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Assessment of risk posed by introduced braconid wasps to *Pieris virginiensis*, a native woodland butterfly in New England

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Abstract

The range of the native butterfly *Pieris virginiensis* Edwards has decreased in New York and Ontario since the 1940s. Loss of habitat and harm from *Cotesia glomerata* (L.), a braconid parasitoid introduced to North America in the late nineteenth century for biological control of the invasive pest butterfly *Pieris rapae* (L.), have been suggested as the causes of this decline. In western Massachusetts, extensive habitat suitable for *P. virginiensis* remains and its principal host plant, *Cardamine diphylla*, is common. We found *P. virginiensis* to be widely present, occurring at 39% of host plant patches. In laboratory tests, we found that both *C. glomerata* and *Cotesia rubecula* (Marshall) are able to parasitize and successfully develop in *P. virginiensis* larvae when these are presented on detached leaves of *C. diphylla*. However, when we exposed laboratory-reared first or second instars of either *P. rapae* or *P. napi* (both suitable hosts for these *Cotesia* spp.) as sentinel larvae on leaves of either *Brassica oleracea* or *C. diphylla* at sites where *P. virginiensis* was present, no parasitism by either *Cotesia* sp. was detected. Some sentinel larvae were parasitized by an unidentified ichneumonid in the genus *Hyposoter*. We conclude that in western Massachusetts, *P. virginiensis* is widely distributed at low densities and that while it is physiologically an acceptable and suitable host for both *C. glomerata* and *C. rubecula*, larvae of field populations of this butterfly are not attacked because these parasitoids do not forage in forested habitats, even when they are locally present in adjacent meadows. Consequently, we report that this butterfly is not threatened by these parasitoids contrary to early suggestions in the literature.

1. Introduction

The West Virginia white, *Pieris virginiensis* Edwards, is a native North American pierid butterfly whose range has contracted historically. The non-native braconid parasitoid *Cotesia glomerata* (L.), introduced for biological control of a congener pest butterfly, the imported cabbageworm (*Pieris rapae* [L.]), has been suggested as the reason for this decline.

Pieris virginiensis is found in wooded habitats in the Appalachian Mts., New York, Pennsylvania, the Great Lakes states, southern Ontario and southwestern New England, including western Massachusetts (Opler and Malikul, 1992; Scott, 1986). It was once considered to be merely the spring brood of *Pieris napi oleracea* (Scudder, 1889), but was later recognized as a distinct species by Klots (1935) (see also Hovanitz (1963) for a history of the species concept for *P. virginiensis*).

Pieris virginiensis is a univoltine spring butterfly whose distribution and life history are closely tied to the range and phenology of two-leaved toothwort (*Cardamine* [formerly *Dentaria*, see Magee and Ahles, 1999] *diphylla*), a woodland spring ephemeral crucifer (Cappuccino and Kareiva, 1985; Klots, 1935; Shapiro, 1971). *Cardamine diphylla* is the only important host plant of this butterfly. This plant occurs widely in western Massachusetts, but is not found in the eastern part of the state, except for a few introduced populations (Sorrie and Somers, 1999). *Dentaria laciniata* (now *Cardamine concaténata* [Michx.]) has also been listed as a suitable host for *P. virginiensis* (Chew, 1980), but Cappuccino and

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Kareiva (1985) found that although this plant was accepted for oviposition, the larvae did not survive. Other plant species have been accepted for oviposition under laboratory conditions or have supported larval development, but no evidence exists for their use in the field (Scott, 1986).

In western Massachusetts, at locations with two-leaved toothwort patches, West Virginia white adults are typically found flying in small numbers in the shade of deciduous forest stands in late April through May, beginning soon after the appearance of C. diphylla foliage. Peak flight occurs in approximately the second and third weeks of May. Females lay eggs singly on toothwort leaves and larvae are able to develop to maturity on just a few leaves. The insect diapauses in the pupal stage until the following May, attached to plants or debris.

Population densities of P. virginiensis adults at any particular site are nearly always very low. However, the species can be widely distributed in regions where stands of two-leaved toothwort are available along streams. In Massachusetts, two-leaved toothwort is common west of the Connecticut River Valley in a 4300 km^2 region that is approximately 70% forested (Dickson and McAfee, 1988; MacConnell, 1975).

During the 20th century, P. virginiensis populations in New York (Shapiro, 1974) and Ontario (Tasker, 1975) declined in some areas, and in Ontario this butterfly was listed as a threatened species. In central New York (near Ithaca), several localized populations of this butterfly disappeared between the 1940s and 1960s for unknown reasons (Shapiro, 1974), but reappeared there in the 1970s and 1980s (Shapiro, pers. comm.). In the Catskill Mountains in southeastern New York, the species is known to have been common since the 1960s (Bob Dirig, Cornell University, pers. comm.).

Natural ecological factors potentially causing fluctuations in P. virginiensis populations include variability in weather during adult flight and the effect of natural enemies on larvae or pupae. Oviposition occurs at a time of year when weather is unpredictable and often too cold or rainy for flight. For the sulfur Colias alexandra Edwards, reduction in adult flight time due to poor weather reduced egg laying and was the principal source of variation in butterfly numbers among study years (Hayes, 1981). P. virginiensis larvae must complete development before senescence of two-leaved toothwort foliage, which occurs about six to eight weeks after the foliage emerges. If emergence of P. virginiensis adults or their egg laying is delayed by as little as five days due to unsuitable weather, synchrony between larvae and host plant foliage may be disrupted (Cappuccino and Kareiva, 1985).

Naturally occurring pathogens and parasitoids might also be important sources of mortality to the life stages of P. virginiensis. Larvae can be killed by a granulosis virus (Cappuccino and Kareiva, 1985), but data are not available on this factor's severity or variability among years. There are no records of parasitism of larvae or pupae of P. virginiensis by any native species.

