

A native and an introduced parasitoid utilize an exotic gall-maker host

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Abstract *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) is non-native to North America and induces formation of galls on petioles and leaves of all chestnut (*Castanea* spp., Fagales: Fagaceae). We investigated the interactions between the gall wasp *D. kuriphilus*, a native parasitoid, *Ormyrus labotus* (Hymenoptera: Ormyridae), and a non-native parasitoid, *Torymus sinensis* (Hymenoptera: Torymidae). Galls were collected monthly from May to August and in January from four locations in the United States consisting of orchard-grown hybrid chestnuts (Hiram, OH and Meadowview, VA), suburban-grown ornamental Chinese chestnuts (*C. mollissima*) (Broadview Heights, OH), or forest-grown American chestnuts (*C. dentata*) (Bowling Green, KY). Parasitoids were removed from galls and *T. sinensis* and *O. labotus* were identified using PCR-markers. The relative abundance of each parasitoid was compared in relation to collection date, habitat, presence of alternative

hosts, and gall characteristics. *T. sinensis* was collected from each location and date, and was dominant in the orchard and suburban locations. However, relatively more *O. labotus* were collected within the forest, which had significant oak component and alternative cynipid hosts. *O. labotus* was only collected in spring and early summer, indicating the use of different summer and winter hosts. Observations suggest that in addition to parasitizing *D. kuriphilus*, *O. labotus* hyperparasitizes *T. sinensis*. *T. sinensis* has a longer ovipositor than *O. labotus*, and parasitized larger galls. This study improves our understanding of interactions between an invasive gall wasp, an introduced parasitoid, and native parasitoids, and illustrates novel relationships that may form as exotic species expand their geographic range.

Keywords Hymenoptera · Torymidae · Ormyridae · Cynipidae · Fagaceae

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Introduction

American chestnut, *Castanea dentata* Marshall (Fagales: Fagaceae), was a dominant/co-dominant canopy tree in eastern North America until its demise from the exotic chestnut blight fungus, *Cryphonectria parasitica* Murr (Diaporthales: Valsaceae) in the first half of the twentieth century. Efforts to breed blight resistance into American chestnut for restoration purposes are progressing (Jacobs 2007; Griffin 2008).

Chinese chestnut (*C. mollissima* Blume) and European chestnut (*C. sativa* Mill.) are also grown commercially as globally important nut crops, and are frequently planted as landscape or ornamental trees. *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is a pest of chestnut (Aebi et al. 2006) that poses a threat to the successful restoration of American chestnut (Anagnostakis 2001) and to profitable chestnut production (Kato and Hijii 1997). *D. kuriphilus* induces the formation of galls on developing buds which can terminate shoot development, prevent flowering, and contribute to tree mortality (Kato and Hijii 1997). A native of Asia, *D. kuriphilus* was first reported in North America in 1974 (Payne et al. 1975), and more recently in Europe (Aebi et al. 2006). Importation biological control efforts using *Torymus sinensis* Kamiyo (Hymenoptera: Torymidae), a generalist parasitoid of galling insects, were largely successful in Japan (Moriya et al. 2003). Therefore, *T. sinensis* was purposefully introduced for *D. kuriphilus* control in North America in the 1970s, and in Europe in the 2000s (Payne 1978; Aebi et al. 2006; Cooper and Rieske 2007; Quacchia et al. 2010).

Gall wasps are ideal models for investigating ecological community interactions because the vast majority of interactions, including parasitization, predation, and mutualisms, occur within the galls, and are therefore quantifiable (Stone et al. 2002). Both basic and applied ecologists are interested in the evolving interactions, and their implications, between native and non-native species. Following a lag time of variable duration, non-native species often become invasive in part due to the lack of natural enemies in the invaded territory (Blossey and Notzold 1995; Sakai et al. 2001). Importation biological control efforts attempt to alter this enemy-free space by introducing natural enemies from native ranges of introduced species (DeBach 1964). The introduction of non-native natural enemies to new landscapes creates novel interactions with native communities (Sakai et al. 2001). These interactions can adversely affect the demographics of native species, which may in turn prevent non-native species from establishing in a new community (Sakai et al. 2001). Such an interaction appears to be evolving in eastern North America with *D. kuriphilus*, *T. sinensis*, and a native parasitoid *Ormyrus labotus* Walker (Hymenoptera: Ormyridae) (Cooper and Rieske 2007; 2010). Adult

parasitoid emergence rates suggest that parasitism by *T. sinensis* is inhibited at locations where recruitment of native parasitoids, particularly *O. labotus*, is high relative to locations in which recruitment of native parasitoids is low (Cooper and Rieske 2007).

We have been investigating the evolving ecological interactions associated with the geographic range expansion of *D. kuriphilus* in eastern North America (Cooper and Rieske 2007, 2009, 2010). Here we focus on interactions between a native parasitoid, *O. labotus*, and an introduced parasitoid, *T. sinensis*, attacking *D. kuriphilus*. Our specific objectives were to investigate (1) parasitism by *T. sinensis* and *O. labotus* in the context of habitat characteristics, (2) interactions between *D. kuriphilus* gall characteristics and parasitism by *O. labotus* and *T. sinensis*, and (3) the use of alternative gall hosts (non-*D. kuriphilus*) by *T. sinensis*.

