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"Chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu): antennal morphology and natural control"

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1 General introduction

1.1 The genus *Castanea*

The chestnut tree *Castanea sativa* Miller is native of South-Eastern Europe and Asia Minor. During the Roman Empire, its cultivation was widespread both for the edible fruits, and for the excellent wood (AA. VV., 2001a).

In the Middle Ages, chestnut cultivation has been associated with various monastic orders that have contributed to its spread and care and, even today, the memories of this type of association remain, like a symbiosis between the trees and ancient monasteries.

Chestnut distribution affects temperate and warm-temperate mountain areas, below the beech altitudinal range. The altitudinal range of chestnut (Castanetum) is between 200-300 m and 800-1000 m. At lower altitudes the vegetation is characterized by oak woods, while the band above is mainly submontane beech (Fagetum, from 800-900 m, in the Apennines its relevance band goes up to 1000 m).

Normally, due to its low tolerance to limestone (the limestone is sustained only with high seasonal rainfall), chestnut grows over arenaceous-pelitic or arenaceous substrates, where they find the conditions of neutrality or sub-acid environment required by the species. Sandy and mineralized soils are appreciated, ensuring ventilation to root system (AA.VV., 2010).

The Genus *Castanea* belongs to the Fagaceae family, and is a monoecious plant with male and female flowers carried by the same plant.

This genus includes a dozen species of shrubs and trees; the most important species belonging to the genus *Castanea*, are the following:

- European species: sweet chestnut (*C. sativa*) (also called "Spanish chestnut" in the US) is the only European species of chestnut, though successfully introduced to the Himalayas and other temperate parts of Asia.
- Asiatic species: *Castanea crenata* (Japanese chestnut), *Castanea mollissima* (Chinese chestnut), *Castanea davidii* (China), *Castanea henryi* (Chinese chinkapin, also called Henry's chestnut – China) and *Castanea seguinii* (also called Seguin's chestnut - China).
- American species: *Castanea dentata* (American chestnut - Eastern states), *Castanea pumila* (American- or Allegheny chinkapin, also known as "dwarf chestnut" - Eastern states), *Castanea alnifolia* (Southern states), *Castanea ashei* (Southern states), *Castanea floridana* (Southern states) and *Castanea paupispina* (Southern states).

C. sativa is a medium-sized to large deciduous tree, that reaches a height of 20–35 m. The oblong-lanceolate, boldly toothed leaves are 16–28 cm (centimeters) long and 5–9 cm broad.
The flowers of both sexes are borne in 10–20 cm long upright catkins, the male flowers in the upper part and female flowers in the lower part. In the Northern hemisphere, they appear in late June to July, and in autumn the female flowers develop into spiny cupules containing 3-7 brownish nuts that are shed during October. The female flowers eventually form a spiky sheath that deters predators from the seed. Some cultivars produce only one large nut per cupule, rather than the average two to four nuts. The bark often has a net-shaped (retiform) pattern with deep furrows or fissures running spirally in both directions up the trunk.

1.2 Chestnut cultivation

1.2.1 - The chestnut orchard in Europe and Italy

Chestnut is economically and environmentally important in Italy. Chestnut orchards are part of traditional farming in many Italian regions and provide a non-negligible additional income to farmers. The widespread distribution of chestnuts, cultivated or naturalized, is crucial for the maintenance of a local biodiversity but also to maintain steep slopes in mountain areas. (Quacchia *et al*., 2008)
Woodland management are mainly chestnut coppice, and meadow chestnut, a sort of high forest with a few scattered individuals (70-150/ha) large and constantly mowed grass undergrowth to make easy fruits collection. The meadow chestnut can be regarded as a kind of orchard, in which cultural practices are typical of this management: pruning, mowing grass, etc.. (AA.VV., 2001a). Chestnut wood represents an excellent building material, while meadow chestnut produces fruits that processed in different ways can provide a wide range of food products (fresh chestnuts, flour, cream, etc...)

- Chestnut for fruit production: world production of chestnuts is concentrated in two large areas, Asia and Europe, representing respectively 80% and 16% of world production (FAO, 2008) . It should be stressed that the Asian production is obtained from chestnut species (*C. crenata* . - Japanese chestnut, *C. mollissima* - Chinese chestnut, and hybrids thereof) different from European one (*C. sativa*) and with different and lower organoleptic characteristics.

In Europe (from Turkey to Portugal) production is largely based on European chestnut (*Castanea sativa* Miller) but in a few areas, such as the South-Western France, also hybrids are cultivated.
Italian production is estimated between 50 000 and 70 000 tons. The part of world production has decreased from 11% to 4% due to China increased production.
European production, after a drastic decline in the 1960s and 1970s, has reached around 170,000 tons. The main European producers are Italy, Turkey and Portugal, which account for 30%, 29% and 15% of European chestnut
Italy is the largest exporter of chestnuts for the quality of trade, while is second for quantity traded; the export price of Italian chestnut is, in fact, higher than that of Asian chestnuts.
The ISTAT data of 2007 show that chestnut production is mainly concentrated in the Central and Southern regions, especially in Campania (13.3 thousand ha), Calabria (10.7 thousand ha), Tuscany (7.8 thousand ha) and Lazio (5.2 thousand ha), while in the Northern regions Piedmont (5.4 thousand ha) and Emilia-Romagna (2.2 thousand ha) are the main producers.

The Italian chestnut farms are small to medium size. In fact, on average, 80% of the farms and 40% of the area is included within SAU 0-5 ha, while the chestnut fruit average area is about 1 ha.
(AA.VV., 2010)

- Chestnut for wood production - The number of timber industry

In the national scene, where 10.5 out of 30 total million ha are occupied by forests, the fraction invested in chestnut takes a very prominent role reaching 2.62% of the Italian land area and 7.53% of the forest.

The extensions of Piedmont, Tuscany and Liguria are no less than 50% of the national heritage. Including the regions that have assets of more than 30,000 ha (Lombardy, Calabria, Campania, Emilia Romagna and Lazio), we get to 90% of the entire national surface, it follows that over 50% of the regions have very low chestnut surface.

Overall, over 75% of Italian chestnut is intended for timber production. (AA.VV, 2010)

1.2.2 - Chestnut orchards in Marche Region

The presence of chestnut in Marche Region is limited by soil characteristics, infact *C. sativa* is a calcifuge plant.

Except of a chestnut area of about 100 hectares is in Pesaro-Urbino province, in particular in high Montefeltro district, we can say that chestnut areas are concentrated in the southernmost provinces of the Region.

According to data shown by IPLA (AA.VV.,2001b), more than half of the chestnut areas of Marche are located on the "Monti della Laga", in high Tronto Valley.

In the Piceno district chestnut distribution is further concentrated in the municipalities of Acquasanta Terme, Arquata del Tronto, Montegallo, Montemonaco and Roccafluvione, for about 2100 ha (about 90%) (AA.VV., 2010).

The organoleptic characteristics of the cultivars widespread on the territory make regional chestnuts particularly precious.

Chestnut areas in the Piceno district are partly subject to recurrent cultural practices.

According to data of "Regional Inventory", chestnut in Marche Region involves 4.6 ha, 1.8% of the total regional surface.

1.3 – Cinipid gall wasps

Cynipid gall wasps consist of six tribes (Tab. 1.1): Aylacini, Diplolepidini, Eschatocerini, Pediaspidini and Cynipini are gall inducers, and Synergini are gall-associated inquilines. They comprise around 1,300 species worldwide, predominantly in temperate regions of the Holarctic.The Eschatocerini are restricted to the Nearctic, but the other five tribes are found in Asia (Abe *et al*., 2007).

The Cynipini includes around 1,000 species, and most of them induce galls on oak *Quercus* trees. Heterogony, cyclical parthenogenesis occurs in many species of this tribe. Female adults of the bisexual and unisexual generations of the same species differ considerably, not only in their morphology but also in the shape of galls that their offspring induce. Therefore, the two generations of the same species have often been described as different species, which confuses the taxonomy of oak gall wasps (Abe, 2010)

Tribes	No. of genera	No. of species	Hosts
Aylacini	18	122	Asteraceae, Rosaceae, Lamiaceae Papaveraceae, Apiaceae, Valerianaceae, Brassicaceae, *Smilax* (Smilaceae)
Diplolepidini	2	50	*Rosa* (Rosaceae)
Eschatocerini	1	3	*Acacia*, *Prosopis* (Fabaceae)
Pediaspidini	3	3	*Acer* (Aceraceae)
Cynipini	27 c.	1,000	Fagaceae (mostly Quercus, also Castanea, Chrysolepis and Lithocarpus)
Synergini	8	159	Inquilines in galls induced by other insects

Tab. 1.1 - Classification, diversity and host associations of Cynipinae (after Abe *et al.,* 2007)

1.4- Galls

Galls are structures composed of plant tissue within which the insect feeds; they can form on roots, fruits, buds, leaves and flowers (Fig. 1.1).

Many groups of insects induce the production of galls in plants.

Associations of galling insect an host are often species-specific, with the insect inducing a highly characteristic type of host differentiation. Some such associations are so specific that they have been used as taxonomic criteria for some insects (Hewett, 1977).

Insect galls are dramatic examples of extended phenotypes: although composed of host plant tissues, their development is largely controlled by insect genes (Stone and Schönrogge, 2003).

It is likely that the altered host differentiation results from a changed metabolism in response to a chemical stimulus from the insect (Hewett, 1977).

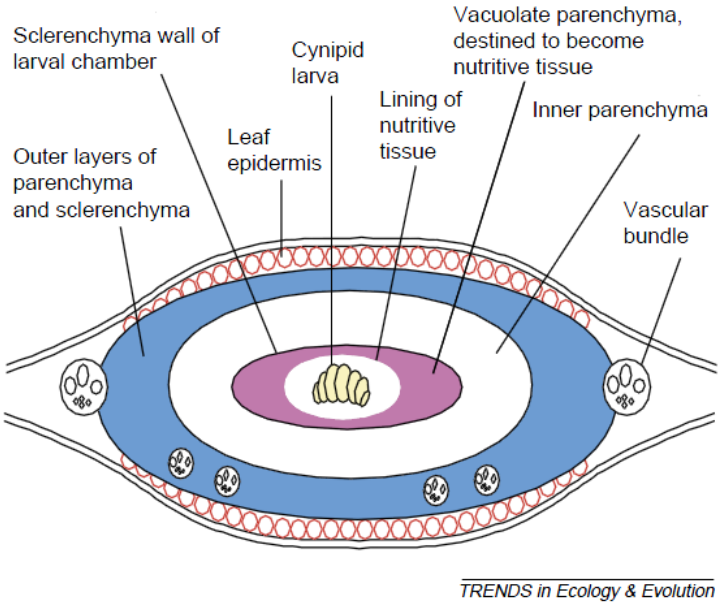


Fig. 1.1 - Distribution of tissues within galls *Diplolepsis rosaefolii* (Hymenoptera: Cynipidae) (Stone and Schönrogge, 2003).

Three general mutually compatible hypotheses for the adaptive significance of insect galls have been proposed: (*a*) The nutrition hypothesis, which states that gall inducers regulate the nutritive value of the plant tissues on which they feed to their own benefit; (*b*) the microenvironment hypothesis, which states that gall occupation protects the gall inducer from external fluctuations in microclimate; and (*c*) the enemy hypothesis, which states that gall structures have been selected to reduce mortality imposed on the gall inducer by natural enemies (Stone *et al.*, 2002).

It's well known, for example, that the gall can act as a metabolic sink for photosynthate, attracting food for the sedentary parasite. One of the physiological mechanisms is mediated by cytokinin, that have also been extracted from the larvae of *D. kuriphilus*. This evidence indicates that, although the hormone may arise from the host, it is being concentrated in the body of the insect, then being excreted and having its effect (Hewett, 1977).

Another physiological process that occours in galling cynipids is manipulation of tannins, both in the inner nutritive tissue and in the external protective one (Ikai and Hijii, 2007).

1.5- *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae)

The chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae), is the most widespread insect pest of chestnut (*Castanea* spp.) (Aebi *et al.,* 2006).

In Europe, it is an exotic species, which induces gall formation on petioles and leaves in chestnut.

Dryocosmus species are known to induce gall formation on *Quercus* spp., except *D. castanopsidis* (Beutenmueller), widespread only in the United States (Melika *et al.,* 2004), which induces gall formation only on *Castanopsis* sp., and *D.kuriphilus*, which causes galls formation only on *Castanea* spp..

1.5.1- Origin and diffusion

The orienta1 chestnut gall wasp is indigenous to China.

In 1941 it was reported in Japan, in the Okayama Prefecture where it was considered as an un-named *Biorhiza* species but later described as a new species (Yasumatsu, 1951).

In the following years it caused serious damages to Japanese chestnut orchards: it spread throughout Japan within 25 years, with a dramatic impact on chestnut production (Aebi *et al*., 2006).

In 1958 it was observed in Korea while in 1974 it was found for the first time in Peach County, Georgia (Payne *et al*., 1975).

In the United States within 10 years it gravely affected local chestnut orchards, based on Chinese and Japanese chestnut varieties (Aebi *et al*., 2006).

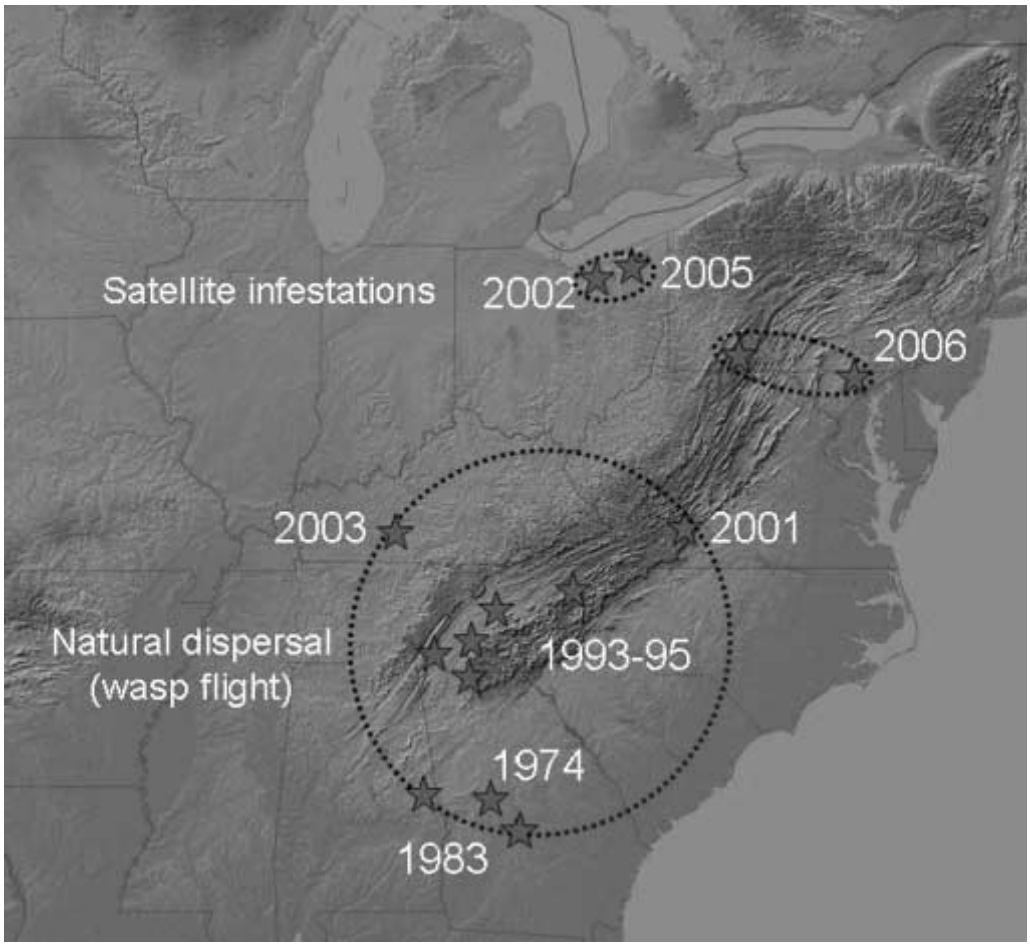


Fig. 1.2- Distribution and dispersal pattern of *D. kuriphilus* in North-Eastern America (Rieske, 2007)

D. kuriphilus was reported from Nepal in 1999 (Abe *et al*., 2007).

In Europe, *D. kuriphilus* was first recorded in 2002, in Piedmont, Italy (Brussino *et al.* 2002). It's believed that the actual introduction date is 1995- 1996, when eight Chinese chestnut cultivars were introduced in the Piedmont region (Aebi *et al*., 2006*)*.

Besides Italy, the gall wasp has been reported in France in 2005, in an orchard (Saint-Dalmas Valdéblore, region of Provence-Alpes-Côte d'Azur) of young plants coming from Cuneo province. Other infestation hotspots have been observed in 2007 in municipal districts of Roya Valley (Tende, La Brigue, Fontan and Saorge) (EPPO RS, 2007). The spread in this area is probably due to the traffic through the Tenda tunnel. In 2005, the gall wasp has been found in Slovenia, after the importation of young infested plants from Piedmont (EPPO RS, 2006). Here, after attempts of eradication, the presence of *D. kuriphilus* has been confirmed in 2007. During the spring of 2009, the insect has been recorded in Switzerland, in chestnut areas in Canton Ticino (www.ti.ch/DT).

Year of first report	Italy	France	Slovenia	Hungary
2002 Piemonte				
2005	Abruzzo, Campania, Lazio, Toscana	Valdéblore (Provence-Alpes-Côte d'Azur region)	Zgornja Pohanca (Spodnjeposavska region), Znojile pri Krki (Osrednjeslovenska region), Renče-Merljaki and Bilje (Goriška region)	
2006	Lombardia			
2007	Liguria, Trentino Alto-Adige, Sardegna, Veneto	Roya valley (4 contaminated communes of Provence-Alpes-Côte d'Azur region); Frouzins (Midi-Pyrénées region) (movement of plants prior to implementation of emergency measures	Sabotin mountain (Goriška region) (trees planted in 2004)	
2008	Emilia-Romagna, Friuli Venezia Giulia	Roya valley (4 + 2 more contaminated communes Provence-Alpes-Côte d'Azur region)	9 other foci (Goriška and Obalno-kraška regions)	
2009	Calabria, Marche, Umbria	Roya valley (4 + 2 more contaminated communes Provence-Alpes-Côte d'Azur region); Maxillysur-Léman (Rhône-Alpes region)	53 locations (Goriška and Obalno-kraška regions	1 tree in Üröm (Budapest)

Tab. 1.2 - A summary of the spread of *Dryocosmus kuriphilus* in Europe (EFSA, 2010)

Spread of *D. kuriphilus* into new countries occurs by introduction of infested twigs or shoots. Local spread occurs through the movement of infested twigs and young plants, or by adult females during their flight period (end of May to the end of July) (http://archives.eppo.org).

Mathematical models have estimated short distance dispersal of adult gall wasps at a rate of 8 km year-1, with a variation comprised in a range of 3–12 km year-1 (EFSA, 2010)

D. kuriphilus could potentially spread throughout the range of *C. sativa* in Europe, but the most threatened areas are Northern Portugal, Northern Spain and South-Western France (EFSA, 2010).

1.5.2 – Morphology

Eggs

D. kuriphilus eggs are deposited by females into the buds of current shoots in June and July. Eggs are oval, milky white, 0.1–0.2 mm long, with a long stalk.

Larva

The larva is 2.5 mm long when fully grown, milky white, without eyes and legs.

Pupa

The pupa is 2.5 mm long, black or dark brown.

Adult

The adult female of *D. Kuriphilus* is 2.5–3 mm long on average, body is black; legs, scapus and pedicels of antennae, apex of clypeus and mandibles are yellow brown; head is finely sculptured; scutum, mesopleuron and gaster are highly polished, smooth; propodeum with 3 distinct longitudinal carinae; propodeum, pronotum (especially above) strongly sculptured; scutum with 2 uniformly impressed and pitted grooves (notaulices) that converge posteriorly; radial cell of forewing opened; antennae 14-segmented with apical segments not expanded into a club (Bosio and Vettorazzo, 2005)

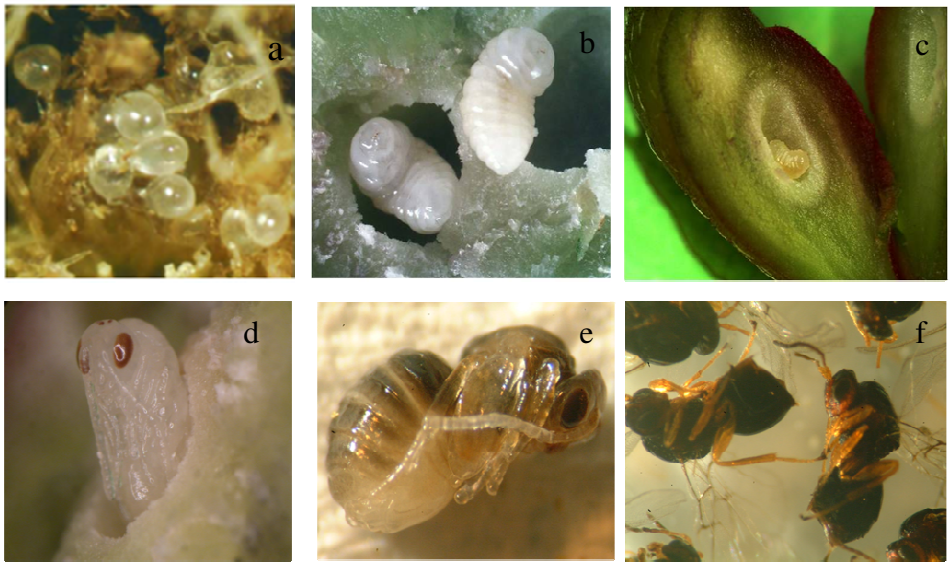


Fig. 1.3 - Pictures of different stages of *D. kuriphilus*: a) eggs - b), c) larvae within the gall niches open longitudinally. d) newly formed pupa, e) pupae in more advanced stage e) adults (Foto: a Melika (2004); c,d F. Riga, b,e E. Moretti).

