Silva Fennica vol. 52 no. 3 article id 9905
Category: research article
https://doi.org/10.14214/sf.9905

SILVA FENNICA

http://www.silvafennica.fi

ISSN-L 0037-5330 | ISSN 2242-4075 (Online)
The Finnish Society of Forest Science

Mercedes M. Fernández[1,2], Diana Bezos[2] and Julio J. Diez[2,3]

Fungi associated with necrotic galls of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in northern Spain

Fernández M.M., Bezos D., Diez J.J. (2018). Fungi associated with necrotic galls of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in northern Spain. Silva Fennica vol. 52 no. 3 article id 9905. 12 p. https://doi.org/10.14214/sf.9905

Highlights

- Presence of *Dryocosmus kuriphilus* in Northern Spain.
- The mycobiota associated to necrotic galls was studied for the first time.
- 7 fungal species were identified.
- The entomopathogenic fungi found could be use as potential biological control agents.
- *Gnomomiopsis smithogilvyi, Fusarium oxysporum* and *F. avenaceum* known by their toxicity against the insect, were found.

Abstract

The Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is one of the most important pests in Castanea species worldwide. In 2012, it was found for the first time in Catalonia (Spain) and a year later, in the north of Spain (Cantabria). Today, it is present in 14 Spanish provinces. In search of biological control against the ACGW, several authors have previously found the relationship between the presence of some *Fusarium* Link species in necrotic galls and wasp mortality due to the production of different types of wall-degrading enzymes and entomopathogenic mycotoxins. The objective of this study was to investigate the mycobiota associated with necrotic galls to find interesting perspectives for biological control of the ACGW. For this purpose, in 2014, 119 necrotic galls of *Castanea sativa* Miller were plated to isolate and identify the associated fungi. The fungal isolates were identified by the morphology of the fruiting bodies and DNA analyses. From necrotic galls, 7 species of fungi were identified. Of these, we highlight three species of *Fusarium* Link as well as the presence of *Gnomoniopsis smithogilvyi* Shuttlew, Liew & Guest due to its toxic capacity. Further studies are required to verify the effectiveness of these fungal species as biocontrol agents against the ACGW.

Keywords Asian chestnut gall wasp; *Castanea sativa*; *Fusarium* spp.; *Gnomoniopsis smithogilvyi*; entomopathogens; fungal diversity
Addresses [1]Dpt. of Agroforestry Sciences, ETSIIAA, University of Valladolid, Av. Madrid 50, 34071 Palencia, Spain; [2]Sustainable Forest Management Research Institute UVa-INIA, ETSIIAA, 34071 Palencia, Spain; [3]Dpt. of Plant Production and Forest Resources, University of Valladolid, Av. Madrid 50, 34071 Palencia, Spain
E-mail mffernan@agro.uva.es
Received 13 November 2017 **Revised** 3 May 2018 **Accepted** 7 May 2018

1 Introduction

The Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), was detected for the first time in Spain in 2012 (Torrell and Heras 2012) specifically in Cataluña (NE). A year later, it was noticed in the northern part of Spain, in Cantabria (Bezos et al. 2013). At present, it has been detected in 9 Autonomic Communities: Cataluña, Cantabria, Galicia, Asturias, Andalucía, Castilla & León, Madrid, Navarra and Basque Country; 14 provinces in total, representing 30% of the Iberian Peninsula territory.

Dryocosmus kuriphilus is a gall-inducing insect, native from China, which was introduced for the first time in Japan, later in North America and more recently, in Europe (2002). After the first record in Italy, it was detected in other 14 neighboring countries. Currently, this pest is the most important on *Castanea* species worldwide with losses of nuts that reach 80% when serious infestations occur (EFSA 2010) and, in addition, the mortality of branches and trees in very severe infestations of young plantlets or weak plants (Cooper and Rieske 2007).

In Europe, it is also the most impactful alien pest for *Castanea sativa* (Sartor et al. 2015) and the level of damage depends on the cultivar (Botta et al. 2006; Panzavolta et al. 2012). The ACGW can reduce the yield in chestnuts by preventing the formation of the female flower when galls are formed in the apical buds of the shoots, stopping the growth and producing floral abortion. Moreover, leaf area, photosynthesis and tree biomass are also reduced (Kato and Hijii 1997; Battisti et al. 2013; Gehring et al. 2017).

Additionally, the gall wasp could also be related to the increase of the chestnut blight disease *Chryphonectria parasitica* (Murrill) Barr as Meyer et al. (2015) pointed out in their work because the abandoned galls of *Dryocosmus kuriphilus* could act as a point of entry and a source of pathogen inoculum. Other authors such as Rigling and Prospero (2017) have also mentioned this possible relationship.

Throughout the world, there have been several attempts at control methods against the ACGW. It has been shown that traditional treatments (pruning methods or protection of immature twigs with nets) are impractical solutions for large-scale use (Maltoni et al. 2012; Payne et al. 1975; Zhang et al. 2009).

Other biological tools such as the breeding of resistant chestnuts varieties were successfully carried out in Japan but only for twenty years, since the resistance of the plants was overcome by a new aggressive biotype of *D. kuriphilus* (Murakami 1981). In addition, Cooper and Rieske (2007) have already seen the inefficiency of the use of chemical pesticides against the immature stages due to the protection of plant tissues (galls). The biological control agents (BCAs) have been recognized as an alternative to the use of chemical products, which are very restricted by the European Union regulations (Directive 2009/128/EC).