Conversion of forests to crop or pasture land may have reduced the range of P. virginiensis (Klots, 1935) because the species' sole host plant (C. diphylla) is an obligate woodland species. Chew (1981) makes the same argument to explain range reductions in the related species P. napi oleracea, which formerly occurred in western Massachusetts. In contrast to most species of Pieris, open habitats provide no host plant resources for P. virginiensis, and, if extensive, may prevent adults from flying between forest fragments. Loss of forest habitat is likely to have been an important cause of the reduction of the range of this butterfly in New York (outside of the Adirondack and Catskill Forest Preserves) and in southern Ontario, both regions in which a high percentage of the land has been converted to agricultural use. In New England, however, forest conversion for agricultural use peaked about 1830 and the proportion of the landscape in native forest has been increasing for nearly 150 years, with a concurrent increase in the amount of P. virginiensis habitat (O'Keefe and Foster, 1998).

A second human-caused threat to native Pieris butterflies in North America may be attack by parasitoids introduced to control P. rapae. Klots (1935) suggested that parasitoids reproducing on the much larger populations of P. rapae might attack P. virginiensis and cause its populations to be reduced. Similarly, Herrera (1982) suggested that native pierines in Chile declined in abundance because of populations of C. glomerata reproducing on the invasive species Pieris brassicae (L.). The term "apparent competition" has been applied when abundance within a group of related species is influenced by shared natural enemies (Holt and Lawton, 1998). Apparent competition can completely exclude the herbivore species least able to sustain its population growth in the presence of a shared parasitoid (Bonsal and Hassel, 1998). Cases of such exclusion have been recorded for other insects (Settle and Wilson, 1990).

Pieris rapae parasitoids that might affect native Pieris species in North America are the larval parasitioids C. glomerata and Cotesia rubecula (Marshall) and the pupal parasitoid Pteromalus puparum L. (Pteromalidae)

Pteromalus puparum is a generalist parasitoid that appears to have had a naturally Holarctic distribution, having been recorded in North America near Hudson Bay, Canada, in the 1840s (Scudder, 1889), well before the invasion of North America by P. rapae in 1860. It has not been recorded parasitizing P. virginiensis pupae, but is likely to do so, given that it attacks P. napi pupae in wooded habitats in Vermont (unpublished data of authors).

Cotesia rubecula is a solitary parasitoid of P. rapae found in Eurasia. Releases of this species from Europe

were made in Missouri and later in Ontario in the 1960s (Puttler et al., 1970), but establishment occurred with difficulty and spread was slow. A second strain, collected from China was first released in Massachusetts in 1988 (Van Driesche and Nunn, 2002). This strain has established in New England and spread as far north as northern Vermont. Compared to *C. glomerata*, *C. rubecula* has a narrower host range and field parasitism records exist only for *P. rapae* (Brodeur et al., 1996; Puttler et al., 1970; Sengonca and Peters, 1993). In laboratory no-choice tests, it has been observed to oviposit in *P. napi* (Brodeur et al., 1996; Van Driesche, unpublished) and *P. brassicae* (Brodeur et al., 1996). There are no data on the suitability of *P. virginiensis* for *C. rubecula*.

Cotesia glomerata is an oligophagous, gregarious larval parasitoid that is known to attack five species in the genus *Pieris* in the field (*P. rapae*, *P. brassicae*, *P. napi*, *Pieris protodice* Boisduval and Leconte, and *Pieris melete Ménétriè*) and one in the genus *Colias* (*C. lesbia* [Fabricius]) (Krombein et al., 1979; Laing and Levin, 1982; Lees and Archer, 1974; Ohsaki and Sato, 1990; Sharkey et al., 2000). Other host records occur in the literature (e.g., Ghosh, 1998 and those cited in Tawfik, 1957), but are likely to be erroneous due to misidentification of the parasitoids reared. There are no records of parasitization of *P. virginiensis* by *C. glomerata* from either natural populations or laboratory experiments.

The origins of the US populations of *C. glomerata* are unclear and perhaps multiple. The species' presence in North America is generally credited to its introduction near Washington, DC, in 1884 for biological control of *P. rapae* (Clausen, 1978). However, *C. glomerata* seems also to have co-invaded North America with *P. rapae*. Scudder (1889) states that he reared *C. glomerata* from *P. rapae* collected near Boston, Massachusetts, in or slightly before 1870, 14 years before the first establishment of the species through deliberate introduction near Washington, DC. Furthermore, Scudder's observation that *P. napi* populations declined in Massachusetts in the 1870s, concurrent with the invasion by *P. rapae*, requires that *C. glomerata* be present in New England then, not a decade later. Alternatively, the species may have had a Holarctic distribution (like *P. puparum*) and been present in North America before the *P. rapae* invasion. Few entomologists worked in North American before 1860 and so the absence of records of *C. glomerata* is not definitive.

Regardless of the mechanism of origin of *C. glomerata*, once *P. rapae* populations became available as hosts, this parasitoid's abundance likely increased significantly. We have found evidence that *C. glomerata* attacks *P. napi* in New England and is likely to have caused the disappearance of this butterfly from Massachusetts (Benson et al., unpub.), but a similar evaluation of the relationship between these introduced parasitoids

and *P. virginiensis* has not been reported. Our study's goal was to make such an evaluation. To do this we measured: (1) the current abundance of *P. virginiensis* and two-leaved toothwort in western Massachusetts; (2) the acceptability and suitability of *P. virginiensis* larvae for oviposition and development of both *C. glomerata* and *C. rubecula* in the laboratory; and (3) levels of parasitism by *Cotesia* spp. in sentinel *Pieris* spp. larvae deployed in *P. virginiensis* habitats.