Materials and methods

Gall collections and assessments

Four locations were used for gall collections: Bowling Green, KY, Broadview Heights, OH, Hiram, OH, and Meadowview, VA, USA (Cooper and Rieske 2007, 2009, 2010). The Bowling Green location was a deciduous forest with naturally occurring forest-grown American chestnut trees infected with the blight fungus. The Broadview Heights location was a suburban area with ornamental Chinese chestnut trees. The Hiram location was a commercial orchard with Chinese chestnut hybrids. The Meadowview location was a chestnut breeding orchard with American and Chinese chestnut and their hybrids.

Galls were collected once a month from each location in May, June, July, and August 2006, and in January 2007. May collections included newly induced and rapidly growing galls, June and July collections included mature galls, August collections included galls excised following *D. kuriphilus* adult emergence, and January collections included dead and desiccating galls (Cooper and Rieske 2010). At Bowling Green, ≈ 10 – 15 galls per tree were collected from two American chestnut trees on each collection date (Table 1). At Broadview Heights, ≈ 10 – 15 galls per tree were collected from three Chinese chestnut trees (Table 1). At Hiram, ≈ 5 galls per tree were

Table 1 Total number of *Dryocosmus kuriphilus* galls collected, number of parasitoids dissected from collected galls, and the number of parasitoids randomly selected for molecular identification

Collection site	May	June	July	August	January
Bowling Green	21/9/9	21/22/11	27/37/19	16/5/5	30/8/8
Broadview Heights	43/93/48	30/104/52	31/123/61	27/90/45	30/89/45
Hiram	55/41/21	47/9/9	62/10/10	44/10/10	50/4/4
Meadowview	37/22/11	51/118/43	36/56/28	51/87/55	50/90/45

collected from ten hybrid chestnuts (Table 1). At Meadowview, ≈ 5 –10 galls per tree were collected from five American \times Chinese hybrid chestnuts. Galls were collected from each infested tree at Bowling Green and Broadview Heights, whereas collection trees were arbitrarily selected from larger populations of infested trees at Hiram and Meadowview. Galls excised from arbitrary locations within each tree were placed in plastic bags and transported to the laboratory where they were stored at -20°C .

Volumes of individual galls were measured (mm^3) after removing associated stem and leaf tissue (Cooper and Rieske 2009, 2010). Gall volume was calculated using the equation for an ellipse volume [$4/3 \times \pi \times \text{radius}_1 \times \text{radius}_2 \times \text{radius}_3$] where radii 1–3 were measured on three different planes across the gall (length and two perpendicular measurements of width) using calipers. Each gall was then dissected under $160\times$ magnification to reveal the number of chambers per gall and the contents of each chamber. All wasps within chambers were identified as either a cynipid gall wasp or parasitoid (Cooper and Rieske 2007, 2010). Gall chambers were cross-sectioned and the thickness of the sclerenchyma layers surrounding the larval chambers was measured using an ocular micrometer.

Parasitoid identification

This research focused on the two most numerous parasitoids of *D. kuriphilus* in North America, *T. sinensis* and *O. labotus* (Cooper and Rieske 2007). A PCR-based approach was used for identification because parasitoid larvae in the Chalcidoidea, which includes *Torymus* and *Ormyrus*, are morphologically indistinguishable (Njentes-Aldry et al. 2005). All wasps regardless of life stage were pulverized using a clean pipette tip and DNA was extracted using an Invitrogen Easy-DNA extraction kit following the

manufacturer's protocol (Invitrogen Corp., Carlsbad, CA, USA, Protocol 1, 2007). Previously described universal primers, ITS2-F (5'-TGTGA ACTGCAGG-ACACATG-3') and ITS2-R (5'-AATGCTTAAATT-TAGGGGTA-3') (Yara 2006) (Invitrogen) were used to amplify the ITS2 region from each wasp. PCR reaction volumes were 50 μl , and reactions included 4 μM MgCl_2 . PCR was performed on an Eppendorf Mastercycler Epigradient thermocycler (Eppendorf, New York, NY, USA) under the following conditions: 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 1 min, followed by 72°C for 5 min. Species-specific size variation in the ITS2 region was determined by direct sequencing of PCR products amplified from DNA extracted from adult wasps previously identified as *D. kuriphilus*, *T. sinensis*, and *O. labotus* (Cooper and Rieske 2007). DNA sequencing was performed in both directions using the primers for ITS2-F and ITS2-R and an ABI 3730xi multi-color fluorescence based DNA sequencer (Applied Biosystems, Foster City CA, USA) at the University of Kentucky Advanced Genetics Technology Center. When total parasitoid counts were >10 at each location on each specific date, half of the parasitoids were randomly selected for identification (PROC SURVEY, SAS 9.01) (Table 1). When total parasitoid counts were ≤ 10 , all parasitoids were used (Table 1). Each selected larva was identified by comparing the estimated size of ITS2 with those of *T. sinensis*, *O. labotus*, or *D. kuriphilus* using gel electrophoresis.

