasps were continually supplied with honey and a sugar solution, but were not exposed to host larvae or host plants. Naïve female wasps, exposed to males for several days for mating, were used when they were 3 to 5 days old. After use in experiments, females were returned to the rearing colony, where they were placed into a separate rearing and exposed to first and second instar *P. rapae* or *P. napi* on fresh collard leaves for parasitoid oviposition. The parasitized *P. rapae* and *P. napi* larvae were then reared on collard leaves to produce *C. glomerata* cocoon masses for future experiments.

*Pieris oleracea* larvae used in our experiments were obtained from our laboratory colony, initiated in 2010 with adult female butterflies collected at the Lenox, Massachusetts site and supplemented by adult females caught in Lenox, Massachusetts during flight of each field butterfly generation over a three year period (2010-2012) (under permit from the State Natural Heritage Program). Butterflies were reared through several generations in the laboratory in the course of the summer (2012) for the experiments reported here. Pupae were stored at about 3°C until needed and were then

moved to 61 cm x 61 cm x 61 cm Bug dorm cages (BioQuip Products, Rancho

Dominguez, CA, USA), where emerging adults were provided with sugar solution

("Instant Hummingbird Nectar," Perky Pet Woodstream Corp. Lititz, PA) and potted

cuckoo flower plants dug from Lenox, Massachusetts. Eggs laid on plants were allowed

hatch and larvae to develop on the cuckoo flower plants until larvae were ready  for

experimentatal use. Excess larvae were returned to the *P. oleracea* colony where they

were transferred from cuckoo flower to collard leaves, both being highly suitable host

plants.


**Olfactometer Physical Design**

A standard four arm olfactometer was used for the olfaction experiments. A

central chamber (55 mm in diameter x 100 mm in height) was connected to four glass

arms (50 mm in diameter x 100 mm long), which were each connected to bait chambers

(100 mm in diameter x 200 mm long) containing an odor source. Wire screens were

placed between the openings of each of the odor source chambers and the arm leading to

the central chamber, to allow air flow, but prevent wasps from entering the odor source

chamber. The odor source chambers were connected to a pressure control valve (allowing

for control of the air flow rate through each arm), a deionized water bubbler to control the

humidity in each arm, and an activated charcoal filter. The central chamber was linked to

a vacuum pump via 10 cm dia plastic tubing. The pump was set at 400 ml min$^{-1}$, to pull

air evenly through all four arms, without affecting the wasps ability to fly through the

arms. Light brown foam boards (430 mm h) were placed on all four sides of the

olfactometer, which was placed on a laboratory table, to prevent directional bias in

lighting. Central fluorescent lighting was located on the ceiling above the center of the olfactometer.

## Olfactometer Experimental Design

As a negative control, tests were run with four empty odor-source chambers in which a single, 3-5 day old, mated female wasp was released into the central chamber, and whose position was recorded after 30 minutes, to confirm lack of directional bias in the unbaited system.

For our experiments, the test protocol was similar to that of Karimzadeh et al. (2012). During each experimental run, one naïve, 3-5 day old, mated female *C. glomerata* wasp was released into the central chamber. Two arms (180° apart) contained the same bait (plant or plant-host complex) and the other two were unbaited controls, giving the wasp a choice between either two plants (or two plant host complexes) and two controls. We tested (1) uninfested collards vs uninfested cuckoo flower and (2) infested collards vs infested cuckoo flower.

As a positivecontrol, two empty arms were tested against *P. oleracea* infested collards in two arms 180 ° from the controls.