1.5.3- Life cycle

D. kuriphilus is univoltine and thelytokus (only female develop from unfertilized eggs); male individuals have never been reported. Thanks to this reproductive way *D. kuriphilus* had a real demographic explosion causing extensive damage to crops.

The adults emerge from the late spring until early summer (the figures vary according to site conditions and weather from May to June, from June to July, etc.) and is immediately ready for oviposition.

The females fly in search of chestnut gems and are able to lay groups of 3 to 5 eggs. Each female may lay more than 100 eggs, with 20-30 eggs found in one bud (Bosio and Vettorazzo, 2005; Ôtake, 1980, Tamura, 1960).

After about 35 to 40 days the eggs hatch. The first larval stage grows slowly and overwinters in the buds. Throughout the fall and winter the presence of the insect is asymptomatic.

Symptoms begin to appear at at bud burst in spring when larval feeding induces the formation of green or rose-coloured galls. The larvae go on developing through the various stages within the galls until, next to flowering of the male catkins of the chestnut tree, they turn into pupae. At the end of chestnut flowering occurs in the adult stage. The females are short-lived (2-10 days) (Yasumatsu, 1951).

1.5.4 - Host plants

D. kuriphilus attacks *Castanea crenata* (Japanese chestnut), *Castanea dentata* (American chestnut), *Castanea mollissima* (Chinese chestnut) and *Castanea sativa* (European chestnut) and their hybrids. It infests also *Castanea seguinii* in China, but is not known to attack the wild North American secies *Castanea pumila* and *Castanea alnifolia* , which are very often grown adjacent to infested chestnuts (Bosio and Vettorazzo, 2005).

1.5.5 - Symptoms and damages

Symptoms are stem, petiole, or leaf galls. These galls provide the developing wasp protection throughout the larval and pupal stages (Cooper and Rieske, 2011b).

Heavy infestations reduce the vigor of the plants, which occur with very sparse foliage, and can cause death. The reductions in production seem to reach 50-70% (Bosio and Vettorazzo, 2005).

Attack by *D. kuriphilus* reduces fruit yield by 50-75% (Payne *et al*., 1983), and heavy attack reduces tree vigor and wood production (Kato and Hijii, 1997) and can kill the tree (Moriya *et al*., 2003).

To date in Italy, neither in chestnut orchards that were experiencing heavy attacks since 2002, no death of trees has been reported (Bosio *et al*., 2009).

Therefore it's very difficult to evaluate the responsibility of *D. kuriphilus* in yield reductions. Infact, if the climatic and agronomic conditions, especially water availability, are favorable to the increase of shoots after the female flight period, the new buds will not be affected by eggs laying. In this situation chestnuts would normally bear fruits in the next spring (Bosio *et al*., 2009).

1.5.6 - Control measures

1.5.6.1 Resistant varieties

Japanese researchers, who faced this problem for the first time in the 1940s, began controlling the pest by means of insecticides and natural enemies without any result, so they focussed their efforts on breeding and propagating resistant varieties, and the gall wasp became a less worrying problem (Quacchia *et al*., 2008).

Subsequent breeding of resistant chestnut varieties enabled chestnut growers to control *D. kuriphilus* for about 20 years, until the appearance of a strain able to attack resistant chestnut varieties (around 1960) (Aebi *et al.*2006).

Moreover, developing resistant varieties of *Castanea* spp. could potentially be a viable management option, but this will only be beneficial for new planting and will not help existing chestnut populations (EFSA, 2010).

1.5.6.2 Chemical control

Since the larval and pupal stages of *D. kuriphilus* are protected within their galls, conventional chemical control is regarded as largely ineffective (EFSA, 2010).

In some cases it has been demonstrated that the use of systemic insecticides is effective on the adult stage of the gall wasp (Tarcali and Radocz, 2009). Anyway, given that these treatments induce side effects on the environment, they have to be avoided in natural environment, especially in forest.

1.5.6.3 Agronomical control

Infestations in small chestnut orchards may be reduced by pruning and destroying the infested shoots, but commercial growers cannot rely on this strategy because of the cost (Bosio and Vettorazzo, 2005).

Even a proper fertilization may be useful, infact manuring treatment at vegetative restart in treated plants showed an evident improvement in the canopy vegetation and efficacy of this kind of treatments for infested chestnut stands (Turchetti *et. al.*, 2012)

1.5.7 - Preventive measures

D. kuriphilus is considered one of the most serious pest of chestnut worldwide but despite this fact it was not included in the European list of quarantine pests (Directive 2002/89/CE and subsequent integrations). In 2003, after the arrangement of a specific Pest Risk Assessment (PRA), it was added to EPPO (European and Mediterranean Plant Protection Organization) A2 action list, which includes pests yet introduced in EU. EPPO member countries are thus recommended to consider it as a quarantine pest to prevent the introduction and the spread in other areas (Bosio *et al*., 2009) .

Control measures such as the regulation of nursery activities and commercialization of chestnut young plants were underlined by a Ministerial Decree issued by the italian Govemment on October 30, 2007, outlining compulsory measures for pest control.

Infact human exchange of infected cultivars and material for grafting among chestnut growers is thought to be the main factor facilitating its dispersal (Aebi *et al*., 2006)

1.5.8 – Native parassitoids

D. kuriphilus does not represent a threaten for chestnut in its native country: this suggests that in China insect populations are controlled by natural enemies.

When it was accidentally introduced in Italy, *D. kuriphilus* found no competitors in its niche but, in a few years, many native parasitoids are gradually moving to the new host and becoming associated with it in its invaded ranges, as best illustrated in Chapter 4.1.

1.5.9 – Exotic Parassitoid – *Torymus sinensis*

Classical insect biological control has been successfully used for more than 120 years, and deployment of more than 2000 species of natural enemies has resulted in the control of at least 165 pest species worldwide (Van Lenteren *et al*., 2006).

T. sinensis Kamijo (Hymenoptera: Torymidae), is univoltine like its host; adults emerge from withered galls in early spring and, after mating, the female lays eggs into newly formed galls, either onto the body surface of the host larva or on the wall of the larval chamber. The parasitoid's larva feeds ectoparasitically on the pest's mature larva and pupates during late winter (Quacchia *et al*., 2008). Among the native parasitoids that control *D. kuriphilus* in China *T. sinensis* was chosen to be introduced by Japanese researchers.

In 1979 and 1981, a total of 260 mated *T. sinensis* females (reared from approximately 5000 *D. kuriphilus* galls imported from China) were released for biocontrol on Japanese chestnut trees at the Fruit Tree Research Station in Ibaraki prefecture (reviewed in Aebi *et al*., 2006).

By 1989 this *T. sinensis* population had grown by 25 times and had become the most common parasitoid reared locally from *D. kuriphilus* (Aebi *et al.*, 2006).

Hybridization between introduced parasitoid *T. sinensis* and indigenous congener *T. beneficus* has drawn attention since *T. sinensis* was first introduced because the biological control would fail if the hybrids were sterile. Such apprehension disappeared as the damage caused by *D. kuriphilus* decreased. Since the initial introduction of *T. sinensis*, however, morphologically intermediate individuals between the two *Torymus* species have appeared in the field. This has led to investigation of the hybridization in the form of examining the non-target effect of biological control in recent years. Molecular markers have been used for hybridization surveys in the field not only because *T. sinensis* and *T. beneficus* are exceedingly similar morphologically but also because at least two emergence types (early- and late-spring) are recognized in *T. beneficus*, for which emergence periods are not defined clearly. Our surveys show that the hybridization between *T. sis*

and early-spring *T. beneficus* occurred at low frequency (around 1%). On the other hand, the hybrid F_1 between *T. sinensis* and late-spring *T. beneficus* were detected at higher frequency (around 20%). Furthermore, individuals with the F_1 genotype were detected even after *T. sinensis* displaced *T. beneficus* and were the dominant species, which suggests that hybrids (including descendants of F_1) interbreed successively with *T. sinensis*. (Yara, 2009)

In 1977, three years after *D. kuriphilus* was first reported in North America, the parasitic wasps *T. sinensis*, *T. tubicola*, and *Megastigmus* sp. (Hymenoptera: Torymidae) which had successfully suppressed damaging gall wasp populations in Japan, were introduced into gall wasp infested orchards in Byron, Georgia. Within a short period, gall wasp populations in central Georgia declined and the incidence of galling dropped below acceptable levels (Rieske, 2007).

Following success in Japan and North America, a classical biological control was initiated in Italy too, in order to reduce the rapid colonisation of *D. kuriphilus* in all the chestnut areas (Bosio *et al*., 2009).

This program was coordinated by entomologists of DIVAPRA, Torino University.
Also in this case the candidate, as a beneficial insect to be inoculated, was *T. sinensis* (Stone *et al*., 2002; Aebi *et al*., 2007; Quacchia *et al*., 2008).

From 2005 on, the parasitoid was released in open fields every spring. A total of 95 release points were achieved in 2009. Establishment of the parasitoid was verified in some sites by the collection of galls during winter. Although the percentage of parasitization by *T. sinensis* varied among sites (from 0.5% to 25.5%), the establishment has been confirmed in each site and the parasitization trend continues (Quacchia *et. al*, 2010).

The introduction of an exotic organism always involves huge risks and must be conducted after carrying out the necessary tests. Preliminary studies on the release of *T. sinensis* in Europe were conducted in 2003 and 2004 in Italy (Quacchia *et al*., 2008). Introduced *T. sinensis* has been placed in contact with galls taken from oak; none of the females put in contact with galls of *M. fagi*, *C. quercusfolii*, and *A. kollari* showed the behavioural components looked for and no oviposition was registered. Five galls of each species were tested (Quacchia *et al.*, 2008).

However, this range of alternative hosts was very limited, and other more logical alternative host galls on other plants (such as *Diplolepis* galls on *Rosa* spp.) were not considered (Gibbs *et al*., 2011). Another aspect to be considered when assessing the effectiveness of *T. sinensis* as a biological control agent is its uncertain systematic status (Quacchia *et al*., 2008).

As suggested by the EFSA Panel on Plant Health (2010) regarding the *T. sinensis*: “further research is needed, particularly on a) the host range of the parasitoid to determine the direct and

indirect non-target effects on closely related oak gall wasps of the Cynipidae; b) the taxonomy and phylogenetic analysis of *T. sinensis* and closely related species and c) the potential of *T. sinensis* for hybridisation with other *Torymus* species".

Actually, about the complex interactions that can be established in the chestnut between native and introduced insects, in the USA it has recently been observed that the native parasitoid *Ormyrus labotus*, in addition to parasitising *D. kuriphilus*, also attacks the introduced biological control agent *T. sinensis,* acting as a hyperparasitoid (Cooper & Rieske, 2011a).

1.5.9.1 - Technical notes on the release of *Torymus sinensis* in Italy

T. sinensis is currently being introduced in most regions across Italy (Quacchia *et al*., 2008).

In the "Chestnut Sector Project 2010/2013" (AA.VV., 2010), a biological control protocol for the introduction of *T. sinensis* is described.

The description of methods of biological control protocol rises from the experience developed by the DIVAPRA of the University of Turin.

The biological control of the chestnut gall wasp is realized through release in the open field of couples of *T.sinensis* obtained from parasitized galls collected in 'areas of multiplication'.

The "multiplication" areas are well-managed chestnut areas in which *T. sinensis*, intentionally introduced, acclimatizes, establishes and multiplies, providing a self-perpetuating supply for the following years. The area can be obtained from a pre-existing chestnut or it can be made *ex novo*. The main feature of the area must be isolation. A distance of at least 2 km away from other chestnut orchard is recommended. The isolation promotes the concentration of the parasitoid and slows the dispersion. Furthermore, when selectioning and setting up a new area, is also very important to consider the Chestnut varieties, infact *Castanea* varieties should be the most susceptible to *D. kuriphilus*.

2 Aim

The purpose of this work was to investigate two different aspects of the cynipid *D. kuriphilus* that can allow to achieve data in a plant protection strategy: from one side, the study of the sensory receptors of the antenna, and, on the other, the community of parasitoids related to the gall maker.

First, an ultrastructure study was carried out on the different types of sensilla. The results of this study associated with future electrophysiological and behavioral tests will allow to understand attraction or avoidance behaviors toward plant pheromones and to develop alternative measures of control and monitoring of *D. kuriphilus* such as or attraction/repulsion.

Then, it was carried out a survey to value the presence of native parasitoids associated to *D. kuriphilus*, in order to value the composition and the amount of potential limiters as biological control agents, for example in augmentation or conservation biological pest control

3 Antennal morphology

3.1 Introduction

3.1.1 Antenna

Among the appendages characterizing the different body parts of insects, antennae are considered those involved primarily in the perception of stimuli from the environment.

Insect antennae occur in a large variety of shapes and sizes, but a general scheme can be outlined considering these appendages divided into three parts: scape (or scapus), pedicel, and flagellum.

Scape, the basal segment, is articulated with the head capsule through the torulus, where it connects via an elastic joint membrane. The base of the scape is inserted into a socket where extrinsic muscles are attached.

Pedicel is articulated proximally with the scape and distally with the rest of the antenna. In most insects, the pedicel houses the Johnston's organ, an auditory organ.

Flagellum, all of the remaining antennal segments, is the main part of the antenna. The flagellum is composed by a different number of segments, termed "flagellomeres".

Historically, insect antennae have been considered the sensory center of the smell and taste, as well as the perception of airborne or contact vibration (Zacharuk, 1985; Keil, 1999). These sensory functions are made possible by the presence of small organs, known as sensilla.

Antennae are not only receivers of various types of signal, but sometimes also emitters of chemical messages (Bin *et al*, 1999).

3.1.2 Definition of sensillum

A sensillum is defined as a well-defined complex of bipolar receptor neurons, auxiliary cells, and cuticular elements (Keil, 1999) that is able to receive and transfer environmental stimuli from the extreme periphery of the insect's body to the main processing centers, located within the head capsule, mainly the protocerebrum and deutocerebrum.

A chemoreceptor receives or senses chemical information in the environment, such as taste and smell, while mechanoreceptors receive mechanical information, such as movement, touch, pressure and vibration.

3.1.3 Type of sensilla

Insect sensilla are based on a stereotyped scheme, and according to this basis, they have been defined as “kleinorgan” (Henke, 1953) or “organule” (Lawrence, 1966). Each sensillum is made up of an external cuticular part and several cellular components.

The cuticular part, that in most cases houses the sensory neurons projections, can have different shapes, although the differentiation in hair or hair-like structures seems to be the most common feature.

The first attempts of classification for insect sensilla were based on light microscopy, and the shape of the external cuticular part was exclusively considered.

More recently, using scanning electron microscopy (SEM) and trasmision electron microscopy (TEM), pore structure and position has been proposed as criteria for sensillar classification, because in many cases these characteristics have been correlated with sensillar function (Altner, 1977).

Based on the wall pore structure, as it can be observed through transmission electron microscope, Altner classifed insect sensilla as:

- No-pore (aporous) sensilla: these sensilla have no permeable pores on the sensilla wall. Different morphological types have been reported, and their functional significance is related with mechanosensitivity.
- Tip-pore (uniporous) sensilla: mostly of these sensilla appear as hairs or pegs, and possess a single pore in apical position. Their function is usually chemosensitivity related with taste stimuli. When they occur in form of hair or pegs, they are inserted on the antenna wall through a flexible socket, thus acting as a bi-modal mechano-chemosensory structure.
- Wall-pore (multiporous) sensilla: the sensilla wall is perforated by numerous pores that are all localized on only some parts of the sensillum or are evenly distributed all over the sensory cuticle. Although the main function of these sensilla is the olfaction, in some cases there have been reports they acted as bi-modal receptors, combining olfaction and thermo-perception (Altner *et al*. 1983, Hansson *et al*. 1996).

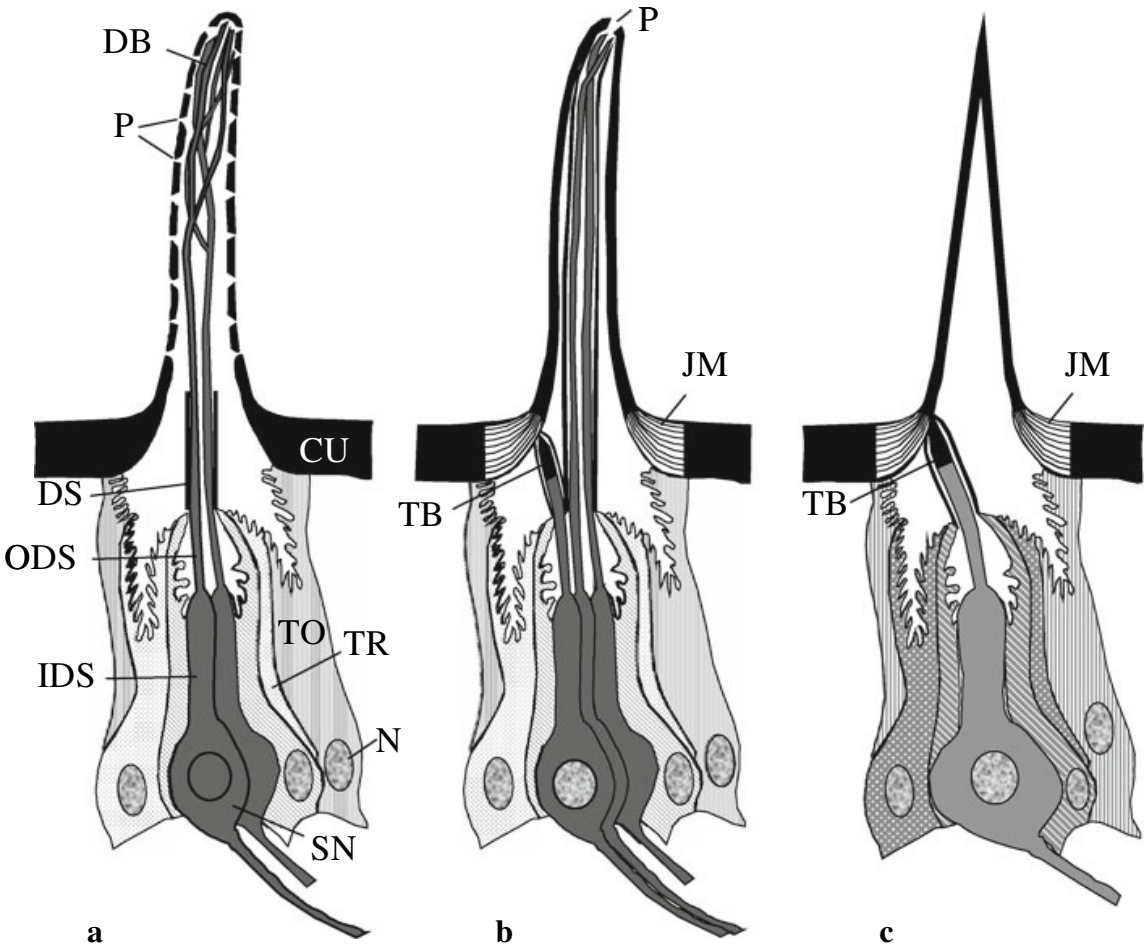


Fig. 3.1 Schematic drawing of different type of sensilla. (**a**) multiporous olfactory sensillum; (**b**) uniporous gustatory sensillum; (**c**) mechanosensory hair. CU – cuticle, DB – dendritic branches, DS – dendrite sheath, IDS – inner dendritic segments, JM – joint membrane, N – nucleus, ODS – outer dendritic segments, P – pores, SN – sensory neurons, TB – tubular body, TH – thecogen cell, TO – tormogen cell, TR – trichogen cell (Romani *et al*, 2010)

3.1.3.1 Aporous sensilla

Aporous sensilla can be represented according to different morphological types, i.e. mechanosensory hairs, styloconic sensilla, campaniform sensilla, and coeloconic sensilla.

Mechanosensory hairs possess a cuticular shaft of varied length that, trough a modified base (socket), allows mechanical distortion and stimulation of the sensory neuron (Keil, 1997); this socket is characterized by specialized cuticular elements that connect to the external hair, a joint membrane, a socket septum and suspension fibers (Gaffal *et al.* 1975).

These sensilla are composed of a single sensory neuron and a number of two to three accessory cells (McIver 1985). The dendritic projection of the sensory neuron is typically modified into a tubular body (Thurm 1964), i.e. a variable number of microtubules closely packed together and

arranged in parallel. Typically, the tubular body is located on the distal end of the outer dendritic segment and it appears circular in a cross section, and very electrondense because of the material lying between the microtubules. The tubular body is accepted as the site of sensory transduction (Gnatzy and Tautz 1980, Thurm 1983).

Mechanosensory hairs usually represent the most abundant class of antennal sensilla, and their function is related to the touching of different surfaces scanned by insect's antennae.

eloconic sensilla (Altner *et al.* 1981, Yokohari 1983, Hansson *et al*. 1996).

3.1.3.2 Uniporous sensilla

Uniporous sensilla are characterized by the presence of a single, in most cases apical, cuticular pore, which opens onto a cuticular shaft usually appearing as a hair or a peg of various lengths. These sensilla are usually innervated by four to six sensory neurons that run unbranched inside the peg lumen, reaching the apical pore (Zacharuk 1985). The lumen is sometimes divided into two chambers: the first chamber (the dendritic chamber) is occupied by the dendrites enveloped by the dendrite sheath, while the other (the sensillar chamber) sends pore tubules or filaments to the external pore. Usually, uniporous sensilla are bi-modal sensilla, since they combine mechanosensory and chemosensory functions in a single sensory unit. In this case one of the sensory neurons does not enter the peg lumen, thus ending at the base of the sensillum where a typical mechanosensory tubular body is formed.

These sensilla are only located in areas that are associated with touching the substrate, host or opposite sex (Isidoro *et al*., 1996). Uniporous sensilla are consider "gustatory" units, i.e. chemical signals are perceived through contact with the substrate.

3.1.3.3 Multiporous sensilla

Multiporous sensilla are characterized by the presence of numerous, well evident cuticular pores opening on the sensory cuticle.

Based on the external appearance multiporous sensilla usually occur in the form of hairs (of various lengths), pegs or plates.

Altner (1977) classified these sensilla as wall-pore sensilla, further divided into single-walled and double-walled.