Data were analyzed using SAS 9.2 (SAS Institute, Cary, NC, USA). The effects of study site, collection date, and their interaction on the relative proportion of *T. sinensis*, *O. labotus*, and unidentified parasitoids were compared using logistic regression (PROC LOGISTIC). The percent compositions of each parasitoid species (*T. sinensis*, *O. labotus*, or unidentified) were the dependent variables, and study site,

date, and their interaction were the fixed effects. The FIRTH option was included to address issues of small sample size and convergence criteria. Differences among collection sites and dates were compared using Wald Confidence Intervals for Odds Ratio. Data were pooled from study sites and collection dates when both *T. sinensis* and *O. labotus* were collected to compare characteristics of galls inhabited by these two parasitoid species. Pooled data were analyzed (PROC GLIMMIX) with gall trait (volume or the thickness of the sclerenchyma layer) as the dependent variable, parasitoid species as the independent variable, and the site \times date interaction as the random variable.

Vegetation surveys

Woody vegetation and site characteristics were surveyed in May 2006 using 0.04-ha fixed-radius plots containing five 0.004-ha and five 0.0004-ha subplots (Coleman et al. 2008a, b). One of each subplot size was positioned at the whole plot center and in each cardinal direction, 7.7 m from the plot center. Three whole plots were used to assess vegetation composition and structure in Bowling Green, Meadowview, and Hiram, but only two whole plots were used in Broadview Heights due to property size restrictions. In Broadview Heights, collection trees were designated as the whole plot centers. In Bowling Green, the two collection trees and an American chestnut tree that was not infested with *D. kuriphilus* were designated as the whole plot centers. Whole plots were separated by ~ 20 m. In Meadowview and Hiram, whole plot centers were arbitrarily placed within the chestnut orchards. Three woody vegetation size classes were surveyed. In the 0.04-ha whole plots, all living trees ≥ 12.7 cm diameter at 1.37 m height were identified and height and diameters were measured. In the 0.004-ha subplots, all live saplings (< 12.7 cm diameter at 1.37 m) and shrubs (≥ 1.37 m height) were identified and measured. Lastly, seedlings, shrubs, and vines (< 1.37 m height) were identified in each of the 0.0004-ha subplots. In addition, all non-*Castanea* woody vegetation within whole plots was visually assessed for galls, and each gall and its contents were analyzed as described for chestnut galls. In Hiram, galls were also collected from a single oak tree located on the border of an

adjacent woodlot ~ 10 m from the orchard's edge, but not within whole plot boundaries.

Ecological indices including species richness and diversity were calculated at each location (Coleman et al. 2008a, b). Diversity was calculated using the Shannon index [$H' = -\sum p_i \ln p_i$] where p_i is relative abundance for species "i" divided by the species richness (Hunter 1996). The chestnut and oak components (%) were determined by dividing the chestnut or oak diameter at 1.37 m height by the total basal area. Proportional data (chestnut and oak compositions) were arcsine square-root transformed for statistical analyses (Zar 1996). Ecological indices describing woody vegetation were compared among sites (PROC GLM) using site as the independent variable with subplots nested within whole plots, and Tukey's HSD post-hoc tests were used when analysis of variance revealed significant differences.

Results

Parasitism

The sizes of the PCR-amplified ITS2 regions of *T. sinensis*, *O. labotus*, and *D. kuriphilus* were distinct from each other and were therefore used as molecular markers to identify the larval forms of *T. sinensis* and *O. labotus*. DNA sequencing of ITS2 of *T. sinensis* indicated a size of ≈ 520 bp, which is consistent with a previous report (GenBank accession number AB200274.1–AB200275.1) (Yara 2006). The ITS2 region of *O. labotus* was ≈ 560 bp in length. It had not been previously described and was submitted to GenBank (accession number 1040293). The length of the *D. kuriphilus* ITS2 region could not be determined by direct sequencing, but estimations from gel electrophoresis indicated a size of ≈ 600 bp in length.

There were significant site \times date interactions for the percent composition of *T. sinensis* (Table 2). The introduced *T. sinensis* was relatively more abundant than other parasitoids at Broadview Heights, Hiram, and Meadowview. *T. sinensis* was present in Meadowview on each collection date, but was not positively identified in galls collected in May from other collection sites (Fig. 1). The relative abundance of *O. labotus* differed among collection dates and

Table 2 Effects of collection site and date on the relative occurrence (% composition) of the introduced parasitoid *Torymus sinensis*, the native parasitoid *Ormyrus labotus*, and unidentified parasitoids of the gall-maker, *Dryocosmus kuriphilus*

Comparison	<i>T. sinensis</i>	<i>O. labotus</i>	Unidentified
Collection site	$\chi^2_3 = 31.4; P < 0.001$	$\chi^2_3 = 40.9; P < 0.001$	$\chi^2_3 = 23.4; P < 0.001$
Date	$\chi^2_4 = 65.9; P < 0.001$	$\chi^2_4 = 9.6; P = 0.048$	$\chi^2_4 = 42.4; P < 0.001$
Site \times date	$\chi^2_{12} = 29.2; P = 0.004$	$\chi^2_{12} = 16.4; P = 0.172$	$\chi^2_{12} = 41.7; P < 0.001$

