Chestnut Growers of America
Promoting Chestnuts and Connecting Chestnut Growers

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# The Chestnut Grower

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# Biology of Chestnut Anthracnose (formerly blossom end rot) in Culinary Chestnut

*By Amy Miller & Melanie L. Lewis Ivey*

## *In This Issue*



**Background**

At harvest time, many chestnut growers in the eastern United States are familiar with nuts with a moldy black tip, that sometimes split open, and often have just a dry black lesion on both the shell and kernel. Because the black tip most often occurs on the stylar or "blossom" end of the nut, this fungal disease has been called blossom end rot (BER), and it has nothing in common, other than the name, with the familiar blossom end rot of tomato.

First described by Fowler and Berry in 1958, BER does not appear in the American nut literature again until Greg Miller's article in *The Chestnut Grower* – "Blossom End Rot of Chestnut: A Small Problem Becomes a Big Problem" (Miller 2017). Even Miller couldn't have known how prescient that report would be as growers in 2018 experienced the worst BER outbreak ever recorded, with some orchards losing half their crop to the disease. Without any existing literature on the disease cycle or effective management strategies, growers in Ohio (OH) turned to The Ohio State University's fruit pathology lab in the Department of Plant Pathology for research and guidance on this emerging threat to the chestnut industry.

Based on G. Miller's observations in 2017 and concerns from OH and Pennsylvania (PA) growers in 2018, several key questions emerged:

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# The Chestnut Grower

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### About Chestnut Growers of America, Inc.

The purpose of Chestnut Growers of America is to promote chestnuts, to disseminate information to growers of chestnuts, to improve communications between growers within the industry, to support research and breeding work, and generally to further the interests and knowledge of chestnut growers. CGA advocates the delivery of only high-quality chestnuts to the marketplace.

CGA began as the Western Chestnut Growers in 1996 in Oregon where about 30 or so chestnut growers understood the need to join forces to promote chestnuts in the U.S. Eventually they realized that they needed to be a national organization and solicited memberships from every grower in the country, which took the membership to over 100. The name of the organization was changed to Chestnut Growers of America, Inc., and it was granted 501(c)(5) status. Annual meetings take place around the country in an effort to make it possible for a maximum number of people to attend. A newsletter, *The Chestnut Grower*, is published quarterly and distributed by mail and/or email. CGA maintains an extensive resource site available only to members containing information helpful in growing and marketing. Visit chestnutgrowers.org for more information.

### Board of Directors



### Committees



### Annual Membership Dues

Single membership, $45; Household membership, $55; Associate membership, $60. Members receive *The Chestnut Grower* quarterly. Emailed newsletters are included. Mailed newsletters are an additional $5 per year. A $10 late fee is applied to membership renewals submitted after April 1.

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## Message from CGA President Roger Blackwell, Chestnut Grower

*Hello Chestnut Growers of America!*

We are hearing that our growers are having a very good year in selling fresh chestnuts.

In this message, I want to let you know the plans for the next annual meeting. We will be meeting jointly with the NNGA and the growers of the American Walnut Council for 2023. The Conference is scheduled for Sunday, July 23 through Wednesday, July 26, 2023, on the University of Missouri campus in Columbia, Missouri and the Horticulture and Agroforestry Research Center in New Franklin, Missouri. Please read this newsletter for additional information about the annual conference. We will have more information in the next issue for everyone's planning schedules.

I have another request for all CGA Members: we are looking for speakers for the annual meeting in July 2023. The talks can be 10 to 15 minutes in length and topics can be anything about chestnuts. We are looking to have your ideas by December 1, 2022 (or as soon as possible). Send your ideas to me at rblackwel@comcast.net.

CGA wants to thank the individuals who have submitted articles for this newsletter, and I encourage others in our organization to provide articles for future newsletters. We are all learning each year something new about growing chestnut trees in orchards throughout the country.

I hope you all have a plentiful harvest this fall and a wonderful holiday season.

Best regards,

Roger R. Blackwell

Roger

# Overview of the Upcoming 2023 Annual Conference

The planning committee for the 2023 joint Northern Nut Growers/ Chestnut Growers of America/Walnut Council Conference has been busy. The Conference is scheduled for Sunday, July 23 through Wednesday, July 26, 2023 on the University of Missouri campus in Columbia and the Horticulture and Agroforestry Research Center in New Franklin, Missouri. At that point in the academic calendar, the University is between sessions, so we will have access to meeting rooms and University housing within a short walking distance of parking and cafeterias. Many major hotel and restaurant chains are located within easy driving distance. Anticipate there will be a CAPs program.