In function of this, the sensory transduction system in olfactory sensilla can be of two different modalities, depending on the specialization of cuticular structures allowing the transport of the volatile molecules from the external aerial environment to the receptor cells.

In single-walled sensilla, the cuticle is perforated by numerous tiny pores. Ultrastructural details of the pores revealed that they consist of a small chamber opening below the external pore, from which several small cuticular channels (pore channels) develop throughout the antennal wall. These pore channels house specialized stimulus-transport structures, known as pore-tubules that reach the sensillar lumen and sometimes establish contact with the olfactory neurons (Steinbrecht and Müller 1971, Keil 1982).

In double-walled sensilla, the sensillum lymph cavity is divided in an innermost and an outermost space, which arise from the external cuticular finger of the sensilla, and are partly fused together (Hunger and Steinbrecht 1998). The sensory neurons are located exclusively in the innermost space, and volatile molecules reach the outer dendritic segment passing through “spoke channels”, i.e. small canals crossing both walls and typically arranged like spokes of a wheel (Altner and Prillinger 1980). In this case, pore-tubules were never observed.

3.2 Aim

The aim of this study is to carry out a detailed morphological investigation, using both scanning and transmission electron microscopy, of the antennae of *Dryocosmus kuriphilus*; this analysis may be important to understand how the perception of chemical or mechanical stimuli takes place and to examine the inner workings of host location.

This can be useful to focus future techniques of lotta/controllo, for example by the use of attractants or repellents in plant/insect interaction.

3.3 Materials and Methods

3.3.1 Sample preparation

3.3.1.1 Scanning Electron Microscopy

Twenty females were immersed in 60% ethanol water solution. After dehydration in a graded ethanol series, the heads with the antennae were dried, gold coated in a Balzer Union SCD 040 unit, and finally examined with a Philips XL 30.

3.3.1.2 Transmission electron microscopy

Ten females each were anesthetized with CO2 and immersed in 2,5% glutaraldehyde in 0.1 M cacodylate buffer + 5 % sucrose, pH 7.2-7.3. Then the antennomeres were detached, to aid fixative penetration, and left at 4°C for 2 hours. After rinsing overnight in cacodylate buffer, the specimens were postfixed in 1% osmium tetroxide at 4°C for 1 hour and rinsed in the same buffer. Dehydratation in a graded ethanol series was followed by embedding in Epon-Araldite with propylene oxide as bridging solvent. Thin sections were taken with a diamond knife on a L.K.B. "Nova" ultramicrotome, and mounted on collodium-coated 50 mesh grids. Finally the sections were examined with a Philips EM 400T, after staining with uranyl acetate (20 minutes, room temperature) and lead citrate (5 minutes, room temperature).

3.4 Result and discussion

D. kuriphilus female antenna is geniculate, composed by 14 antennomeres, and can be divided into three parts (Fig. 3.2): scape, pedicel and flagellum.

Scape is the basal segment articulated with the head capsule through the trorulus, where it connects via an elastic joint membrane.

The second antennal segment is the pedicel, articulated proximally with the scape and distally with the rest of the antenna.

All the remaining 12 antennal segments, termed "flagellomeres", form the flagellum that bears most of the sensilla.

While the structure of scape and pedicel is characterized by a reduced length and a greater development in diameter, flagellomeres appear isodiametric, so that the antenna seems threadlike.

However, total of six proximal flagellomeres have a greater development in length than the distal six. The overall length of the antenna is about 1.5 mm. SEM observation of the antennal surface allowed the identification of 5 types of sensilla (chaetica, trichoidea, placoidea, caeloconica and styloconica). These sensilla are described below in detail: their ultrastructural characterization is achieved through SEM and transmission electron microscopy (TEM) techniques.

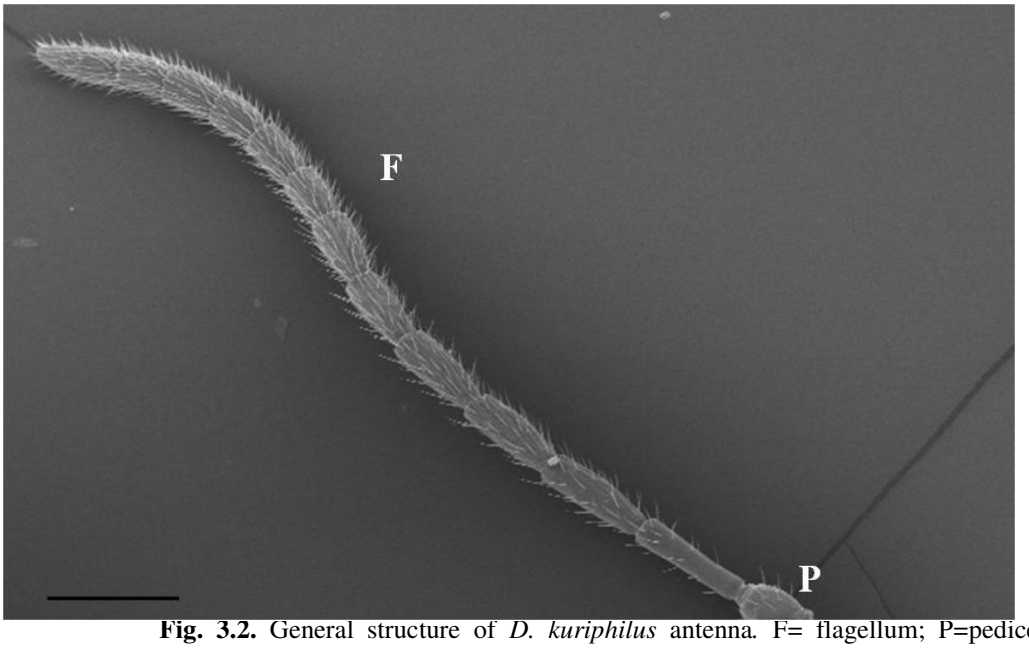


Fig. 3.2. General structure of *D. kuriphilus* antenna. F= flagellum; P=pedicel. Scale bar: 200µm.

3.4.1 Sensilla Trichoidea (ST)

They are uniformly present over the entire surface of the antenna (Fig. 3.3a). They are characterized by a elongated cuticular shaft decreasing in diameter toward the apex. ST are characterized by the absence of evident cuticular pores. Their cuticular shaft is 25 µm long. The basal part of the sensillum is inserted through a visible socket on the wall; how suggested by Keil (1997), the cuticolar shaft allows the perception of the flexible basal socket relative distortion through which it is inserted into the antennal wall. The cuticular shaft appear empty (Fig. 3.3c).

TEM investigations show that these sensilla are composed of a single sensory neuron and three accessory cells. As described by Thurm (1964) the dendritic projection of the sensory neuron is typically modified into a tubular body (Fig. 3.3b). The tubular body is located on the distal end of the outer dendritic segment and it appears circular in a cross section and very electrondense due to the material lying between the microtubules. The tubular body is accepted as the site of sensory transduction (Gnatzy and Tautz, 1980; Thurm, 1983). All these features suggest a mechanosensory function for these sensilla.

Sensilla trichoidea were reported on the antennae of several species belonging to different families (Amornsak *et al*., 1998; Cônsoli *et al*., 1999; Van Baaren *et al*., 1999) where represent the most abundant class of antennal sensilla, and their function is related to the touching of the different surfaces scanned by insect's antennae.

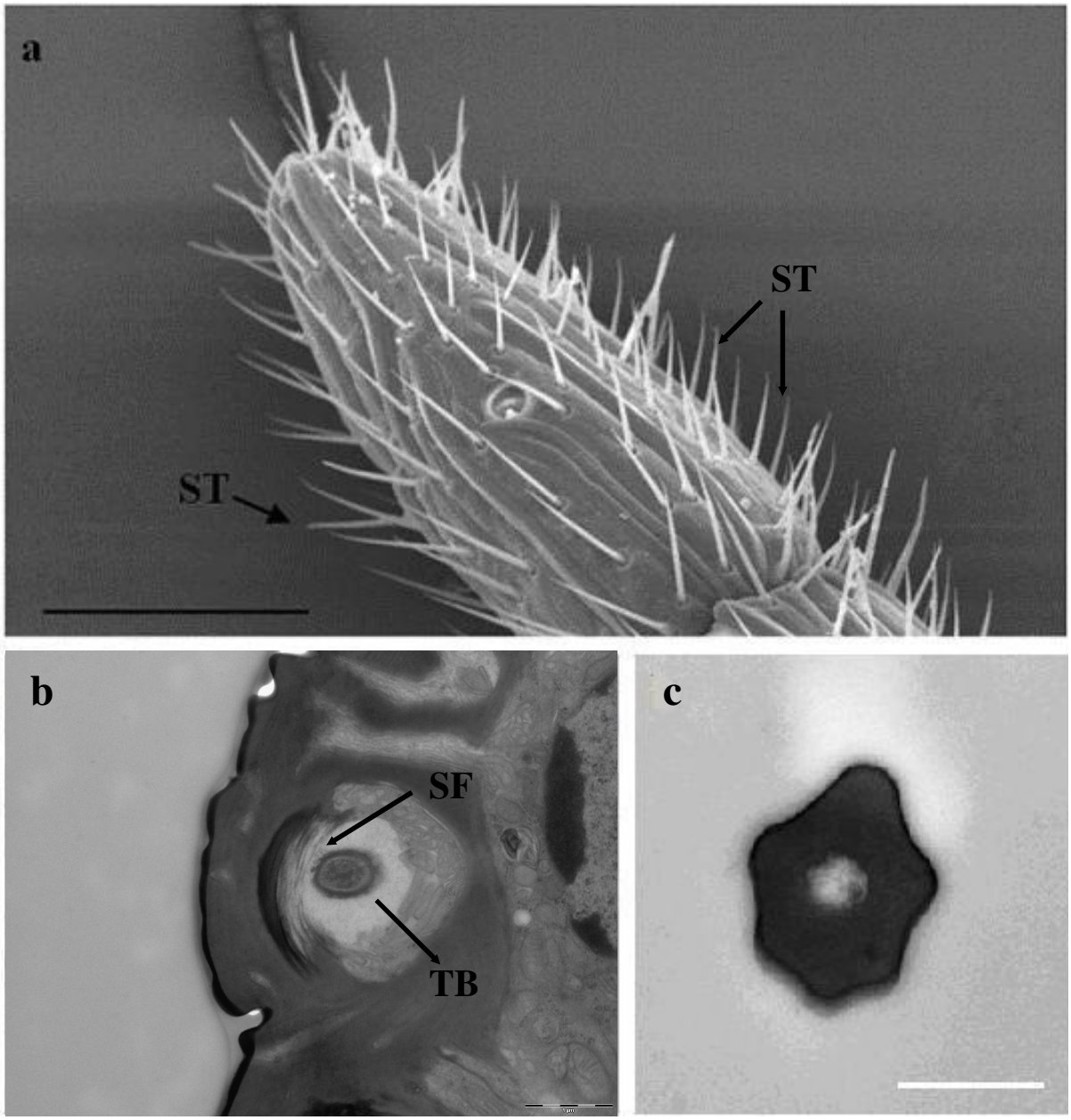


Figure 3.3 Sensilla trichoidea (ST). a) SEM pictures of the last antennomere; b) TEM oblique section at socket level showing tubular body (TB) and suspension fibres (SF); c) TEM shaft cross section close to the apex. Scale bar: a=50 µm; b=1 µm; c=500 nm.

3.4.2 Sensilla chaetica (SCH)

They are long sensilla, ending in a fine, sharp tip (Fig. 3). The SCH are positioned radially in the tip of the A14, in number of 5, and distally from A3 to A13, in number of 4. They are characterized by a elongated cuticular shaft decreasing in diameter toward the round apex pierced by few pores not visible in SEM pictures. The length of the sensilla is about 28-30 µm. They show a flexible socket. These sensilla usually possess a thick wall.

TEM investigations show that the cellular components consist of five sensory neurons and three accessory cells (Fig. 3.4b). The bundle of four dendrites is surrounded by a common dendritic sheath. The dendritic projection of one sensory neuron is modified into a tubular body located on the distal end of the outer dendritic segment, connected to suspension fibers of the socket. The others four dendrites, after entering the peg lumen reach unbrunched the tip of the shaft (Fig. 3.4c).

Sensilla with similar ultrastructural features were reported in several groups of insects (Romani *et al*, 2010; Zacharuk,1985). Mechanosensory associated to a gustatory function is hypothesized for them.

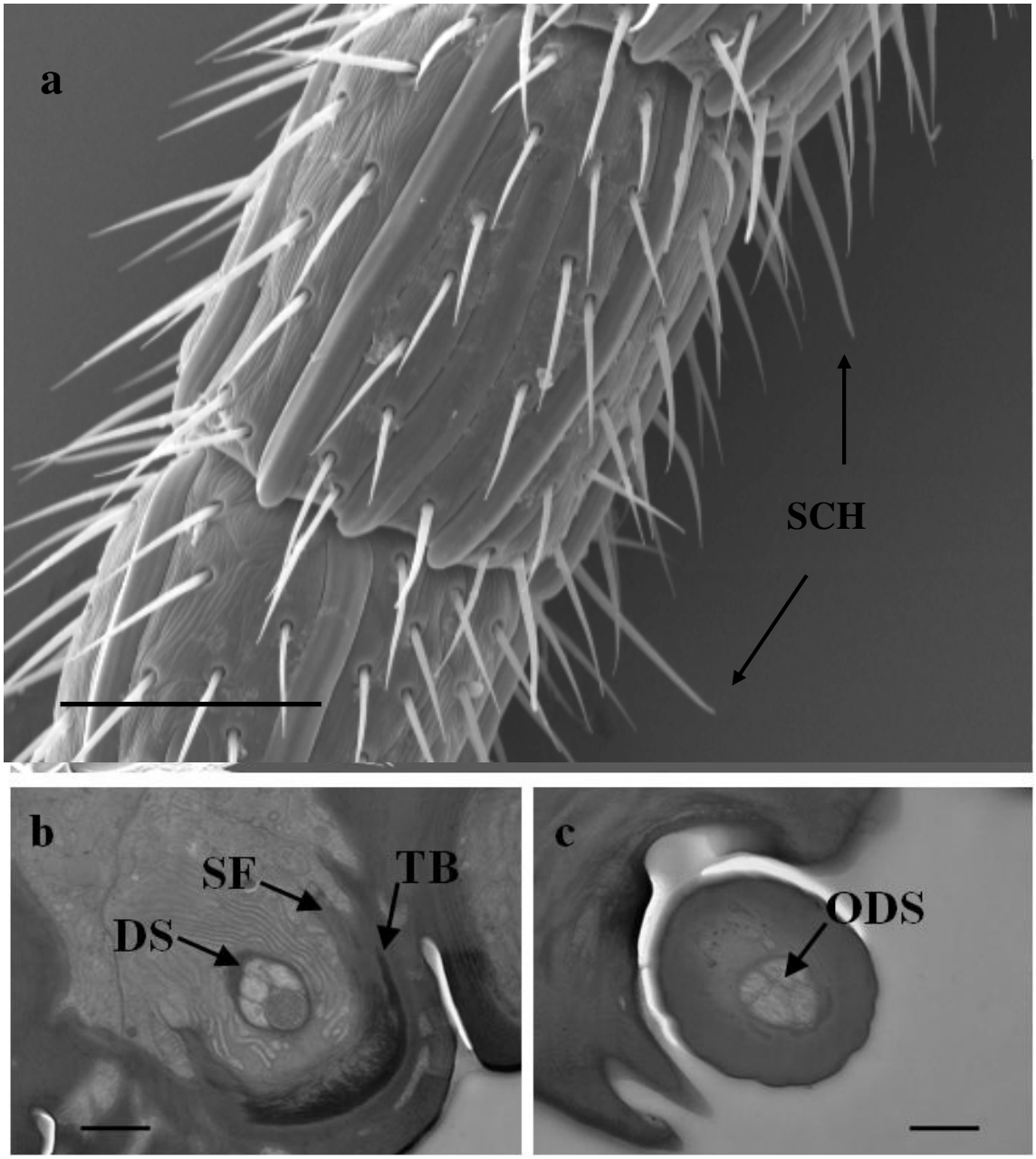


Figure 3.4 Sensilla chaetica (SCH). a) SEM pictures of the two last antennomere; b) TEM oblique section at socket level showing five dendrites enveloped in dendritic sheat (DS); c) TEM shaft cross section showing four dendrite inside (ODS). TB=tubular body; SF=suspension fibres. Scale bar: a=50 µm; b=1 µm; c=500 nm.

3.4.3 Multiporous Plate Sensilla (MPS)

This sensory structures are found on all antennal segments, with exception for scape and pedicel. MPS extend longitudinally, often occupying the entire length of antennomere, with the distal part slightly free. The number of MPS decreases from apical to basal antennomere, from A6 to A14 in number of eight, while from A3 to A5 in number of four. Their distribution is uniform for all the entire surface of the segments. The length of MPS varies according to antennomere's length and ranges from 70-75 in A3 and A14 to about 125 µm in the pedicel central antennomere . They are characterized by a thin cuticular structure, in the form of a long plaques slightly arising from antennal wall. The cuticle is perforated by numerous tiny pores distributed onto the entire sensillum cuticular surface (Fig.3.5a).

TEM investigations show that these sensilla are composed of 25 sensory neuron and 3 accessory cells (Fig. 4d). The bundle of dendrites is surrounded by a common dendritic sheath. In cross sections, the sensillar lumen presents two cuticular reinforcements, which divide the lumen into two chambers, with one of them occupied by the outer dendritic segments (Fig. 3.5c). After entering within the lumen, in contact with the pores, the dendrites greatly branch. A sheath cell completely envelops all the sensory cell bodies.

These sensilla perceive volatile chemical stimula, an olfactory function is attribuited.

These were mainly described in Hymenoptera (Navasero and Elzen 1991, Basibuyuk and Quicke 1998, Barlin and Vinson, 1981) and Coleoptera (Allsopp, 1990). For these sensilla, an olfactory function has been proved (Lacher, 1964).

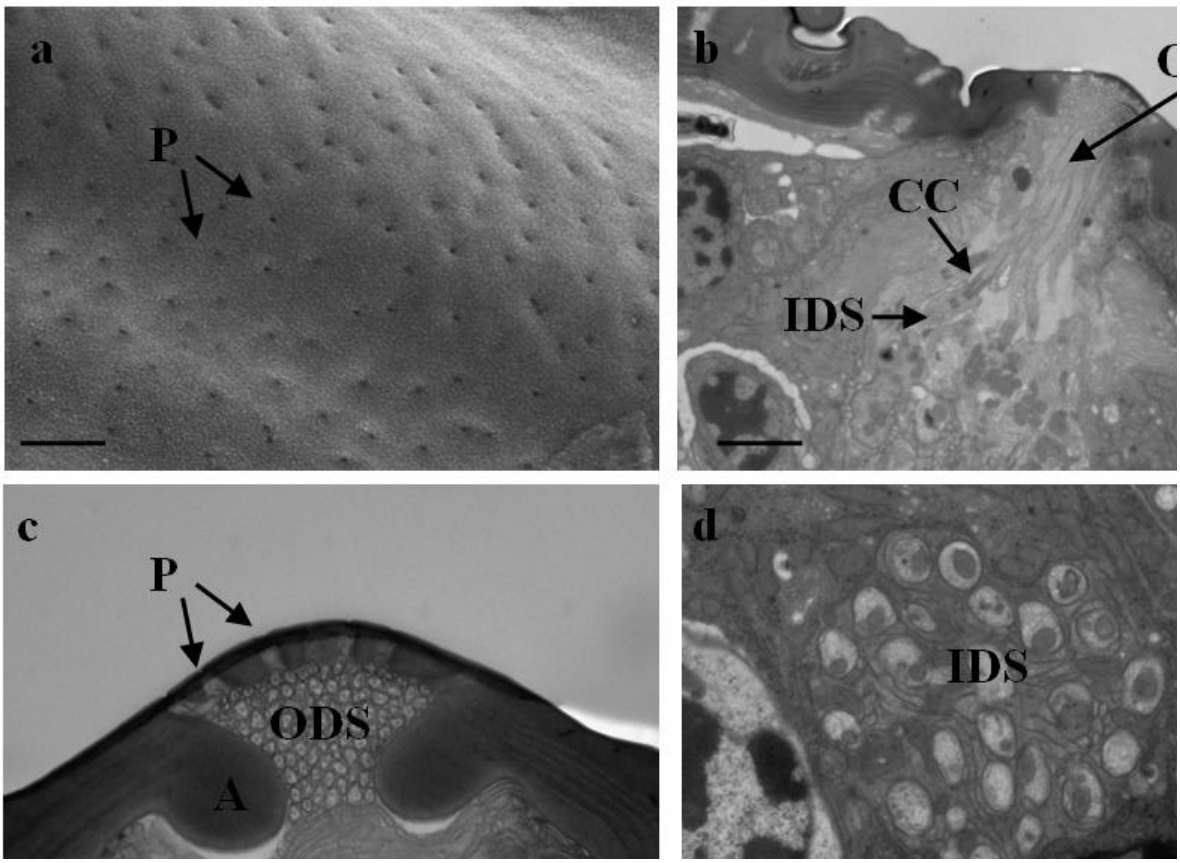


Figure 3.5 Multiporous Plate Sensilla (MPS). a) SEM pictures multiporous surface; b) TEM cross section showing cellular and cuticola components; c) TEM cross section showing the dentritic branches adjacent to the porous surface; d) cross section showing inner dendritic segments (IDS), ciliar constriction (CC) and outer dendritic segments (ODS). P=pore; A=apodema. Scale bar: a=400nm; b,d=2 µm; c=1 µm

3.4.4 Coeloconic Sensilla (CS)

As already described by Romani *et al*. (2010) coeloconic sensilla are usually in relatively low numbers when compared with the other antennal sensory structures, with a defined distribution on the antennal segments. In fact in this work 7 CS are counted, one each segment (Fig. 3.6a), on the distal external part from A8 to A13 and halfway of A14, where is possible to observe a suture line. The cuticular parts have a small and clavate peg with walls proximally smooth, distally grooved and multiporous along the groves except on the tip (Fig. 3.6c), and set in round, shallow pit wider at the bottom than at its opening (Fig. 3.6b). The peg, 6,25 µm long, is completely embedded within the antennal wall, in an ellipsoidal shape of maximum amplitude of 8 µm.