Fig. 1 Mean percent parasitism (+SE) of *Dryocosmus kuriphilus* by *Torymus sinensis*, *Ormyrus labotus*, and unidentified parasitoids in Bowling Green, KY, Broadview Heights, OH, Hiram, OH, and Meadowview, VA. For each parasitoid species, different capital letters denote statistical differences among collection months while lower-case letters denote significant differences among collection sites ($\alpha = 0.05$) according to Wald Confidence Intervals for Odds Ratios. The number of each parasitoid species identified from each collection site and date are given as values

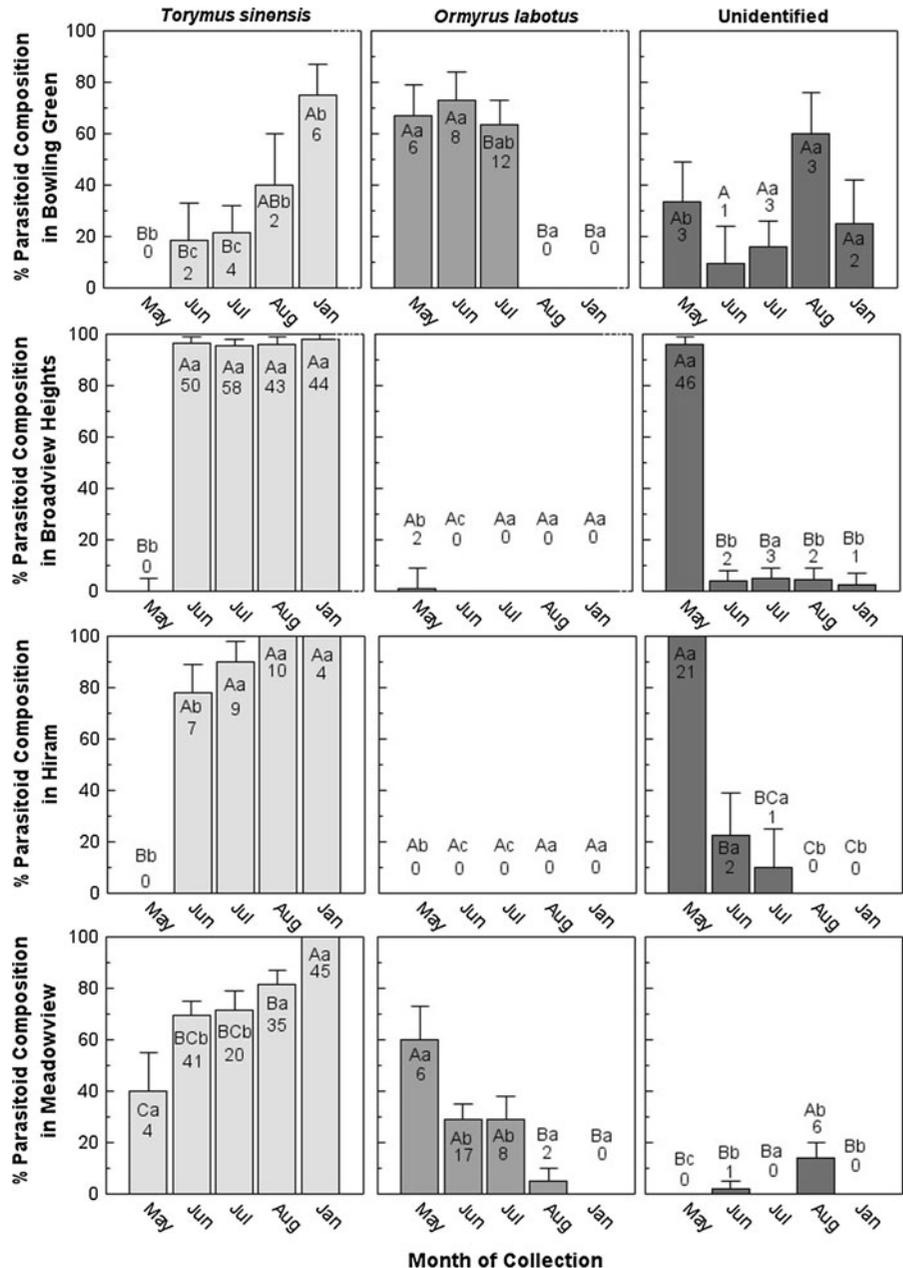


Table 3 Gall volume and sclerenchyma layer thickness (mean \pm SE) of *Dryocosmus kuriphilus* galls parasitized by *Torymus sinensis* or *Ormyrus labotus*