With respect to the BCAs, it has been shown that the use of natural enemies such as the native parasitoid *Torymus sinensis* Kamijo is an effective control agent, but on a medium or long time scale (Moriya et al. 1989; Quacchia et al. 2008; Colombari and Battisti 2016; Matosevic et al. 2017). However, it should be taken into account as several authors have already mentioned (Yara et al. 2010; Gibbs et al. 2011; Cooper and Rieske 2011; Ferracini et al. 2017) that there may be a negative impacts on local fauna parasitoids due to the risk of hybridizations, hyperparasitations or displacements.

The recruitment of native parasitoids needs at least two or three years (Matosevic and Melika 2013; Palmeri et al. 2014) to be effective in reducing the gall wasp population. It should be also taken into account that the parasitization rate can be very variable depending on the different geographical locations. This rate has been classified as medium or low by authors such as Santi and Maini (2011), García (2013) or Quacchia et al. (2013). However, some species of Torymidae such

as *Torymus flavipes* (Walker) could be a potential and effective tool in the control of the ACGW in the future (Panzavolta et al. 2013). Other authors such as Iskender et al. (2017) have even proposed other biological control techniques based on the use of the properties and possibilities of the bacteria associated with the ACGW.

Another potential alternative tool against the ACGW could be the use of entomopathogenic fungi. This was already investigated by authors such as Cooper and Rieske (2007, 2010) a few years ago in the USA. These authors found within the galls, an unknown endophyte causing up to 14% of the mortality of the pupae.

In addition, in Italy, several studies have been developed in recent years to find endophytes that could be used as biological control agents, such as *Gnomoniopsis* Stoneman (Magro et al. 2010; Vanini et al. 2012, 2016; Vinale et al. 2014; Seddaiu et al. 2017), *Colletotrichum* Corda, (Graziosi and Rieske 2015; Gaffuri et al. 2015) or species of *Fusarium* Link such as *F. proliferatum* (Matsush.) Nirenberg, *F. incarnatum-equiseti* complex, *F. oxysporum* Schltdl. and *F. verticilloides* (Sacc.) Nirenberg (Addario and Turchetti 2011; Tosi et al. 2014).

Several authors have previously found the relationship between the presence of some species of *Fusarium* in the necrotic galls and the mortality of the ACGW. This mortality is due to the production of different types of wall-degrading enzymes (e.g. cellulases, glucanases or glucosidases) as well as entomopathogenic mycotoxins such as beauvericin, moniloformin or fumonisins (Blaney et al. 1985; Lorgrieco et al. 1998; Monzón 2001; Mirete et al. 2003; Addario and Turchetti 2011; Summerell and Leslie 2011; Tosi et al. 2014; Stepien et al. 2016). These compounds cause the death of larvae, pupae and adults. Moreover, other secondary metabolites such as abscisic acid ((+)-ABA) can also act as phytotoxins (Vinale et al. 2014). In addition, in previous studies (Bezos et al. 2013) *Fusarium* spp. was detected in 15% of the necrotic galls of *Dryocosmus kuriphilus* in Northern Spain. The objective of this work was to identify the fungal species associated with the necrotic galls of the ACGW as a first step for the use of entomopathogenic tools for the future control of the pest.

2 Material and methods

In 2014, two *C. sativa* trees (C4 and C5) affected by the ACGW were selected in Vejorís (Cantabria, Northern Spain) to carry out a biweekly sampling from July to September. ACGW galls were collected and classified in necrotic or green galls. Subsequently, data regarding the position of the gall in the branch (petiole or leave) was recorded. Moreover, before plating the necrotic galls, the following data were recorded: number of chambers made by the wasp, presence/absence of the wasp inside the chambers, the development stage of the insect (larvae, pupae or adult) as well as the state of the insect (dead or alive).

One hundred nineteen necrotic galls were plated on Potato Dextrose Agar (PDA, previously autoclaved for 20 min at 121 °C) after surface sterilization (1 min soaked in tap water, 1 min in ethanol 70%, 1 min in sodium hypochlorite 20% and finally another min in destilled sterilized water) with the aim of isolating the associated fungi species. Cultures were incubated in darkness at 23 °C. After 4 days, all outgrowing fungi were transferred by taking a ca. 9 mm^2 piece of agar from the edge of each colony to fresh medium, and during 1 month, a weekly check was carried out in order to find new colonies. Fungal isolates were counted and stored at 4 °C. Finally, assemblages were grouped according to colony morphology on PDA. Fungal isolates were classified into “colonial morphotypes” (CMSs) attending to macromorphological features based on colony color, size, texture, and presence of aerial hyphae (Wang et al. 2005).

