CAN OUR CHESTNUT SURVIVE ANOTHER INVASION?

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Abstract.—Plant breeders and land managers have been actively pursuing development of an American chestnut with desirable silvicultural characteristics that demonstrates resistance to the chestnut blight fungus. As progress towards development of a blight-resistant chestnut continues, questions arise as to how these plants will interact with pre-existing stresses. The Asian chestnut gall wasp is an introduced invader exploiting chestnut in eastern North America and contributing to the complex of stressors facing chestnut restoration efforts. The gall wasp is a potentially devastating pest that causes globular galls on actively growing shoots of all *Castanea* species. Galling reduces tree vigor, prevents normal shoot development, reduces or eliminates nut production, and can cause tree mortality. The persistent spread of this exotic, invasive insect threatens chestnut production and restoration efforts throughout the eastern United States. We have been characterizing associates of the Asian chestnut gall wasp in eastern North America to more fully understand gall development and what factors regulate gall wasp populations. The natural enemy complex has been characterized, and interactions between a native parasitoid and an exotic parasitoid that was introduced for Asian chestnut gall wasp control are being evaluated. We are also evaluating the extent to which surrounding vegetation influences natural enemy occurrence. Our ultimate goal is to gain an understanding of the ecological interactions, dispersal patterns, and mechanisms regulating gall wasp populations in eastern North America.

INTRODUCTION

Historically, American chestnut, *Castanea dentata* (Marsh.), was a dominant component of the northern and central hardwood forests of eastern North America, and at one time was among the most abundant tree in portions of the Appalachians (Braun 1950). However, reports of chestnut mortality attributable to black ink disease, *Phytophthora cinnamomi*, occurred as early as the mid-1800s (Crandall and others 1945), and the accidental introduction of the chestnut blight fungus, *Cryphonectria (Endothia) parasitica*, in 1904 virtually eliminated American chestnut from its former range. Nevertheless, extensive efforts at developing disease-resistant varieties have recently met with some success (Griffin 2000).

Selecting for resistant relic American chestnut, and/or hybridizing susceptible American chestnut with resistant Chinese chestnut, *C. mollissima*, followed by repeated selection of resistant progeny and backcrossing with American chestnut, produces blight-resistant products with many of the desirable growth and morphological characteristics of the American chestnut (Jaynes and Dierauf 1982, Griffin 2000). Blight-resistance is not straightforward, however, and can be adversely affected by altitude (Jaynes and Dierauf 1982), cold temperatures (Griffin and others 1993), xeric conditions (Gao and Shain 1995), and vegetative

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competition (Griffin and others 1991). Additionally, resistance to one stressing agent may affect susceptibility to a second stressor (Chapin 1991), suggesting that blight-resistance could alter chestnut's susceptibility to herbivory.

As progress towards development of a blight-resistant chestnut continues and the prospects for the return of chestnut to the eastern North American landscape become more tangible, questions arise as to how pre-existing stresses will affect these plants. Hybrids developed in response to the chestnut blight fungus have not been assessed for herbivore resistance (Rieske and others 2003, Kellogg and others 2005). Such novel herbivores as the Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), further threaten efforts to deploy disease-resistant chestnut in eastern North America (Payne and others 1975).

The Asian chestnut gall wasp is a potentially devastating exotic insect that causes globular twig, shoot, and leaf galls on actively growing shoots of all *Castanea* species. Galling reduces fruiting and nut yield, suppresses shoot elongation and twig growth, reduces tree vigor and wood production, and can kill trees (Yasumatsu 1951, Payne and others 1975, Dixon and others 1986, Anagnostakis and Payne 1993, Kato and Hijii 1997). Galling also prevents infested shoots from producing new shoot growth and reproductive flowers, thereby reducing or eliminating nut production. Chestnut production and chestnut restoration efforts throughout the eastern United States are threatened by the persistent spread of this exotic, invasive insect.

The gall wasp was initially introduced into North America in Byron, GA, in 1974 (Payne and others 1975); within 7 years of its introduction, it had devastated the fledgling chestnut industry in Georgia (Anagnostakis and Payne 1993). The gall wasp has since expanded its distribution north-northeasterly. Since its accidental introduction into North America on Chinese chestnut in 1974, ACGW has expanded its host range to include five *Castanea* species, and its geographic range to encompass 11 eastern states (Stehli 2003, Cooper and Rieske 2007, Rieske 2007, Rieske unpubl. data). Additional infestations are likely to be reported as the gall wasp continues to expand its range.