TEM investigations show that each sensillum is innervated by four sensory neurons. One of them, near the peg base, tapers and terminates, while the other three enter the peg lumen and, still

enclose in a thick dendritic sheath originating at ciliary constriction's level and terminating where the peg grooves and porosity begin, run the peg lumen up to almost the tip without branching (Fig. 3.6d). Three accessory cells are present.

Morphologically speaking, these sensilla might be considered olfactory for their general configuration and for having a mutiporous peg (Roux *et al.*,2005),

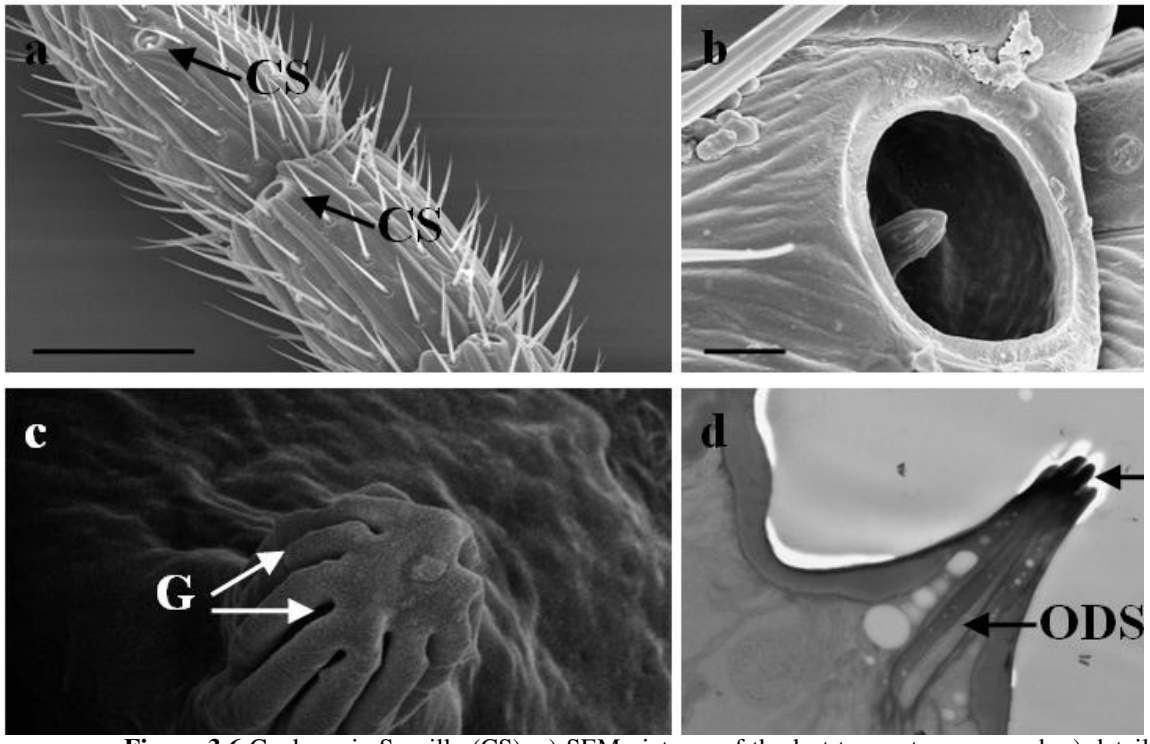


Figure 3.6 Coeloconic Sensilla (CS). a) SEM pictures of the last two antennomere; b-c) detail of CS; d) TEM longitudinal section of CS. G=groove-, ODS=outher dendritic segment. Scale bar: a=50 µm; b,d=2 µm, c=400 nm.

3.4.5 Styloconic Sensilla (SS)

There is one in the last seven antennomeres (Fig. 3.7), these sensilla are not articulated and their sensory portion is sunken and surrounded by the cuticular surface that comes in relief in an ellipsoidal shape of maximum amplitude of 8 µm (Fig. 3.7b). SEM observations do not show pores. A possible thermo-hygrosensitivity is associated, in agreement with other Hymenoptera previously observed (Roux *et al*. 2005; Altner and Prillinger, 1980).

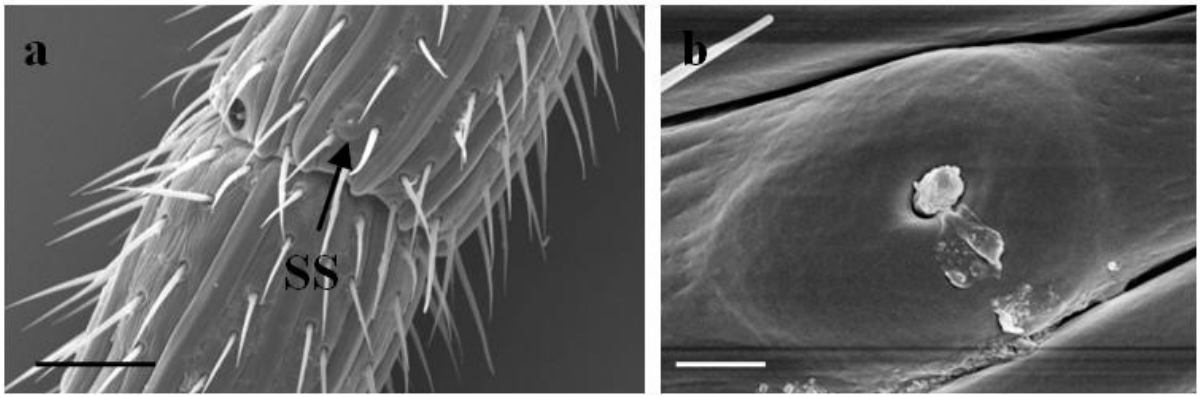
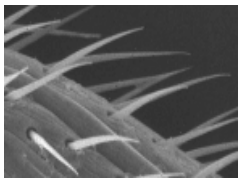
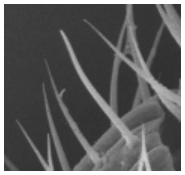
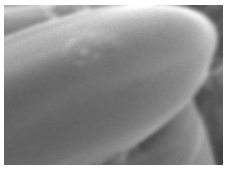
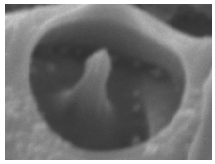
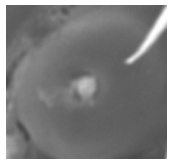


Figure 3.7 SEM micrograph of Styloconic Sensilla. a) A11 and A12 showing SS position; b) detail of SS. Scale bar: a=25 µm; b=2 µm.

A summary of some morphological properties of examined sensilla is shown in the following table:

					
	Sensilla Tricoidea ST	**Sensilla Chetica SCH**	**Multiporous Plate Sensilla MPS**	**Coeloconic Sensilla CS**	**Styloconic Sensilla CS**
Lenght	20-25 µm	25-30 µm	70-75 / 100-125 µm	6.25 µm	-
Diameter	-	-	-	8 µm	8 µm
Pores	aporous	probably one or few	multiporous	multiporous	aporous
Neurons	1	5 (4+1)	25	4 (3+1)	undetected

Tab. 3.1 Summary of morphological features of antennal sensilla in *Dryocosmus kuriphilus*

3.5 Conclusions

Characterization of *D. kuriphilus* female antenna through SEM and transmission electron microscopy (TEM) techniques permitted to highlight five kinds of sensilla:

- sensilla with a mechanosensory function (ST);
- sensilla suitable to perceive volatile chemical stimula, with an olfactory function (MPS and CS);
- sensilla with a mechano-gustatory function (SCH);
- sensilla with a possible thermo-hygrosensitivity (SS).

4 Natural control

4.1 Introduction

4.1.1 Natural enemies of cynipid gallwasps: chalcid parasitoid wasps

The natural enemies causing the highest degree of mortality in cynipid galls are chalcid parasitoid wasps (Hymenoptera, the superfamily Chalcidoidea) (Askew, 1984; Stone *et al*., 2002).

Six families of chalcids attack Western Palaearctic oak galls: Pteromalidae, Eurytomidae, Eupelmidae, Eulophidae, Ormyridae and Torymidae (Askew, 1984; Stone *et al*., 2002; Csóka *et al*., 2005).

Chalcids oviposit either close to or onto their host so larvae can feed either after prohibiting further growth by stinging (idiobionts) or as the host continues to develop (koinobionts) (Godfray, 1994; Hayward and Stone, 2005).

Most chalcids reared from cynipid galls are solitary idiobiont ectoparasitoids that feed suctorially on their host (Askew, 1961; Schönrogge *et al*., 1999). Chalcid species commonly attack a range of possible hosts inside the galls, i.e. gall formers and inquilines, but they also act as facultative hyperparasitoids and autoparasitoids attacking other chalcids of different or sometimes the same species (Askew, 1961; Askew and Shaw, 1974).

Some exceptions to these rules include *Sycophila biguttata* and the gregarious *Baryscapus berhidanus*, both idiobionts (Askew, 1984; Schönrogge *et al*., 1995). A range of parasitoid species are also at least partially herbivorous, feeding on gall tissues either before they feed on the host larva, for example, the phyto-entomophagous koinobiont, *Torymus cyanaeus*, or feeding on gall tissues as they move between host larvae within a single gall, for example, *Eurytoma brunniventris* (Askew, 1984).

The majority of chalcids exploit hosts in a wide range of oak gall types; few are restricted to just one oak gall type. Some chalcids that have broader host gall ranges over a wide geographical area can appear locally host gall specific. For example, *Aulogymnus skianeuros* in Britain (and probably over much of Western Europe) is specific to sexual generation galls of *Biorhiza pallida*, but in Eastern Europe it has also been reared from some 15 additional cynipid species, 9 of which also occur in Britain. *Aulogymnus trilineatus* is at the edge of its range and rare in Britain where it is known to attack only asexual generation *Andricus foecundatrix*, but on the continent it is recorded from a broad range of 29 types, 8 of which are found in Britain (Fulmek, 1968).

Most of the parasitoid species found in cynipid galls on oaks will not attack hosts even in cynipid galls on other plants, i.e. this community is relatively closed. However, a minority of species, such as *Eupelmus urozonus* and *E. vesicularis* (Eupelmidae), are exceptionally polyphagous and attack a range of endophytic hosts in a number of insects (Askew, 1984). A few chalcid species also occasionally attack cynipid galls on plants other than oak (often *Rosa*) (Askew, 1984).

For example, *Torymus flavipes*, *Eurytoma pistacina*, *Hobbya stenonota*, *Mesopolobus amaenus* and *Baryscapus pallidae* have also been recorded from rose galls of *Diplolepis mayri* (Pujade-Villar, 1992; Askew *et al*., 2006). Some parasitoids, particularly eulophid species, are occasionally reared from oak galls despite being more generally associated with communities centered on leaf-mining hosts (e.g. *Cirrospilus* species, *Pediobius pyrgo*, *P. saulius*, *Closterocerus trifasciatus*, *Minotetrastichus frontalis*).

However, these records appear to result from rare and opportunistic ovipositions as are those of a few ichneumonid (Ichneumonoidea) species that only occur sporadically (Fulmek, 1968; Schönrogge *et al*., 1996).

4.1.2 Natural control of *D. kuriphilus* in Asia

D. kuriphilus in its native country is controlled by many natural enemies.

A total of 11 species, belonging to 5 chalcid families (Torymidae, Ormyridae, Eurytomidae, Eulophidae and Eupelmidae), are known parasitoids of *D. kuriphilus* in China (Murakami *et al.*, 1980).

A summary of the natural enemies associated with *D. kuriphilus* in its native area and in the closer asian States is hereby provided. The table 2 shows the parasitoid species attacking *D. kuriphilus* in Asia (China, Japan and Korea).

	China	Japan	Korea
Chalcids			
Amblymerus sp.		X	
Arthrolytus usubai		X	
Caenacis peroni		X	X
Cecidostibafushica		X	
Cecidostiba semifascia		X	
Cynipencyrtus flavus		X	
Cynipencyrtus sp.		X	
Eupelmus urozonus	X	X	X
Eupelmus sp.		X	X
Eurytoma brunniventris	X	X	X
Eurytoma schaeferi		X	
Eurytoma setigera	X	X	X
Megastigmus maculipennis	X	X	X
Megastigmus nipponicus	X	X	X
Mesopolobus yasumatsui		X	
Ormyrus flavitibialis		X	
Ormyrus pomaceus (=O. punctiger)	X	X	X
Pteromalus apantelophagus			
Sycophila concinna		X	
Sycophila variegata	X		
Tetrastichus sp.	X	X	X
Torymus beneficus	X	X	
Torymus geranii		X	
Torymus sinensis local strain	X	X	X
Torymus koreanus	X	X	X
Torymus sp.		X	X
Non-chalcid parasitoids		X	X
Aspilota yasumatsui			
Cynipid inquiline		X	
Synergus sp.			
		X	

Tab. 1 Parasitoids attacking *D. kuriphilus* in Asia (Modified from Aebi *et al*., 2006 and Matsuo *et al.*, 2011)

Native *T. sinensis* was recorded both from Japan and from Korea (where it has never been imported). However, the local population of *T. sinensis* of both States differs in adult emergence phenology from wasps imported from China and it's thought to be native to its country (Aebi *et al*., 2006).

In Japan and Korea this *T. sisnensis* local has not been effective in controlling *D. kuriphilus*, so as to make useful the introduction of the Chinese strain.

4.1.3 Natural control of *D. kuriphilus* in Italy

Some transpalaearctic species of parasitoids that are also effective parasitoids of *D. kuriphilus* in Asia are very common and widespread in oak cynipid galls in Europe.

Some of them have already shifted on the new host and infact they have been reared from galls of *D. kuriphilus* in Italy, as shown in the table below.

Species	Family	Aebi *et.al.*, 2007	Speranza *et al.*, 2009	Santi and Maini, 2012)	Quacchia *et al.*, 2012
Aprostocetus sp.	Eulophidae				X
Aulogymnus arsames	Eulophidae				X
Aulogymnus sp.	Eulophidae				X
Baryscapus pallidae	Eulophidae	X			
Baryscapus sp.	Eulophidae				X
Pediobius chilaspidis	Eulophidae				X
Pediobius saulius	Eulophidae				X
Pediobius sp.	Eulophidae				X
*Eupelmus annulatus**	Eupelmidae				X
*Eupelmus spongipartus**	Eupelmidae				X
Eupelmus splendens	Eupelmidae				X
Eupelmus urozonus	Eupelmidae	X	X		X
Eupelmus fulvipes	Eupelmidae				X
Eurytoma brunniventris	Eurytomidae	X	X		X
Eurytoma adleriae	Eurytomidae				X
Eurytoma pistaciae	Eurytomidae	X			X
Sycophila variegata	Eurytomidae	X			X
Sycophila biguttata	Eurytomidae	X	X		X
Sycophila iracemae	Eurytomidae	X			
Ormyrus nitidulus	Ormyridae				X
Ormyrus pomaceus	Ormyridae	X	X		X
Cecidostiba sp.	Pteromalidae				X
Mesopolobus amaenus	Pteromalidae				X
Mesopolobus mediterraneus	Pteromalidae	X			
Mesopolobus sericeus	Pteromalidae	X	X		X
Mesopolobus tarsatus	Pteromalidae	X			X
Mesopolobus tibialis	Pteromalidae				X
Megastigmus dorsalis (sp1)	Pteromalidae	X	X		X
Megastigmus dorsalis (sp2)	Pteromalidae				X
Torymus auratus	Torymidae	X			
Torymus flavipes	Torymidae	X	X	X	X
Torymus scutellaris	Torymidae	X			
Torymus erucarum	Torymidae		X		X
Torymus geranii	Torymidae			X	X

Tab. 2 Parasitoids attacking *Dryocosmus kuriphilus* in Italy

In few years parasitoid communities based around invading *D. kuriphilus* have developed, involving species shared with local populations of oak gallwasps - typically those with broad host ranges - and have reached a remarkable level of species richness.

In comparison with the invading oak cynipids, *D. kuriphilus* is recruiting natural enemies rather rapidly. For example, the asexual generation galls of the invader *Andricus quercuscalicis* in Britain were free of parasitoids and inquilines for about 20 years (Schonrogge *et al*., 2000).

In addition to the rapid recruitment of parassitoids, an other striking feature of the *D. kuriphilus* community is the absence of cynipid inquilines - a major component of the communities associated with most oak cynipid galls (Stone *et al.* 2002; Aebi *et al*., 2006).

The cynipid inquilines (tribe Synergini) feed only on gall tissue. Although unable to induce their own gall, they have some ability to modify the plant tissue that immediately surrounds them (Stone *et al.*, 2002). If these important constituents of the oak gall community were to start utilising chestnut galls, we could also expect them to bring along greater parasitoid diversity (Schönrogge *et al*., 1996) and hence tighten further the trophic links between oak and chestnut gall communities (Quacchia *et al*., 2012)

While in its native distribution *D. kuriphilus* populations are kept at low densities by natural enemies; in regions where it has invaded (Japan, South Korea and the USA), the attack rates of indigenous parasitoid species have remained low many years after the arrival of the pest (typically <2%) (Murakami *et al*., 1995; Stone *et al*., 2002; Aebi *et al*., 2007).

In Italy galls of *D. kuriphilus* were reared to census natural enemy recruitment immediately after its discovery in 2002, 2003, 2004 and 2005, and community richness has grown steadily over the years.

However, studies of parasitoids in various Italian regions showed a low impact on the overall population dynamics of gall wasp chestnut, rarely reaching the threshold of 2% of parasitization (Aebi *et al*, 2007).

In Piedmont, where the percentage of parasitization (number of parasitoids / number of larval gall cells) was very low, the most abundant species were *Megastigmus dorsalis* (F.) and *Eupelmus urozonus* Dalman (Quacchia *et al*, 2011).

Instead, in Emilia Romagna *Torymus flavipes* (Walker) soon appeared as the dominant species of parasitoids of *D. kuriphilus*, arriving in 2011 at a rate of parasitization checked in the gall of 31.75% (Santi and Maini, 2011).

The rapid recruitment of oak cynipid parasitoids to *D. kuriphilus* suggest that, despite current low attack rates, there may be some value in an augmentative or conservation biological control programme using native species (Aebi *et al*., 2007)

4.1.4 Notes on Calcidoidea recruited in Italian chestnuts

Here I briefly introduce the families of parasitoids found in Italy from galls of *D. kuriphilus*.More detailed elements on the genera and species found in the present work are given in the Appendix.

Family Torymidae is a family of wasps that consists of attractive metallic species with enlarged hind legs, and generally with a long ovipositor. Many are parasitoids on gall-forming insects, and some are phytophagous species. In the Torymidae only two subfamilies are recognised: Megastigminae and Toryminae.

A number of *Torymus* species appears to be monophagous and associated with only one host-plant. Some attack several hosts upon one host-plant, or on different host-plants belonging to the same genus (*T. auratus* on *Quercus* spp.). In general, a given species tends to limit its attacks to hosts of a given family, e.g. Diptera Cecidomyiidae or Hymenoptera Cynipidae but some cases are known of species that attack hosts in both groups, usually on the same plant species Askew (1961)

The genus *Torymus* comprises phytophagous species and parasitoids of many Orders (Coleoptera, Diptera, Hemiptera, and Hymenoptera). It is associated with a large number of plants. Some species are hyperparasitoids of Cinipidae, Eulophidae and Ichneumonidae. Can also be host of parasitoids. (see Appendix Tab 1)

Some species belonging to the genus *Megastigmus* can be phytophagous (mainly seed-infesting of conifers); others are parasitoids of insects belonging to the orders Diptera, Hymenoptera and Hemiptera (families Eulofidae, Eurytomidae and Pteromalidae); and sometimes they act as hyperparassitoids (for example of the genus *Torymus*, of *Eupelmus urozonus* and of *Eurytoma brunniventris*) (see Appendix Tab 6).

Family Pteromalidae is a very wide and diverse chalcidoid family, with more than 31 subfamilies and around 4,200 described species: It is completely artificial, composed of numerous, distantly related groups (polyphyletic).

Genus *Mesopolobus* is cosmopolitan genus, widespread in the Western Palearctic, were it counts 40 known species (Boucek , 1988).

It includes species with different diet specializations: some are parasitoids (of Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera), others are hyperparasitid (of Diptera and

Hymenoptera, even of species of genus *Mesopolobus*). It can be prey of parasitoids such as *Eupelmus urozonus*, *Mesopolobus fasciventris* and *Torymus auratus* (see Appendix Tab 9).

Family Eurytomidae is a family within the superfamily Chalcidoidea. The group is apparently polyphyletic, though the different subfamilies may each be monophyletic, and may be elevated to family status in the near future. As presently defined, there are some 1420 described species in 87 genera (en.wikipedia.org).

The genus *Eurytoma* comprises phytophagous species and parasitoids of many orders of insects; some species are hyperparasitoids. It is associated with a large number of plants (on the contrary *E. brunniventris* is associated only with plants of Fagaceae) (for details see Appendix Tab 20)

Thr Genus *Sycophila* includes species associated with cynipid galls, especially on *Quercus* spp., but even phytophagous species or associated with parasitoids, both as hyperparasitoid and as pray (for example *Sycophila* spp. is parasitized by *Ormyrus brunneipes* and *Eurytoma* sp. see Appendix Tab 17).

Family Ormyridae is a small family of chalcidoid wasps, represented by 2 genera and 74 species all over the world (Noyes, 2003): 13-15 species, belonging to the genus *Ormyrus* Westwood, live in the Palearctic region (Gomez *et al.,* 2006.) (for details see Appendix Tab 15).

Family Eupelmidae is a chalcidoid family counting around 900 species, which parasitize Hymenoptera, Coleoptera, Lepidoptera and other insect and spider orders (Nieves-Aldrey & Fontal-Cazalla, 1999). In the Palearctic region this family is represented by 2 genera and 14 species (Gomez *et al*., 2006.).

Eupelmus spp. is the most widespread genus, with about 265 species all over the world (Gibson,1995): 13 species are represented in the Palearctic region. This species are generally polyphagous, widespread but also belonging to the parasitoid community associated with cynipid gall wasps (Askew & Nieves-Aldrey, 2000; Gomez *et al*., 2006). (for details see Appendix Tab 21).