One isolate from each CMS was selected for DNA extraction following the protocol described by Vainio et al. (1998). Once the DNA was extracted, the polymerase chain reaction (PCR) was run to amplify the Internal Transcribed Spacer region (ITS) of the rDNA with primers ITS1F (5′-TTG-GTCATTTAGAGGAAGTAA-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) (Gardes and Bruns 1993). For amplification, the thermal cycling program was: 10 min denaturation at 95 °C followed by 13 cycles of 35 sec at 95 °C, 55 sec at 55 °C and 45 sec at 72 °C; 13 cycles of 35 sec at 95 °C, 55 sec at 55 °C and 2 min at 72 °C; 9 cycles of 35 sec at 95 °C, 55 sec at 55 °C and 3 min at 72 °C; and a final elongation 7 min at 72 °C. PCR product was sent to sequencing (Macrogen Europe) after purification (Nucleo Spin Gel and PCR Clean up, Macherey Nagel). The ITS region sequences were corrected with Genious Pro 6.1.5 software package for proper search with Blast in the Gen Bank data base.

3 Results

During the months of July, August and September 2014, 477 galls from the selected chestnuts trees were collected. 45% of the galls were located on the petiole and 55% on the leaves of the branches. 119 galls showed necrosis, which represents 25% of the total galls collected (Table 1).

In relation to the gall occupancy by the insect (Table 1), 2% of galls were found with 3 chambers and 9% with 2 chambers, with most of the galls (81%) presenting a single chamber. The adult wasps began to leave the galls before the month of July. In that month and in both chestnuts, more than half of the galls were already empty. This percentage increased in the following months, reaching its maximum in August in C4 and at the end of September in C5, respectively. As for the presence of the insect within the gall, the larvae and pupae were observed until the end of July and, as of this date, only adults were detected.

Only 16% of the wasps died and more than half were adults. It was not possible to establish any direct relationship between the fungi isolated from these necrotic galls and the dead insects inside them. We were able to isolate *Fusarium oxysporum* and *Epiccocum nigrum* Link from 3 galls with dead adult insects.

Table 1. Number of collected galls, number of necrotic galls and number of insects died as larval stage, pupa or adult within the necrotic galls and percentage of empty chambers from each collecting date in both chestnuts (C4 and C5).

	Number of collected galls		Number of necrotic galls		Nº of insect died inside the necrotic galls of chestnut C4				Nº of insects died inside the necrotic galls of chestnut C5				% of empty chambers in the necrotic galls	
Date	C4	C5	C4	C5	Larva	Pupa	Adult	Total	Larva	Pupa	Adult	Total	C4	C5
09-Jul-14	52	54	18	1	2	4	0	6	1	0	0	1	55.6	66.7
23-Jul-14	35	21	9	8	0	1	1	2	1	0	2	3	66.7	50.0
7-Aug-14	47	26	12	10	0	0	1	1	0	0	2	2	91.7	80.0
22-Aug-14	67	25	12	11	0	0	0	0	0	0	2	2	100.0	81.8
12-Sept-14	43	35	13	11	0	0	0	0	0	0	1	1	100.0	91.7
29-Sep-14	44	28	0	14	0	0	0	0	0	0	1	1	-	93.3
Total	288	189	64	55	2	5	2	9	2	0	8	10		

Table 2. Fungal species isolated from the morphotypes of the necrotic galls.

Sample	Month	Tissue	Fungal taxa	Accession number
1	July	Necrotic gall	*Pestalotiopsis* sp.	KU095868
2	July	Necrotic gall	*Epicoccum nigrum*	KU095869
3	July	Necrotic gall	*Epicoccum nigrum*	KU095870
4	July	Necrotic gall	*Penicillium ramulosum*	KU095871
5	July	Necrotic gall	*Epicoccum nigrum*	KU095872
6	August	Necrotic gall	*Epicoccum nigrum*	KU095873
7	September	Necrotic gall	*Epicoccum nigrum*	KU095874
8	September	Necrotic gall	*Fusarium oxysporum*	KU095875
9	September	Necrotic gall	*Gnomoniopsis smithogilvyi*	KU095876
10	September	Necrotic gall	*Fusarium avenaceum*	KU095877
11	September	Necrotic gall	*Fusarium* sp.	KU095878

From a total of 125 cultures, seven fungal taxonomic units were identified from the 11 selected CMSs isolated from the necrotic galls: *Pestalotiopsis* Steyaert, *Epicoccum nigrum*, *Penicillium ramulosum* Visagie & K. Jacobs, *Fusarium oxysporum*, *Gnomoniopsis smithogilvyi*, *Fusarium avenaceum* (Fr.) Sacc., and *Fusarium* sp. (Table 2). The sequences from the 11 isolates identified were uploaded to the Genbank database with accession numbers from 095868 to 095878. The frequency data for each morphotype are shown in Table 3. *Gnomoniopsis smithogilvyi* and *Fusarium avenaceum* were the fungi more frequent with 25 and 24 isolates correspondingly. *Epicoccum nigrum* and *Pestalotiopsis* spp. were quite rare with only one isolate each.

Table 3. Relative abundance for each morphotype among the isolated cultures.

Morphotypes	Fungal taxa	Nº of isolates	Relative abundance
1	*Pestalotiopsis* sp.	1	0.008
2	*Epicoccum nigrum*	1	0.008
3	*Epicoccum* sp.	18	0.144
4	*Penicillium ramulosum*	2	0.016
5	*Epicoccum nigrum*	9	0.072
6	*Epicoccum nigrum*	8	0.064
7	*Epicoccum nigrum*	12	0.096
8	*Fusarium oxysporum*	11	0.088
9	*Gnomoniopsis smithogilvyi*	25	0.20
10	*Fusarium avenaceum*	24	0.192
11	*Fusarium* sp.	14	0.112

4 Discussion

Of the seven species of fungi found in this study, we highlight the three species of *Fusarium*, as well as the presence of *Gnomoniopsis smithogilvyi* the most isolated fungus species in our study.