For tests with uninfested plants, collard leaves were cut at the end of the petiole nearest the leaf and weighed; Cuckoo flower leaves were then cut along the petiole to match the weight of the collard leaves. This was done because cuckoo flower leaves are pinnate and thus easier than collards to downsize to a desired weight without cutting the actual leaf blade (which would release green leaf volatiles attractive to parasitoids). Uninfested leaves were placed in test chambers 10 minutes before each experiment. For

experimental runs with infested leaves, 10 second instar *P. oleracea* larvae were placed

on each collard leaf, or group of cuckoo flower leaves. All leaves were pre-weighed and

sized to hold foliar weight equal between treatment. Foliage with larvae was placed in the

odor source chambers and larvae were allowed to feed for 1h in each experimental run to

allow time for plants to respond to feeding by releasing both constituent and de novo

induced compounds. Tests ran until wasps made a decision and entered an arm or if

wasps were unresponsive, a run was terminated after 30 minutes. Wasps entering and

remaining in an olfactometer arm beyond a line 4 cm into the arm were counted as having

made a decision and those remaining in the center were considered unresponsive. Wasps

that reversed direction within an arm were counted as unresponsive. The position of odor

sources was rotated 90° after each run (= the trial of one wasp). The odor source

chambers were removed after each run, rotated, and placed onto a new arm. After each

group of replicates run on a given day (ca 15-20 per day), the odor source chambers were

removed and washed in an odorless detergent and rinsed with water and allowed to air-

dry overnight. In total, there was a minimum of 30 replicates per treatment (each

consisting of the trial of one wasp).


## Overtopping Vegetation Field Cage Experiment

To determine if overtopping by other vegetation affected the rate of host detection

by *C. glomerata*, field experiments were run on the University of Massachusetts Amherst

campus in a meadow using open bottom cube cages (0.6 m$^3$) with black mesh fabric

sides. In each run of the experiment, we placed four such cages at the field site. In total, 8

experimental runs were performed from June to August 2012, one each per day. Each

cage was a single replicate of one of four treatments being tested: (1) collards and clipped native vegetation, (2) collards and unclipped vegetation, (3) cuckoo flower and clipped native vegetation, and (4) cuckoo flower and unclipped vegetation. Test plants of each species were 10 cm tall. Clipped vegetation was cut with scissors within 2--5 cm of the soil. Unclipped vegetation was ca 35 cm tall. Each test plant was baited with five first instars of *P. oleracea* (= 10 larvae per cage). Then two 3-5 day old *C. glomerata* females, which had been given the opportunity to mate and had had no previous exposure to *C. pratensis* or *B. oleracea* volatiles, were released into each cage. The larvae were exposed to parasitoids for 24 hours. The test was then terminated and the larvae removed and dissected to detect parasitoid oviposition.

## Statistical Analyses

All statistical analyses were conducted using the program R (version 2.13.1). Binomial exact tests were used to compare female wasp choices in the olfactometer. Cage experiment percent parasitism data were arcsine transformed in order to meet the assumption of normality. Parasitism data were analyzed with an ANOVA followed by a Tukey HSD test.

## Results

### Olfactometer Tests

*Negative controls*. Wasps (n = 32) placed in the olfactometer with four empty odor chambers showed no directional bias in the olfactometer set up ($X^2 = 0.25$, df = 3, $P = 0.969$).

***Postive control to infested foliage***. Females given the choice of arms baited with collard foliage infested with *P. oleracea* larvae vs empty (unbaited) arms consistently chose the arms with the infested collards (Fig. 1). Twenty-three of the 25 wasps tested chose infested collards over empty arms. A two-tailed binomial exact test shows this to be significant at the 0.05 level ($P = 0.56$) and this demonstrates that the wasps and olfactometer system we used functioned normally as expected.

***Exp. #1. Host plants without larvae***. *Cotesia glomerata* females, when offered two arms with collards and two with cuckoo flower (all plants unifested), were equally attracted to both treatments, with no significant difference in attraction in a two-tailed binomial exact test at the 0.05 level ($P = 0. 51$) (Fig. 1).

***Exp. #2. Infested host plants***. *Cotesia glomerata* females were equally attracted to *P. oleracea*-infested cuckoo flower plants and *P. oleracea*-infested collards. Of the 105 resonsive female wasps tested, 59 chose infested collards  and 46 chose infested cuckoo flower, which was not statistically significant in a two-tailed binomial exact test at the 0.05 level ($P = 0.24$).