Sunday is set aside during the day for board meetings of all organizations. They will be followed in the evening by a welcoming reception. We anticipate lining one of the hallways with tables for exhibits and poster boards. Presenters should be able to set up their posters Sunday afternoon or evening.

Monday will be an all-day field tour at the Horticulture and Agroforestry Research Center (HARC). Plan to see new and established research plantings of Chinese chestnut, hazelnut, black walnut, northern pecan, pawpaw, and elderberry. Because there is ample parking at HARC, those of us who drive will be asked to drive and carpool. In the evening, a live auction is being planned to support the research grant programs for the NNGA and Walnut Council. Along with the live auction, the Walnut Council Foundation will be conducting a silent auction from Sunday evening through Tuesday evening. We hope members of all three organizations will participate in both the live and silent auctions.

Tuesday will be filled with invited presentations on Chinese chestnut, hazelnut, black walnut, northern pecan, and other topics determined by the planning committee. Instead of concurrent sessions, we anticipate having poster sessions with a time designated for presenters to be by their posters. In the evening, we will have a joint social and banquet. After the banquet each organization will have time to entertain the group with fun activities including the Big Nut crowning, honor and service awards, the Walnut Achievement Award, Pass the Hat, and elections.

Wednesday morning will be filled with additional invited presentations and continuation of the poster session. Closing the conference at noon allows for scheduling of post-conference tours in the afternoon.

If you have wanted to volunteer to do a how-to presentation, let Roger Blackwell know ASAP so he can communicate that information to the local planning committee. The planners anticipate a mix of short talks and traditional technical presentations. Because of the costs associated with recording, editing, and posting of presentations, it is unlikely there will be a virtual option. That means mark your calendars now and plan to physically attend.

## Mark Your Calendars!

*For the 2023 Annual Meeting, a joint meeting with the Northern Nut Growers Association (NNGA) and the Walnut Council.*

**July 23-26, 2023**

*Columbia, Missouri*

*More information above and detailed schedule coming soon.*

## NNGA CGA 2022 Joint Meeting Presentation Recordings

*From Sara Fitzsimmons:* Many thanks to you all for supporting our 2022 joint conference of the Northern Nut Growers Association and Chestnut Growers of America! The event was a great success both because of the hard work of all the organizers as well as those of you who came to join us, both in person and virtually. If you missed it, a playlist of all the presentations from meeting can be found here: psu.mediaspace.kaltura.com/playlist/dedicated/1_pe5gwwzs

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- **Why is the disease so much worse now?** Blossom end rot has occurred in culinary chestnut crops in the eastern U.S. for at least the last 80 years, historically appearing as a minor pathogen affecting <2% of the crop. Since 2011 growers in OH and PA have seen disease incidence rise to 10-50%.
- **What fungus is responsible for causing BER?** In 2014, Dennis Fulbright at Michigan State University putatively identified the causal agent of chestnut BER as *Colletotrichum gloeosporioides*. Advances in gene and whole genome sequencing provide an opportunity to confirm the identity of the fungal pathogen.
- **Is there a relationship between chestnut BER and other diseases caused by *Colletotrichum*?** *Colletotrichum gloeosporioides* is also known to cause disease in many small fruit crops and apples. In 2018, an outbreak of bitter rot in OH apple orchards caused substantial losses of the 'Honey Crisp' crop. This outbreak coincided with the BER outbreak, leading to the possibility that the bitter rot disease cycle could be used as a model to decipher the BER disease cycle.
- **What is the BER disease cycle?** Deciphering the disease cycle is key to identifying strategies to prevent and control BER.
- **How can BER be prevented or effectively managed?** Most diseases caused by *Colletotrichum* require an integrated approach, starting with host resistance.
- **Does the fungus that causes BER cooccur with the Asian chestnut gall wasp?** In 2015, Lynne Rieske-Kinney at the University of Kentucky explored the invasion of the Asian chestnut gall wasp in OH chestnut orchards and found that a fungus in the genus *Colletotrichum* acted as a biocontrol for the wasp.

While it may take decades to fully answer the questions above, we have embarked on foundational research using the commercial orchards belonging to the Route 9 Cooperative in Carroll County, OH as our study site. Our work has focused on BER in Chinese chestnut (*Castanea mollissima*) and hybrids, but we have also started to explore this disease in Allegheny chinkapin (*C. pumila*) and American chestnut (*C. dentata*). In 2019-2020, we identified twig cankers caused by the same fungus that causes BER, leading us to reclassify BER into a larger disease complex called **chestnut anthracnose** (Miller and Lewis Ivey 2022).