2. Materials and methods

2.1. Host plant survey

In 1998, we estimated the abundance of two-leaved toothwort in western Massachusetts by visiting 50 randomly selected riparian areas along brooks in mesic deciduous forests. The survey was conducted in a 359 km² section of western Massachusetts defined by 10 contiguous 7.5 min (or equivalent) USGS topographic maps ("quadrats"), an area roughly bounded by Greenfield, North Adams, Pittsfield and Northampton, Massachusetts (east–west from 72°15′ to 72°45′ longitude and north–south from 42°25′ to 42°40′N latitude). The survey area was typical of the western portion of the state, which is approximately 70% forested, generally hilly and drained by a large number of small brooks. Topographic quadrats defining the survey area were the US Geological Survey maps named North Adams, Rowe, Bernardston, Cheshire, Ashfield, Greenfield, and Goshen, some of which are 15 min maps. To choose sample locations, we examined 10 maps, each covering an area equivalent to a 7.5 min area. On each map, we marked the location of all intersections of a road (including unpaved automobile roads) with a stream or river in forested habitat (as suggested by green color on map). We numbered all marked locations and used random numbers to select 10 per quadrat, five as sample locations and five as alternates if needed. Sites were rejected only if they were no longer forested, if we could not reach them by car, or if access to the land was legally restricted.

At each sample site, the surveyor walked a 500 m transect parallel to the brook, stopping at each of 25 predetermined, random distances along the stream and noting the presence or absence of two-leaved toothwort plants within one meter forward or backward along the transect, and as far to the sides as the plants could be seen and recognized (approximately 5 m). In addition, any *Pieris* spp. butterflies seen during the visit to a site were recorded and captured for identification if possible.

2.2. Butterfly survey

Within the area where the 1998 two-leaved toothwort survey was run, we conducted butterfly surveys in both

1999 and 2000. We measured the proportion of sites with *C. diphylla* at which we saw *P. virginiensis* adults. In 1999, we chose the towns of Ashfield and Buckland (the eastern half of the Ashfield 15 min topographic map) as the butterfly survey area because our 1998 survey indicated that sites with two-leaved toothwort were common in that area. During the 1999 flight period of *P. virginiensis* (late April through early May), we visited all the forested road-stream intersections in the survey area, some each on 27, 28, 30 April or 1, 3, 6 May. At each site, a surveyor walked along the stream for five min in each direction from the road, for a 10-min total sample time, and noted whether *C. diphylla* and any *Pieris* spp. butterflies were present. We caught all the butterflies we saw, if possible, and identified them to species. We limited the survey from 1000 to 1500 h on sunny, warm days with little wind so that survey times were suitable for *P. virginiensis* flight. We recorded separately the numbers of *Pieris* butterflies that we could not net for identification, but we considered these to be *P. virginiensis* if they were seen flying in the woods. *Pieris rapae* rarely enters wooded areas and *P. napi* is now either absent or extremely rare in western Massachusetts (based on our unsuccessful attempts to locate this species in western Massachusetts in 1997–1999, during which only two *P. napi* specimens were ever recovered during more than 10 day-long surveys of multiple sites at appropriate times). From these observations we calculated the proportion of sites at which we saw *P. virginiensis* adults, as a fraction of all sites where patches of two-leaved toothwort were present.

In 2000, we resurveyed the same area examined in 1999, but changed our methods because *P. virginiensis* adults appeared to visit sites only in small numbers and to remain only briefly. To improve the survey, we increased the time spent at each site to 60 min and conducted the whole survey on a single warm, sunny day near the date of peak *P. virginiensis* flight. Using data from our 1998 two-leaved toothwort survey, we chose 23

sites in the Buckland–Ashfield areas, where two-leaved toothwort was present. On one date (13 May 2000), we recruited 13 observers and stationed them one per site for 60 min turns between 1100 and 1400 h. After one h, observers moved to a new site; most observers made observations at two sites over the course of the day. At the time of the survey, the air temperature was 18–24 °C and it was generally sunny. The survey date was chosen because it was a Saturday (when volunteers were available) and because frequent observations by Van Driesche at one site in Huntington, MA (approximately 35 km due south of the survey area) indicated that *P. virginiensis* was nearing peak flight (see Table 1). Observations at the Huntington site were made approximately weekly from late April through the end of May. Each weekly observation consisted of one person walking or bicycling for one h along a dirt road through an area with many two-leaved toothwort patches, counting *Pieris* spp. butterflies seen flying in the woods. Once butterflies were seen in large numbers at this one location, we conducted our 23-site butterfly survey.

2.3. Parasitoid oviposition and development in P. virginiensis in the laboratory

We conducted laboratory experiments to determine if *P. virginiensis* would be accepted by the two *Cotesia* parasitoids for oviposition, be suitable for complete development of their larvae, and (for *C. glomerata* only) produce normal sized F_1 adults likely to have normal fecundity. Such host range testing was not conducted for either of these species prior to their release in the United States. We used a "no-choice" cage test to assess the acceptability of *P. virginiensis* and then reared parasitized hosts on two-leaved toothwort foliage to see if *P. virginiensis* was a suitable host. Similar approaches have been used to estimate host ranges of other parasitoids (e.g., Barratt et al., 1997; Luhring et al., 2000; Porter and Alonso, 1999).

Table 1
Observations on the timing of the 2000 flight of *Pieris virginiensis* at the Knightville Dam Tract in Huntington, Massachusetts

Date	Physical conditions and sampling activity	Status	
		Two-leaved toothwort	*P. virginiensis*[a] (# seen)
April 9	Last snow of season (2.5 cm)		
April 16	Surveyed 1 h (10:30–11:30 am); partly sunny, 16–18 °C	Foliage, buds only, no flowers	0
April 17–23	Cold (2–10 °C) and rainy each of last 7 days; not suitable for flight		
April 30	1 h survey (4–5 pm)	In bloom	1
May 3	1 h survey (3–4 pm)	In bloom	3 (all collected)
May 6	2 h survey; hot (27–32 °C), humid and calm;	In bloom	14 (8 collected)
May 13	Pv survey done in Ashfield area		20, over 9 sites
May 18–25	8 cold, rainy days, unsuitable for Pv flight		
May 29	1 h survey; warm (16–21 °C), partial sun, light wind; five other species of butterflies seen flying	Toothwort overgrown by other plants	0; flight appears to have ended

[a] All netted butterflies were confirmed as *P. virginiensis*.

