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Invasion by the chestnut gall wasp in Italy causes significant yield loss in *Castanea sativa* nut production

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Abstract

1 The Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae) is an invasive species in chestnut forests and orchards in many parts of the world.

2 Nuts produced by the European chestnut (*Castanea sativa* Miller) are important in human food and culture, and as a component in food webs in forest ecosystems.

3 Severe infestations are reported to reduce nut yield, although precise data are lacking because of large natural year-to-year variability in yield.

4 The recent colonization of chestnut orchards in north-eastern Italy, where nut yield has been continuously and precisely recorded for several years, offered an opportunity to calculate the impact of gall wasp infestation level on yield.

5 The nut yield of *C. sativa* chestnut trees was negatively related to the gall wasp infestation level, with losses as high as 80% being reported when the number of current-year galls was above six galls per 50-cm twig.

6 Yield losses can be explained by direct and indirect factors related to gall formation, and a fuller understanding of the mechanisms involved could identify possible mitigation measures.

Keywords Cynipidae, *Dryocosmus kuriphilus*, gall, invasion, management, yield.

Introduction

The Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae) is an invasive species in chestnut forests and orchards in various parts of the world. Indigenous to China, ACGW was introduced first in Japan and then North America and, most recently, in Europe, where it was first found in north-western Italy in 2002 (Anonymous, 2005, 2013; Rieske, 2007; Haack *et al.*, 2011). ACGW has now spread throughout Italy and is also considered established in Croatia, France, Slovenia, and Switzerland, whereas it is under official control in Austria, Czech Republic, Germany, Hungary and The Netherlands as of September 2013 [Anonymous, 2005; European Food Safety Authority (EFSA) Panel on Plant Health, 2010; European and Mediterranean Plant Protection Organization, 2013]. Although gall-forming cynipids are primarily associated with oaks (*Quercus* spp.), a few other genera of the beech family Fagaceae, including sweet chestnut (*Castanea* spp.), are also colonized by cynipids (Stone *et al.*, 2002; Ács *et al.*, 2007; Buffington & Morita, 2009).

European chestnut (*Castanea sativa* Miller) and other *Castanea* species are exploited for nut production worldwide. Chestnuts have been used for thousands of years by humans as a staple food (Pezzi *et al.*, 2011) and nowadays are still important as typical elements of local food. They are also important in the heritage of many human cultures. For example, *C. sativa* was often associated with early farming settlements in southern Europe (Anderson *et al.*, 2011). Chestnuts are also an important component in food webs and in the evolution of forest ecosystems (Vander Wall, 2001; Dalgleish & Swihart, 2012).

Severe ACGW infestations are reported by growers associations to cause nut yield reduction in chestnut orchards in the order of 60–80% (Anonymous, 2005; EFSA Panel on Plant Health, 2010). These reports, however, are not precise because large natural year-to-year variability in nut yield makes it difficult to achieve reasonable estimates. In addition, cultivars of *C. sativa* show large variation in susceptibility to ACGW, which likely results in differential impact on nut yield

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(Panzavolta *et al.*, 2012; Bernardo *et al.*, 2013). The ACGW may reduce yield in chestnut through both direct and indirect mechanisms. ACGW galls can directly prevent the formation of the female flower when galls are formed on the apical buds of the shoot, which stops shoot growth and causes flower abortion. In addition, the yield can be indirectly affected as a result of reduced leaf area, photosynthesis and tree biomass, which can extend into future years after the initial ACGW attack (Kato & Hijii, 1997). An additional, although little explored possibility of interaction between ACGW and chestnut trees is via possible metabolic changes in the host tissues in response to the release of compounds by ACGW larvae or the associated gall tissues, as shown by differential gene activation in gall tissues (Botta *et al.*, 2009). Given that gall formation responds to certain plant hormones (Cooper & Rieske, 2011), it is also possible that galls release molecules associated with female flower differentiation and growth.

The recent colonization of chestnut orchards by ACGW in an area of north-eastern Italy, where nut yield has been continuously and precisely recorded for several years on a number of small farms, has offered the opportunity to calculate the yield reduction soon after ACGW arrival on farms with different levels of ACGW infestation. Given that the farms have similar conditions of soil, climate, management and chestnut cultivar, we hypothesize that differences in yield are mainly explained by the level of ACGW infestation.