4.2 Aim

The aim of this work is to investigate, in the chestnut orchards considered and in the spontaneous vegetation surrounding them, the presence of a community related to the chestnut gall wasp *D. kuriphilus*, so that could be evaluated the composition and the amount of the parasitoids present.

Some of these parasitois could be used as biological control agents, for example in augmentation biological pest control.

4.3 MATERIALS AND METHODS

4.3.1 Description of sites

Parasitoids were reared from galls of *D. kuriphilus* collected during years 2010 and 2011. Galls were collected from four sites in central Italy consisting of private fruit chestnut.

Two sites belong to Marche region; they are regarded as "Settlement Areas" by the Plant Protection Service (D.D. n. 579 – 06/08/2010; D.D. n.171 -24/02/2012).

They are: Site A - San Pietro, hamlet of Ascoli Piceno municipality (AP) and Site B – Montemonaco (AP).

Site A:
GPS coordinates*:*
Lat. 42.8295694002
Lon. 13.5850990736
Altitude*:* 597 s.l.m.
Exposure: Nord-Est.

Site B
GPS coordinates**:**
Lat. 42.9143182454
Lon. 13.3338691371
Altitude**:** 797 m s.l.m.
Exposure: Nord-Ovest.

The other two sites were located until 2009 in Regione Marche too, in Pesaro- Urbino district, but after Act n. 117 of 03/08/2009 the town of Novafeltria have been aggregated, together with the municipality of Casteldelci, Maiolo, Pennabilli, San Leo and Sant' Agatha Feltria and Talamello, in Emilia Romagna. Now they are subject to phytosanitary regulations by Emilia-Romagna region (D.R. n. 1735 – 23/02/2010) and they belong to a "Settlement Area" too.
They are Site C – Uffogliano, Novafeltria (RN) and Site D - "Piedimonte," Novafeltria (RN).

Site C

GPS coordinates:

Lat. 43.9563361651,

Lon. 12.3268215899

Altitude: 417 m s.l.m.

Exposure: Ovest, Nord-Ovest.

Site D:

GPS coordinates**:**

Lat. 43.90993599061048

Lon. 12.259540557861338

Altitude**:** 572 m s.l.m.

Exposure**:** Nord-Est, Nord-Ovest

All four sites are chestnut orchards with large solitary elements mutually spaced to form a sparse wood . The distance between trees is big enough to allow the growth plants in their “solitary” shape. There is the presence of some new chestnuts. The undergrowth is only herbaceous because of the periodic mowing grass. Slope less than 5%. The orchards are sourrounded by chestnut forest and mixed hardwood forest for timber.

The chestnut orchards are of privately owned. The owner allows, during the period of ripening, self-managed collecting by the customers. Management of the site is typical of the orchard, rather than a forest. Frequent works are held such as pruning, mowing - during the fruit harvesting- and grafting. Phytosanitary treatment are not carried out.

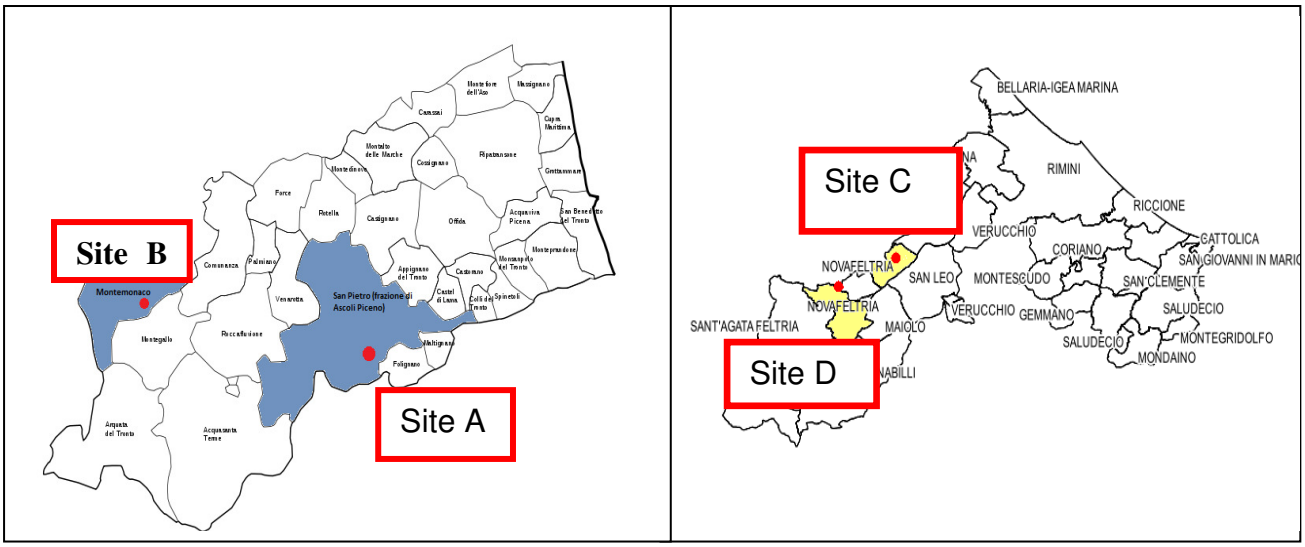


Fig. 4.4. Location of survey sites in the provinces of Ascoli (Site A and B) and Rimini (Site C and D).

4.3.2 Collection of *D. kuriphilus* galls on plants of *C. sativa* - Year 2010

The collection of galls was carried out randomly inside the chestnuts.
It took places on 30/06/2010 in site A - San Pietro, on 02/07/2010 in site B – Montemonaco and in Site C – Uffogliano, and finally on 24/06/2010 in site D – Piedimonte.

In each chestnut 1000 galls were randomly collected by hand from low branches and with the aid of lopping shears from the medium-high canopy. The galls were first stored in plastic bags up to the laboratory. They were separated from any non-gall plant material to avoid contamination by other insects and then were put in cardboard boxes (14 cm x 22 cm x 12 cm) with extractable skylights. Multiple galls (up to 40) were kept in every container. They were maintained in the laboratory to natural light and at room temperature. The tubes were inspected weekly until the first parasitoid emerged, and daily afterwards. The emerged parasitoids were collected using a brush, photographed at stereomicroscope and divided in males and females and in groups on the bases of morphological characteristics. Then they were placed in eppendorf with absolute ethanol and finally stored in freezer at -20 ° C.The parasitoids were then sent to Prof. Alberto Alma of DIVAPRA - Entomology and Zoology applied to the Environment "C. Vidano", University of Turin, for the identification.

The number of emerged adults of each parasitoid species was counted and a standardised emergence rate was calculated by dividing the number of emerged adults by the number of galls sampled in that collecting period. As *D. kuriphilus* galls are multilocular, emergence rate is not a measure of the exact rate of parasitism.
In order to evaluate the rate of parasitism, it has been considered that a gall may contain from 1 to 35 larvae of *D. kuriphilus*, with an average of 3.5 larvae/gall (Aebi *et al*., 2011).

4.3.3 Collection of galls on wild vegetation – Year 2010

In the survey sites I searched for galls on border vegetation.

Galls collection was made with the ausilum of lopping shears when I needed to reach the highest galls in the canopy.

The galls were stored in plastic bags up to the laboratory.Then were put in cardboard boxes (14 cm x 22 cm x 12 cm) with extractable skylights.

The emerged parasitoids were collected using a brush. Then they were placed in eppendorf with absolute ethanol and finally stored in freezer at -20 ° C. The parasitoids were then sent to Prof. Alberto Alma of DIVAPRA - Entomology and Zoology applied to the Environment "C. Vidano", University of Turin, for the identification.

4.3.4 Collection galls of *D. kuriphilus* on plants of *C. sativa* - Year 2011

The collection of galls was carried out twice a year , the first time at the end of May (hereinafter referred to as “spring collection”) and the second in the second decade of June (hereinafter referred to as “summer collection”).

In each of the four sites and in both periods have been collected 1000 galls (except for Site D in which has been carried out the only spring collection.

Spring collection has been made in site A on 23/05/11, in site B on 20/05/2011 and in sites C and D on 26/05/2011.

Summer investigations have been carried out on 16/06/2011 in site A and B, and on 14/06/11 in site C.

The emerged parasitoids were first divided in groups on the bases of morphological characteristics, Then they were sent to Prof. Alberto Alma of University of Turin, for the identification, as made in year 2010.

Emergence rate and rate of parasitism have been calculated as shown above.

4.3.5 Collection of galls on wild vegetation- Year 2011

In 2011 too I searched for galls on border vegetation.

Collection and storage were done with the same modalities of year 2010.

4.3.6 Vegetational surveys

In correspondence of every site, vegetational surveys were made around the border vegetation on trees and shrubs following the cover-abundance scale of Braun-Blanquet (1932).

According to the Braun-Blanquet floristic-sociological approach plant-community types are conceived as units recognized by their total floristic composition.

In detail, this method develops in different stages (Pignatti, 1982):

1. Choice of sample plots. Within a landscape representative element, an area with floristic, geomorphologic and ecological homogeneous features was chosen. The minimum representative surface is called “minimal area”.

In the present case study, the vegetation next to the edge of every chestnut orchard was characterized.

2. Location data. In addition to the serial number of the survey, name of the locality and date, also height, slope rate, exposure were recorded.

3. Vegetation features. This phase consists in the observation and recording of vegetation structure, dominant species, presence of different vegetation layers, height and cover percentage of the layers, and total cover percentage of the sample plot.

4. Floristic list. Plants were collected in the field, and conserved in nylon bags in a cooler, until arrival at the laboratory. There, plants were dried in absorbent paper under a press. Plants were then positioned in a herbarium until their identification with the taxonomic keys of the “Flora d’Italia” from Pignatti (Pignatti, 1982). A table with the complete floristic list was then composed.

5. Cover-abundance coefficients assignment. Two phytosociological values were assigned to every species: the first represents abundance-dominance, the second sociability. The abundance is an estimate of the number of individuals of every species present in the survey; dominance (or coverage) is an evaluation of the surface covered by a species. In multilayered groupings, abundance-coverage has to be evaluated layer by layer.

For every species, the cover-abundance value can have the following values:

r : rare and sparse individuals;

+ : not abundant with low coverage (< 1%) individuals;

1 : more abundant with weak coverage (1-5%) individuals;

2 : abundant with coverage between 5 and 25%;

3 : abundant with coverage between 25 and 50%;

4 : abundant with coverage between 50 and 75%;

5 : abundant with coverage between 75 and 100%.

Sociability represents the disposition of each species in a population, and is linked to the biological type of every species. The sociability scale has five values:

1 : isolated individuals;

2 : grouped individuals;

3 : individuals in small colonies;

4 : populations extended on more than half of the sample plot;

5 : pure (or almost pure) populations.

6. Phytosociological Table. Plant species were then grouped by families in tables (one for every site and every relief), each one reporting the corresponding cover-abundance coefficients and all the location data previously mentioned.

During the vegetational surveys particular attention was made to the presence of species belonging to Fagaceae and Rosaceae families, since they are potential hosts of gall inducers.

4.3.7 Infestation rate – Year 2011

Infestation rate was recorded on one year old braches as a percentage of infected buds (i.e. buds affected by the presence of a gall) over total amount of buds (Aebi *et al*., 2011).

In each area, 10 chestnut trees were randomly chosen. For each tree, 10 branches were randomly selected and galls and uninfected buds counted. Recorded data included length of the one year old branch, total number of buds originally produced, amount of infected buds and qualitative differentiation of the gall types. Galls were divided into four types: sessile gall (S) when there was no axis extension; foliar gall (F), when the gall was grown on a leaf; terminal gall (T) when there wasn't any further axis extension; median gall (M) when the branch had furher axis extension.

The chosen method is identical to the one used by many other authors (Aebi *et al*, 2011).

On the buds of these branches *D. kuriphilus* oviposition may have taken place during summer 2009 leading to gall formation in 2010.

4.3.8 Statistical analysis

Secondary sex ratios (the sex ratio of emerging adults) for the dominant species of parassitoid (H_0: 50:50 males:females) in each season, and infestation rates of the four sites (H_0: no difference between sites) were tested using a chi-square test.

4.4 Results and discussion

4.4.1 Collection of chestnut galls - Year 2010

The total number of parasitoids emerged in 2010 from chestnut galls collected in the Summer has been of 20 specimens overall, belonging to three chalcid (Hymenoptera: Chalcidoidea) families: Torymidae, Pteromalidae and Eupelmidae (Tab. 4.3).
The parasitoid species emerged more frequently was *Torymus flavipes* (six specimens).

Tab. 4.3 - Number of parasitoid specimens emerged from chestnut galls collected during Summer period, year 2010. Place A= Ascoli Piceno; Place B= Montemonaco (AP); Place C= Uffugliano (RN); Place D= Piedimonte (RN).

Parasitoid species	Family	Summer				
		Places				
		A	B	C	D	Total no
Eupelmus annulatus	Eupelmidae	0	0	1	1	2
Megastigmus dorsalis	Torymidae	1	0	0	0	1
Mesopolobus tarsatus	Pteromalidae	1	0	0	1	2
Mesopolobus tibialis	Pteromalidae	1	0	0	1	2
Mesopolobus sp.	Pteromalidae	1	0	0	2	3
Torymus auratus	Torymidae	1	0	0	0	1
Torymus flavipes	Torymidae	0	0	0	6	6
Torymus sp.	Torymidae	1	0	0	2	3
TOTAL SPECIMEN NUMBER		6	0	1	13	20

Emergence rate was calculated by dividing the number of emerged adults by the number of galls sampled in that collecting period.
The emergence rate was 0.5%.
Then, as *D. kuriphilus* galls are multilocular and it has been estimated an average of 3.5 larvae/gall (Aebi *et al*, .2011), the rates of estimated parasitism was 0,14%.

All of these parasitoid species have already been recruited in Italy by others authors (Aebi *et al*., 2006; Quacchia *et al.,* 2012).

4.4.2 Collection of chestnut galls - Year 2011

In total 1,923 native specimen parasitoids from the Superfamily Chalcidaidea emerged during 2011 year from 7,000 collected galls.

The total number of parasitoid specimens achieved in 2011 from the Spring collected chestnut galls (4,000) has been of 1,508. In detail 378 specimens emerged from galls collected at the site of Ascoli Piceno, 287 specimens emerged from galls collected at the site of Montemonaco and, respectively, 529 specimens and 314 specimens from the sites of Uffogliano and Piedimonte (Rimini province). The recorded specimens belongs to 6 chalcid genera: *Torymus*, *Mesopolobus*, *Megastigmus*, *Ormyrus*, *Sycophila* and *Eurytoma* (Tab. 4.4).

Tab. 4.4 - Year 2011 - Place A= Ascoli Piceno; Place B= Montemonaco (AP); Place C= Uffugliano (RN); Place D= Piedimonte (RN)

	Spring					Summer			
	Places					Places			
	A	B	C	D	Total no	A	B	C	Total no
Torymus flavipes	313	253	422	265	1253	90	166	28	284
Torymus sp.	24	5	57	35	121	11	6	4	21
Torymus geranii	0	0	2	0	2	0	1	0	1
Torymus erucarum	3	0	1	0	4	3	10	2	15
Mesopolobus tibialis	19	10	11	7	47	0	0	0	0
Mesopolobus spp.	10	15	14	0	39	3	3	14	20
Mesopolobus lichtensteini	0	0	0	0	0	1	0	0	1
Mesopolobus mediterraneus	0	0	1	0	1	0	0	1	1
Mesopolobus amaenus	4	0	0	0	4	0	0	1	1
Megastigmus dorsalis	1	0	0	1	2	3	0	16	19
Ormyrus nitidulus	0	0	5	0	5	0	0	0	0
Ormyrus pomaceus	3	0	14	3	20	8	0	16	0
Sycophila spp.	1	2	0	2	5	0	0	0	0
Sycopila biguttata	0	0	0	1	1	2	2	0	4
Sycophila variegata	0	0	0	0	0	2	0	0	2
Eurytoma brunniventris	0	2	2	0	4	6	8	8	22
TOTAL NUMBER	378	287	529	314	1508	129	196	90	415

The number of parasitoids emerged from the summer collected chestnut galls has been lower of spring collected one: 129 specimens from Ascoli Piceno site, 196 specimens from Montemonaco

site and 90 specimens from Secchiano site, for a total of 415 parasitoid specimens emerged from 3,000 chestnut galls. They belong to the same genera recruited in Spring collection: *Torymus*, *Mesopolobus*, *Megastigmus*, *Ormyrus*, *Sycophila* and *Eurytoma*.

The total parasitoid species recovered belong to four Families of Chalcidoidea Superfamily, as shown in the following table (Tab. 4.5).

Tab. 4.5 - Fiveteen species of native parasitoids were found associated to *Dryocosmus kuriphilus*

Superf. Chalcidoidea	Fam. Torymidae	*Torymus flavipes*
		Torymus erucarum
		Torymus geranii
		Torymus sp.
		Megastigmus dorsalis
	Fam. Pteromalidae	*Mesopolobus* spp.
		Mesopolobus tibialis
		Mesopolobus mediterraneus
		Mesopolobus amaenus
		Mesopolobus lichtensteini
	Fam. Ormyridae	*Ormyrus pomaceus*
		Ormyrus nitidulus
	Fam. Eurytomidae	*Sicophila biguttata*
		Sicophila variegata
		Eurytoma brunniventris

In both collections have been achieved the same species except for *Ormyrus nitidulus*, that has emerged only from Spring collection galls.

The species *Mesopolobus lichtensteini* was not recorded from other studies (Aebi *et.al*., 2007; Speranza *et al*., 2009; Santi and Maini, 2012; Quacchia *et al*., 2012), so can represent a recent addition to the parasitoid community attacking *D. kuriphilus* in Italy.

For each site and for each collection period have been calculated the emergence rate and the rate of estimated parasitism (Tab. 4.6).

Tab. 4.6 - Emergence rate (number of parasitoid specimens/gall number) and parasitism rates (emergence rate/3.5). Place A= Ascoli Piceno; Place B= Montemonaco (AP); Place C= Uffugliano (RN); Place D= Piedimonte (RN). Ns= no sampling.

	A	B	C	D	AVERAGE
	SPRING				
Emergence rate	37.80	28.70	52.90	31.40	37.70
Rate of estimated parasitism	10.80	8.20	15.11	8.97	10.77
	SUMMER				
Emergence rate	12.90	19.60	9.00	ns	13.83
Rate of estimated parasitism	3.69	5.60	2.57	ns	3.95

The general emergence rate has been 37.70 % (Spring) and 13.83 % (Summer).

These data strongly differ from most of those found on bibliography (Stone *et al*., 2002; Aebi *et al.*, 2007; Quacchia *et al*., 2012) which arrange the emergence rate under or across 2-3% threshold.

The rates of estimated parasitism rates has been of 10.77 % for the Spring collected galls and 3.95 % for the Summer collected galls.

From Spring collected galls have emerged parassitoids of the following species:

- *T. flavipes*: 313 specimens (223 f, 90 m) (f= females; m=males) from site A, 252 (197 f, 56 m) from site B, 422 (351 f, 71 m) from site C and 265 (224, 41) from site D;
- *Torymus* sp.: 24 specimens (5 f, 19 m) from site A, 5 (1f, 4m) from site B, 57 (3 f, 54m) from site C and 35 (14 f, 21 m) from site D;
- *T. erucarum*: 3 specimens from site A (2f, 1m), none from site B, only a female from site C and none from site D;
- *T. gerani*: have emerged only two females from site C;
- *Mesopolobus* spp.: emerged 10 females from site A, 15 from site B,14 from site C and none from site D;
- *Mesopolobus tibialis*:19 males from site A, 10 from site B, 11 from site C and 7 from site D;
- *M. lichtesteini*: no specimen has emerged;
- *M. mediterraneus*: only a male emerged from site C;
- *M. amaenus*: emerged only from site A (4 m);

- *Megastigmus dorsalis*: a male from site A and a female from site D;
- *Ormyrus nitidulus*: only from site C (1f, 4m);
- *O. pomaceus*: 3 specimens from site A (females), none from site B, 14 from site C, 3 females from site D;
- *Sicopyla* spp.: emerged a female from site A, 2 specimen from site B (1f, 1m), none from site C and 2 females from site D;
- *Sycophila biguttata*: emerged only a female from site D;
- *Eurytoma brunniventris*: emerged 2 males from site B and 2 females from site C.