**Overtopping Vegetation Field Cage Experiment**

Overtopping vegetation had a significant effect on rates of parasitism of *P. oleracera* by *C. glomerata* for both cuckoo flower and collards (F = 12.8, df = 3, P <0.001). *Pieris oleracea* larvae on both collards and cuckoo flower with overtopping vegetation experienced significantly less parasitism by *C. glomerata* ($23 \pm 0.16$ % on collards, $22 \pm 0.15$ % on cuckoo flower) than *P. oleracea* larvae on collards and cuckoo flower plants without overtopping vegetation ($77 \pm 0.25$% on collards, $68 \pm 0.22$ % on

cuckooflower). There was no significant difference in parasitism by *C. glomerata* between plants within a given vegetation height treatment (based on the Tukey HSD test) (Table 1).

## Discussion

*Cotesia glomerata* is able to detect hosts even from long distances via olfactory signals from the plant-host complex, which is a combination of volatiles released by the plant due to either mechanical damage or induced effects caused by saliva of *Pieris* larvae (Steinberg et al. 1993). *Cotesia glomerata* is not only able to detect its host from long distances, but is also able to detect how many hosts are present on a host plant, and the age of the plant and preferentially choose the most profitable patches with the highest number of hosts onyounger host plants (Mattiacci and Dicke 1995; Geervliet et al. 1998). We know that *C. glomerata* is present at the Lenox site, at least at low levels (M.H., unpub. data), yet there is no evidence of parasitism during experimental trap host exposures of Pieris larvae on suitable host plants (M.H. unpub. data). The goal of the experiments we report here was to determine whether this olfactory signaling system from the plant-host complex was equally efficacious for detecting *P. olearcea* larvae feeding on *C. pratensis* plants. Also, there is evidence from the literature that complex plant architecture, including overtopping vegetation, decreases parasitoid host finding and provides an enemy free space (Sato and Ohsaki 1987; Meiners and Obermaier 2004; Obermaier et al. 2008). There is also evidence that some host-plant combinations produce volatiles that parasitioids donot detect or do not recognize as a signal of their host's

presence, thereby providing enemy free space for the host. For example the Japanese subspecies of *Pieris napi* uses a nutritionally inferior host plant *Arabis sp.* in order to avoid parasitism by *C. glomerata* (Ohsaki and Sato 1990). The results of our olfactometer experiments, however, suggest that *C. glomerata* does not distinguish between *C. pratensis* and *B. oleracea* when plant types were both uninfested or both infested with *P. oleracea*, suggesting that in the field *C. glomerata* should be able detect volatiles produced when *P. oleracea* feed on *C. pratensis*. This implies that something else, such as plant architecture or resource abundance, may be providing protection against parasitism by *C. glomerata*.

Our data show that overtopping vegetation greatly decreases parasitism of *P. oleracea* feeding on either *C. pratensis* or *B. oleracea* (to levels only about one third of controls without overtopping vegetation). Overtopping vegetation likely physically impedes or confuses the wasps during their search process and therefore likely is an important factor under field conditions, at least in the summer and fall generation, when cuckoo flower plants are masked by other vegetation. Also, in our experiment, in cages without the physical barrier of overtopping vegetation, *C. glomerata* wasps were able to easily detect, locate, and parasitize *P. oleracea* larvae on both *C. pratensis* and *B. oleracea*, showing that the pinnate foliage of *C. pratensis* per se does not impede or reduce the efficiency of the wasps' searching ability once on the plant.

In summary, in our view, the population of *P. oleracea* at our Lenox, Massachusetts site was likely able to survive despite presumed high parasitoid pressure at the site before 2000 by switching host plants in the summer and fall from tall crucifer species in meadows such as hedge mustard to the low stature basal rosettes of *C.*

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*pratensis* which were overtopped and thus partly hidden by other vegetation. Based on

our data this layer of over topping vegetation likely protects *P. oleracea* by impeding  or

confusing *C. glomerata*'s search efforts, despite the wasps' ability to detect the volatiles

of the host-plant complex as shown in the olfactometer experiments as well as in the

clipped treatments of the cage experiments. Also, we must acknowledge that we could

not test our fourth hypothesis. The fact that cuckoo flower provides a large resource for

the whole growing season may well have enhanced the population growth rate of the

butterfly, which in turn may have allowed the population to sustain itself despite some

significant levels of parasitism.  Since data on parasitism were not being taken during this

critical period historically, this possibility can neither be confirmed nor ruled out.