Parasitoid	Volume (mm ³)	Sclerenchyma thickness (mm)
<i>T. sinensis</i>	483 \pm 107	4.6 \pm 0.6
<i>O. labotus</i>	396 \pm 87	4.7 \pm 0.6
	$F_{1,83} = 219.4; P < 0.01$	$F_{1,83} = 0.1; P = 0.78$

sites (Table 2). The lack of a significant site \times date interaction indicated that the effects of date on the relative abundance of *O. labotus* were similar among collection sites (Table 2; Fig. 1). The native *O. labotus* was relatively more abundant at Bowling Green compared to other collection sites, but was also prevalent at Meadowview. *O. labotus* was mostly collected from chestnut galls in May, June, and July, and was not collected from galls in January (Fig. 1). *O. labotus* was not collected from Hiram, and only two specimens were collected from Broadview Heights (Fig. 1). Many parasitoids collected at Broadview Heights and Hiram in May were small, and could not be identified using our PCR-based methods (Fig. 1). Additionally, many parasitoid PCR product sizes were not consistent with the sizes of *T. sinensis* or *O. labotus*. These parasitoids were collected from each location, but were less abundant than *T. sinensis* or *O. labotus* (Fig. 1). Both parasitoids that were too small for identification and parasitoids that produced PCR product sizes not

consistent with *T. sinensis* and *O. labotus* are reported as unidentified (Fig. 1).

Both *T. sinensis* and *O. labotus* were present in May, June, and July in Meadowview and in June and July in Bowling Green (Fig. 1). Analysis of the characteristics of galls inhabited by *T. sinensis* or *O. labotus* revealed significant differences in gall size (Table 3). On average, *T. sinensis* inhabited larger galls compared to *O. labotus*, but there was no difference in the thickness of sclerenchyma layers of galls inhabited by *T. sinensis* or *O. labotus* (Table 3).

Alternative gall hosts

T. sinensis was not observed from non-*D. kuriphilus* galls collected from our study sites. Non-*D. kuriphilus* galls had a mean (\pm SE) size of 476 (\pm 85) mm³ and ranged from 27 to 1267 mm³. One unidentified parasitoid larva was collected from a non-*D. kuriphilus* gall from Broadview Heights, and 11 unidentified parasitoid larvae were collected from non-*D. kuriphilus* galls from Bowling Green (Table 4). Electrophoresis of ITS2 PCR products derived from these 12 specimens did not reveal visible bands. All other gall contents were gall formers in the families Cynipidae or Eriophyiidae (Table 4).

Vegetation surveys

There were significant differences in woody plant species richness among collection sites ($F_{3,40} = 20.3; P < 0.01$) (Table 5). The deciduous forest at Bowling

Table 4 Gall chamber contents and gall characteristics of non-*Dryocosmus kuriphilus* galls collected in May 2006

Collection site	Host plant (n)	Gall former	No. of galls	Gall type	Gall chamber contents
Broadview Heights	<i>Quercus robur</i> (2)	<i>Andricus</i> sp. (Roly-Poly Gall) Hymenoptera: Cynipidae	30	Stem	Cynipidae (12 larvae, 17 pupae) 1 non-Chalcidoid ^a larva
Hiram ^b	<i>Q. alba</i> (1)	<i>Amphibolips confluenta</i> Hymenoptera: Cynipidae	2	Stem	Empty
Bowling Green	<i>Q. montata</i> (1)	<i>Callirhytis quecuspuncata</i> Hymenoptera: Cynipidae	4	Bud	2 emergence holes 8 Chalcidoid ^a larvae
	<i>Q. velutina</i> (2)	<i>Callirhytis cornigera</i> Hymenoptera: Cynipidae	3	Leaf	1 emergence hole 3 Chalcidoid ^a larvae
	<i>Amelanchier laevis</i> (5)	<i>Eriophyes amelanchieri</i> Acari: Eriophyiidae	18	Leaf	Gall former

^a Refers to the Hymenoptera superfamily Chalcidoidea, which includes the genera *Torymus* and *Ormyrus*

^b Galls were collected outside of the whole-plot samples

Table 5 Vegetative composition and structure at four *Dryocosmus kuriphilus* gall collection sites used in our study

Location	Description	<i>Castanea</i> species	Species richness	Shannon diversity index	% <i>Castanea</i> diameter ^a	% <i>Quercus</i> diameter ^a
Bowling Green, KY	Forest mosaic	<i>C. dentata</i>	5.2 ± 0.4 a	1.1 ± 0.1 a	4 ± 7 b	10 ± 3 a
Broadview Heights, OH	Suburban ornamental	<i>C. mollissima</i>	2.5 ± 0.6 b	0.8 ± 0.2 a	18 ± 11 b	4 ± 4 ab
Hiram, OH	Commercial	Various hybrids	1 ± 0.7 b	0 ± 0.2 b	100 ± 12 a	0 b
Meadowview, VA	Blight resistance breeding	<i>C. dentata</i> , <i>C. mollissima</i> , and hybrids	1.7 ± 0.3 b	0.3 ± 0.1 b	77 ± 5 a	0 b

Means of ecological indices within columns followed by the same letter do not differ

Each value presents mean ± SE

^a Percentage of the total basal diameter of woody vegetation measured at 1.37 m height

Green contained the greatest species richness, but there were no differences in species richness among Broadview Heights, Hiram, and Meadowview. There were also significant differences in woody plant diversity among collection sites ($F_{3,40} = 16.8$; $P < 0.01$) (Table 5). Diversity at Bowling Green and Broadview Heights did not differ, but these sites had greater woody plant diversity compared to Hiram and Meadowview. The percentage of total basal diameter composed of chestnut or oak differed among collection sites ($F_{3,40} = 28.1$; $P < 0.01$ and $F_{3,40} = 3.1$; $P = 0.04$, respectively) (Table 5). Chestnut composition was greatest in Hiram and Meadowview, and lowest in Bowling Green and Broadview Heights, whereas oak composition was greatest in Bowling Green and Broadview Heights, but was absent from Hiram and Meadowview.