2.3.1. Source of P. virginiensis larvae

In early May 2000, *P. virginiensis* adults were collected in Huntington, Massachusetts, and larvae from eggs laid by these butterflies on potted two-leaved toothwort plants were used to assess parasitism by *C. glomerata* under laboratory conditions. In May 2001, we repeated the process in order to conduct the same test with *C. rubecula*. In each year, butterflies were caged with locally collected, potted *C. diphylla* plants and the cage placed partly in natural light to obtain oviposition. Eggs were allowed to hatch on these plants and when larvae were first or second instars, they were removed and exposed to parasitoids.

2.3.2. Source of parasitoids

Cotesia glomerata was colonized by collecting cocoons or parasitized *P. rapae* larvae in 1999 or 2000 from organic vegetable plots in Hadley or Northampton, Massachusetts. Both *P. rapae* or *P. napi* were used as rearing hosts to support the colony. We exposed larvae to parasitoids either individually (placing one larva at a time into an oviposition cage and then withdrawing it after a single oviposition event) or in groups (by placing adult parasitoids into cages with host plants bearing various numbers of larvae). We reared parasitized larvae on potted collard plants in wooden cages (46 × 46 × 61 cm) with glass tops at 21–25 °C and 16:8 (L:D) h photoperiod. We provided larvae with fresh collard plants as needed and collected parasitoid cocoons as they formed. We grouped cocoons by collection date and held them in cups for wasp emergence, so that we could standardize the age and history of the wasps we used in the experiment.

The *C. rubecula* colony was started from the same local sources and reared in our laboratory using the same methods as described above, except that only *P. rapae* was used as a rearing host.

2.3.3. Parasitization arena and method of parasitization

In 2000, to determine if *P. virginiensis* larvae were acceptable for *C. glomerata* oviposition, we placed one young larva (first or second instar) on a small piece (2 × 2 cm) of *C. diphylla* leaf that had *P. virginiensis* feeding damage. Then we attached this leaf to the end of an artist's paint brush and introduced the leaf and larva through a sleeve into a plexiglass cage with ca. five adult parasitoids (of which 2 or 3 were female). Cages were 28 cm on all sides, ventilated on three sides with organdy panels or sleeves for access. *Cotesia glomerata* parasitoids at the start of the experiment were 2–4-day-old and had no previous host contacts. We held the leaf with the larva near individual parasitoids and allowed females to walk onto or land on the leaf and forage for a host. Sequentially over a 1–2 h period, we exposed 43 *P. virginiensis* larvae to *C. glomerata* wasps. We withdrew each larva from the cage immediately after the first

oviposition. This procedure prevented multiple ovipositions into a host larva, which can easily occur if a larva is exposed to groups of *C. glomerata* adults. We needed to avoid multiple ovipositions as this is often a cause of increased rates of death in attacked larvae shortly after oviposition due to the physical trauma of multiple ovipositor insertions. For comparison, a group of 22 first or second instar *P. napi* larvae were also parasitized using these same procedures, using larvae from our laboratory colony.

In 2000, we repeated these procedures and, on 16 May, we exposed 39 first or second *P. virginiensis* instars to recently emerged, naïve *C. rubecula* adults. Larvae were introduced (on a two-leaved toothwort leaf with feeding damage, held on the tip of an artist's paint brush) one at a time into a cage with several *C. rubecula* adults and then withdrawn after being stung a single time.

2.3.4. Rearing of parasitized larvae

In 2000, we placed individual larvae of *P. virginiensis* or *P. napi* that had been parasitized by *C. glomerata* in unventilated petri dishes (9 cm diameter) on excised *C. diphylla* leaves resting on moist filter paper and held them in a growth chamber at 24 °C, 16:8 (L:D) h photoperiod, and 50–60% RH. We examined larvae every 1–2 days, adding new leaves and moistening the filter papers as needed. For comparison, a group of 126 *P. virginiensis* larvae that had not been exposed to parasitoids, but which were from the same source as the larvae used in the experiment, were also reared in petri dishes on excised *C. diphylla* leaves. These larvae were held under continuous light at room temperature (in an attempt to prevent the normal induction of diapause). Petri dishes of the control group were opened twice daily and new leaves and moisture added as needed.

In 2001, we reared larvae parasitized by *C. rubecula* on foliage of intact, potted two-leaved toothwort plants rather than on excised leaves as in the previous year. Plants with larvae were held in cages (plastic cubes, 25 cm on a side, ventilated with organdy on two faces) in a growth chamber at 24 °C, 16:8 (L:D) photoperiod, 60–80% RH until larvae died, pupated or yielded a parasitoid cocoon.

2.3.5. Rate of adult parasitoid emergence and parasitoid size

In both 2000 and 2001, we examined parasitized larvae periodically to detect parasitoid cocoons. *P. virginiensis* suitability as a rearing host was judged by the proportion of stung larvae from which parasitoids successfully emerged.

In 2000 only, we held the parasitoid cocoons reared from *P. virginiensis* (under the same conditions as used for rearing parasitized hosts) to obtain adult emergence. We allowed emerged adults to die and then opened the

dishes and counted the numbers of *C. glomerata* from each host larva. We then chose 20 emerged adults at random and measured the length of their hind tibia as a proxy measure for likely adult fecundity (since body size of Hymenoptera correlates directly with fecundity, Jervis and Kidd, 1996). We compared hind tibial lengths of 20 *C. glomerata* adults reared from *P. virginiensis* to 20 *C. glomerata* reared in the species' usual host, *P. rapae*.