The gall wasp has one generation per year and reproduces asexually (Payne and others 1976, Payne 1978). Adult females lay eggs inside buds in early summer. Eggs hatch soon after, but larvae remain inactive. Larval growth begins at budbreak the following spring, when larvae induce rapid formation of conspicuous stem, petiole, or leaf galls. These galls provide the developing wasp protection throughout the larval and pupal stages. Adults emerge from galls in the early summer and locate new chestnut shoots to lay eggs for the next generation (Payne 1978). Once the wasps emerge, galls become woody and dry, and can remain on the tree for several years. Small leaves remain attached to the galls during the winter; these leaves are highly visible and are useful in detecting infestations.

Shortly after the gall wasp was first reported in North America, the parasitic wasps *Torymus sinensis*, *T. tubicola*, and *Megastigmus* spp., which had successfully suppressed damaging gall wasp populations in Japan (Murakami and others 1980, 1994; Moriya and others 1989, 2003), were introduced into gall wasp-infested orchards in Byron, GA (Payne 1978). Within a short period, gall wasp populations in central Georgia declined and the incidence of galling dropped to acceptable levels. *Torymus sinensis* has moved to a limited extent with expanding gall wasp populations in eastern North America, and several native parasitoids, apparently recruited from native oak gallmakers, have adapted to exploit the gall wasp (Cooper and Rieske 2007). *T. sinensis* and the native *Ormyrus labotus* occur in frequencies great enough to influence gall wasp populations, but the extent to which these natural enemies are able to keep up with expanding gall wasp populations is unknown (Cooper and Rieske 2007, 2009). Several additional parasitoid species have been identified, as well as a potentially pathogenic fungus that causes external lesions that may cause mortality and aid in regulating gall wasp populations (Cooper and Rieske 2007). Interestingly, galling by the nonnative gall wasp may facilitate populations of the native lesser chestnut weevil, *Curculio sayi* (Coleoptera: Curculionidae), through an enhanced resource base, as weevils utilize galls as a resource (Cooper and Rieske 2007).

As we document the expansion of the gall wasp's range in eastern North America, we have been investigating evolving ecological associations, population regulators, and impacts. We have been working to characterize associates of the gall wasp in its invaded North American range to more fully understand what factors regulate gall wasp populations. Here we report on relative abundance of and interactions between the native parasitoid, *O. labotus*, and the introduced parasitoid, *T. sinensis*, assess their effects on populations of the Asian chestnut gall wasp, and evaluate how surrounding vegetation may affect natural enemy occurrence and activity. Our specific objectives were to: 1) evaluate natural enemy recruitment as the ACGW expands its geographic range; 2) determine the primary mortality factor(s) for ACGW in North America; and 3) evaluate the extent to which surrounding vegetation influences natural enemies.

METHODS

We collected galls from four distinct geographic locations: naturally occurring American chestnuts in a forest (Bowling Green, KY), Chinese chestnut, *C. mollissima*, in a landscape setting (Broadview Heights, OH), Chinese chestnut and hybrids in an orchard setting (Hiram, OH), and American and Chinese chestnut and their associated hybrids in a plantation (Meadowview, VA). At each collection site, surrounding vegetation was characterized to evaluate the extent to which associated vegetation can influence natural enemy occurrence and gall wasp mortality. Vegetation data were collected in accordance with the protocols of the U.S. Department of Agriculture (USDA), Forest Service's Natural Resource Information System and its Field Sampled Vegetation Module (FSVeg) and associated Common Stand Exam procedures (USDA Forest Service 2003). The height, diameter, and basal area of all woody vegetation were measured. Canopy structure, ground surface cover, slope, slope position, elevation, and aspect were assessed in each plot. In each 0.04-ha plot, all large trees (≥12.7 cm diameter at 1.5 m high; d.b.h.) were measured. Each whole plot contained five 0.004-ha subplots in which saplings (<12.7 cm d.b.h.) and larger shrubs (≥137 cm height) were measured, as well as five 0.0004-ha subplots for seedlings, smaller shrubs (<137 cm height), and woody vines. The 0.0004-ha subplots were nested within the 0.004-ha subplots, which were located at the center point of each whole plot and 7.8 m from the center in each cardinal direction (Coleman and others 2008). Collection sites were then characterized as "landscape," containing few *Castanea* of the same species and age within a diverse landscape; "orchard," containing few *Castanea* species or varieties and of relatively uniform age; "plantation," containing several to many *Castanea* species and their hybrids, of mixed age; and finally, "natural forest," containing lingering American chestnut with an abundant oak component and of mixed age.