Pestalotiopsis sp. is a ubiquitous genus that acts as an endophyte, saprotroph or pathogen in different hosts throughout the world (Jeewon et al. 2004). In addition, Lv et al. (2011) have also considered it as a useful entomopathogen in *Pinus halepensis* Mill. affected by the pine needle hemiberlesian scale.

Epicoccum nigrum the less isolated fungus in our study, together with the previous one, are well known for the production of secondary metabolites that frequently act as antibiotics and could be good candidates for the biological control of some pathogenic fungi such as *Monilinia* spp. Honey (Larena et al. 2005; Pascual et al. 1996).

Penicilium ramulosum was also isolated but like other species of the genus, it could be a saprotroph in the tree because it rarely appears as an endophyte in healthy tissues (Zamora et al. 2008). On the other hand, Chaoyang et al. (2015) found this fungus in decaying wood.

Species of *Gnomoniopsis* on *Castanea* spp. are documented as endophytes and are associated with rotten chestnuts in Italy, being in some cases, the most abundant fungus in the old galls (Gentile et al. 2009; Magro et al. 2010; Vettraino et al. 2011; Visentin et al. 2012; Vannini et al. 2012, 2017; Ugolini et al. 2014; Lione et al. 2016). This fungus was also found in New Zealand (Sogonov et al. 2008) in chestnut blight cankers in India (Dar and Rai 2013). *Gnomoniopsis smithogilvyi* appears on dead burrs and branches of *Castanea sativa* (Sogonov et al. 2008) and was also isolated as an endophyte from symptomatic flowers, leaves and stems (Shuttleworth 2012). Maresi et al. (2013) and Vinale et al. (2014) found *Gnomoniopsis castanea* in necrotic galls and explained its entomophatogenic capacity due to the toxicity of abcisic acid production. More recently, Vanini et al. (2017) described a severe impact of *G. castanea* on the vitality of *D. kuriphilus*, mainly affecting the adult stage within the gall.

The potential entomophatogenic capacity of *Fusarium* spp. as well as the production of dangerous mycotoxins for humans and animals is well known and has already been mentioned by several authors such as Teetor-Barsch and Roberts (1983), Bottalico and Perrone (2002) and Logrieco et al. (2002). Lorgrieco et al. (1998) as well as Blaney et al. (1985) also noted the ability to produce insecticidal toxins by *F. incarnatum* (Roberge) Sacc. and *F. equiseti* (Corda) Sacc. In addition, Addario and Turchetti (2011) demonstrated the effectiveness of these two fungal species to cause the death of individuals of the ACGW with a time of action of seven days penetrating directly into the tissues of the gall. The potential entomopathogenicity of *Fusarium proliferatum* against *Dryocosmus kuriphilus* has already been demonstrated by Tosi et al. (2014) in Italy reaching mortality rates of 33 to 97% in laboratory tests. Moreover, it was also seen in Argentina, that other species of the genus, *Fusarium verticilloides* is an effective entomopathogen against grasshoppers (Pelizza et al. 2011).

In this work, three *Fusarium* taxa were isolated: *Fusarium oxysporum*, *F. avenaceum* and *Fusarium* sp. Prakash et al. (2010) already observed that *Fusarium oxysporum* was an entomopathogenic fungus against Diptera larvae and that the production of (+)-ABA was responsible for the toxicity of the fungus. Other authors such as Bustillo et al. (2002) already mentioned it as a natural enemy of the coffee berry borer (Coleoptera: Scolytinae) while Asensio et al. (2007) showed its entomopathogenic capacity against the red scale (Hemiptera: Pseudoccocidae).

Fusarium avenaceum is a cosmopolitan plant pathogen with a wide and diverse range of host and is responsible for diseases in more than eighty genera of plants (Leach and Hobbs 2013) due to its capacity to produce toxins such as enniatins, moniliformin and beauvericin (Morrison et al. 2002; Kokkonen et al. 2010). Batta (2012) demonstrated for the first time its entomopathogenic

capacity against the rice weevil (Coleoptera: Curculionidae) but other authors such as Strongman et al. (1987) had previously found this toxicity in the spruce budworm larvae (Lepidoptera: Tortricidae) due to the production of enniatins. In addition, Wenda-Piesik et al. (2006) found it as a colonizer in cadavers of sawflies (Hymenoptera: Symphita) larvae.

The detection of these potentially entomopathogenic species in the necrotic galls of *D. kuriphilus* opens the way for its use as BCAs. However, deeper studies are required to verify the applicability of this potential tool, testing it on fresh galls to see the ability of these fungi to penetrate the galls, leaves and twigs and cause the death of the insect at its different stages of development (larvae, pupae and adults).

Acknowledgments

The authors want to thank Milagros Vallejo and Juan Blanco (Gobierno de Cantabria) for helping in the collection of the galls.

References

Addario E., Turchetti T. (2011). Parasitic fungi on *Dryocosmus kuriphilus* in *Castanea sativa* necrotic galls. Bulletin of Insectology 64: 269–273.