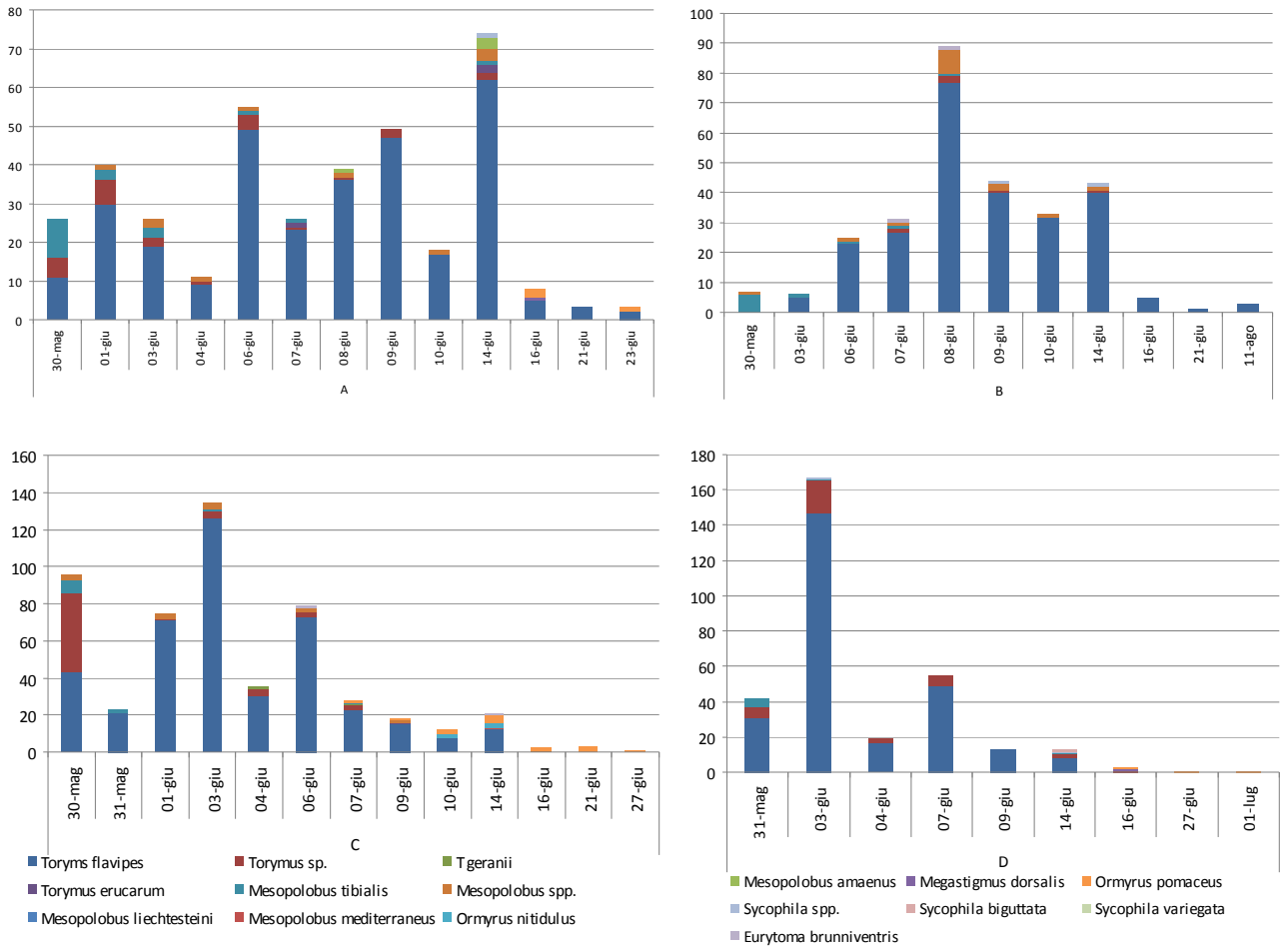


Fig 4.5 Parassitoids emerged from site A, B, C and D and emergency time, in Spring collection.

From Summer collection have emerged parassitoids of the following species:

- *T. flavipes*: 90 specimens 87 f, 3 m) from site A, 166 (143 f, 23 m) from site B, and 28 (26 f, 2 m) from site C;
- *T.* sp.: 11 specimens (6f, 5m) from site A, 6 (3f, 3m) from site B, and 4 (3f, 1m) from site C;
- *T. erucarum*: three females from site A, 10 females from site B and 2 from site C;
- *T. gerani*: have emerged only a female from site B;
- *Mesopolobus* spp.: three females emerged from site A, 3 from site B, and 14 from site D;
- *M. lichtesteini*: only a male emerged from site A;
- *M. mediterraneus*: only a male was found from site C;
- *M. amaenus*: only a male emerged from site C;
- *Megastigmus dorsalis*: three specimens from site A (1f, 2m), none from site B 16 (8f, 8m) from site C;
- *O. pomaceus*: 8 specimens from site A (females), none from site B, 16 from site C;
- *Sycophila biguttata*: two females emerged from site A and 2 from site B, none from site C;
- *Sycophila variegata*: two females emerged from site A only;
- *Eurytoma brunniventris*: 6 specimens (4 f, 2 m) from site A, 8 (5 f, 3 m) from site B, and 8 (8 f) from site C.

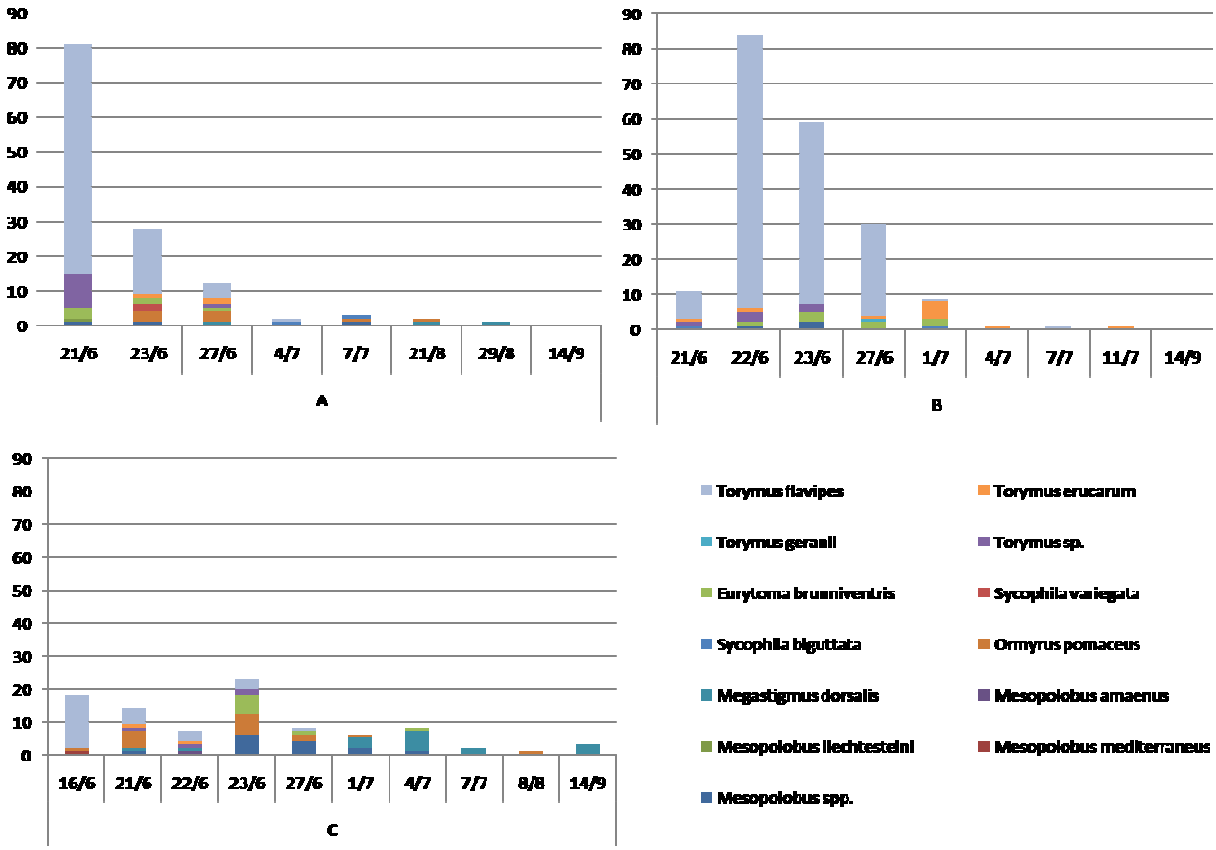


Fig 4.6 Parassitoids emerged from site A, B, C and D and emergency time, in Summer collection.

Both in Spring and in Summer period *T. flavipes* was the most frequent species recognized. In Spring its presence was assessed in 83.09 % of total parasitoid specimens (1253 specimens/1508 total emerged parasitoids), and was 68.43 % of total in Summer period (284 specimens/415 total emerged parasitoids).

In Spring collection the second most aboundant species was *Torymus* sp. (7.57 % , 121 specimens), followed by *Mesopolobus tibialis* (2.94 %, 47 specimens).

In Summer collection the second most aboundant species was *Eurytoma brunniventris* (5.31 %, 22 specimens) and then *Torymus s*p. with a percentage of 5.07 (21 specimens).

Torymus flavipesis has been the most abundant parasitoid species of *D. kuriphilus* in chestnut galls even in the Bologna area, as lightened by Santi and Maini (2011), where the parasitisation of *D. kuriphilus* larvae reached 31.75 % in 2011.

On the contrary in Norh-western Italy *T. flavipes* has been collected only occasionally (Quacchia *et al*., 2012).

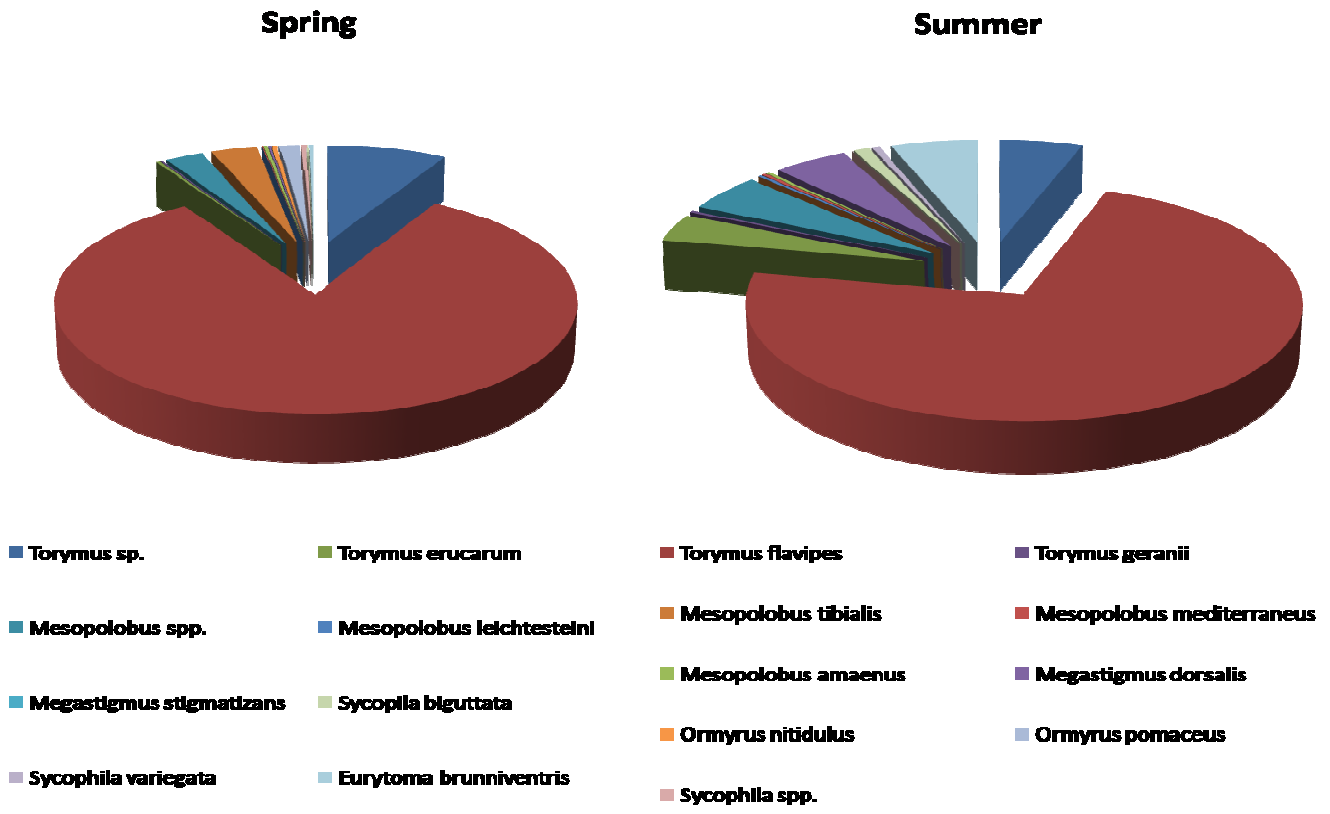


Fig 4.7 Graphs of parasitoid species abundance during 2011: Spring and Summer collections.

Moreover I have analyzed the *T. flavipes* female and male flight in all sites regarding Spring and Summer collections.

In Site A female emergence occurred from 30/05 to 04/07 with a series of peaks, the highest of which on 21/06 (63 specimens) (Fig. 4.8); male emergence occurred from 30/05 to 21/06, with a peak of 19 specimens on 06/06. In Site B there was flight of females from 03/06 to 07/07 with a single specimen emerged subsequently (on 11 August). The peak occurred on 22/06 with 63 females. For the males flight took place June 3 to 27, with a peak of 26 males on 8 June. From galls Site C we see the flight of the females from 30/05 to 27/06 with a maximum of 103 specimens emerged on 03/06. For males there was flight from 30/05 to 23/06, the peak was again on 03/06 (23 specimens). In Site D the flying time considered is shorter because it lacks the summer collection: female flight was began on 31/05 and ended on 14/06 with a peak on 03/06 (127 specimens), the flight of the males begins 31/05 and ends on 07/06 with a maximum of 20 units on 03/06.

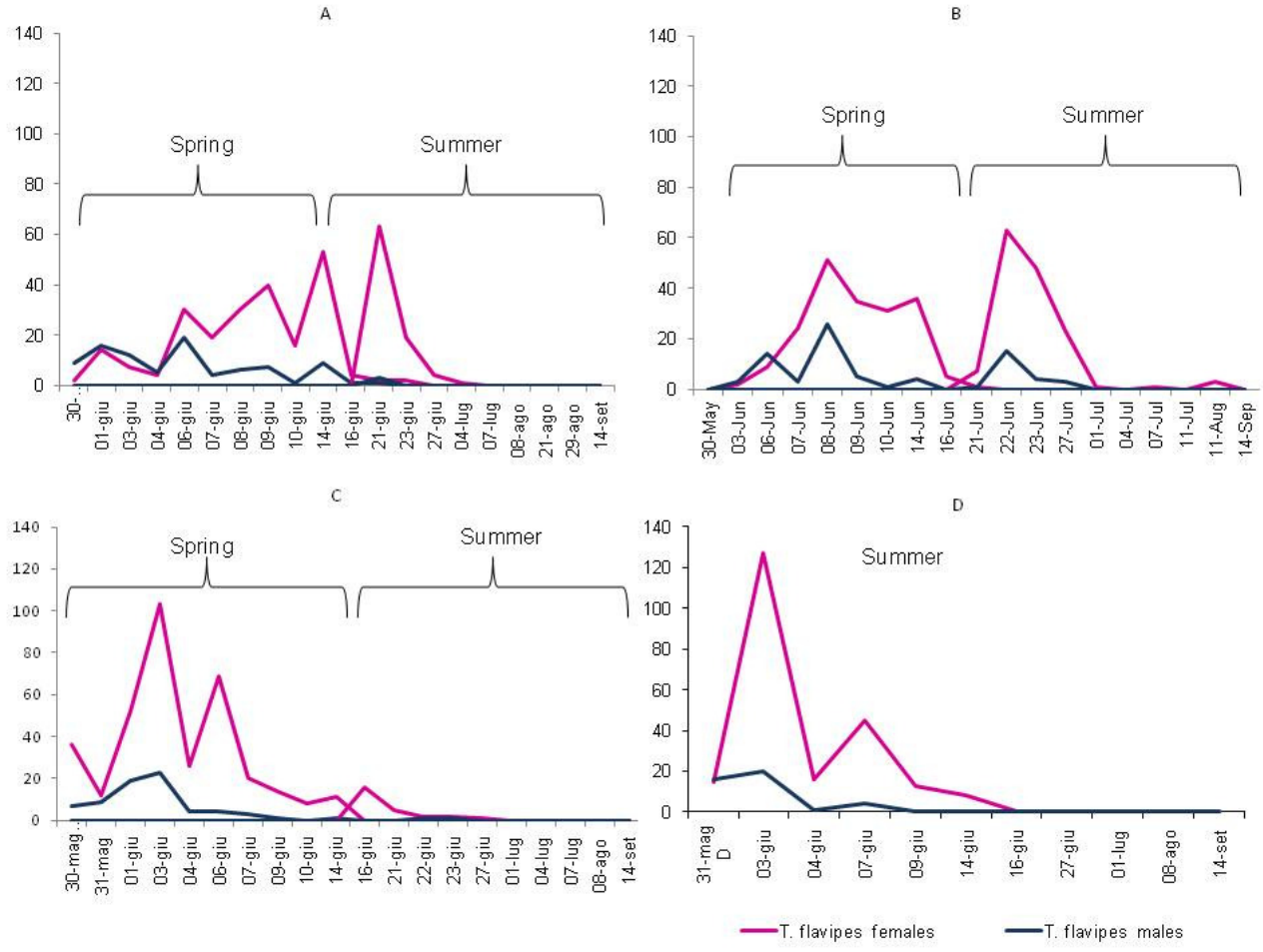


Fig 4.8 Population dinamic of *Torymus flavipes* males and females.

The greater emergence of female than of males of *T. flavipes* it is confirmed by sex ratio analysis that gave the following results:

- Spring total sex ratio (female/males: 995/258) = 3.86
- Summer total sex ratio (female/males: 257/27)= 9.52

Tab 4.7 - *Torymus flavipes* sex ratio observed during 2011 year. F= female number; M= male number. Place A= Ascoli Piceno; Place B= Montemonaco (AP); Place C= Uffugliano (RN); Place D= Piedimonte (RN). Ns = no sampling.

Places	Spring			Summer		
	F	M	χ^2	F	M	χ^2
A	223	90	$56{,}51^{***}$	87	3	$78{,}40^{***}$
B	197	56	$78{,}58^{***}$	144	22	$89{,}66^{***}$
C	351	71	$185{,}78^{***}$	26	2	$20{,}57^{***}$
D	224	41	$126{,}37^{***}$	ns	ns	-

The observed sex ratios were tested against an expected 50:50 ratio (all.d.f. = 1, ***P< 0.001).

We observed a *T. flavipes* female-biased sex ratio both in spring and summer period and it may be seen as a good fitting to the new host, in fact parasitoid mothers oviposit a greater proportion of daughters when a fitness increase is expected (Jones, 1982; King, 1995).

4.4.3 Collection of galls from wild vegetation - Year 2010

In the survey places I searched for galls on wild vegetation and I collected galls from oack plants (*Quercus* spp.), in order to study the parasitoids associated with these gall insects. The study has produced the following results:

In site A – Ascoli and site D – Piedimonte (respectly on 22/07 and 20/07) haven't been found galls; in site B – Montemonaco were kept 13 galls of *Andricus caputmedusae* from oaks on 30/07 ;

in Site C – Uffugliano have been found 1145 galls of *Neuroterus quercusbaccarum,* one gall of *A. kollari* aborted, 110 galls of *Andricus numismalis* and 5 galls of *Andricus caputmedusae.*

From galls collected emerged only two parasitoid specimens from the galls collected at place C (Uffugliano, RN). In particular: one *Torymus sp.* specimen emerged from an aborted gall of *Andricus kollari* and one *T. flavipes* emerged from a *Neuroterus quercusbaccarum* gall.

4.4.4 Collection of galls from wild vegetation - Year 2011

The collection of galls on wild vegetation at end May-middle June 2011has permitted to obtain some specimen of cynipid galls from oaks.

In Site B have been collected 19 specimens of *Andricus caputmedusae*, 3 of *A. quercuscustozae*, and 4 of *A. kollari.*

From Site C galls have emerged 7 *Andricus ostrea* and 20 *Neuroterus lanuginosum*

From site D have emerged 8 specimens of *Andricus caputmedusae* and 2 of *A. ostrea.*

On the contrary in Site A no oaks galls have been found.

From the 19 galls of *A. caputmedusae* collected in the place B (Montemonaco, AP) emerged 3 parasitoid specimens belonging to two species: *Megastigmus stigmatizans* (two specimens) and *Ormyrus nitidulus* (one specimen).

Megastigmus stigmatizans hasn't yet been found associated with *D. kuriphilus* on chestnut.

O. nitidulus has also emerged from chestnut galls of *D. kuriphilus* from place C (Uffugliano, RN) from chestnut gall collected during the Spring.

O. nitidulus has been reported associated to *D. kuriphilus* in North-Western Italy (Quacchia *et. al.*, 2012).

4.4.5 Infestation rate – Year 2011

As shown in the following Table 4.8, infestation rate was recorded on 10 trees in each study site in Autumn 2011 and gave the following results: in site A, the infestation rate was 79.95% and the total number of collected galls was 572; the most widespread kind of gall was foliar (33.57%).

In Site B the percentage of infestation was 63.04 with 215 total galls, the most frequent galls are the foliar one (85/215).

In site C the percentage of infestation was 67.77 with 287 galls, of which 130 of the median type.

In Site D the infestation rate was 81.14% with the prevalence of median galls (153/357).

	tree	healthy buds	infected buds	bud total number	gall position				gall total number	infestation rate
					S	F	T	M		
Site A	1	5	27	32	7	15	10	12	44	84.38
20/09/12	2	17	11	28	6	6	3	7	22	39.29
	3	9	23	32	7	17	0	16	40	71.88
	4	7	40	47	18	4	10	27	59	85.11
	5	8	36	44	13	24	0	21	58	81.82
	6	5	30	35	5	17	16	23	61	85.71
	7	4	26	30	0	37	0	24	61	86.67
	8	14	40	54	12	36	10	19	77	74.07
	9	2	36	38	0	30	16	34	80	94.74
	10	5	34	39	0	6	34	30	70	87.18
	tot	76	303	379	68	192	99	213	572	79.95
Site B	1	5	12	17	0	7	5	6	18	70.59
27/09/12	2	5	18	23	0	25	2	2	29	78.26
	3	9	9	18	0	4	2	5	11	50.00
	4	7	19	26	0	1	7	14	22	73.08
	5	10	15	25	0	3	7	5	15	60.00
	6	14	21	35	0	29	6	6	41	60.00
	7	8	15	23	0	1	6	10	17	65.22
	8	11	15	26	0	2	5	14	21	57.69
	9	14	16	30	0	4	10	2	16	53.33
	10	12	22	34	0	9	8	8	25	64.71
	tot	95	162	257	0	85	58	72	215	63.04
Site C	1	9	17	26	3	11	0	8	22	65.38
26/10/12	2	10	17	27	1	7	5	15	28	62.96
	3	11	18	29	6	2	5	13	26	62.07
	4	14	15	29	2	4	1	11	18	51.72
	5	13	10	23	1	5	1	4	11	43.48
	6	2	31	33	13	5	1	14	33	93.94
	7	12	17	29	5	10	3	4	22	58.62
	8	7	32	39	22	3	11	15	51	82.05
	9	9	28	37	8	2	9	34	53	75.68
	10	10	19	29	5	3	3	12	23	65.52
	tot	97	204	301	66	52	39	130	287	67.77
Site D	1	13	38	51	7	20	6	12	45	74.51
26/10/12	2	5	34	39	2	10	4	22	38	87.18
	3	11	31	42	4	11	0	22	37	73.81
	4	1	27	28	3	15	4	10	32	96.43
	5	3	31	34	8	25	1	11	45	91.18
	6	12	25	37	6	8	4	15	33	67.57
	7	4	20	24	5	3	8	10	26	83.33
	8	5	28	33	0	11	6	20	37	84.85
	9	6	26	32	1	9	2	17	29	81.25
	10	6	24	30	0	17	4	14	35	80.00
	tot	66	284	350	36	129	39	153	357	81.14

Tab.4. 8 – Infestation rate recorded on 10 chestnut trees randomly chosen in sites A, B, C and D.