2.4. Survey for parasitoids attacking Pieris spp. larvae at sites occupied by P. virginiensis

Laboratory experiments on parasitoid host ranges may yield false positives because environmental factors, such as habitat, are not included. To compensate for this limitation in our laboratory data, we directly sampled to detect the presence of *Cotesia* spp. in *P. virginiensis* habitats. Adult parasitic Hymenoptera are difficult to directly observe. Some estimates can be made by use of sweep netting, Malaise traps, or deployment of yellow sticky cards. For internal parasitoids such as *Cotesia* spp., detection of immature parasitoids inside their larval hosts provides a convenient alternative survey method. Eggs and larvae of both *Cotesia* spp. can be readily recognized in dissected *Pieris* spp. larvae. (*Cotesia glomerata* is gregarious, with 20 or more immature stages per host. *C. rubecula* is solitary and the first instar larva is mandibulate, whereas that of *C. glomerata* is not). However, larvae of field populations of *P. virginiensis* are too scarce to collect to measure rates of parasitism. As a substitute, laboratory-reared larvae can be deployed as sentinels to detect parasitism (Jervis and Kidd, 1996; Van Driesche and Bellows, 1996). Because *P. virginiensis* cannot be reared continuously in the laboratory due to an obligatory diapause, we used laboratory-reared larvae of two related species (*P. rapae* and *P. napi*), both of which are suitable hosts for *C. glomerata* (Benson et al., unpub.; Laing and Levin, 1982; Lees and Archer, 1972; Richards, 1940). For *C. rubecula*, only *P. rapae* is a suitable field host (Richards, 1940). Both of these species can readily be reared in the laboratory. In both 1999 and 2000, we placed *Pieris* spp. first or second instars (the only stages subject to parasitoid oviposition) in *P. virginiensis* habitats to detect *Cotesia* spp. parasitism to determine if adults of either *C. glomerata* or *C. rubecula* were present.

2.4.1. Source of larvae used as sentinels

Larvae used as sentinels were reared in our laboratory. We started a colony of *P. rapae* in 1997 with adults collected near Amherst, Massachusetts, and supplemented with material from Ithaca, New York (courtesy of Dr. A. Renwick, Boyce Thompson Institute, Cornell University). We started a colony of *P. napi* in 1998 with material from A. Renwick (from a culture originally collected in Vermont), and we supplemented our *P. napi*

colony each year with butterflies collected near Craftsbury, Vermont. We reared *P. rapae* larvae on potted collards in large cages and *P. napi* larvae on open benches in a small rearing room. To obtain eggs, we provided butterflies of each species with seedling collards or kale in plexiglas cages (either 28 cm on a side or $105 \times 50 \times 50$ cm) under natural light supplemented with one incandescent bulb to ensure a minimum 16 h of light daily. We replaced oviposition plants every 2 days to produce synchronized larval cohorts. We allowed larvae that were to be used in field experiments to feed for 2–24 h after egg hatch. To inoculate collard plants, we used a fine artist's paintbrush to place 30 first instars (six larvae on each of five leaves) on each of a series of potted plants with 5–7 leaves. Plants were taken to field sites within a 2–4 h after being inoculated with larvae and deployed. To inoculate two-leaved toothwort plants, we took first instars of *P. rapae* or *P. napi* to field sites and used brushes to place larvae directly on field plants.

2.4.2. Study sites

In 1999, we placed potted collard plants bearing first or second instars of *P. napi* or *P. rapae* at four wooded sites in western Massachusetts where two-leaved toothwort was present along streams and *P. virginiensis* butterflies were seen flying in 1999. Site #1 (72°53′ longitude by 42°38′N latitude) was located in Charlemont, MA, along a stream on the north side of Hog Mountain along a very steep slope that was well shaded by a canopy of mature, hardwood forest. Site #2 (72°50′ longitude by 42°33′N latitude) was located in Buckland, MA, on the relatively level flood plain of Smith Brook, which is a substantial-sized, fast-running brook with *C. diphylla* along both banks, but rarely in dense patches. The forest at site #2 consisted of 10–30-year-old mixed deciduous and coniferous trees, with a relatively open canopy. Site #3 (72°38′ longitude by 42°32′N latitude) was located in Deerfield, MA, along Hawk's Brook, a small stream running through hardwood forest. Two-leaved toothwort grew in large, dense patches in small openings along the stream. Site #4 (72°38′ longitude by 42°32′N latitude) consisted of very dense patches of *C. diphylla* along a wooded stream on a dairy farm in Shelburne, Massachusetts (quite near site #3).

2.4.3. Design of 1999 experiment

At each study site, we chose three sampling points (separated from each other by 20 m) and at each point we placed one potted collard plant with *P. rapae* larvae and one with *P. napi*. We placed wire-mesh (2.5 cm dia. spacings) cages over collard plants and staked the cages to the ground to protect the plants from deer and other vertebrates. To protect the plants from slugs, we removed the debris and vegetation from a narrow ring around each cage and sprinkled salt on the ground.

Larvae were left on plants at these sites for 3 days and then returned to laboratory and dissected to detect parasitism.

Because foraging parasitoids can potentially be influenced by the plant on which a larva is feeding (e.g., Geervliet et al., 1998; McCall et al., 1993), we also exposed sentinel larvae on the natural host plant of *P. virginiensis* at two of the four study sites. At these two sites, we used an artist's paint brush to place first instars of both *P. rapae* and *P. napi* on leaves of two-leaved toothwort at each of the three sample points where we placed potted collard plants. At each sample point, we selected six or seven toothwort plants randomly and placed one larva of a given species on each of the three leaflets of a single toothwort leaf. We tied colored tape to the leaf petioles to mark each location and to identify the species of *Pieris* larvae. *Pieris* larvae of the age used in this experiment are small in relation to leaf size and remained on the leaves on which they were placed.