***Figure 1.*** *Chestnut anthracnose caused by* Colletotrichum henanense. *Disease symptoms on the shell (top left), kernel (bottom left), and twig (right). Photos by Amy Miller.*

The short-term goals of our research are to determine the species of *Colletotrichum* implicated in disease in OH chestnut orchards, to evaluate for natural host resistance in commercially important chestnut cultivars, and to determine the optimal environmental conditions that favor disease development and progression. The long-term goals are to decipher the chestnut anthracnose disease cycle and identify time points and/or host tissues that may be targeted for management practices.

Anthracnose affects the kernels, shells, and twigs of chestnut (Figure 1). On the kernel, the disease is recognized by blackening of the kernels that may or may not be visible on the stylar end of the chestnut shell. The most common location for the lesion is on the tip; however, lesions are occasionally found on other parts of the kernel. The disease presents as one lesion per kernel, and multiple lesions per kernel have never been observed/recorded for this disease. Lesions tend to be black but sometimes are partially light brown. Lesions appear dry, have a dehydrated texture compared to healthy tissue, and do not have a discernible odor or flavor from the healthy kernel tissue. Lesions may occupy anywhere from 1-100% of the kernel area, but most commonly infect 30- 50% of the area. While rare, orange spore masses can form on the shell lesions.

Typically, kernel lesions that occupy more than 10% of the kernel area also exhibit a black lesion on the shell. Often when the lesion on the kernel covers less than 10% of the area, black lesions are not apparent on the shell, and thus it can be difficult to identify an infected kernel from the outside. Because of the dry nature of the lesions, anthracnose can be confirmed in fresh chestnuts by pressing on the tip with a finger. If the tip or suspected lesion area is soft, disease is present. If the tip is firm and turgid, the tissue is healthy. The squeeze test only works on fresh chestnuts that are fully hydrated.

Any anthracnose lesion renders a fresh chestnut unsaleable. If anthracnose nuts are not culled, they exhibit increased postharvest molds in cold storage compared to healthy nuts. In addition, anthracnose can ruin a chestnut's ability to germinate and grow because the lesions typically occur on the nut tip where the embryo and radicle meristem occur (Miller et al. 2013). This can

greatly impact seedling production for commercial chestnut nurseries.

Cankers form on 1-yr twigs of chestnut seedlings. Cankers are brown with a purplish and/or orangish hue, sunken, and approximately 1 cm in length. In some seedlings, distal portions of infected twigs may die, while in other seedlings only shoots and leaves within the cankered areas die. Black acervuli (spore-bearing structures) have been observed erupting from canker tissues. The biology of anthracnose cankers is still under investigation, but we hypothesize that spores from twig cankers could be the source of kernel infections.

## CHARACTERIZATION OF THE PATHOGEN CAUSING CHESTNUT ANTHRACNOSE IN OHIO

### Species Identification

*Colletotrichum* species are fungal pathogens of fruits, nuts, and vegetables worldwide, especially in tropical regions. Until recently, the species *Colletotrichum gloeosporioides* and *C. acutatum* were identified based on morphology and recognized as the main pathogenic species in fruits and nuts in temperate North America (Dowling et al. 2020). Advances in genomics techniques have revealed many species in the *C. gloeosporioides* and *C. acutatum* species complexes. Genomic tools allowed us to identify *Colletotrichum* species much more accurately than in the past, and improve the specificity of *Colletotrichum* species distribution, population dynamics, and ecology. We identified the causal agent of chestnut anthracnose as *Colletotrichum henanense* (Miller and Lewis Ivey, 2022). *Colletotrichum henanense* is a distinct species within the highly variable *C. gloeosporioides* species complex.

To identify the chestnut anthracnose pathogen, we isolated and cultured *Colletotrichum* spp. on potato dextrose agar (PDA) from surface-sterilized tissue of both chestnut kernel lesions and chestnut twig cankers (Figure 2). Isolates from both tissue types produced grey aerial mycelia, pink sporodochia, and cylindrical conidia with rounded ends ranging in size from 12-20 um long by 5-8 um wide. Isolates were preliminarily identified as belonging to the *C. gloeosporioides* species complex based on these morphological characteristics. To identify the species molecularly, genomic DNA was extracted from isolates grown in culture on PDA.