Discussion

We assessed the biology and ecology of both an introduced and a native parasitoid of the invasive Asian chestnut gall wasp, *D. kuriphilus*, at four locations in eastern North America. *T. sinensis* is the dominant parasitoid of *D. kuriphilus* in Japan (Moriya et al. 2003) and was introduced to North America and Europe as a biological control against *D. kuriphilus*. Our study confirms our previous report that *T. sinensis* is a primary parasitoid of *D. kuriphilus* in the eastern United States, and that *D. kuriphilus* galls are also frequently parasitized by *O. labotus*, which is a native parasitoid of oak-galling cynipids (Cooper and Rieske 2007).

Consistent with previous research (Otake 1989), *T. sinensis* was present in chestnut galls throughout the year and overwintered in dead galls. Most of the parasitoids collected from Broadview Heights and Hiram in May that were too small to be identified (Fig. 1) were likely early instars of *T. sinensis*. This parasitoid is a generalist of cynipid gall wasps in China, but acts as a specialist in Japan where *D. kuriphilus* galls are abundant on chestnut (Stone et al. 2002). *T. sinensis* is univoltine. Adults emerge in early-April consistent with the timing of bud break and gall initiation, and parasitize newly developing galls for a period of several weeks (Cooper and Rieske 2007, 2010). This partial synchrony with the *D. kuriphilus* life-cycle contributes to the efficacy of *T. sinensis* as a parasitoid of *D. kuriphilus*, and may explain why *T. sinensis* acts as a specialist outside of its native range. Many other cynipid parasitoids have short generation times compared with that of *T. sinensis* (Washburn and Cornell 1981; Hanson 1992). In general, the generation time of many parasitoids of galls are shorter than the generation time of the host cynipids, and thus the parasitoids utilize a suite of potential hosts (Washburn and Cornell 1981; Hanson 1992). *T. sinensis* was not present in oak galls collected from Broadview Heights, Bowling Green, and Hiram. Although few alternative hosts were collected from each site, those that were collected were found in close proximity to *T. sinensis* populations on chestnut, and would have been present during the *T. sinensis* flight and oviposition period. As observed in other regions where *T. sinensis* was introduced for *D. kuriphilus* control (Stone et al. 2002), *T. sinensis* may act as a specialist on

D. kuriphilus in North America. However, further research is required to confirm this hypothesis.

Unlike *T. sinensis*, *O. labotus* emerged from chestnut galls between July and August and was absent from chestnut galls in August and January at Bowling Green and in January at Meadowview. Presumably, *O. labotus* requires other cynipid host(s) throughout the remaining summer and winter months. *O. labotus* exploits more hosts than any other *Ormyrus* species (Hanson 1992). It is the most abundant parasitoid in galls formed by *Callirhytis cornigera* Osten Sacken (Hymenoptera: Cynipidae), and overwinters as pupae within *C. cornigera* stem galls on *Q. palustris* Muenchhausen (Eliason and Potter 2000). *D. kuriphilus* was introduced to North America in the 1970s, and is therefore a relatively new host for *O. labotus* (Cooper and Rieske 2007). The Bowling Green collection site is a forest mosaic with the greatest woody plant diversity and highest oak component of our study sites. The diversity of woody plant species corresponds with the presence of multiple cynipid host species (see Table 4). The Meadowview site did not contain oak within our sample plots, but the surrounding forests and woodlots may provide suitable source populations of *O. labotus*; these were not sampled because of property size restrictions. In contrast to these sites, almost no *O. labotus* specimens were collected from Broadview Heights and Hiram.

Our studies provide evidence that *T. sinensis* is hyperparasitized by *O. labotus*. *O. labotus* emerged from dead galls collected from Meadowview in the spring of 2005 (Cooper and Rieske 2007), but were not identified from galls collected in January of our current study. Dead and desiccated galls do not house living *D. kuriphilus*, and only contain parasitoids such as *T. sinensis* (Cooper and Rieske 2010). This suggests that in addition to parasitizing *D. kuriphilus* during gall initiation, *O. labotus* hyperparasitizes *T. sinensis* prior to *T. sinensis* emergence from overwintering locations within dead galls. Hyperparasitism by *O. labotus* could potentially suppress *T. sinensis* populations in some locations, and may explain the relatively low number of *T. sinensis* collected in Bowling Green, where *O. labotus* was abundant (Cooper and Rieske 2007; current study). This is consistent with our previous report suggesting potential antagonistic interactions among recruitment of native parasitoids

and *T. sinensis* populations within *D. kuriphilus* galls (Cooper and Rieske 2007).