During the period when sentinel larvae were exposed, larvae remained either first or second instars, the stages susceptible to parasitism. We placed sentinel larvae of each butterfly species at the study sites four times between 15 May and 4 June, a period corresponding to when we expected *P. virginiensis* larvae to be present, based on timing of adult flight. At one site (#4), we monitored for parasitism in both the woods and the adjacent meadow for a longer period of time (May–September).

2.4.4. Detecting parasitism in sentinel larvae

After the 3-day exposure period, we brought the potted collard plants back to our laboratory and examined them to locate remaining larvae. We placed the surviving larvae from each plant on a collard leaf in a sealed petri dish and held them at 4 °C until they were dissected, within one week, to detect parasitoid eggs or larvae. We collected the larvae exposed on two-leaved toothwort directly in the field at the end of the 3-day periods and then treated these larvae the same as the larvae from potted collards.

Sentinel larvae that had been attacked at the study sites by *C. glomerata* were recognized in dissection by the presence of multiple eggs or larvae (20–40). At 2 days post-oviposition eggs of *C. glomerata* range from 0.2 to 0.32 mm in length (Van Driesche, 1988a) and are slightly curved. Larger eggs (0.56–0.76 mm) that occurred singly in sentinel larvae were later found (based on rearing) to be those of an unidentified ichneumonid wasp in the genus *Hyposoter*. Eggs of this species could be distinguished from those of *C. rubecula* by greater curvature of the *Hyposoter* egg. To obtain adult *Hyposoter* sp. wasps, we placed additional sentinel larvae at our field sites and reared these larvae. Specimens were sent to Dr. David Wahl of the American Entomological Institute in Gainesville, Florida, but could not be iden-

tified to species because the genus is in need of revision and half of the nearly 60 North American species are undescribed.

2.4.5. Design of 2000 experiment

In the second year of the field study to detect parasitism at *P. virginienis* sites, we exposed sentinel *P. napi* larvae on potted collards at three of the 1999 sites (#1, 2, and 3, as above) over a seven week period (11 May–16 June) that spanned the whole time when field *P. virginiensis* larvae would have been young enough for parasitoid oviposition. Larvae were deployed using the same methods as in 1999. At site #3, several wild-caught *P. virginiensis* collected on 6 May were caged over toothwort plants. These plants were checked periodically thereafter to determine the local timing of occurrence of young larvae (instars 1 and 2), the stages susceptible to *Cotesia* spp. parasitism.

3. Results

3.1. Host plant survey

We found two-leaved toothwort at $54 \pm 14\%$ (95% CI) (27/50) of the wooded, road-stream intersections examined in our survey. At the 27 sites where two-leaved toothwort was present, we found toothwort at $31 \pm 4\%$ (95% CI) (208/675) of the points along the sample transects. Given the generally wooded (> 70%) nature of western Massachusetts and the abundance of small streams due to the hilly nature of the region, these data indicate that the two-leaved toothwood abundance in the region is high.

3.2. Butterfly survey

3.2.1. 1999 Survey

We found two-leaved toothwort at 32 of 41 forested, road-stream intersections marked on the eastern half of the Ashfield topographic map. At these 32 sites, we observed *Pieris* spp. butterflies flying in the woods at four sites (12.5%) during a 10 min observation period per site. In total we saw eight *Pieris* butterflies at the 32 sample sites, and we were able to capture four of these, all of which were *P. virginiensis*. The remaining four butterflies could not be netted or observed closely enough for identification.

3.2.2. 2000 Survey

In the weekly observations made at the Knightville dam property in Huntington, MA, we first saw adults of *P. virginiensis* on 30 April (one specimen). Adults of *P. virginiensis* were numerous on 6 May and flight appeared to have ended by 29 May (Table 1). These observations indicate that 13 May, the date chosen for our

2000 *P. virginiensis* survey, was approximately the middle of the flight period. On 13 May, we saw *P. virginiensis* adults at nine (39%) of the 23 sites. The species identification of the butterflies was confirmed by netting or close observation of one or more specimens at all 9 sites; in total, 20 butterflies were identified as *P. virginiensis*.

3.3. Laboratory host specificity trials

3.3.1. Cotesia glomerata

Parasitism of *P. virginiensis* was the same (100%, 43/43) as for *P. napi* (100%, 22/22), a known field host. We obtained cocoons of *C. glomerata* from 58% (25/43) of the parasitized *P. virginiensis* larvae 10–16 days later, compared to 41% (9/22) for *C. glomerata* on *P. napi*. Of the 18 *P. virginiensis* larvae that did not yield parasitoid cocoons, 15 died, 1 was lost and 2 pupated successfully. The mortality rate of parasitized *P. virginiensis* larvae during rearing to causes other than parasitism, while high (31%, 15/48), was lower than that of the unparasitized control group of *P. virginiensis* larvae (71%, 90/126). While rearing conditions were not optimal, parasitism itself did not increase mortality before the immature parasitoid completed its lifespan, indicating that larvae of *P. virginiensis* are physiologically suitable for this parasitoid's development. Numbers of adult wasp progeny per host were as high for *C. glomerata* reared in *P. virginiensis* (21.0 ± 2.3 SE, $n = 23$) as for ones reared in *P. napi* (18.1 ± 1.2 SE, $n = 9$). Similarly, progeny of C. glomerata reared in *P. virginiensis* were as large (0.78 mm ± 0.012 SE, $n = 20$, measured as hind tibia length) as those reared in *P. rapae* (0.79 mm ± 0.008 SE, $n = 20$, hind tibia length). These values indicate that *P. virginiensis* is as suitable a host for *C. glomerata* as is *P. napi*.