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, β-tubulin (TUB2) gene, and the intergenic spacer known as ApMat between the DNA lyase gene and MAT1-2-1 mating type gene were amplified using the primers and protocols described by Dowling et al. (2020). These genes are suitable for identifying species within the *C. gloeosporioides* species complex according to Eaton et al. (2021). The sequences were compared with published sequences in an international nucleotide sequence database (GenBank). The *Colletotrichum* isolates from kernels and twigs were identified as *C. henanense* based on a 98-99% sequence identity with fungal isolates MT513015.1, MT513080.1, and MT512917.1 (Miller and Lewis Ivey, 2022). To date, only one species of *Colletotrichum*, *Colletotrichum henanense*, has been identified as the causative agent of chestnut anthracnose. In other nut and fruit crops, multiple species of *Colletotrichum* cause anthracnose, making the chestnut anthracnose pathosystem unique.

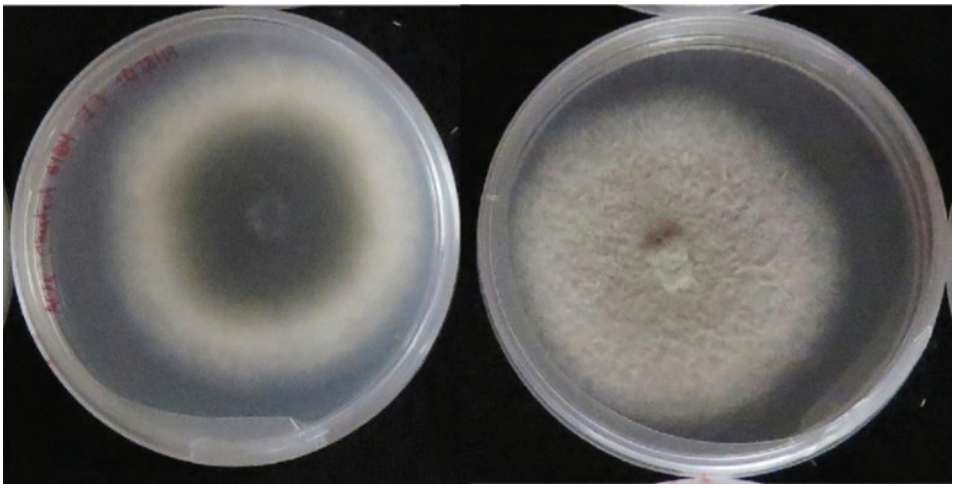

***Figure 2.*** Colletotrichum henanense *cultured on potato dextrose agar (PDA). View of the bottom (left) and top (right) of the isolate. Photo by Amy Miller.*

### Pathogenicity Tests

Pathogenicity tests were developed to use nuts at various stages of development and one-year-old chestnut seedlings grown in containers. Developing chestnuts in burs were gathered from the field and disinfested with 70% ethanol. Inoculation was attempted through an invasive method (stabbing nuts with a sterile hypodermic needle and injecting 50 uL inoculum) and a non-invasive method (spraying the nut tufts where they meet the outside of burs with 100 uL of inoculum). All bur samples were incubated at room temperature in a moist chamber for 21 days.

Twig nodes were also disinfested with 70% ethanol and inoculated via three invasive methods and three non-invasive methods. Invasive methods included wounding with a sterile probe and 1) carefully dropping 10 uL of inoculum directly onto the wound, 2) spritzing 100 uL of inoculum on and around the wound area, and 3) securing a mycelial compress from an agar plug directly onto the wound. Non-invasive methods were the same as the invasive ones, but instead of wounding, inoculum was applied to the non-wounded leaf scar at each node. Seedlings were incubated at room temperature for 60 days and monitored for symptom development.

Chestnut kernels that were inoculated by wounding developed characteristic brown/black lesions at the wound site while nuts inoculated using a non-invasive technique showed no symptoms. Like the nuts, only the invasive techniques resulted in disease on the twigs. Within five days, brown/purple cankers were developing, and by 30 days canker growth had stopped and acervuli had begun to form. Acervuli were still present 60 days after inoculation. No sporulation, as indicated by oozing acervuli, was observed within that time window.

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For many hosts, *Colletotrichum* does not require a wound to infect as it produces specialized structures called appresoria that can puncture through the host surface. Because inoculation through wounds was the only way we successfully caused disease on kernels and twigs in the lab, it is likely that wounds have ecological relevance to the disease cycle. Although chestnut twig cankers can be induced in healthy trees, the cankers do not necessarily cause shoot death. However, shoot death associated with anthracnose twig cankers has been observed in trees that are also stressed in other ways. In our studies we observed that otherwise healthy trees were able to heal around the cankers and maintain healthy shoots distally from the cankers. Even if anthracnose cankers do not kill shoots, the fungus can sporulate from cankers and potentially be a primary source of inoculum in the orchard.