Galls were collected approximately monthly April-August to evaluate wasp development and gall associates, with a single January collection date. In Meadowview (plantation), two shoots per tree, each containing 20-30 galls per tree, were collected from five American and five Chinese chestnut trees. In Bowling Green ("natural forest"), three shoots, each containing 20-30 galls, were collected from relic American chestnut infected with the blight fungus. The Bowling Green site consisted of only a single infested tree, so collections were replicated by shoot. In Hiram (orchard), two shoots per tree, each containing 20-30 galls per tree, were collected from five Chinese chestnut trees. In Broadview Heights ("landscape"), three shoots per tree, each with approximately 20-30 galls, were collected from three Chinese chestnut trees. Shoots were clipped at the second apical growth scar to ensure only 2 years of growth. Clipped shoots were bagged and kept cool during transport to the laboratory, where current growth was separated from the previous years' growth to delineate gall age. The fresh weight and volume of collected galls were measured. A subsample (10 percent) was dissected; the number of chambers per gall was counted and the thickness of the protective schlerenchyma layer surrounding larval chambers was measured. The contents of each were identified as gall former, parasitoid, fungus, or empty chamber.

Larval hymenopteran parasitoids are morphologically indistinguishable, so we used a molecular approach to identify the two most numerous parasitoids of the gall wasp in North America, the native *O. labotus*, and the introduced *T. sinensis* (Cooper and Rieske 2007). DNA was extracted from parasitoid larvae, and DNA primers were used to amplify the ribosomal ITS2 region for each wasp. Species-specific size variation in ITS2 PCR products was determined by direct sequencing of DNA extracted from known *D. kuriphilus*, *O. labotus*, and *T. sinensis* adults collected and identified previously (Cooper 2007). Each unknown larval parasitoid was identified by molecular comparison with adults of known specimens.

RESULTS

Our collections confirmed that parasitism accounts for the greatest amount of gall wasp mortality (Table 1) (Cooper and Rieske 2007). Using a molecular approach, we were able to identify parasitoids of the ACGW that were previously indistinguishable. The most abundant parasitoid species present was *T. sinensis*, which was introduced into the United States in the mid-1970s for gall wasp suppression (Payne 1978). *T. sinensis* was present at each collection site, but was most abundant at orchard and plantation settings, which had the greatest relative density of chestnut. The native parasitoid *O. labotus*, which historically utilizes oak gallmakers, was most abundant within chestnut galls in a forested setting containing a significant oak component. Presumably the oaks provide an abundance of closely related gallmaking hosts that historically sustain *O. labotus* populations (Cooper and Rieske 2007, 2009). The majority of nearctic *Ormyrus* are oak-associates (Hanson 1992).

Fungi within the gall interior also caused mortality (Table 1), as did failure of adults to emerge for undetermined reasons. Additionally, feeding by the lesser chestnut weevil and black lesions on the gall exterior

Table 1.—Incidence of factors influencing populations of the Asian chestnut gall wasp, April-August, across four sites in eastern North America.

			Parasitization (%)				
Site	Setting	Chestnut present	*T. sinensis* (introduced)	*O. labotus* (native)	Interior fungus[a]	Weevil feeding[b]	Exterior lesions[c]
Meadowview, VA	Plantation	Am., Chi., hybrids	65.5	25.3	3.6	0.3	0.17
Bowling Green, KY	Forest	American	19.3	50.5	1.6	0.04	0.10
Broadview Hts., OH	Landscape	Chinese	95	0	2.1	0.12	0.27
Hiram, OH	Orchard	Chinese, hybrids	90	10	1.0	0	0.11

[a]Percent chambers affected
[b]Average number of feeding scars per gall
[c]Average number of fungal lesions per gall

caused by an unidentified fungus, were positively correlated with gall wasp mortality (Table 1). In spite of their co-occurrence with gall mortality, we cannot be certain whether this relationship is causative or simply correlative.

DISCUSSION

We evaluated ecological interactions and mortality agents impacting populations of the ACGW as it expands its geographic range in eastern North America. Parasitization accounted for the majority of gall wasp mortality; we found relatively high rates at all our collection sites. The prevalence of *T. sinensis* suggests that this introduced parasitoid is moving with expanding gall wasp populations in eastern North America, likely in infested plant material. *T. sinensis* is univoltine; adults emerge in early spring and parasitize newly developing galls. *T. sinensis* occurs in galls throughout the year and overwinters in dead galls as pupae and larvae (Payne 1978, 1981; Cooper and Rieske 2009). It is a generalist parasitoid of cynipid gall wasps in China, but acts as a specialist on Asian chestnut gall wasp in Japan, and possibly North America, where *D. kuriphilus* galls are abundant in surviving chestnut (Stone and others 2002). This synchrony with the ACGW life cycle contributes to the effectiveness of *T. sinensis* as a parasitoid of the gall wasp, and may explain why *T. sinensis* appears to act as a specialist on the Asian chestnut gall wasp in North America.