3.3.2. Cotesia rubecula

All 39 first or second instars of *P. virginiensis* presented to female *C. rubecula* were rapidly accepted for oviposition. Five proved not to be successfully parasitized and yielded a normal host pupa. Of the remaining 34 larvae, 16 died in rearing and the remaining 18 produced a *C. rubecula* cocoon, giving a survival rate of immature parasitoids of 54% (18/34).

3.4. Survey for parasitoids attacking Pieris sp. larvae at sites occupied by P. virginiensis

3.4.1. 1999 Experiment

Of 894 larvae recovered after field exposure, 600 were *P. napi* and 294 were *P. rapae*. Of these, 682 were exposed on collards and 212 on two-leaved toothwort. None of the *P. rapae* or *P. napi* larvae recovered and dissected after exposure in the field for 3 days were parasitized by *C. glomerata* or *C. rubecula* at any of the four wooded sites occupied by *P. virginiensis*, on either collards or *C. diphylla* in any time period. However, at three sites, *P. napi* and/or *P. rapae* were parasitized by an ichneumonid wasp (*Hyposoter* sp.) in three exposure periods, 15–18 May, 25–28 May, and 1–4 June (Table 2).

Parasitism by *Hyposoter* sp. was greatest on *P. napi* on two-leaved toothwort in the last week of the experiment (June 1–4) (62%, $n = 37$). The overall rate of parasitism of *P. napi* on *C. diphylla* was 29% ($n = 90$), which was significantly higher than for *P. napi* on collards (7%, $n = 510$) ($\chi^2 = 21.46$, df = 1, $P < 0.0001$). However, for *P. rapae*, there was no statistically significant difference between rates of parasitism of larvae on two-leaved toothwort (5%, $n = 122$) versus collards (6%, $n = 172$) ($\chi^2 = 0.009$, df = 1, $P > 0.10$). Summed over both plant species and all dates, *P. napi* larvae were attacked at a significantly higher rate (10%, $n = 600$) than *P. rapae* larvae (5%, $n = 294$) ($\chi^2 = 6.32$, df = 1, $P < 0.025$). All observed parasitism was due to an unidentified *Hyposoter* sp. wasp.

At one study site (Shelburne), we also deployed sentinel larvae in an adjacent meadow and found that larvae of both *Pieris* species were parasitized by *C. glomerata* (2.4% [12/504] parasitism of *P. napi* and 0.6% [6/938] of *P. rapae* larvae), indicating that this parasitoid was present in the area in which the main experiment was run.

3.4.2. 2000 Experiment

Pieris virginiensis adults were observed to be present in 2000 at all three study sites. At Hawk's Brook, several larvae hatched from eggs laid by *P. virginiensis* caged over two-leaved toothwort on 6 May and these were

Table 2
Percent parasitism in 1999 by *Hyposoter* sp. (Ichneumonidae) of *Pieris napi* and *Pieris rapae* larvae exposed as sentinel larvae at four wooded, riparian sites in western Massachusetts with *Cardamine diphylla* and *Pieris virginiensis* populations

Exposure dates	Collards		Toothwort	
	P. napi	*P. rapae*	*P. napi*	*P. rapae*
May 15–18	0% (51)[a]	0% (39)	–	8% (24)
May 18–21	0% (118)	0% (23)	0% (9)	0% (34)
May 25–28	13% (191)	20% (51)	7% (44)	3% (45)
June 1–4	7% (150)	0% (59)	62% (37)	16% (19)
All dates	7% (510)	6% (172)	29% (90)	5% (122)

[a] Number of larvae dissected after exposure at field sites.

third instars by 4 June. This instar is not a suitable host for *C. glomerata* in *P. rapae* (Van Driesche, 1988b), and we assume the same would be true for *P. virginiensis*. Our sentinel larval exposures, which continued until 16 June, thus completely covered the period during which field *P. virginiensis* larvae susceptible to *C. glomerata* parasitism would have been present. No parasitism was detected in any of the 487 sentinel larvae retrieved and dissected after exposure at the three study sites between 11 May and 16 June (site 1, 0/98; site 2, 0/194; and site 3, 0/195).

4. Discussion

Habitat for *P. virginiensis* (i.e., riparian patches of two-leaved toothwort) was abundant in our study area, occurring at 54% of the sites examined. We believe that our study area is representative of most of Massachusetts west of the Connecticut River, of which approximately 70% is forested. Western Massachusetts (ca. $4300\,km^2$) thus provides substantial habitat with suitable host plants for *P. virginiensis*.

The commonness of this butterfly at these habitat patches is under appreciated because adults, the only readily visible stage, are in flight for a very limited period (3–4 weeks per year) and occur at low densities (sighted typically one or two at a time). However, we found in our 2000 survey that *P. virginiensis* butterflies were present at more than a third (39%) of the habitat patches in our study area. These observations suggest that this species is a low density but widely distributed species found in many, perhaps most, patches of its commonly occurring host plant over a large area.

In our laboratory host preference tests, we found that *P. virginiensis* is a suitable host for both *C. glomerata* and *C. rubecula*. *P. virginiensis* is readily accepted for oviposition by both parasitoids and the larvae provide good nutrition for the developing parasitoids of both species. For *C. glomerata*, clutch size in *P. virginiensis* is as large as in *P. napi*, a known field host. The suitability of *P. virginiensis* as a host for *C. glomerata* is consistent with literature records that show that *C. glomerata* attacks several species or subspecies within the genus *Pieris*, including *P. napi japonica* and *P. napi nesis* in Japan (Sato, 1976; Sato et al., 1999), *P. napi microstriata* in California (Shapiro, 1981), *P. napi* and *P. brassicae* in Europe (Brodeur et al., 1996), and *P. rapae* in Europe, Japan, and North America (Brodeur et al., 1996, Sato, 1976; Van Driesche, 1988a). The suitability of *P. virginiensis* for *C. rubecula* is less predictable from the literature, which generally characterizes *C. rubecula* as a specialist on *P. rapae*.