Asensio L., López-Jiménez J.A., López-Llorca L.V. (2007). Mycobiota of the date palm phylloplane: description and interactions. Revista Iberoamericana de Micología 24(4): 299–304. https://doi.org/10.1016/S1130-1406(07)70060-8.

Batta Y. (2012). The first report on entomopathogenic effect of *Fusarium avenaceum* (Fries) Saccardo (Hypocreales, Ascomycota) against rice weevil (*Sitophilus oryzae* L.: Curculionidae, Coleoptera). Journal of Entomological and Acarological Research 44(3): 51–55. https://doi.org/10.4081/jear.2012.e11.

Battisti A., Benvegnu I., Colombari F., Haack R.A. (2013). Invasion by the chestnut gall wasp in Italy causes significant yield loss in *Castanea sativa* nut production. Agricultural and Forest Entomology 16(1): 75–79. https://doi.org/10.1111/afe.12036.

Bezos D., Diez J.J., Fernández M.M. (2013). Posibilidades del control biológico de *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) con hongos endófitos. [Possibilities of the biological control of *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) with endophytic fungi]. Second International meeting on *Dryocosmus kuriphilus* Yasumatsu. Biocastanea. Ponferrada (León).

Blaney B., Green P., Connole M. (1985). Fungal metabolites with insecticidal activity: relative toxicity of extracts of fungal cultures to sheep blowfly, *Lucilia cuprina* (Wied.). General and Applied Entomology 17: 42–46.

Botta R., Mellano M., Bounous G. (2006). Valutazione della sensibilità a *Dryocosmus kuriphilus* in *Castanea* spp. Atti del IV Convegno Nazionale Castagno. Parretti, Firenze, Italy. p. 211–213.

Bottalico A., Perrone G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-cereals in Europe. European Journal of Plant Pathology 108(7): 611–624. https://doi.org/10.1023/A:1020635214971.

Bustillo A.E., Cardenas R., Posada F.J. (2002). Natural enemies and competitors of *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in Colombia. Neotropical entomology 31(4): 635–639. https://doi.org/10.1590/S1519-566X2002000400018.

Chaoyang L., Zhicheng S., Tingheng Z., Wensheng Q. (2015). Newly Isolated *Penicillium ramulosum* N1 is Excellent for Producing Protease-Resistant Acidophilic Xylanase. Journal of Molecu-

lar Microbiology and Biotechnology 25(5): 320–326. https://doi.org/10.1159/000439170.

Colombari F., Battisti A. (2016). Native and introduced parasitoids in the biocontrol of *Dryocosmus kuriphilus* in Veneto (Italy). Bulletin OEPP/EPPO 46(2): 275–285.

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(2): 236–244. https://doi.org/10.1603/0013-8746(2007)100[236:CAOAEG]2.0.CO;2.

Cooper W.R., Rieske L.K. (2010). Gall structure affects ecological associations of *Dryocosmus kuriphilus*. Environmental Entomology 39(3): 787–797. https://doi.org/10.1603/EN09382.

Cooper W.R., Rieske L.K. (2011). A native and an introduced parasitoid utilize an exotic gall-maker host. BioControl 56: 725–734. https://doi.org/10.1007/s10526-011-9350-1.

Dar M., Rai M. (2013). Biological and phylogenetic analyses, evidencing the presence of *Gnomoniopsis* sp. in India, causing canker of chestnut trees: a new report. Indian Forester 139(1): 37–42.

Directiva 2009/128/CE del Parlamento Europeo y del Consejo, de 21 de octubre de 2009, por la que se establece el marco de la actuación comunitaria para conseguir un uso sostenible de los plaguicidas. [Directive 2009/128 / EC of the European Parliament and of the Council of 21 October 2009, establishing the framework for Community action to achieve sustainable use of pesticides].

EFSA Panel on Plant Health (2010). Risk assessment of the oriental chestnut gall wasp, *Dryocosmus kuriphilus* for the EU territory on request from the European Commission. Efsa Journal 8. 1619 p.

Ferracini C., Ferrari E., Pontini M., Hernández L., Saladini M., Alma A. (2017). Post-release evaluation of non-target effects of *Torymus sinensis*, the biological control agent of *Dryocosmus kuriphilus* in Italy. BioControl 62(4): 445–456. https://doi.org/10.1007/s10526-017-9803-2.

Gaffuri F., Maresi G., Pedrazzoli F., Longa C., Boriani M., Molinari M., Tantardini A. (2015). *Colletotrichum acutatum* associated with *Dryocosmus kuriphilus* galls on *Castanea sativa*. Forest Pathology 45(2): 169–171. https://doi.org/10.1111/efp.12178.

García J. (2013). La agalla de *Dryocosmus kruriphilus* en *Castanea sativa*; estudio de los parasitoides en Catalunya y análisis de los posibles métodos de control de esta plaga de origen asiático.[The gall of *Dryocosmus kruriphilus* in *Castanea sativa*; study of parasitoids in Catalonia and analysis of the possible methods of control of this plague of Asian origin]. UB-Tesis Fin Master.

Gardes M., Bruns T.D. (1993). ITS primers with enhanced specificity for basidiomycete's application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.

Gehring E., Bellosi B., Quacchia A., Conedera M. (2017). Assessing the impact of *Dryocosmus kuriphilus* on the chestnut tree: branch architecture matters. Journal of Pest Science 91(1): 189–202. https://doi.org/10.1007/s10340-017-0857-9.