Materials and Methods

Two chestnut growers associations in north-eastern Italy (Combai and Monfenera) were asked to provide names of local growers who deliver their entire annual crop to the association and for whom a reliable time series of nut yield was available. An initial list of 30 growers was screened with on-site visits targeted at assessing farm size, growing conditions and year of initial ACGW infestation. The nut yield level, given in total weight of nuts delivered to the association, was obtained and checked for consistency with interviews of the farmers and by comparing the farm yields with the mean historical yield per tree recorded in the area (personal communication with the Combai Growers Association was calculated at 17.8 kg of nuts per tree for the period 1998–2008). Of the 30 initial farms screened, we selected 15 growers with reliable crop estimates and negligible levels of other nut pests, such as *Cydia fagiglandana* (Zeller) (Lepidoptera: Tortricidae), and various nut weevils, such as *Curculio* spp. (Coleoptera: Curculionidae), and obtained 4 years of yield records (2007–2010) for each orchard. It was also reported that no ACGW infestation was known to be present before 2007 at any of the 15 selected orchards, whereas ACGW was present in all 15 orchards in 2010.

In addition, given that chestnut production can be significantly affected by summer weather, especially heavy rains at flowering and drought during the nut ripening period (Pereira *et al.*, 2011), we first compared the summer weather pattern of 2010 with the three previous years, which is similar to how we tested the chestnut yield data (see below). For the period June to September, we compared total rainfall (mm) and the daily mean air temperature (°C) using data from a weather station located inside the study area (Valdobbiadene-Bigolino, ARPAV Centro Meteorologico di Teolo, Regione del Veneto).

Between December 2010 and February 2011, we visited each of the 15 orchards and selected 10 chestnut trees at random. In this area of Italy, productive trees are generally between 40 and 120 years old, well-spaced and result from grafting the local cultivar 'Marrone' on wild seedlings. The susceptibility of this cultivar to ACGW appears to be average (Botta *et al.*, 2010). From each tree, 10 twigs from lateral branches, each approximately 50 cm in length and including the growth of the last 2–3 years, were randomly selected and clipped with a telescopic shears from all sides of the trees up to a maximum height of 7 m. At this time of the year, the twigs carry only galls produced during the previous spring. The mean number of galls per twig was first estimated for each sampled tree by counting all galls produced during the spring of 2010 (and considering multiple galls that had coalesced as a single gall) on the 10 sampled twigs and then dividing by 10. Then, the mean number of galls per twig was calculated at the orchard level by averaging the mean values for each of the 10 sampled trees per orchard. We acknowledge that counting galls in winter slightly underestimates the real infestation level because it did not include galls produced on leaves, which had fallen by the time of our census. The galls on leaves, however, are usually a minor portion of the total number of galls on a tree and do not prevent shoot growth (Maltoni *et al.*, 2012b). The impact of the ACGW was assessed for each orchard using a yield ratio by comparing the 2010 yield (i.e. the year when the highest ACGW infestation was observed) with the mean yield of the previous three years (2007–2009), when the ACGW infestation level was zero or negligible (personal communication with the Combai and Monfenera Growers Association: galls were first detected at very low densities in four out of 15 farms during 2007–2009, and first at the other 11 orchards in 2010). The slow build-up of an infestation after initial invasion is typical of *D. kuriphilus* (EFSA Panel on Plant Health, 2010) and so we are confident that the information obtained from the farmers was correct. We used linear regression to explore the relationship between yield ratio for each of the 15 orchards and the corresponding mean ACGW infestation level (number of ACGW galls per 50 cm of shoot) in 2010 for the same orchard. The root mean square error of the regression was also provided (Sokal & Rohlf, 1995).