## RESERVOIRS AND INOCULUM SOURCES OF *C. HENANENSE*

To identify pathogen reservoirs and sources of inoculum in the chestnut orchard, we collected and cultured from leaves, twigs, burs of Chinese chestnut, American chestnut, and Allegheny chinkapin, and galls of the Asian chestnut gall wasp (*Dryocosmus kuriphilus*), throughout the year and at key developmental points during the growing season (bloom, bur growth, and fruit fill). Samples were collected from an orchard with a history of anthracnose. We also collected leaf and twig tissue from apple trees in an orchard adjacent to the Chinese chestnut orchard. Isolates with a morphology consistent with *Colletotrichum* were identified to species using the same genomic methods described above.

We successfully isolated *C. henanense* on leaves, twigs, and burs, and at all developmental stages of the three chestnut types during the growing season. This suggests that *C. henanense* is ubiquitous in chestnut orchards following an outbreak of anthracnose and that its presence in the orchard does not necessarily result in disease. However, additional research is needed to determine the length of time that *C. henanense* persists on these tissues in the orchard. Although *C. henanense* was isolated from asymptomatic tissues in American chestnut and Allegheny chinkapin, anthracnose disease has never been observed on kernels of these species. More research is needed to determine whether those *Castanea* species native to North America are resistant to chestnut anthracnose, despite hosting *C. henanense.*

**Figure 3.** *Sporulating fungal lesion of* Colletotrichum fiorinae *on gall caused by Asian chestnut gall wasp. Photo by Amy Miller.*

Interestingly, *C. henanense* was not isolated from lesions on galls of the Asian chestnut gall wasp or from apple leaves or twigs. The only species isolated from lesions on wasp galls was *C. fiorinae* (Figure 3), which belongs to the *C. acutatum* species complex and has antagonistic activity against the Asian chestnut gall wasp (Graziosi and Rieske 2015). On apple fruit, bitter rot disease is commonly caused by *C. fioninae*, *C. fructicola*, *C. nymphaeae*, and *C. chrysophilum* (Martin et al. 2022). While *C. henanense* has rarely been implicated in bitter rot in apple, an extensive survey of other tissues (I.e., leaves, twigs, blossoms) in apple orchards has not been done but it would be prudent to do so to determine if apple orchards are a source of C. *henanense*.

## CULTIVAR SUSCEPTIBILITY TO CHESTNUT ANTHRACNOSE

To explore whether there is variation in disease susceptibility among commercially important cultivars of Chinese chestnut, we evaluated disease incidence and severity in nuts over three years. Bloom windows were assessed for eight chestnut cultivars ('Qing', 'Perry', 'Gideon', 'ACE', 'Peach', 'Szego', 'Liu', and 'AU Homestead') and regional weather data (average daily rainfall, free moisture, maximum and minimum daily temperature) were collected during the bloom periods. Disease intensity was assessed for each cultivar at harvest time. Nuts from each cultivar were sorted and counted based on the presence or absence of anthracnose symptoms, and anthracnose severity was determined by cutting nuts in half and measuring the length of the necrotic lesion. Simple linear regression was used to compare disease incidence with weather variables, and chi square tests of homogeneity were used to compare disease intensity (incidence and severity) among cultivars.

Significant differences in disease incidence were observed among the cultivars, with consistently low incidence in 'Qing', 'Liu', and 'AU Homestead' (<2% each), moderate incidence in 'Gideon', 'Perry', and 'ACE' (2-10% each), and high incidence in 'Peach' and 'Szego' (>10% each) (Figure 4).