**Table 3.1.**

Rates (± 95% CI) of parasitism of *Pieris oleracea* larvae by *Cotesia glomerata* in field-cage experiments, comparing the effects of the presence or absence of overtopping vegetation. Parasitism was detected via larval dissection. Statistical differences are denoted by different lowercase letters.

| Plant type | Proportion parasitized |
|---|---|
| **Collards** | |
| Clipped | 0.77 ± 0.11 (52) a |
| Overtopping vegetation | 0.23 ± 0.12 (44) b |
| **Cuckoo flower** | |
| Clipped | 0.68 ± 0.16 (34) a |
| Overtopping vegetation | 0.22 ± 0.13 (37) b |
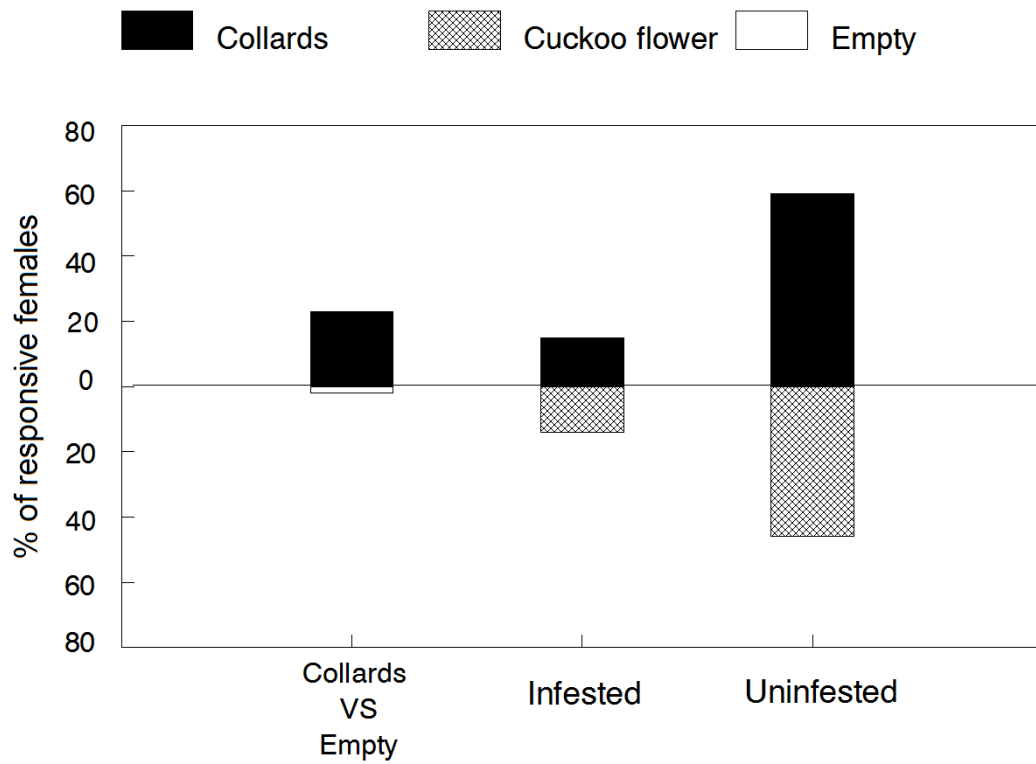
**Figure 3.1** Proportions of choices made by female *Cotesia glomerata* wasps inside the olfactometer. From left to right (1) % of females choosing either empty arms or uninfested collards (2) % of females choosing either *Pieris oleracea* infested cuckooflower or *P. oleracea* infested collards (3) % of females choosing either uninfested cuckooflower or uninfested collards.

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