Gentile S., Valentino D., Visentin I., Tamietti G. (2009). *Discula pascoe* infections of sweet chestnut fruits in North-West Italy. Australian Nutgrower 12/2009: 23–25.

Gibbs M., Schönrogge K., Alma A., Melika G., Quacchia A., Stone G., Aebi A. (2011). *Torymus sinensis*: a viable management option for the biological control of *Dryocosmus kuriphilus* in Europe? BioControl 56(4): 527–338. https://doi.org/10.1007/s10526-011-9364-8.

Graziosi I., Rieske L.K. (2015). A plant pathogen causes extensive mortality in an invasive insect herbivore. Agricultural and Forest Entomology 17(4): 366–374. https://doi.org/10.1111/afe.12116.

Iskender N., Algur O., Aksu Y., Saral A. (2017). Isolation, identification and characterization of biotechnologically important bacteria from microflora of *Dryocosmus kuriphilus* Yasumatsu

(Hymenoptera: Cynipidae). Biotechnology & Biotecnological equipment 31(3): 505–510.
Jeewon R., Liew E., Hyde K. (2004). Phylogenetic evaluation of species nomenclature of *Pestalotiopsis* in relation to host association. Fungal Diversity 17: 39–55.
Kato K., Hijii N. (1997). Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hym., Cynipidae) on the growth of chestnut trees. Journal of Applied Entomology 121: 1–15. https://doi.org/10.1111/j.1439-0418.1997.tb01363.x.
Kokkonen M., Ojala L., Parikka P., Jestoi M. (2010). Mycotoxin production of selected *Fusarium* species at different culture conditions. International Journal of Food Microbiology 30(1–2): 17–25. https://doi.org/10.1016/j.ijfoodmicro.2010.07.015.
Larena I., Torres R., De Cal A., Liñán M., Melgarejo P., Domenichini P., Bellini A., Mandrin J., Lichou J., de Eribe X.O. (2005). Biological control of postharvest brown rot (*Monilinia* spp.) of peaches by field applications of *Epicoccum nigrum*. Biological Control 32(2): 305–310. https://doi.org/10.1016/j.biocontrol.2004.10.010.
Leach M.C., Hobbs S. (2013). Plant wise knowledge bank: delivering plant health information to developing country users. Learned Publishing 26: 180–185.
Lione G., Giordano L., Ferracini C., Alma A., Gonthier P. (2016). Testing ecological interactions between *Gnomoniopsis castaneae* and *Dryocosmus kuriphilus*. Acta Oecologica 77: 10–17. https://doi.org/10.1016/j.actao.2016.08.008.
Lorgrieco A., Moretti G., Castella M., Kostecki P., Golinski A., Ritieni J., Chelkowski J. (1998). Beauvericin production by *Fusarium* species. Applied and Environmental Microbiology 64(8): 3084–3088.
Lorgrieco A., Rizzo A., Ferracane R., Ritieni A. (2002). Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. Applied and Environmental Microbiology 68: 82–85. https://doi.org/10.1128/AEM.68.1.82-85.2002.
Lv C., Huang B., Qiao M., Wei J., Ding B. (2011). Entomopathogenic Fungi on *Hemiberlesia pitysophila*. PLoS ONE 6(8): e23649. https://doi.org/10.1371/journal.pone.0023649.
Magro P., Speranza S., Stacchiotti M., Martignoni D., Paparatti B. (2010). *Gnomoniopsis* associated with necrosis of leaves and chestnut galls induced by *Dryocosmus kuriphilus*. Plant Pathology 59: 1171. https://doi.org/10.1111/j.1365-3059.2010.02336.x.
Maltoni A., Mariotti B., Tani A. (2012). Case study of a new method for the classification and analysis of *Dryocosmus kuriphilus* Yasumatsu damage to young chestnut sprouts. iForest 5: 50–59. https://doi.org/10.3832/ifor0598-008.
Maresi G., Oliveira C.M., Turchetti T., 2013. Brown rot on nuts of *Castanea sativa* Mill: an emerging disease and its causal agent. iForest 6(5): 294–301. https://doi.org/10.3832/ifor0952-006.
Matosevic D., Melika G. (2013). Recruitment of native parasitoids to a new invasive host: first results of *Dryocosmus kuriphilus* parasitoid assemblage in Croatia. Bulletin of Insectology 66(2): 231–238.
Matosevic D., Lackovic N., Kos K., Kriston E., Melika G., Rot M., Pernek M. (2017). Success of classical biocontrol agent *Torymus sinensis* within its expanding range in Europe. Journal of Applied Entomology 141(9): 758–767. https://doi.org/10.1111/jen.12388.
Meyer J., Gallien L., Prospero S. (2015). Interaction between two invasive organisms on the European chestnut: does the chestnut blight fungus benefit from the presence of the gall wasp? FEMS Microbiology Ecology 91(11): 1–10. https://doi.org/10.1093/femsec/fiv122.
Mirete S., Patino B., Vázquez C., Jiménez M., Hinojo M., Soldevilla C., González-Jaén M. (2003). Fumonisin production by *Gibberella fujikuroi* strains from *Pinus* species. International Journal of Food Microbiology 89(2–3): 213–221. https://doi.org/10.1016/S0168-1605(03)00150-8.
Monzón A. (2001). Producción, uso y control de calidad de hongos entomopatógenos en Nicaragua. Avances en el fomento de productos fitosanitarios no-sintéticos. [Production, use and quality

control of entomopathogenic fungi in Nicaragua. Advances in the promotion of non-synthetic phytosanitary products]. Manejo Integrado de Plagas 63: 95–103.