A small number of parasitoids (<10 per site and date) produced PCR products of sizes which were not consistent with those of *T. sinensis*, *O. labotus*, or *D. kuriphilus*. In 2005, several native parasitoids other than *O. labotus* were reared from chestnut galls including *Sycophila mellea* Walker (Hymenoptera: Eurytomidae), *Pnigalio minio* Walker (Hymenoptera: Eulophidae), *Eupelmus* sp. (Hymenoptera: Eupelmidae) and *Pteromalus* sp. (Hymenoptera: Pteromalidae) (Cooper and Rieske 2007). Two native parasitoids, *Torymus tubicola* Osten Sacken and *T. advenus* Osten Sacken, were reared from dry galls collected from Byron, GA, USA in 1976 (Payne 1978). Combined with our current findings, these observations suggest that a suite of native parasitoids are capable of parasitizing *D. kuriphilus* galls in eastern North America. However, these other parasitoid species are considerably less abundant in chestnut galls than *T. sinensis* or *O. labotus*.

Two hypotheses address the delay in recruitment of natural enemies by invasive species (Grabenweger et al. 2010). The “Geographical-Spread Hypothesis” predicts that the number of parasitoids which attack an invasive species increases as the geographic range of the invader increases (Schonrogge et al. 1995, 1996; Stone et al. 2002; Grabenweger et al. 2010). The “Adjustment Hypothesis” predicts a lag between introduction of an exotic species and recruitment of native parasitoids as parasitoids adapt to the novel host (Grabenweger et al. 2010). In our opinion, the two hypotheses are not necessarily mutually exclusive. *D. kuriphilus* and its associated parasitoids provide a unique system to investigate the Geographical-Spread and Adjustment Hypotheses in North America. Parasitism by *O. labotus* or other native parasitoids may increase over time at the non-forest collection sites as native parasitoids adapt to galls of the exotic *D. kuriphilus*. The Adjustment Hypothesis may also be relevant to parasitism of native gall wasps by the introduced *T. sinensis*. Although *T. sinensis* was not observed in native galls collected from our collection sites, long-term studies may indicate adaptations by *T. sinensis* to parasitize native cynipids.

In addition to investigating interactions between parasitoids, we investigated the relationships between gall traits (volume and sclerenchyma thickness) and

species-specific parasitism. Larger gall volume and thicker sclerenchyma layers are negatively correlated with overall parasitism and are generally considered gall defenses (Stone and Schonrogge 2003; Cooper and Rieske 2010). However, following the emergence of *D. kuriphilus*, thicker sclerenchyma layers and larger galls are positively correlated with the presence of parasitoids and negatively correlated with mortality of the gall inhabitants. Potentially, thicker sclerenchyma layers provide parasitoids protection from certain mortality factors such as hyperparasitism, but successful parasitization of *D. kuriphilus* galls may be limited to galls of intermediate size with sclerenchyma of intermediate thickness (Cooper and Rieske 2010). *T. sinensis* parasitized larger galls than *O. labotus*, and also has a longer ovipositor compared to parasitoids in other genera (Washburn and Cornell 1981), including *O. labotus* (Cooper and Rieske 2007). This longer ovipositor likely facilitates penetration of larger galls. *O. labotus* was observed in developing galls earlier than *T. sinensis*, and may either parasitize smaller galls, or parasitize galls before they fully mature. This could explain why *O. labotus* did not parasitize mature *D. kuriphilus* galls after they emerged as adults in July. These potential differences in gall parasitoid strategies may create a mechanism for either spatial or temporal niche partitioning between *T. sinensis* and *O. labotus* based on gall size and/or gall maturity. These patterns in parasitoid–gall interactions warrant further investigation.

Our study provides insight into the ecological interactions between two important parasitoids of *D. kuriphilus* and provides evidence that a native parasitoid, *O. labotus*, utilizes an invasive gall wasp and its introduced parasitoid as hosts. The recruitment of native natural enemies may provide American chestnut with protection against *D. kuriphilus* galling where oak and oak galls are persistent. However, hyperparasitism of *T. sinensis* by *O. labotus* could limit the establishment of *T. sinensis* in forest habitats. This study also provides evidence that the two most prevalent parasitoids of *D. kuriphilus* in North America, *T. sinensis* and *O. labotus*, may compete for higher quality galls and may partition this resource as a consequence of variations in wasp/chestnut phenology and ovipositor length. The use of an exotic gall wasp by the native parasitoid, and the interspecific competition between the native and introduced parasitoid, provides an opportunity to

investigate the evolutionary consequences of invasive species using importation biological control. Collectively, our findings improve our understanding of the interactions between an invasive insect, its introduced parasitoid, and native parasitoid recruitment, and could contribute to the development of *D. kuriphilus* management strategies in cultivated chestnut and American chestnut restoration.