Results

Mean ACGW infestation levels and nut yields are presented in Table 1. The relationship between the ACGW infestation level in 2010 and the yield ratio 2010/2007–2009 (Fig. 1) indicates that there was a significant yield reduction with increasing ACGW infestation level ($F_{1,13} = 10.3$, $P = 0.007$, $r^2 = 0.47$). Significant r^2 values were also observed when the yield ratios were calculated for 2010 and each individual year during 2007–2009 (2007: $r^2 = 0.44$; 2008: $r^2 = 0.45$; 2009: $r^2 = 0.23$). A crop loss of approximately 50% resulted from mean infestation levels of four to six current-year galls per 50-cm long twig, and as high as 80% when the mean number of

Table 1 Summary data for the 15 selected chestnut farms in north-eastern Italy, including geographical location, number of chestnut trees, mean Asian chestnut gall wasp (ACGW) galls per twig in 2010, chestnut yield per farm in 2010, mean chestnut yield per year during 2007–2009, and their ratio

Growers association	Municipality and farm	Number of trees	Mean ± SE number galls/twig	Yield in 2010 (kg)	Mean ± SE annual yield (2007–2009) (kg)	Yield ratio 2010/2007–2009
Combai	Cison di Valmarino	45	1.4 ± 0.25	509.0	515.5 ± 185.7	0.99
Combai	Follina a	30	0.71 ± 0.13	273.0	258.1 ± 102.2	1.06
Combai	Follina b	120	1.59 ± 0.18	1506.0	1348.3 ± 596.2	1.12
Combai	Miane a	78	2.91 ± 0.64	504.5	610.8 ± 270.4	0.83
Combai	Miane b	27	0.71 ± 0.25	97.5	154.0 ± 69.2	0.63
Combai	Miane c	25	0.52 ± 0.07	145.0	118.2 ± 50.5	1.23
Combai	Segusino	30	2.97 ± 0.48	114.0	424.1 ± 142.4	0.27
Combai	Vittorio Veneto	50	0.2 ± 0.13	712.0	281.0 ± 29.7	2.53
Monfenera	Cavaso – Costalunga[a]	53	5.36 ± 0.64	161	505.0 ± 135.1	0.32
Monfenera	Cavaso – Rizzele[a]	114	4.08 ± 0.66	1025	1106.0 ± 242.3	0.93
Monfenera	Pederobba – Fenaroi a	39	6.44 ± 0.73	122	544.0 ± 171.4	0.22
Monfenera	Pederobba – Fenaroi b	110	1.93 ± 0.31	495	743.33 ± 216.1	0.67
Monfenera	Pederobba – Fenaroi c[a]	42	2.85 ± 0.43	166	368 ± 82.1	0.46
Monfenera	Pederobba – S. Margherita	37	3.57 ± 0.42	995	1250.0 ± 380.5	0.80
Monfenera	Possagno – Val de la Geda[a]	42	3.66 ± 0.56	233	1024.0 ± 524.5	0.23

[a]Orchards that were known to be infested with ACGW for the first time during 2007–2009. ACGW was first observed in the other 11 orchards in 2010.

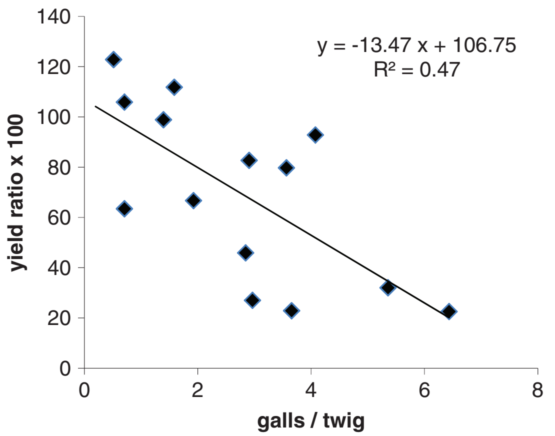

Figure 1 Linear relationship between chestnut yield ratio (2010 yield/mean annual yield during 2007–2009) and the mean number of Asian chestnut gall wasp galls per 50-cm long twig formed in 2010 in 15 chestnut farms in Italy. The root mean square error of the regression is 23.7. For details, see text.

galls exceeded six galls per twig. It should be noted, however, that the root mean square error amounted to 23.7% of the yield ratio, indicating a limited spread of the original points from the regression line.

Rainfall during June to September in 2010 amounted to 510 mm compared with 543 mm (SD 135.8) during 2007–2009. Similarly, June to September mean daily air temperature was 20.8 °C (SD 2.8) in 2010 and 21.1 °C (SD 2.4) in 2007–2009. We thus concluded that 2010 was an ordinary year for chestnut production, and this was also confirmed through interviews with the growers. Similarly, variation in weather did not explain the year-to-year differences in yield during 2007–2009, as revealed by the high standard error values in Table 1, which was consistent among orchards and related to normal annual fluctuations of yield in these Italian orchards.