The second most abundant parasitoid, *O. labotus*, is a native species previously confined to oak gallmakers (Cooper and Rieske 2007). Our results suggest that this parasitoid is effectively making a host jump from oak gallmakers to utilize the novel resource provided by the chestnut gallmaker. *O. labotus* produces multiple generations per year and exploits more hosts, including cynipid gall wasps and cecidomyiid gall midges (flies), than do other known *Ormyrus* species (Hanson 1992). *O. labotus* was present in chestnut galls only in late spring and early summer, indicating that this native parasitoid requires additional gallmaking hosts for subsequent generations through the remaining year (Cooper and Rieske 2009).

The Asian chestnut gall wasp is a relatively new host for *O. labotus* (Cooper and Rieske 2007). Our data suggest an evolving competitive interaction between the native *O. labotus* and the introduced *T. sinensis*; this evolving competitive interaction can be influenced by habitat type. *T. sinensis* is the primary parasitoid where chestnut is numerically dominant, whereas *T. sinensis* and *O. labotus* are both common in the more vegetatively diverse study sites. *O. labotus* appears to utilize the chestnut gall wasp and its introduced parasitoid, *T. sinensis*, as hosts within chestnut galls. This finding is consistent with our previous work suggesting potential antagonistic interactions between the native and introduced parasitoids (Cooper and Rieske 2007).

Our results provide important insight into ecological interactions between two important parasitoids of *D. kuriphilus*, and provide evidence for a native parasitoid's host shift to an invasive gall wasp and its introduced parasitoid. Collectively, this study improves our understanding of interactions between native and introduced gall parasitoids and could lead to improved management of *D. kuriphilus* in forest chestnut plantings and cultivated chestnut.

LITERATURE CITED

Anagnostakis, S.L.; Payne, J.A. 1993. **Oriental chestnut gall wasp.** NA-PR-02-93. Asheville, NC: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry.

Braun, E.L. 1950. **Deciduous forests of eastern North America.** Philadelphia, PA: The Blakiston Co. 595 p.

Chapin, F.S., III. 1991. **Effects of multiple environmental stresses on nutrient availability and use.** In: Mooney, H.A.; Winner, W.E.; Pell, E.J., eds. Response of plants to multiple stresses. New York, NY: Academic Press: 67-88.

Coleman, T.W.; Clarke, S.R.; Meeker, J.R.; Rieske, L.K. 2008. **Forest composition following overstory mortality from southern pine beetle and associated treatments.** Canadian Journal of Forest Research. 38: 1406-1418.

Cooper, W.R. 2007. **Chestnut defenses affect generalist and specialist herbivore responses and influence community dynamics.** Lexington, KY: University of Kentucky. 58 p. Ph.D. dissertation.

Cooper, W.R.; Rieske, L.K. 2007. **Community associates of an exotic gallmaker, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in eastern North America.** Annals of the Entomological Society of America. 100: 236-244.

Cooper, W.R.; Rieske, L.K. 2009. **Woody stem galls interact with foliage to affect community associations.** Environmental Entomology. 38: 417-424.

Crandall, B.S.; Gravatt, G.F.; Ryan, M.M. 1945. **Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*.** Phytopathology. 35: 162-180.

Dixon, W.L.; Burns, R.E.; Strange, L.A. 1986. **Oriental chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae).** Entomology Circular No. 287. Gainesville, FL: Florida Department of Agriculture and Consumer Service, Division of Plant Industry.

Gao, S.; Shain, L. 1995. **Effects of water stress on chestnut blight.** Canadian Journal of Forest Research. 25: 1030-1035.

Griffin, G.J.; Kahn, M.A.; Griffin, S.L. 1993. **Superficial canker instability during winter and virulence of *Endothia parasitica* associated with managed forest clearcut and plantation American chestnut trees.** Canadian Journal of Plant Pathology. 15: 159-167.

Griffin, G.J.; Smith, H.C.; Dietz, A.; Elkins, J.R. 1991. **Importance of hardwood competition to American chestnut survival, growth, and blight development in forest clearcuts.** Canadian Journal of Botany. 69: 1804-1809.

Griffin, G.J. 2000. **Blight control and restoration of the American chestnut.** Journal of Forestry. 98: 22-27.