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References

- Aebi CY, Schonrogge K, Melika C, Alma A, Bosio G, Quacchia A, Picciau L, Abe Y, Moriya A, Yara K, Seljak G, Stone GM (2006) Parasitoid recruitment to the globally invasive chestnut gall wasp. In: Ozaki K, Yakawal Y, Ohgushi T, Price PW (eds) Ecology and evolution of galling arthropods and their associates. Springer Verlag, Tokyo, pp 103–121
- Anagnostakis SL (2001) The effect of multiple importations of pests and pathogens on a native tree. Biol Invasions 3:245–254
- Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants—a hypothesis. J Ecol 83:887–889
- Coleman TW, Meeker JR, Clarke SR, Rieske LK (2008a) Forest composition following overstory mortality from southern pine beetle and associated treatments. Can J For Res 38:1–13
- Coleman TW, Meeker JR, Clarke SR, Rieske LK (2008b) The suppression of *Dendroctonus frontalis* and subsequent wildfire have an impact on forest stand dynamics. Appl Veg Sci 11:231–242
- Cooper WR, Rieske LK (2007) Community associates of an exotic gallmaker, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Eastern North America. Ann Entomol Soc Am 100:236–244
- Cooper WR, Rieske LK (2009) Woody stem galls interact with foliage to affect community associations. Environ Entomol 38:417–424

- Cooper WR, Rieske LK (2010) Gall structure affects ecological associations of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae). *Environ Entomol* 39:787–797
- DeBach P (ed) (1964) Biological control of insect pests and weeds. Chapman and Hall, London, p 844
- Eliason AE, Potter DA (2000) Biology of *Callirhytis cornigera* (Hymenoptera: Cynipidae) and the arthropod community inhabiting its galls. *Environ Entomol* 29:551–559
- Grabenweger G, Kehrli P, Zweimüller I, Augustin S, Avtzis N, Bacher S, Freise J, Girardoz S, Guichard S, Heitland W, Lethmayer C, Stolz M, Tomov R, Volter L, Kenis M (2010) Temporal and spatial variations in the parasitoid complex of the horse chestnut leafminer during its invasion of Europe. *Biol Invasions* 12:2797–2813
- Griffin G (2008) Recent advances in research and management of chestnut blight on American chestnut. *Phytopathology* 98:S7
- Hanson P (1992) The Nearctic species of *Ormyrus* Westwood (Hymenoptera, Chalcidoidea, Ormyridae). *J Nat Hist* 26:1333–1365
- Hunter ML (1996) Fundamentals of conservation biology. Blackwell Science, Cambridge
- Jacobs DF (2007) Toward development of sivil strategies for forest restoration of American chestnut (*Castanea dentata*) using blight-resistant hybrids. *Biol Conserv* 137:487–506
- Kato K, Hijii N (1997) Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hym., Cynipidae) on the growth of chestnut trees. *Jpn J Appl Entomol* 121:9–15
- Moriya S, Masakazu S, Ishizue A (2003) Classical biological control of the chestnut gall wasp in Japan. In: 1st international symposium on biological control of arthropods, Waikiki, Japan, pp 407–415
- Njentes-Aldry JU, Vardal H, Ronquist F (2005) Comparative morphology of terminal-instar larvae of Cynipoidea: phylogenetic implications. *Zool Scr* 34:15–36
- Otake A (1989) Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae)—analyses of records on cell contents inside galls and on emergence of wasps and parasitoids outside galls. *Appl Entomol Zool* 24:193–201
- Payne JA (1978) Oriental chestnut gall wasp: new nut pest in North America. In: Proceedings of the American chestnut symposium. West Virginia University, 4–5 January 1978, Morgantown, WV, pp 86–88
- Payne JA, Menke AS, Schroeder PM (1975) *Dryocosmus kuriphilus* Yasumatsu, (Hymenoptera: Cynipidae), an oriental chestnut gall wasp in North America. U.S. Department of Agriculture Cooperative Economic Insect report 25, pp 903–905
- Quacchia A, Ferracini C, Moriya S, Alma A (2010) Italian experience in biological control of *Dryocosmus kuriphilus*. In: Moriya S (ed) A global serious pest of chestnut trees, *Dryocosmus kuriphilus*: yesterday, today and tomorrow. Proceedings of Japan-Italy joint international symposium, Tsukuba, Japan, 24–25 November 2009. National Agricultural Research Center, Tsukubap, pp 14–17
- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand MC, McCauley DE, O’Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. *Annu Rev Ecol Syst* 32:305–332
- Schonrogge K, Stone GN, Crawley MJ (1995) Spatial and temporal variation in guild structure—parasitoids and inquilines of *Andricus quercuscalicis* (Hymenoptera, Cynipidae) in its native and alien ranges. *Oikos* 72:51–60
- Schonrogge K, Stone GN, Crawley MJ (1996) Abundance patterns and species richness of the parasitoids and inquilines of the alien gall-former *Andricus quercuscalicis* (Hymenoptera: Cynipidae). *Oikos* 77:507–518
- Stone GN, Schonrogge K (2003) The adaptive significance of insect gall morphology. *Trends Ecol Evol* 18:512–522
- Stone GN, Schonrogge K, Atkinson RJ, Bellido D, Pujade-Villar J (2002) The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annu Rev Entomol* 47:633–664
- Washburn JO, Cornell HV (1981) Parasitoids, patches, and phenology: their possible role in the local extinction of a cynipid gall wasp population. *Ecology* 62:1597–1607
- Yara K (2006) Identification of *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae), introduced and indigenous parasitoids of the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), using the ribosomal ITS2 region. *Biol Control* 36:15–21
- Zar JH (1996) Biostatistical analysis. Prentice-Hall, Englewood Cliffs