However, our field study of parasitism of sentinel *Pieris* spp. larvae exposed in wooded habits with populations of *C. diphylla* and *P. virginiensis* strongly sug-

gest that *P. virginiensis* is not attacked in nature by *C. glomerata*. Examination of 1381 sentinel larvae, of either *P. rapae* or *P. napi*, exposed in *P. virginiensis* habitats on 11 occasions in either 1999 or 2000 detected no cases of attack by *C. glomerata* or *C. rubecula*, despite the demonstration that *C. glomerata* was present nearby our study sites, in meadow habitats. Furthermore, we demonstrated that the timing of exposure of sentinel larvae coincided correctly with the phenology of the larval stages of *P. virginiensis* that we assume are the ones susceptible to *C. glomerata* parasitism, based on monitoring adult flight of *P. virginiensis* and development of *P. virginiensis* larvae in field cages at one of our study sites. That such sentinel larvae are readily parasitized by *Cotesia* spp. parasitoids is amply demonstrated by use of the same technique in meadow habitats, where we have commonly encountered attack by these parasitoids in sentinels, at times of up to 100% (as reported in Benson et al., unpub.). Consequently, we conclude that these negative data for sentinel larvae exposed in the habitat of *P. virginiensis* demonstrate that neither *C. glomerata* nor *C. rubecula* forage significantly in wooded habitats. This implies that populations of this butterfly, contrary to some previous suggestions are not being affected by *Cotesia* spp. parasitism.

These findings are consistent with studies of *C. glomerata* in Japan that showed that the related butterfly *P. napi japonica*, while generally susceptible to *C. glomerata* parasitism, remains unparasitized if its larvae occur on food plants that grow along forest edges in shade or if the food plants are overtopped by growth of other types of plants (Ohsaki and Sato, 1994; Sato and Ohsaki, 1987). Similarly, Benson et al. (unpub.) found that *C. rubecula* parasitism was minimal on *P. rapae* larvae deployed as sentinels in woods adjacent to agricultural fields with high levels of this parasitoid, suggesting that it too prefers sunny meadow habitats over wooded areas.

Apart from direct habitat effects, lack of *C. glomerata* parasitism in *P. virginiensis* habitats in May and June may also be due partially to spatial and temporal factors. Because *P. virginiensis* is a spring univoltine species, its larvae only occur early in the season, when population densities of *C. glomerata* are at their seasonal low because of population reductions from overwintering mortality. However, the same cannot be said for *C. rubecula*, for which parasitism levels are typically highest in May (Benson et al., unpub.).

Assuming that *C. glomerata* densities are sharply reduced away from agricultural areas, where the larger *P. rapae* populations feeding on crops would support larger *C. glomerata* populations, it would seem likely that the restriction of *P. virginiensis* to hilly, forested areas with stands of *C. diphylla* would confer some spatially based protection from parasitism by *C. glomerata*. In this regard, it is perhaps of interest that the few tooth-

wort sites in the Connecticut River Valley that do occur in close proximity to agriculture do not appear to be occupied by *P. virginiensis* (Benson, Van Driesche, per. obs.). Exposure of such isolated forest fragments to greater penetration by *C. glomerata* from adjacent meadows may explain the absence of *P. virginiensis* from such sites, acting together with the effect of habitat fragmentation, which restricts recolonization of tooth-wort patches by adult butterflies (Cappuccino and Kareiva, 1985). However, recent studies by the authors (unpublished) have shown that parasitism by *C. glom-erata* of *P. napi* sentinel larvae exposed for one week in the *P. virginiensis* study region can be extremely high (100%, 59/59) in August in meadows. This indicates that while habitat and time of year may reduce *P. virginien-sis'* exposure to this parasitoid, it is generally present in the region at densities able to cause high levels of mor-tality under favorable circumstances. Finally, our study suggests that another parasitoid, a presumably native, unidentified species of ichneumonid in the genus *Hy-posoter*, is associated with populations of *P. virginiensis* in its habitat and causes moderate to high levels of larval parasitism (assuming this parasitoid's responses to the sentinel species and *P. virginiensis* are similar).

We conclude that habitat type protects *P. virginiensis* from attack by the non-native braconids *C. glomerata* and *C. rubecula*. For the former species, seasonal phe-nology of larvae may add further protection. Lack of attack in nature occurs despite the fact that this but-terfly's larvae are physiologically suitable hosts for both parasitoids. This illustrates that, in some cases, labora-tory estimations of parasitoid host ranges may be greater than realized host ranges in the field because in the field additional factors, such a habitat differences or lack of seasonal synchrony, can act to prevent a par-ticular species from being exploited.

In addition to the above conclusions concerning *P. virginiensis* specifically, we suggest that this case also provides support for use of surrogate species as probes to assay effects on rare species not numerous enough for direct sampling and support for assessing the native range habitat preferences of parasitoids proposed for introduction to new areas, as a means of predicting likely impacts on native species.

Use of surrogate species as tools to study effects on other species is not required when projects focus on abundant species, such as agricultural pests. Studies of insects that are the objects of conservation interest, however, may focus on species too rare to obtain ade-quate samples, or legal restrictions on their taking may apply. In such cases, use of a closely related species that may be treated similarly to the target species by a source of mortality of interest, may provide a solution.

Additionally, studies, in their native range, of the habitat preferences of parasitoids being considered for introduction as biological control agents can improve estimates of likely host ranges in the area of introduc-tion. Such an approach has been taken, for example, with parasitoids of mirid bugs in Europe, in which field studies in the European native range provide informa-tion on which parasitoid species are likely to forage in which mirid habitats, which in turn suggests which North American mirids (the region of proposed intro-duction) will likely come into contact with particular European mirid parasitoids (Kuhlmann et al., 2000).

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