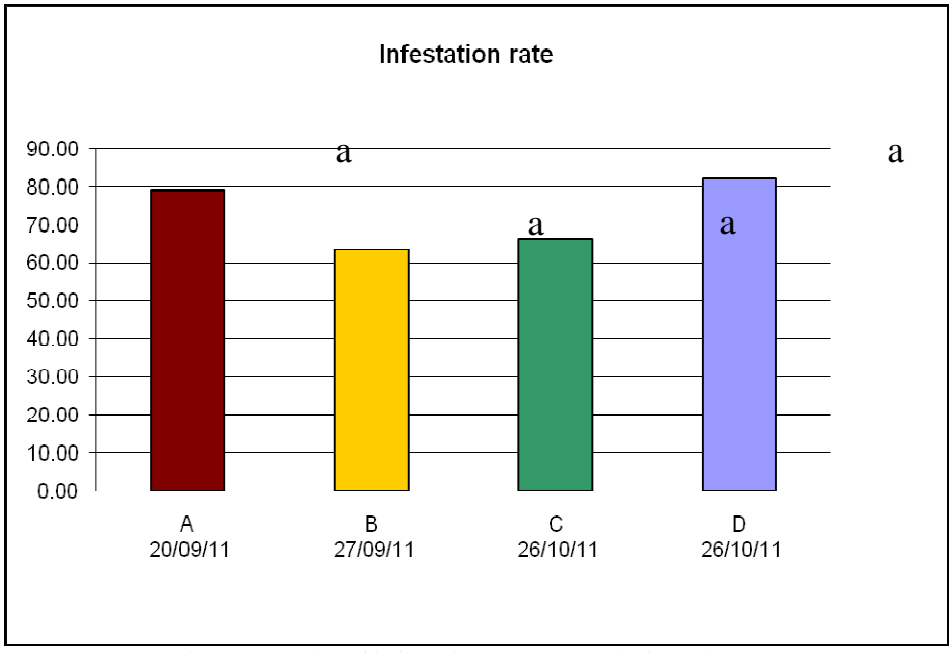


Fig 4.9 Graphs of infestation percentage in four sites

From previous fig. 4.9 can be seen a difference among the four sites, in fact sites A and D (79.95% and 81.14% respectively) have an higher percentage of infestation than the other two sites (site B: 63.04%; site C: 67.77%), although this is not stressed from the statistical analysis (results: χ^2= 0.068, P= 0.995).

2.4.6 Vegetational surveys

As can be deepen on subsequent Tab. 4.9, the most abundant species of the arboreal level of the site A are the hop hornbeam (*Ostrya carpinifolia* Fam. Corilaceae), the downy oak (*Quercus pubescens*, Fam. Fagaceae), the mountain elm (*Ulmus glabra*, Fam. Ulmaceae), and the blackthorn (*Prunus spinosa*, Fam. Rosaceae). Follow in order of frequency the flowering ash (*Fraxinus ornus*, Fam. Oleaceae), the black locust (*Robinia pseudacacia*, Fam. Fabaceae) the field maple (*Acer campestre*, Fam. Aceraceae) and the service tree *(Sorbus domestica*, Fam. Rosaceae). The shrub level is composed mainly of common dogwood (*Cornus sanguinea*, Fam. Cornaceae), less frequently there are blackberry (*Rubus fruticosus*, Fam. Rosaceae), the scorpion vetch (*Coronilla valentina* Fam. Fabaceae) and the old man's beard (*Clematis vitalba* , Fam. Ranunculaceae).

Most of the arboreal level of the forest around Site B consists of hornbeam (*Ostrya carpinifolia*, Fam. Corilaceae) and oak (*Quercus pubescens*, Fam. Fagaceae). Important is the presence of Turkey oak (*Quercus cerris*, Fam. Fagaceae), of italian maple (*Acer opalus* subsp. *obtusatum*), and field maple (*Acer campestre*), the last two belong to Aceraceae, family, of the flowering ash (*Fraxinus ornus*, Fam. Oleaceae) and of the black locust (*Robinia pseudoacacia*, Fam. Fabaceae). Presence of white willow (*Salix alba*, Fam. Salicaceae). In the shrub level characteristics are the common dogwood (*Cornus sanguinea*, Fam. Cornaceae), elm leaf blackberry (*Rubus ulmifolius*, Fam. Rosaceae) and the common laburnum (*Laburnum anagyroides*, Fam. Fabaceae). It is recorded the presence of common juniper (*Juniperus communis*, Fam. Cupressaceae) and of common european hawthorn *Crataegus monogyna*.

In Site C the arboreal level consists mainly of elm *Ulmus minor*, hop hornbeam (*Ostrya carpinifolia*, Fam. Corilaceae), downy oak (*Quercus pubescens*) , turkey oak (*Quercus cerris*) both of the Fagaceae family and of the blackthorn (*Prunus spinosa*, Fam. Rosaceae). Abundant are the flowering ash (*Fraxinus ornus*, Fam. Oleaceae) and italian maple (*Acer opalus* subsp. *obtusatum*). More sporadic the service tree (*Sorbus domestica*) and wild service tree (*Sorbus torminalis*), of the Rosaceae family. The shrub level is composed mostly of elm leaf blackberry (*Rubus ulmifolius*), broom tree heath (*Erica arborea*, Fam. Ericaceae) and dogwood (*Cornus sanguinea*, Fam. Cornaceae), followed by hawthorn (*Crataegus monogyna*, Fam. Rosaceae), the honeysuckle (*Lonicera etrusca,* Fam. Caprifoliaceae) and wayfaring tree (*Viburnum lantana*, Fam. Caprifoliaceae) Has been detected the presence of old man's beard (*Clematis vitalba*, Fam. Ranunculaceae) and of dog rose (*Rosa canina*) both of the Family Rosaceae.

The arboreal level of site D is mainly consists of the black locust (*Robinia pseudoacacia*, Fam. Fabaceae), downy oak (*Quercus pubescens*) and turkey oak (*Quercus cerris*), both of the

Fagaceae family. Important is the presence of hop hornbeam (*Ostrya carpinifolia*, Fam Corilaceae). Were recorded individuals of white poplar (*Popolus alba*) and black poplar (*Popolus nigra*), both of the Salicaceae family, and of english oak (*Quercus robur*, Fam. Fagaceae). The shrub level is composed of elm leaf blackberry (*Rubus ulmifolius*), dogwood (*Cornus sanguinea*, Fam. Cornaceae), elderberry (*Sambucus nigra*, Fam. Caprifoliacee), hawthorn (*Crataegus monogyna*, Fam. Rosaceae), and greenbriar (*Smilax excelsa*, Fam Liliaceae).

We can say that the four sites do not greatly differ with regards to the vegetational composition; however, the phytosociological analysis allows us to highlight in site C an higher presence of oaks. In order to obtain parasitoids of cynipids this is an important data because oaks are “reservoirs” of galls and consequently of community focused on gall makers..

SITE	LEVEL		A-D/ S		LEVEL		A-D/ S
A				**Site B**			
Ostrya carpinifolia	Arboreal	border	4.3	*Ostrya carpinifolia*	Arboreal	board	3.1
Ostrya carpinifolia	Arboreal	Chestnut	3.3	*Quercus pubescens*	Arboreal	board	2.1
Ulmus glabra	Arboreal	chestnut	3.3	*Quercus cerris*	Arboreal	board	1.1
Quercus pubescens	Arboreal	board	3.1	*Acer campestre*	Arboreal	board	+.1
Prunus spinosa	Arboreal	chestnut	2.2	*Acer opalus* subsp. *obtusatum*	Arboreal	board	1.2
Acer campestre	Arboreal	chestnut	1.1	*Robinia pseudoacacia*	Arboreal	board	1.1
Fraxinus ornus	Arboreal	board	1.1	*Fraxinus ornus*	Arboreal	board	1.2
Robinia pseudacacia	Arboreal	board	1.1	*Salix alba*	Arboreal	board	+.1
Sorbus domestica	Arboreal	chestnut	1.1	*Cornus sanguinea*	Shrub	board	2.1
Cornus sanguinea	Shrub	board	3.1	*Rubus ulmifolius*	Shrub	board	2.1
Rubus fruticosus	Shrub	board	2.5	*Laburnum anagyroides*	Shrub	board	1.1
Clematis vitalba	Shrub	board	2.3	*Juniperus communis*,	Shrub	board	+.1
Coronilla valentina,	Shrub	board	2.4	*Crataegus monogyna*	Shrub	board	+.1
C				**D**			
Ulmus minor	Arboreal	East board	3.2	*Robinia pseudacacia*	Arboreal	board	3.3
Ostrya carpinifolia	Arboreal	North board	3.1	*Quercus pubescens*	Arboreal	board	2.1
Quercus pubescens	Arboreal	North board	3.1	*Quercus cerris*	Arboreal	board	2.1
Quercus cerris	Arboreal	East board	3.1	*Ostrya carpinifolia*	Arboreal	board	2.1
Prunus spinosa	Arboreal	East board	2.3	*Populus nigra*	Arboreal	board	1.1
Fraxinus ornus	Arboreal	East board	2.1	*Populus alba*	Arboreal	board	+.1
Acer opalus subsp. *obtusatum*	Arboreal	North board	2.1	*Quercus robur*	Arboreal	board	+.2
Salix alba	Arboreal	North board	+.1	*Rubus ulmifolius*	Shrub	board	5.5
Sorbus domestica	Arboreal	North board	+.1	*Cornus sanguinea*	Shrub	board	3.2
Sorbus torminalis	Arboreal	North board	+.1	*Sambucus nigra*	Shrub	board	2.2
Rubus ulmifolius	Shrub	East board	4.3	*Crataegus monogyna*	Shrub	board	2.1
Erica arborea	Shrub	East board	4.2	*Smilax excelsa*	Shrub	board	1.1
Cornus sanguinea	Shrub	East board	4.1 3.3	ABUNDANCE - DOMINANCE		SOCIABILITY	
Rubus ulmifolius	Shrub	North board	2.3				
Cornus sanguinea	Shrub	North board	2.2	+ : < 1%		1 : isolated individuals;	
Crataegus monogyna	Shrub	North board	2.1	1 : 1-5%		2 : grouped individuals;	
Lonicera etrusca	Shrub	East board	2.1	2 5 - 25%;		3 : individuals in small colonies;	
Viburnum lantana	Shrub	North board	2.1	3 : 25 -50%;		4 : populations extended on more than half of the sample plot;	
Rosa canina	Shrub	North board	1.1	4 : - 50 - 75%;		5 : pure populations.	
Clematis vitalba	Shrub	North board	1.1	5 : 75 - 100%.			

Tab. 4.9 Vegetational surveys on trees and shrubs near the study sites

4.5 Conclusions

In this study, *D. kuriphilus* parasitoid general emergence rate was of 0.5% in 2010 and of 25.76% in 2011 (37.70% in spring and 13.83% in summer). However the rate of estimated parasitism was of 0.14% in 2010 and of 7.36% in 2011. (10.77 % in spring and 3.95 % in summer).

In 2011 data are far above the italian average values found in the bibliography, which arrange the emergence rate under or across 2-3% threshold.

T. flavipes was the most abundant species collected both in Spring and in Summer period with an average parasitism rate of 75.75%.

In this parasitoid species we observed a female-biased sex ratio and it may be seen as a good fitting to the new host, in fact adaptative sex ratio manipulation in response to host quality cause offspring production of daughters (Jones, 1982; King, 1995).

Further studies are needed to deepen its possible use in biological control programs.

Furthermore this work showed a new association parasitoid/*D.kuriphilus* in Italy: *Mesopolobus lichtensteini* (Mayr, 1903) (Chalcidoidea: Pteromalidae).

The collection of galls on wild vegetation has permitted to obtain from oak a parasitoid that has never found associated with *D. kuriphilus* on chestnut (*Megastigmus stigmatizans*) and some others that have already been recruited on *Dryocosmus kuriphilus* (*Torymus* sp., *T. flavipes*, *Ormyrus nitidulus*). The last has recently been reported associated to *D. kuriphilus* in North-Western Italy (Quacchia *et al*., 2012).

Concerning phytosociological analysis, we evidenced a higher presence of oaks in site C. In this site, a greater parasitoid emergence rate has been detected: we can hypothesize a relationship between the abundance of oak trees and parasitoids, which could be attributed to the high presence of oak cynipids. A host shift may occur from oak cynipids to *D. kuriphilus* on chestnut; however, further studies are necessary to assess the cynipid fauna in our study area.

APPENDIX (Universal Chalcidoidea Database - The Natural History Museum)

Tab. 1 - *Torymus* spp.

Primary hosts	**Plant hosts**
Order: Coleoptera	**Order: not specified**
Family: Bruchidae	**Family: Aceraceae**
Bruchidius fasciatus	*Acer* sp.
Bruchus baudoni	**Family: Pinaceae**
Bruchus lividimanus	*Picea* sp.
Bruchus rufipes	**Parasitoid hosts**
Family: Curculionidae	**Order: Hymenoptera**
Smicronyx fulvus	**Family: Cynipidae**
Family: Rhynchitidae	*Synergus pallicornis*
Rhynchites bacchus	**Family: Eulophidae**
Family: Scolytidae	*Aprostocetus brevicornis*
Scolytus rugulosus	*Aulogymnus aceris*
Order: Diptera	*Olynx skianeuros*
Family: Cecidomyiidae	**Family: Ichneumonidae**
Anisostephus betulinum	*Casinaria atrata*
Asphondylia sp.	**Parasitoids**
Asphondylia calycotomae	**Family: Pteromalidae**
Asphondylia rudbeckiaeconspicua	*Mesopolobus fasciiventris*
Asphondylia sarothamni	**Associates**
Cecidomyia sp.	**Order: Diptera**
Cecidomyiidae unspecified sp.	**Family: Tephritidae**
Celticecis japonica	*Tephritis joanae*
Contarinia constricta	**Order: Hymenoptera**
Contarinia medicaginis	**Family: Cynipidae**
Contarinia oregonensis	Cynipidae unspecified
Dasineura sp.	**Family: Eulophidae**
Dasineura affinis	*Pediobius sasae*
Dasineura albovittata	**Plant associates**
Dasineura gleditchiae	**Order: not specified**
Dasineura hebefolia	**Family: Asteraceae**
Dasineura laricis	*Bebbia juncea*
Dasineura mali	*Ericameria pinifolia*
Dasineura rachiphaga	*Grindelia hirsutula*
Dasineura ulmea	*Lactuca viminea*
Giraudiella sp.	*Rudbeckia laciniata*
Isosandalum noxium	**Family: Betulaceae**
Kaltenbachiola strobi	*Betula* sp.
Lasioptera sp.	*Betula pendula*
Lasioptera vernoniae	**Family: Chenopodiaceae**
Mayetiola destructor	*Atriplex halimus*
Mayetiola piceae	**Family: Cupressaceae**

Mayetiola thujae	*Juniperus thurifera*
Mayetiola tumidosae	*Thuja plicata*
Phytophaga thujae	**Family: Ericaceae**
Psectrosema reticulatum	*Arctostaphylos glauca*
Rabdophaga rosaria	**Family: Fabaceae**
Rabdophaga salicisrhodoides	*Medicago sativa*
Rabdophaga swainei	*Sarothamnus scoparius*
Rabdophaga terminalis	**Family: Fagaceae**
Rabdophaga triandraperda	*Castanea* sp.
Rhopalomyia californica	*Castanea crenata*
Family: Tephritidae	*Castanea mollissima*
Goedenia stenoparia	*Quercus* sp.
Goedenia steyskali	*Quercus ilex*
Terellia ruficauda	*Quercus rubra*
Trupanea imperfecta	**Family: Myrtaceae**
Trupanea wheeleri	*Psidium guineense*
Order: Hemiptera	**Family: Pinaceae**
Family: Psyllidae	*Larix* sp.
Pachypsylla celtidisvesicula	*Picea abies*
Pachypsylla venusta	*Picea mariana*
Order: Hymenoptera	*Pseudotsuga menziesii*
Family: Cynipidae	**Family: Poaceae**
Amphibolips sp.	*Sasa nipponica*
Andricus eldoradensis	**Family: Rosaceae**
Biorhiza pallida	*Malus pumila*
Callirhytis lanata	*Family:* **Rubiaceae**
Cynipidae unspecified	*Rubus caesius*
Cynips divisa	*Rubus fruticosus*
Diastrophus rubi	**Family: Salicaceae**
Dryocosmus kuriphilus	*Salix* sp.
Timaspis phoenixopodos	**Family: Tamaricaceae**
Family: Eurytomidae	*Tamarix* sp.
Eurytoma sp.	**Family: Ulmaceae**
Prodecatoma sp.	*Celtis* sp.
Family: Tenthredinidae	
Euura amerinae	
Order: Lepidoptera	
Family: Coleophoridae	***Torymus* Dalman, 1820**
Coleophora pruniella	
Family: Gelechiidae	***[* Torymidae : Toryminae : Torymini]**
Dichomeris juniperella	
Gelechia muscosella	
Family: Lasiocampidae	
Dendrolimus punctatus	
Family: Tortricidae	

Ancylis comptana	
Rhopobota naevana	

Tab. 2 - *Torymus geranii*

Primary hosts	**Plant associates**
Order: Diptera	**Order: not specified**
Family: Cecidomyiidae	**Family: Fagaceae**
Helicomyia saliciperda	*Castanea* sp.
Order: Hymenoptera	*Castanea sativa*
Family: Cynipidae	*Quercus* sp.
Andricus curvator	*Quercus cerris*
Andricus kollari	*Quercus petraea*
Andricus quercuscalicis	*Quercus pyrenaica*
Biorhiza pallida	*Quercus robur*
Cynipidae unspecified	**Family: Rosaceae**
Cynips divisa	*Rosa* sp.
Cynips longiventris	***Torymus geranii* Walker, 1833** **[Torymidae : Toryminae : Torymini]**
Diplolepis nervosa	
Dryocosmus kuriphilus	
Trigonaspis synaspis	

Tab. 3 - *Torymus flavipes*

Primary hosts	**Parasitoid hosts**
Order: Diptera	*Periclistus brandtii*
Family: Cecidomyiidae	*Synergus sp.*
Cecidomyia baccharicola	*Synergus albipes*
Dasineura asperulae	***Synergus gallaepomiformis***
Kiefferia pimpinellae	*Synergus nervosus*
Putoniella marsupialis	**Family: Eulophidae**
Rabdophaga dubia	*Olynx arsames*
Family: Tephritidae	*Pediobius lysis*
Chaetostomella onotrophes	**Family: Eurytomidae**
Urophora jaceana	*Eurytoma brunniventris*
Urophora quadrifasciata	**Family: Pteromalidae**
Order: Hymenoptera	*Mesopolobus* sp.
Family: Cynipidae	*Mesopolobus tibialis*
Andricus sp.	**Family: Torymidae**
Andricus conglomeratus	*Syntomaspis notata*
Andricus curvator	*Torymus notatus*
Andricus inflator	**Plant associates**
Andricus multiplicatus	**Order: not specified**

Andricus quadrilineatus	**Family: Apiaceae**
Andricus quercusramuli	*Daucus carota*
Andricus seminationis	*Pastinaca* sp.
Aphelonyx cerricola	*Pimpinella* sp.
Biorhiza sp.	*Seseli* sp.
Biorhiza pallida	*Torilis* sp.
Chilaspis nitida	**Family: Fagaceae**
Cynipidae unspecified	*Fagus sylvatica*
Cynips disticha	*Quercus* sp.
Cynips divisa	*Quercus cerris*
Cynips longiventris	*Quercus coccifera*
Cynips quercusfolii	*Quercus faginea*
Diplolepis eglanteriae	*Quercus pedunculiflora*
Diplolepis mayri	*Quercus petraea*
Diplolepis nervosa	*Quercus pubescens*
Diplolepis rosae	*Quercus pyrenaica*
Diplolepis spinosa	**Family: Rosaceae**
Neuroterus sp.	*Rosa sp.*
Neuroterus albipes	***Torymus flavipes* (Walker, 1833)** [**Torymidae** : Toryminae : Torymini]
Neuroterus numismalis	
Neuroterus quercusbaccarum	
Neuroterus tricolor	
Plagiotrochus ilicis	
Plagiotrochus quercusilicis	

Tab. 4 - *Torymus erucarum*

Primary hosts	**Parasitoid hosts**
Order: Diptera	**Order:** Hymenoptera
Family: Cecidomyiidae	**Family:** Cynipidae
Mikiola fagi	*Synergus incrassatus*
Order: Hymenoptera	**Plant associates**
Family: Cynipidae	**Order:** not specified
Andricus sp.	**Family:** Fagaceae
Andricus inflator	*Fagus* sp.
Andricus kollari	*Fagus sylvatica*
Andricus quercusradicis	*Quercus* sp.
Andricus testaceipes	*Quercus faginea*
Aphelonyx cerricola	*Quercus pyrenaica*
Biorhiza pallida	**Family:** Rhamnaceae
Cynipidae unspecified	*Rhamnus alaternus*
Diastrophus rubi	***Torymus erucarum* (Schrank, 1781)**
Family: Diprionidae	
Diprion pini	

Order: Lepidoptera	[**Torymidae** : Toryminae : Torymini]
Family: Tortricidae	
Carpocapsa pomonella	

Tab. 5 - *Torymus auratus*

Primary hosts	**Parasitoid hosts**
Order: Hymenoptera	*Synergus pallicornis*
Family: Cynipidae	*Synergus reinhardi*
Andricus aestivalis	*Synergus umbraculus*
Andricus conglomeratus	**Family: Eurytomidae**
Andricus fecundator	*Eudecatoma biguttata*
Andricus hispanicus	*Eurytoma brunniventris*
Andricus kollari	**Plant associates**
Andricus lignicolus	**Order: not specified**
Andricus mayri	**Family: Fagaceae**
Andricus quercuscalicis	*Quercus* sp.
Andricus quercustozae	*Quercus cerris*
Aphelonyx cerricola	*Quercus faginea*
Biorhiza pallida	*Quercus pubescens*
Chilaspis nitida	*Quercus pyrenaica*
Cynipidae unspecified	*Quercus robur*
Cynips sp.	**Family: Rosaceae**
Cynips divisa	*Rosa canina*
Cynips longiventris	**Family: Rubiaceae**
Cynips quercus	*Rubus* sp.
Cynips quercusfolii	
Diastrophus rubi	
Diplolepis fructuum	***Torymus auratus* (Müller, 1764)**
Diplolepis quercusfolii	
Neuroterus quercusbaccarum	[**Torymidae** : Toryminae : Torymini]
Synergus gallaepomiformis	

Tab. 6 – *Megastigmus* spp.