Significant differences in disease incidence among the same cultivar across

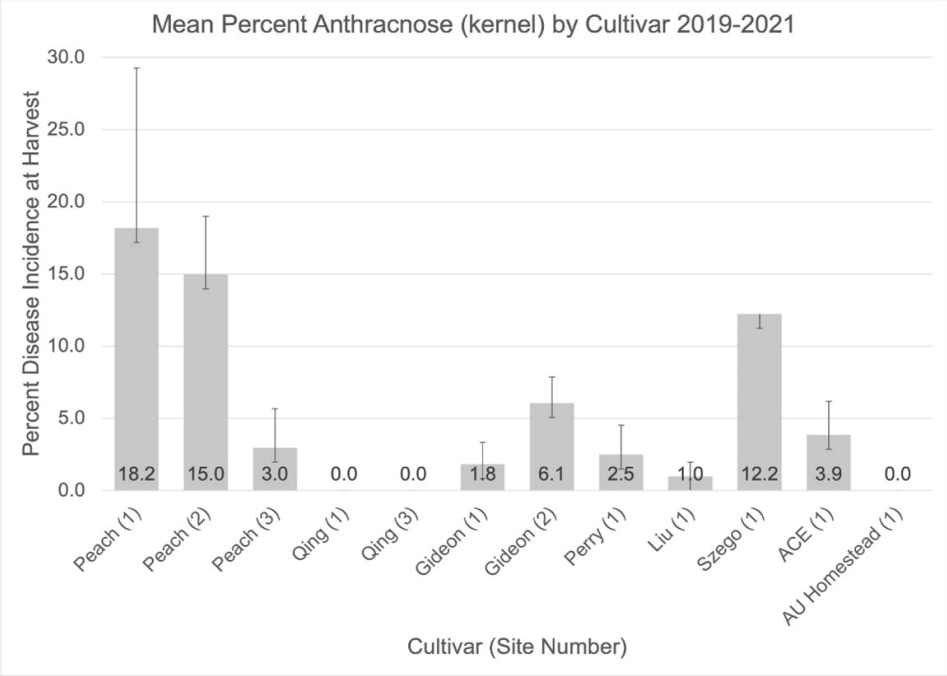


***Figure 4.*** *Incidence of chestnut anthracnose on kernels by cultivar averaged over the 2019-2021 harvest seasons at three sites in the Route 9 Cooperative orchards. Incidence is expressed as a percent of diseased kernels out of all kernels harvested per cultivar. Error bars indicate standard deviation in disease incidence for all cultivars with 2+ years of data.*

different sites suggest that environmental factors also contribute to cultivar susceptibility; however, no correlation was detected between incidence and total precipitation, free moisture, or temperatures from bloom to harvest within the scope of this study. Mean severity per cultivar, expressed as percent of kernel infected, ranged from 52%-64%, with no significant differences in severity among cultivars. To continue deciphering the disease cycle, disease incidence and severity over several more years will need to be compared with weather data.

**Acknowledgements:**

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**ABOUT THE AUTHORS**

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*Dr. Melanie L. Lewis Ivey is an Associate Professor in the Department of Plant Pathology at The Ohio State University, College of Food, Agricultural and Environmental Sciences – Wooster Campus. She is also the OSU Extension Fruit Pathologist and Fresh Produce Safety Specialist. Her research focuses on the sustainable and safe production of fruit, nut, and hop crops.*

# The Asian Chestnut Gall Wasp in North America

*By Carol C. Mapes*

The Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) is an invasive gall-forming cynipid wasp that is a global pest of *Castanea* spp. Mill. (Gibbs et al. 2011; Borowiec et al. 2014). Native to China, the ACGW spread to Japan in 1941, Korea in 1958, the United States in 1974, Nepal in 1999, Italy in 2002 with subsequent spread to many other countries in Europe, Canada in 2012, and Russia in 2016 (Avtzis et al. 2019). Galls caused by the ACGW in North America are found on the Chinese chestnut (*Castanea mollissima* Blume), Japanese chestnut (*C. crenata* Siebold & Zucc.), and European chestnut (*C. sativa* Mill.), as well as on the American chestnut (*C. dentata* (Marshall) Borkh.). ACGW galls are rarely found on native Allegheny chinquapins (*C. pumila* (L.) Mill.) and native Ozark chinquapins (*C. ozarkensis* Ashe) (Rieske 2007; Anagnostakis 2014).

**Life History of the ACGW**

Larvae of the ACGW spend the winter "hidden" in buds of *Castanea* spp. After overwintering in the buds, the larvae form green and/or red galls (Figure 1) that are 5 to 20 mm (sometimes up to 30 mm) in diameter, on developing petioles, leaves, and stems (Figures 2 and 3) of *Castanea* spp. (EPPO 2005; Cooper and Rieske-Kinney 2007). Galls contain from 1 to 25 larval chambers or locules (Figure 4) (EPPO 2021).

Larvae pupate in the galls (Cho and Lee 1963), and adult female wasps emerge from fully formed galls in late spring or early to mid-summer. The time of emergence is site-specific and is affected by factors such as latitude and altitude (Bosio et al. 2010, EPPO 2021).

There are no male wasps, no sexual reproduction, and females develop from unfertilized eggs (Zhu et al. 2007). Adult ACGWs (Figure 5) are only 2.5 to 3.0 mm long and live from 2 to 10 days (Yasumatsu 1951; EPPO 2021). Female wasps lay unfertilized eggs in *Castanea* spp. buds, and each female wasp can lay up to 300 eggs (EPPO 2021).

It takes just one female to start an infestation (EFSA 2010). Individual buds can contain up to 30 eggs that are 0.1-0.2 mm long (Payne 1978, EPPO 2021). Larvae hatch from eggs in the buds in 30 to 40 days, and larvae overwinter in the buds.