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). Journal of Applied Entomology and Zoology 24(2): 231–233. https://doi.org/10.1303/aez.24.231.

Morrison E., Kosiak B., Ritieni A., Aastveit A.H., Uhlig S., Bernhoft A.J. (2002). Mycotoxin production by *Fusarium avenaceum* strains isolated from Norwegian grain and the cytotoxicity of rice culture extracts to porcine kidney epithelial cells. Agricultural and Food Chemistry 50(10): 3070–3075. https://doi.org/10.1021/jf011532h.

Murakami Y. (1981). Comparison of the adult emergence periods between *Torymus* (*Syntomaspis*) *beneficus* a native parasitoid of the chestnut gall wasp and a congeneric parasitoid imported from China (Hymenoptera: Torymidae). Proceedings of the Association for Plant Protection of Kyushu 27: 156–158. https://doi.org/10.4241/kyubyochu.27.156.

Palmeri V., Cascone P., Campolo O., Grande S., Laudani F., Malacrino A., Guerrieri E. (2014). Hymenoptera wasps associated with the Asian gall wasp of chestnut (*Dryocosmus kuriphilus*) in Calabria, Italy. Phytoparasitica 42(5): 699–702. https://doi.org/10.1007/s12600-014-0411-8.

Panzavolta T., Bracalini M., Croci F., Campani C., Bartoletti M., Miniati G., Benedetelli S., Tiberi R. (2012). Asian chestnut gall wasp in Tuscany: gall characteristics, egg distribution and chestnut cultivar susceptibility. Agricultural and Forest Entomology 14(2): 139–145. https://doi.org/10.1111/j.1461-9563.2011.00551.x.

Panzavolta T., Bernardo U., Bracalini M., Cascone P., Croci F., Gebiola M., Iodice L., Tiberi R., Guerrieri E. (2013). Native parasitoids associated with *Dryocosmus kuriphilus* in Tuscany, Italy. Bulletin of Insectology 66: 195–201.

Pascual S., Magan N., Melgarejo P. (1996). Improved biological control of peach twig blight by physiological manipulation of *Epicoccum nigrum*. Proceedings of the Brighton Crop Protection. Conference Pests and Diseases. p. 411–412.

Payne J., Menke A., Schroeder P. (1975). *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), an oriental chestnut gall wasp in North America. Cooperative Economic Insect Report 25(49–52): 903–905.

Pelizza S., Stenglein S., Cabello M., Dinolfo M., Lange C. (2011). First record of *Fusarium verticillioides* as an entomopathogenic fungus of grasshoppers. Journal of insect Science 11(1): 70. https://doi.org/10.1673/031.011.7001.

Prakash S., Singh G., Soni N., Sharma S. (2010). Pathogenicity of *Fusarium oxysporum* against the larvae of *Culex quinquefasciatus* (Say) and *Anopheles stephensi* (Liston) in laboratory. Parasitology Research 107(3): 651–655. https://doi.org/10.1007/s00436-010-1911-1.

Quacchia A., Moryia S., Bosio G., Scapin I., Alma A. (2008). Rearing, release and the prospect of establishment of *Torymus sinensis*, biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*, in Italy. BioControl 53(6): 829–839. https://doi.org/10.1007/s10526-007-9139-4.

Quacchia A., Ferracini C., Nicholls J., Piazza E., Saladini M., Tota F., Melik G., Alma A. (2013). Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in northwestern Italy. Insect Conservation and Diversity 6(2): 114–123. https://doi.org/10.1111/j.1752-4598.2012.00192.x.

Rigling D., Prospero S. (2017). *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. Molecular Plant Pathology 19(1): 7–20. https://doi.org/10.1111/mpp.12542.

Santi F., Maini S. (2011). New association between *Dryocosmus kuriphilus* and *Torymus flavipes*

in chestnut trees in the Bologna area (Italy): first results. Bulletin of Insectology 64: 275–278.

Sartor C., Dini F., Torello Marinoni D., Mellano M.G., Beccaro G.L., Alma A., Quacchia A., Botta R. (2015). Impact of the Asian wasp *Dryocosmus kuriphilus* (Yasumatsu) on cultivated chestnut: yield loss and cultivar susceptibility. Scientia Horticulturae 197: 454–460. https://doi.org/10.1016/j.scienta.2015.10.004.

Seddaiu S., Cerboneschi A., Sechi C., Mello A. (2017). *Gnomoniopsis castaneae* associated with *Dryocosmus kuriphilus* galls in chestnut stands in Sardinia (Italy). iForests 10: 440–445. https://doi.org/10.3832/ifor2064-009.

Shuttleworth L. (2012). The biology and management of chestnut rot in southeastern Australia. PhD thesis. The University of Sydney, Faculty of Agriculture and Environment, Eveleigh, Australia.

Sogonov M., Castlebury L., Rossman A., Mejia L., White JF. (2008). Leaf-inhabiting genera of the *Gnomoniaceae*, *Diaporthales*. Studies in Mycology 62(1): 1–77. https://doi.org/10.3114/sim.2008.62.01.