Hanson, P. 1992. **The nearctic species of *Ormyrus* Westwood (Hymenoptera, Chalcidoidea, Ormyridae).** Journal of Natural History. 26: 1333-1365.

Jaynes, R.A.; Dierauf, T.A. 1982. **Hybrid chestnuts at the Lesesne Forest, Virginia.** In: Smith, H.C.; MacDonald, W.L., eds. Proceedings of the American chestnut symposium; 4-5 January 1978; Morgantown, WV. Morgantown, WV: West Virginia University: 68-73.

Kato, K.; Hijii, N. 1997. **Effects of gall formation by *Dryocosmus kuriphilus* on the growth of chestnut trees.** Japanese Journal of Applied Entomology. 121: 9-15.

Kellogg, S.K.; Hebard, F.V.; Rieske, L.K. 2005. **Interactions between a fungal pathogen, foliar properties, and generalist herbivores.** Entomologia Experimentalis et Applicata. 117: 209-219.

Moriya, S.; Inoue, K.; Otake, A.; Shiga, M.; Mabuchi, M. 1989. **Decline of the chestnut gall wasp population, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae), after the establishment of *Torymus sinensis* Kamijo (Hymenoptera, Torymidae).** Japanese Journal of Applied Entomology and Zoology. 24: 231-233.

Moriya, S.; Masakazu, S.; Ishizue, A. 2003. **Classical biological control of the chestnut gall wasp in Japan.** In: Proceedings of the 1st international symposium on biological control of arthropods; Honolulu, HI: 407-415.

Murakami, Y.; Ao, H.B.; Chiang, C.H. 1980. **Natural enemies in the chestnut gall wasp in Hopei Province, China (Hyemenoptera: Chalcidoidea).** Japanese Journal of Applied Entomology. 15: 184-186.

Murakami, Y.; Hiramatsu, T.; Maeda, M. 1994. **Parasitoid complexes of the chestnut gall wasp (Hymenoptera, Cynipidae) in two localities before introduction of *Torymus (syntomaspis) sinensis* (Hymenoptera, Torymidae), with special reference to prediction of results after release of the parasitoid.** Japanese Journal of Applied Entomology and Zoology. 38: 29-41.

Payne, J.A. 1978. **Oriental chestnut gall wasp: new nut pest in North America.** In: MacDonald, W.L.; Cech, F.C.; Luchok, J.; Smith, H.C., eds. Proceedings of the American chestnut symposium; 4-5 January 1978; Morgantown, WV. Morgantown, WV; West Virginia University: 86-88.

Payne, J.A. 1981. **Asian chestnut gall wasp in North America.** In: Smith, H.C. Proceedings of the U.S. Forest Service American Chestnut cooperators' meeting. Gen. Tech. Rep. NE-64. Broomall, PA: U.S. Department of Agriculture, Northeastern Research Station: 14.

Payne, J.A.; Menke, A.S.; Schroeder, P.M. 1975. ***Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), an oriental chestnut gall wasp in North America.** USDA Cooperative Economic Insect Report 25: 903-905.

Payne, J.A.; Green, R.A.; Lester, C.D. 1976. **New nut pest: an oriental chestnut gall wasp in North America.** Annual Report of the Northern Nut Growers Association. 67: 83-86.

Rieske, L.K. 2007. **Success of an exotic gallmaker, *Dryocosmus kuriphilus*, on chestnut in the USA: a historical account.** European Plant Protection Organization Bulletin. 37: 172-174.

Rieske, L.K.; Rhoades, C.C.; Miller, S.P. 2003. **Foliar chemistry and gypsy moth herbivory on pure American chestnut, *Castanea dentata* (Fam: Fagaceae), and a disease-resistant hybrid.** Environmental Entomology. 32: 359-365.

Stehli, B. 2003. **Oriental chestnut gall wasp found in N.E. Ohio.** Nutshell: Newsletter of the Northern Nut Growers Association. Available at www.nutgrowing.org/chstwasp.htm.

Stone, G.N.; Schonrogge, K.; Atkinson, R.J.; Bellido, D.; Pujade-Villar, J. 2002. **The population biology of oak gall wasps (Hymenoptera: Cynipidae).** Annual Review of Entomology. 47: 633-634.

USDA Forest Service. 2003. **Natural Resource Information Service (NRIS): Field sampled vegetation (FSVeg) Common Stand Exam. V1.5.1.** Fort Collins, CO: Rocky Mountain Research Station.

Yasumatsu, K. 1951. **A new *Dryocosmus* injurious to chestnut trees in Japan (Hym., Cynipidae).** Mushi. 22: 89-93.