It is not possible to visually determine which buds contain eggs or larvae without the aid of a stereomicroscope (EPPO 2021). Molecular techniques (PCR) can however be used to detect the presence of the ACGW in buds (Sartor et al. 2012).

After overwintering, larvae become active again in the spring and induce gall formation (Borowiec et al. 2014). After galls are fully developed, adult female wasps emerge from the current season galls, and the cycle repeats. Interestingly, there is a newly described species, *Dryocosmus zhuili* Liu & Zhu, 2015 that has been shown to form galls on Chinese chinquapins (*C. henryi* (Skan) Rehd. & Wils.) that are morphologically indistinguishable from ACGW galls. Unlike the case for the ACGW, there are both male and female wasps of *D. zhuili*. As of yet, *D. zhuili* has only been reported in China and only on Chinese chinquapins (Zhu et al. 2015).

**Damage Caused by the ACGW**

Infestation with ACGW galls results in inhibited shoot elongation, reduction in flowering and fruiting/nut production, premature leaf death (Figure 6), premature leaf abscission resulting in leaf area loss, twig dieback, reduction in winter bud development, reduction in shoot and tree vigor and in some cases tree mortality, particularly of smaller trees when other stressors are present (Payne 1978; Avtzis et al. 2019). Heavy infestations of the ACGW have been implicated in making European chestnuts (*C. sativa*) trees more susceptible to blight (Turchetti et al. 2010).

**Dispersal of the ACGW in North America**

Since its initial introduction in Georgia in 1974, *D. kuriphilus* has been reported in 16 additional states: Alabama, Tennessee, South Carolina, North Carolina, Kentucky, Virginia, West Virginia, Ohio, Maryland, Pennsylvania, New York, New Jersey, Delaware, Michigan, Connecticut, and Massachusetts (USDA Forest Service 2019; Mapes et al. 2020). *D. kuriphilus* was reported in Niagara-on-the-Lake, Ontario, Canada in 2012 (Huber and Read 2012).

The ACGW disperses by natural and artificial means. Natural means consist of dispersal by active flight and transport by the wind (EPPO 2021). ACGWs are not strong flyers; they are induced to fly at low wind speeds but at high wind speeds, the wasps are blown by the wind. The direction of the prevailing winds plays an important role in dispersal (Oho and Shimura 1970, EFSA 2010).

Artificial means of dispersal include human transport of infested plant material (EPPO 2021) including seedlings, saplings, or scionwood. Early range expansion in the Eastern United States was reported at rates of about 23 km/year, with a range of 15.2 to 25 km/year (Rieske 2007) and 15 miles/year (24.1 km/year) (Payne 1981). The early expansion of the range of the ACGW in the Eastern United States was thought to be due to natural means (Rieske 2007), with some of the more recent dispersal events most likely due to the introduction of infested plant material (Cooper and Rieske 2007; Mapes et al. 2020).

**Control of the ACGW**

Resistant Japanese chestnut (*C. crenata*) cultivars that were developed after the introduction of the ACGW into Japan in 1941, were grown successfully for many years until the 1960's and 1970's when galls were found more and more frequently on those cultivars (Moriya et al. 2003). Additional resistant Japanese chestnut cultivars have subsequently been developed. In Europe, the Japanese chestnut (*C. crenata*) × European chestnut (*C. sativa*) "Bouche de Bétizac" hybrid cultivar that was developed, is used by growers as it is resistant to the ACGW (Dini et al. 2012). In North America, crosses have been done using native Ozark chinquapins (*C. ozarkensis*) that are rarely galled or Chinese chinquapins (*C. henryi*) that also show some resistance. Offspring of crosses of American chestnut with Ozark chinquapin x Chinese chestnut have shown some resistance to gall formation (Anagnostakis 2014), and research involving crosses of commercial chestnuts

***Figure 1.*** *Galls of the Asian chestnut gall wasp (Photo: Mark Giambrone).*

***Figure 4.*** *The interior of a gall of the Asian chestnut gall wasp showing two larval cavities/locules (Photo: Mark Giambrone).*

***Figure 7.*** *The biological control parasitoid* Torymus sinensis *(Photo: Gregory Setliff).*

with Chinese chinquapin or Ozark chinquapin are also showing promise (Anagnostakis 2019).

The use of insecticides against the ACGW has not been considered a particularly suitable control option for several reasons. Larvae are well protected within the galls early in the season, and bud scales provide protection of larvae within buds later in the season (Moriya et al. 2003; Aebi et al. 2007; Bosio et al. 2010). Use of insecticides against emerging adults would require precise timing and would also require potentially large quantities of insecticides adding to other environmental and toxicity concerns (Bosio et al. 2010; Avtzis et al. 2019; EPPO 2021).