Stepien L., Waskiewicz A., Urbaniak M. (2016). Wildly growing asparagus (*Asparagus officinalis* L.) hosts pathogenic *Fusarium* species and accumulates their mycotoxins. Microbial ecology 71(4): 927–937. https://doi.org/10.1007/s00248-015-0717-1.

Strongman D., Strunz G., Giguere P., Yu C., Calhoun L. (1987). Enniatins from *Fusarium avenaceum* isolated from balsam fir foliage and their toxicity to spruce budworm larvae, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Journal of Chemical Ecology 14(3): 753–764. https://doi.org/10.1007/BF01018770.

Summerell B.A., Leslie J.F. (2011). Introducing the genus *Fusarium*. In: Alves-Santos F.M., Diez J.J. (eds.). Control of *Fusarium* diseases. Research signpost, Kerala, India. p. 1–16.

Teetor-Barsch G.H., Roberts D.W. (1983). Entomogenous *Fusarium* species. Mycopathologia 84(1): 3–16. https://doi.org/10.1007/BF00436991.

Torrell A., Heras J. (2012). Vespeta del castanyer; *Dryocosmus kuriphilus*. [Wasp of the chestnut; *Dryocosmus kuriphilus*]. Servei de Gestió Forestal i el Servei de Sanitat Vegetal. Generalitat de Cataluña, Departament d´Agricultura, Ramadería, Pesca, Alimentanció i Medi natural.

Tosi L., Beccari G., Rondoni G., Covarelli L., Ricci C. (2014). Natural occurrence of *Fusarium proliferatum* on chestnut in Italy and its potential entomopathogenicity against the Asian chestnut gall wasp *Dryocosmus kuriphilus*. Journal of Pest Science 88(2): 369–381. https://doi.org/10.1007/s10340-014-0624-0.

Ugolini F., Massetti L., Pedrazzoli F., Tognetti R., Vecchione A., Zulini L., Maresi G. (2014). Ecophysiological responses and vulnerability to other pathologies in European chestnut coppices, heavily infested by the Asian chestnut gall wasp. Forest Ecology and Management 314(1): 38–49. https://doi.org/10.1016/j.foreco.2013.11.031.

Vainio E.J., Korhonen K., Hantula J. (1998). Genetic variation in *Phlebiopsis gigantea* as detected with random amplifies microsatellite (RAMS) markers. Mycological Research 102(2): 187–192. https://doi.org/10.1017/S0953756297004577.

Vannini A., Martignoni D., Bruni N., Tomassini A., Aleandri M., Vettraino A., Caccia R., Speranza S., Paparatti B. (2012). New notes on the biology of the chestnut fungus *Gnomoniopsis* sp. and its possible use as a biocontrol agent of oriental chestnut gall wasp. V International Chestnut Symposium. p. 235–238.

Vannini A., Vettraino A.M., Maritgnoni D., Morales-Rodriguez C., Contarini M., Cacci R., Paparatti B., Speranza S. (2017). Does *Gnomoniopsis castanea* contribute to the natural biological control of chestnut gall wasp? Fungal Biology 121(1):44–52. https://doi.org/10.1016/j.funbio.2016.08.013.

Vettraino A., Aleandri M., Martignoni D., Bruni N., Vannini A. (2011). Endophytism of *Gnomoniopsis* sp. in chestnut tissues. GenBank accessions JN793529–JN793536. Department of

Innovation in Biological, Agro-food and Forest Systems, University of Tuscia,Viterbo, Italy.

Vinale F., Ruocco M., Manganiello G., Guerrieri E., Bernardo U., Mazzei P., Piccolo A., Sannino F., Caira S., Woo S.L. (2014). Metabolites produced by *Gnomoniopsis castanea* associated with necrosis of chestnut galls. Chemical and Biological Technologies in Agriculture 1(8): 1–3. https://doi.org/10.1186/s40538-014-0008-y.

Visentin I., Gentile S., Valentino D., Gonthier P., Tamietti G., Cardinale F. (2012). *Gnomoniopsis castanea* sp. nov. (Gnomoniaceae, Diaporthales) as a causal agent of nut rot in sweet chestnut. Journl of Plant Pathology 94(2): 411–419.

Wang Y., Guo L., Hyde K.D. (2005). Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences. Fungal Diversity 20: 235–260.

Wenda-Piesik A., Morrill W.L., Grey W.E., Weaver D.K. (2006). Entomopathogenic capacity of *Fusarium* crown rot on wheat stem sawfly larvae. Progress in Plant Protection 46: 380–387.

Yara K., Ssawaki T., Kunimi Y. (2010). Hybridization between introduced *Torymus sinensis* (Hymenoptera: Torymidae) and indigenous *T. beneficus* (late-spring strain), parasitoids of the Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae). Biological Control 54(1): 14–18. https://doi.org/10.1016/j.biocontrol.2010.03.006.

Zamora P., Martínez-Ruiz C., Diez J. (2008). Fungi in needles and twigs of pine plantations from northern Spain. Fungal Diversity 30: 171–184.

Zhang Z., Tarcali G., Radocz L., Feng Y., Shen Y. (2009). Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu in China and in Hungary. Journal of Agricultural Sciences 38: 123–128.

Total of 79 references.