Discussion

Overall, we found a significant negative linear relationship between ACGW infestation level and nut yield of *C. sativa* chestnut trees, although we recognize that local orchard management and environmental conditions can result in substantial variation in the response. To our knowledge, this is the first numerical evidence to substantiate yield losses in relation to gall density. Losses are often claimed by nut producers after invasion of ACGW, and were recently estimated to reach between 65% and 85% in Piedmont, Italy, based on a 9-year record (1999–2006) of yield in two orchards that became infested in 2004 (Bosio *et al.*, 2013). In another location in Italy, a few years after the initial ACGW invasion, yield reductions were estimated between 20% and 90%, although these losses were in combination with the nut pest *C. fagiglandana* (Speranza & Paparatti, 2010). In south-eastern and eastern China, where ACGW is a native pest on Chinese chestnut *Castanea mollissima* Blume, yield losses are reported at between 15% and 30% (Zhang, 2009), although they can rise as high as 80% (EFSA Panel on Plant Health, 2010). Reliable yield impact estimates are not available for North America, where ACGW has been present and spreading from the 1970s onward. Although it has been suggested that losses could reach 50–75% in commercial Chinese chestnut orchards in the U.S.A. (Payne *et al.*, 1983), no data have been published to support this claim. Moreover, in Ohio, where ACGW was first found in 2002, local growers of primarily Chinese chestnut cultivars reported no consistent pattern between ACGW severity and yield levels (G. Miller and R. Stehli, personal communication). However, in Michigan, which neighbours Ohio, there is great concern about the potential arrival of ACGW because many Michigan growers have planted Japanese–European chestnut cultivars, which are considered to be more susceptible to ACGW than Chinese chestnut cultivars (Fulbright *et al.*, 2010; Haack *et al.*, 2011). Similarly, in preliminary studies conducted in Italy on individual trees of a susceptible cultivar of European chestnut ('Marsol'), reductions in nut yield have been recorded with increasing numbers of galls per shoot, with a slope similar to that observed under field conditions in the present study (Botta *et al.*, 2010).

The magnitude of direct impact from ACGW in Europe on yield reduction in *Castanea* was estimated as moderate in the last available review, although with a high level of uncertainty as a result of the absence of quantitative data (EFSA Panel on Plant Health, 2010). Our results provide the first evidence of a relationship between gall density and yield loss in individual orchards, and can thus be used as a reference for managers when predicting losses from ACGW. We admit, however, that our estimates refer to local conditions in north-eastern Italy and should be validated in other situations, such as intensively managed orchards. If our results are confirmed, it would provide justification to growers to adopt management strategies aiming to reduce ACGW numbers and maintain nut yield. Given that direct pest control is difficult and troublesome (Bernardo *et al.*, 2013) and classical biocontrol is a long-term option (Quacchia *et al.*, 2008), relatively simple cultural measures could be adopted by growers to reduce the number of buds that are susceptible to ACGW oviposition at the time of female emergence. Interestingly, a new pruning technique based on the removal of shoots of ACGW-infested trees at peak time of oviposition (green pruning) has been suggested to restore growth in young chestnut orchards (Maltoni *et al.*, 2012a,b), alone or in combination with organic fertilizers (Turchetti *et al.*, 2012). Restoring growth in trees that have been subjected to repeated ACGW infestations appears to be a requisite to maintain nut yield, although it may largely depend on water availability in traditionally non-irrigated orchards. In addition, some growers in North America suggest that, by promoting shoot growth into late summer, the effects of ACGW can be reduced because late-season buds are seldom infested by ACGW, which oviposits primarily in early summer (G. Miller, personal communication). Such management options are achievable if environmental conditions are conducive to summer shoot growth, which are generally associated with high soil water availability. Variation in summer shoot growth in chestnut trees could thus be an important factor affecting both the success of ACGW, as well as nut yield. This hypothesis needs further testing in situations where chestnut cultivar, flower bud formation and environmental conditions can be controlled.

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