***Figure 2.*** *A stem gall of the Asian chestnut gall wasp (Photo: Carol Mapes).*

***Figure 5.*** *The Asian chestnut gall wasp,* Dryocosmus kuriphilus *(Photo: Gregory Setliff).*

Another avenue of control that has been investigated is hot water treatment (HWT) of dormant buds to kill the ACGW larvae within buds. Immersion of Qing Chinese chestnut scions in water at 52°C for 10 minutes was shown to kill *D. kuriphilus* larvae in buds and resulted in successful grafts. It was shown that conditions need to be carefully controlled as slightly higher temperatures (53°C for 10 minutes) caused injury and much lower graft union success (Warmund 2014). Immersion of European chestnut (*C. sativa*) scions in water at 49°C for 10 minutes killed *D. kuriphilus* larvae in buds and resulted in a high percentage of successful grafts (Ciordia et al. 2020). More research is needed to determine the effectiveness and parameters required for HWT of scions of other *Castanea* spp. and cultivars, as well as more research on HWT of seedlings.

Biological control with the parasitoid *Torymus sinensis* Kamijo, 1982 (Hymenoptera: Torymidae) (Figure 7) is considered the most effective method of

***Figure 3.*** *A leaf gall of the Asian chestnut gall wasp (Photo: Carol Mapes).*

***Figure 6.*** *Premature leaf death caused by a gall of the Asian chestnut gall wasp (Photo: Carol Mapes).*

ACGW control. Populations of *T. sinensis*, native to China, are now established in Japan, Europe, and North America (Avtzis et al. 2019). The life cycle of *T. sinensis* is closely aligned with that of the ACGW. *T. sinensis* adults emerge from overwintering ACGW galls in the spring. After mating, females lay eggs on ACGW larvae or on the walls of larval chambers in developing galls. A larva of *T. sinensis* feeds on an ACGW larva, eventually killing it. Larvae of *T. sinensis* overwinter and pupate in ACGW galls, and adults emerge in the spring (Quacchia et al. 2008).

Pruning of branches with newly formed galls in the spring before adults emerge, and disposal of the galls in a manner that kills the wasps, is a practice that can provide control on a small scale (Payne and Johnson 1979). Removal of current season galls after ACGW departure will result in the removal of *T. sinensis* parasitoids that are still present in the galls. Therefore, if one wishes to help maintain levels of the parasitoid *T. sinensis*, recently vacated current season galls (Figure 8, next page), should not be removed, as *T. sinensis* overwinters in the galls.

The states of Michigan and Oregon have issued quarantines for *D. kuriphilus* (MDA

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***Figure 8.*** *Galls after the emergence of adult Asian chestnut gall wasps (Photo: Carol Mapes).*

2010; Oregon Administrative Rule § 603-052-0075, 2014). Michigan's Chestnut Gall Wasp Quarantine was established in 2010. Among other things, it prohibits importing *Castanea* spp. plants and scionwood from infested states unless the place of production has passed inspections as specified and is certified "pest free". *Castanea* spp. plant material must be enclosed when transported through an infested county between May 1 and July 1 (MDA 2010). The Oregon Administrative Rule § 603-052-0075 (2014) protects against chestnut blight and all chestnut pest insects including the chestnut gall wasp. *Castanea* spp. & *Castanopsis* spp. plant material as specified, is not allowed into the state from defined eastern states, except by special permit. For defined western states, plant material and the site of production need to be certified disease and pest free as specified (Oregon Administrative Rule § 603-052-0075, 2014). Warmund et al. (2017) have proposed that additional states consider implementing quarantines to delay what may be the inevitable spread of the ACGW into non-infested states.

**Summary**

The Asian chestnut gall wasp, a global pest of *Castanea* spp., has spread throughout much of the Eastern United States since it was first inadvertently introduced into Georgia in 1974. The ACGW spreads by natural means (by flight and wind) and by artificial means through the transport of contaminated plant material. The ACGW "hides" in dormant buds in late summer and through the winter, making it easily spread through contaminated plant material. Hot water treatment (HWT) of buds has shown promise in treating contaminated buds. Some resistant chestnut cultivars have been developed, and there is ongoing research to develop others. The most effective method of control has been biocontrol by the introduced parasitoid *Torymus sinensis*. Two states have issued quarantines for the ACGW, and other states may follow. Greater awareness of the ACGW and additional research are needed to help control the spread of this invasive pest species in North America.

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