Primary hosts	Parasitoid hosts
Order: Diptera	**Order: Hymenoptera**
Family: Cecidomyiidae	**Family: Cynipidae**
Psectrosema manii	*Synergus sp.*
Family: Fergusoninidae	**Family: Eupelmidae**
Fergusonina sp.	*Eupelmus urozonus*
Order: Hemiptera	**Family: Eurytomidae**
Family: Asterolecaniidae	*Eurytoma brunniventris*
Asterolecanium variolosum	**Family: Torymidae**
Order: Hymenoptera	*Syntomaspis cyanea*
Family: Cynipidae	*Torymus sp.*
Cynipidae unspecified	*Associates*
Cynips divisa	**Family: Eulophida***e*
Dryocosmus kuriphilus	*Ophelimus maskelli*
Family: Eulophidae	*Plant associates*
Leptocybe invasa	**Order: not specified**
Family: Eurytomidae	**Family: Cupressaceae**
Bruchophagus mellipes	*Juniperus sp.*
Family: Pteromalidae	**Family: Fagaceae**
Trichilogaster acaciaelongifoliae	*Castanea crenata*
Plant hosts	*Quercus sp.*
Order: not specified	**Family: Myrtaceae**
Family: Anacardiaceae	*Eucalyptus camaldulensis*
Schinus terebinthifolius	*Melaleuca quinquenervia*
Family: Cupressaceae	**Family: Pinaceae**
Cupressus dupreziana	*Abies sp.*
Juniperus sp.	*Pinus ponderosa*
Family: Fabaceae	*Pseudotsuga sp.*
Acacia pendula	**Family: Rosaceae**
Sesbania grandiflora	*Rosa sp.*
Family: Myrtaceae	
Eucalyptus sp.	
Eucalyptus camaldulensis	
Family: Pinaceae	***Megastigmus Dalman, 1820***
Abies amabilis	
Abies delavayi	[Torymidae : Megastigminae]
Abies religiosa	
Larix laricina	
Picea sp.	
Picea jezoensis	
Picea koraiensis	
Pinus albicaulis	
Pinus monticola	
Pseudotsuga sinensis	

Family: Tamaricaceae
Tamarix sp.

Tab. 7 - *Megastigmus dorsalis*

Primary hosts	*Cynips kollari*
Order: Hymenoptera	*Cynips lignicola*
Family: Cynipidae	*Cynips longiventris*
Andricus sp.	*Cynips quercusfolii*
Andricus aestivalis	*Diplolepis rosae*
Andricus albopunctatus	*Neuroterus macropterus*
Andricus aries	*Neuroterus quercusbaccarum*
Andricus caputmedusae	*Synergus pallicornis*
Andricus conglomeratus	*Synophrus politus*
Andricus coriarius	**Family: Torymidae**
Andricus corruptor	*Syntomaspis sp.*
Andricus corruptrix	**Plant hosts**
Andricus curvator	**Order: not specified**
Andricus fecundator	**Family: Fagaceae**
Andricus grossulariae	*Quercus semecarpifolia*
Andricus hispanicus	**Parasitoid hosts**
Andricus hungaricus	**Order: Hymenoptera**
Andricus infectorius	**Family: Cynipidae**
Andricus inflator	*Synergus reinhardi*
Andricus kollari	*Synergus umbraculus*
Andricus lignicolus	**Plant associates**
Andricus lucidus	**Order: not specified**
Andricus multiplicatus	**Family: Fagaceae**
Andricus peyerimhoffi	*Fagus sylvatica*
Andricus quercuscorticis	*Quercus sp.*
Andricus quercusradicis	*Quercus cerris*
Andricus quercusramuli	*Quercus faginea*
Andricus stefani	*Quercus frainetto*
Andricus testaceipes	*Quercus pedunculiflora*
Andricus vindobonensis	*Quercus persica*
Aphelonyx cerricola	*Quercus pubescens*
Biorhiza sp.	*Quercus pyrenaica*
Biorhiza pallida	*Quercus robur*
Callirhytis erythrocephala	*Quercus semecarpifolia*
Callirhytis glandium	*Quercus suber*
Cynipidae unspecified	
Cynips sp.	***Megastigmus dorsalis***
Cynips conglomerata	***(Fabricius, 1798)***
Cynips coriaria	
Cynips divisa	***[Torymidae : Megastigminae]***

Tab. 8 - ***Megastigmus stigmatizans***

Primary hosts	***Megastigmus stigmatizans*** ***(Fabricius, 1798)*** ***[Torymidae : Megastigminae]***
Order: Hymenoptera	
Family: Cynipidae	
Andricus sp.	
Andricus caputmedusae	
Andricus coriarius	
Andricus curvator	
Andricus hispanicus	
Andricus kollari	
Andricus lignicolus	
Andricus lucidus	
Andricus quercuscalicis	
Andricus quercustozae	
Cynipidae unspecified	
Cynips divisa	
Neuroterus numismalis	
Parasitoid hosts	
Family: Torymidae	
Torymus nigricornis	
Plant associates	
Order: not specified	
Family: Fagaceae	
Quercus sp.	
Quercus cerris	
Quercus faginea	
Quercus pyrenaica	
Quercus robur	
Quercus suber	

Tab. 9 - *Mesopolobus* spp.

Primary hosts	*Leucoma salicis*
Order: Coleoptera	**Family: Noctuidae**
Family: Chrysomelidae	*Heliothis armigera*
Plagiodera versicolora	**Family: Tischeriidae**
Family: Curculionidae	*Tischeria* sp.
Bothynoderes punctiventris	**Family: Tortricidae**
Ceutorhynchus assimilis	*Archips cerasivoranus*
Ceutorhynchus floralis	*Archips conflictana*
Cleonus confluens	*Archips fumiferana*
Cleonus subfuscus	*Barbara colfaxiana*
Cylindrocopturus adspersus	*Choristoneura* sp.
Cylindrocopturus furnissi	*Pseudotomoides strobilellus*
Hypera variabilis	**Family: Yponomeutidae**
Tychius medicaginis	*Yponomeuta malinellus*
Family: Scolytidae	**Parasitoid hosts**
Dendroctonus adjunctus	**Order: Diptera**
Hylurgopinus rufipes	**Family: Tachinidae**
Polygraphus poligraphus	*Actia pilipennis*
Order: Diptera	*Anthomyiopsis plagioderae*
Family: Cecidomyiidae	**Order: Hymenoptera**
Asphondylia sp.	**Family: Braconidae**
Cecidomyiidae unspecified	*Apanteles californicus*
Chamaediplosis nootkatensis	*Apanteles solitarius*
Clinodiplosis pucciniae	*Meteorus versicolor*
Cystiphora schmidti	**Family: Cynipidae**
Dasineura brassicae	*Synergus gallaepomiformis*
Dasineura gleditchiae	*Synergus nervosus*
Dasineura ignorata	**Family: Encyrtidae**
Dasineura oleae	*Copidosoma deceptor*
Dasineura saliciperda	**Family: Eurytomidae**
Diarthronomyia hypogaea	*Eudecatoma biguttata*
Isosandalum dentipes	*Eurytoma brunniventris*
Mayetiola destructor	**Family: Ichneumonidae**
Mayetiola piceae	*Aethecerus pinifolii*
Mayetiola rigidae	**Family: Pteromalidae**
Psectrosema indicum	*Mesopolobus* sp.
Psectrosema parvum	**Family: Torymidae**
Psectrosema reticulatum	*Megastigmus spermotrophus*
Rabdophaga swainei	**Parasitoids**
Rhopalomyia californica	**Family: Eupelmidae**
Family: Chloropidae	*Eupelmus urozonus*
Dicraeus ingratus	**Family: Pteromalidae**
Dicraeus pallidiventris	*Mesopolobus fasciiventris*
Dicraeus tibialis	**Family: Torymidae**

Dicraeus vagans	*Torymus auratus*
Family: Simuliidae	**Plant associates**
Simulium pseudequinum	**Order: not specified**
Family: Tephritidae	**Family: Agavaceae**
Aciurina thoracica	*Nolina parryi*
Ceratitis capitata	**Family: Asteraceae**
Goedenia setosa	*Acamptopappus sphaerocephalus*
Neaspilota alba	*Artemisia tridentata*
Neaspilota footei	*Aster chilensis*
Oxyna aterrima	*Aster occidentalis*
Procecidochares blanci	*Baccharis pilularis*
Trupanea actinibola	*Baccharis sarothroides*
Trupanea imperfecta	*Bebbia juncea*
Trupanea jonesi	*Chondrilla juncea*
Trupanea wheeleri	*Ericameria brachylepis*
Order: Hemiptera	*Hypochaeris radicata*
Family: Diaspididae	*Isocoma acradenia*
Carulaspis juniperi	**Family: Brassicaceae**
Family: Margarodidae	*Brassica napus*
Xylococculus macrocarpae	*Capsella bursa-pastoris*
Family: Psyllidae	**Family: Chenopodiaceae**
Pachypsylla venusta	*Beta vulgaris*
Order: Hymenoptera	**Family: Cupressaceae**
Family: Cynipidae	*Calocedrus decurrens*
Andricus callidoma	*Chamaecyparis nootkatensis*
Andricus curvator	*Juniperus excelsa*
Andricus hispanicus	**Family: Fabaceae**
Andricus kollari	*Cyamopsis tetragonoloba*
Andricus quercuscalicis	*Gleditsia triacanthos*
Andricus vindobonensis	*Medicago sativa*
Biorhiza pallida	**Family: Fagaceae**
Cynipidae unspecified	*Quercus* sp.
Cynips divisa	*Quercus cerris*
Cynips quercusfolii	*Quercus coccifera*
Dryocosmus australis	*Quercus faginea*
Pediaspis aceris	*Quercus ilex*
Phanacis hypochoeridis	*Quercus pedunculiflora*
Plagiotrochus britaniae	*Quercus petraea*
Plagiotrochus coriaceus	*Quercus pubescens*
Plagiotrochus quercusilicis	*Quercus robur*
Family: Eurytomidae	*Quercus suber*
Bruchophagus gibbus	*Quercus vaccinifolia*
Eurytoma onobrychidis	**Family: Hippocastanaceae**
Family: Torymidae	*Aesculus hippocastanum*
Megastigmus pinus	**Family: Oleaceae**

Order: Lepidoptera	*Olea europaea*
Family: Bucculatricidae	**Family: Pinaceae**
Bucculatrix variabilis	*Abies* sp.
Family: Coleophoridae	*Larix* sp.
Coleophora sp.	*Picea excelsa*
Coleophora dahurica	*Picea mariana*
Coleophora laricella	*Pinus jeffreyi*
Family: Gelechiidae	*Pseudotsuga menziesii*
Coleotechnites sp.	**Family: Rhamnaceae**
Coleotechnites piceaella	*Ceanothus greggi*
Evagora milleri	**Family: Salicaceae**
Evagora starki	*Populus* sp.
Family: Geometridae	*Salix fragilis*
Philtraea elegantaria	**Family: Tamaricaceae**
Family: Gracillariidae	*Tamarix* sp.
Cameraria sp.	
Cameraria ohridella	***Mesopolobus***
Family: Incurvariidae	
Mesepiola specca	***[*** **Pteromalidae : Pteromalinae**]
Family: Lymantriidae	

Tab. 10- *Mesopolobus mediterraneus* (Mayr, 1903)

Primary hosts	**Parasitoid hosts**
Order: Coleoptera	**Order: Hymenoptera**
Family: Apionidae	**Family: Braconidae**
Apion fuscirostre	*Apanteles glomeratus*
Apion ulicis	*Braconidae unspecified sp.*
Family: Bruchidae	*Macrocentrus thoracicus*
Bruchidius ater	**Family: Pteromalidae**
Family: Curculionidae	*Habrocytus sequester*
Stereonychus fraxini	**Parasitoids**
Order: Diptera	**Family: Aphelinidae**
Family: Cecidomyiidae	*Azotus chionaspidis*
Cecidomyiidae unspecified	**Plant associates**
Dasineura affinis	**Order: not specified**
Dasineura gleditchiae	**Family: Asteraceae**
Dryomyia lichtensteini	*Artemisia herba-alba*
Rhopalomyia producticeps	*Hypochaeris radicata*
Schmidtiella gemmarum	**Family: Cupressaceae**
Order: Hemiptera	*Juniperus communis*
Family: Pseudococcidae	*Juniperus thurifera*
Ferrisia oleae	**Family: Fabaceae**
Order: Hymenoptera	*Cytisus scoparius*
Family: Cynipidae	*Genista scorpius*
Andricus sp.	*Sarothamnus scoparius*
Andricus pseudoinflator	*Ulex europaeus*
Andricus quercusramuli	**Family: Fagaceae**
Biorhiza pallida	*Quercus* sp.
Cynipidae unspecified	*Quercus coccifera*
Cynips sp.	*Quercus faginea*
Neuroterus sp.	*Quercus ilex*
Phanacis hypochoeridis	*Quercus suber*
Plagiotrochus sp.	**Family: Oleaceae**
Plagiotrochus amenti	*Olea europaea*
Plagiotrochus britaniae	**Family: Pinaceae**
Plagiotrochus cardiguensis	*Abies alba*
Plagiotrochus ilicis	*Abies nordmanniana*
Plagiotrochus quercusilicis	*Pinus halepensis*
Order: Lepidoptera	**Family: Rosaceae**
Family: Coleophoridae	*Malus communis*
Coleophora serratella	**Family: Tamaricaceae**
Family: Gelechiidae	*Tamarix canariensis*
Coleotechnites piceaella	**Family: Vitaceae**
Family: Lepidoptera indet.	*Vitis vinifera*
Lepidoptera unspecified sp.	
Family: Tortricidae	***Mesopolobus mediterraneus* (Mayr, 1903)**

Archips murinana	[**Pteromalidae** : Pteromalinae]
Lobesia botrana	
Rhyacionia buoliana	
Tortrix viridana	
Family: Yponomeutidae	
Argyresthia fundella	
Prays oleellus	
Yponomeuta sp.	
Yponomeuta malinellus	
Yponomeuta padellus	

Tab. 11- *Mesopolobus tarsatus*

Primary hosts	***Mesopolobus tarsatus* (Nees, 1834)** [**Pteromalidae** : Pteromalinae]
Order: Hymenoptera	
Family: Cynipidae	
Andricus burgundus	
Andricus curvator	
Plagiotrochus quercusilicis	
Plant associates	
Order: not specified	
Family: Fagaceae	
Quercus sp.	
Quercus faginea	
[illegible]	
Quercus pyrenaica	
Quercus suber	

Tab. 12- *Mesopolobus tibialis*

Primary hosts	**Parasitoid hosts**
Order: Diptera	**Order: Hymenoptera**
Family: Cecidomyiidae	**Family: Cynipidae**
Kaltenbachiola strobi	*Synergus albipes*
Order: Hymenoptera	*Synergus gallaepomiformis*
Family: Cynipidae	**Family: Eulophidae**
Andricus sp.	*Pediobius lysis*
Andricus albopunctatus	**Family: Pteromalidae**
Andricus burgundus	*Caenacis divisa*
Andricus curvator	**Parasitoids**
Andricus grossulariae	**Family: Torymidae**
Andricus kollari	*Torymus auratus*
Andricus legitimus	**Plant associates**
Andricus lignicolus	**Order: not specified**
Andricus malpighi	**Family: Apiaceae**
Andricus ostreus	*Eryngium maritimum*
Andricus pseudoinflator	**Family: Fabaceae**
Andricus quercuscalicis	*Medicago* sp.
Andricus quercusramuli	**Family: Fagaceae**
Biorhiza pallida	*Fagus sylvatica*
Cynipidae unspecified	*Quercus* sp.
Cynips sp.	*Quercus cerris*
Cynips flosculi	*Quercus coccifera*
Neuroterus sp.	*Quercus faginea*
Neuroterus albipes	*Quercus ilex*
Neuroterus anthracinus	*Quercus pedunculiflora*
Neuroterus baccarum	*Quercus petraea*
Neuroterus numismalis	*Quercus pyrenaica*
Neuroterus politus	*Quercus robur*
Neuroterus quercusbaccarum	*Quercus suber*
Neuroterus tricolor	**Family: Pinaceae**
Plagiotrochus sp.	*Pinus halepensis*
Plagiotrochus amenti	**Family: Rosaceae**
Plagiotrochus australis	*Prunus* sp.
Plagiotrochus cardiguensis	***Mesopolobus tibialis* (Westwood, 1833)** [**Pteromalidae** : Pteromalinae]
Plagiotrochus ilicis	
Plagiotrochus quercusilicis	
Plagiotrochus razeti	
Synergus albipes	
Synergus gallaepomiformis	
Trigonaspis megaptera	
Order: Lepidoptera	
Family: Tortricidae	
Tortrix viridana	

Tab. 13- *Mesopolobus amaenus*

Primary hosts	**Parasitoid hosts**
Order: Diptera	**Order: Hymenoptera**
Family: Cecidomyiidae	**Family: Cynipidae**
Dryomyia circinnans	*Synergus umbraculus*
Dryomyia lichtensteini	**Associates**
Order: Hymenoptera	Cynipidae unspecified
Family: Cynipidae	*Synergus umbraculus*
Andricus sp.	**Plant associates**
Andricus amenti	**Order: not specified**
Andricus coriarius	**Family: Fagaceae**
Andricus corruptrix	*Quercus* sp.
Andricus curvator	*Quercus cerris*
Andricus grossulariae	*Quercus faginea*
Andricus inflator	*Quercus ilex*
Andricus kollari	*Quercus persica*
Andricus lignicolus	*Quercus pyrenaica*
Andricus multiplicatus	*Quercus robur*
Andricus pictus	**Family: Rosaceae**
Andricus pseudoinflator	*Rosa* sp.
Andricus quercuscalicis	***Mesopolobus amaenus* Walker, 1834** ***[* Pteromalidae : Pteromalinae**]
Andricus solitarius	
Andricus vindobonensis	
Biorhiza pallida	
Cynipidae unspecified	
Diplolepis mayri	
Diplolepis quercusfolii	
Dryocosmus kuriphilus	
Neuroterus quercusbaccarum	
Neuroterus saltans	
Plagiotrochus ilicis	
Synergus gallaepomiformis	
Trigonaspis synaspis	
Order: Lepidoptera	
Family: Lymantriidae	
Leucoma wiltshirei	

Tab. 14- ***Mesopolobus lichtensteini***

Primary hosts	***Mesopolobus lichtensteini* (Mayr, 1903)** [**Pteromalidae** : Pteromalinae]
Order: Diptera	
Family: Cecidomyiidae	
Dryomyia circinnans	
Dryomyia lichtensteini	
Order: Hymenoptera	
Family: Cynipidae	
Andricus burgundus	
Andricus grossulariae	
Neuroterus saliens	
Plagiotrochus sp.	
Plagiotrochus amenti	
Plagiotrochus australis	
Plagiotrochus britaniae	
Plagiotrochus cardiguensis	
Plagiotrochus coriaceus	
Plagiotrochus quercusilicis	
Plagiotrochus razeti	
Plant associates	
Order: not specified	
Family: Fagaceae	
Quercus sp.	
Quercus cerris	
Quercus coccifera	
Quercus ilex	
Quercus suber	

Tab. 15- ***Ormyrus nitidulus***

Primary hosts	*Cynipidae unspecified sp.*
Order: Diptera	*Cynips* sp.
Family: Cecidomyiidae	*Cynips conglomerata*
Oligotrophus bergenstammi	*Cynips conifica*
Order: Hymenoptera	*Cynips lignicola*
Family: Cynipidae	*Cynips longiventris*
Adleria sp.	*Cynips quercus*
Andricus sp.	*Cynips quercusfolii*
Andricus aestivalis	*Diplolepis* sp.
Andricus caputmedusae	*Diplolepis rosae*
Andricus conglomeratus	*Rhodites rosae*
Andricus conificus	*Trigonaspis megaptera*
Andricus coronatus	**Associates**
Andricus curvator	**Family: Eupelmidae**
Andricus fecundator	*Macroneura vesicularis*
Andricus galeata	**Family: Pteromalidae**
Andricus gallaetincticorne	*Pteromalus papaveris*
Andricus glutinosus	**Plant associates**
Andricus grossulariae	**Order: not specified**
Andricus hartigi	**Family: Fagaceae**
Andricus hispanicus	*Quercus* sp.
Andricus kollari	*Quercus cerris*
Andricus lignicolus	*Quercus faginea*
Andricus lucidus	*Quercus pubescens*
Andricus mayri	*Quercus pyrenaica*
Andricus mitratus	*Quercus robur*
Andricus multiplicatus	**Family: Rosaceae**
Andricus polycerus	*Rosa* sp.
Andricus quercuscalicis	***Ormyrus nitidulus* (Fabricius, 1804)** [**Ormyridae**]
Andricus testaceipes	
Aphelonyx cerricola	
Biorhiza sp.	
Biorhiza pallida	

Tab. 16- *Ormyrus pomaceus*

Primary hosts	*Diastrophus mayri*
Order: Hymenoptera	*Diplolepis* sp.
Family: Cynipidae	*Diplolepis divisa*
Andricus sp.	*Diplolepis folii*
Andricus aestivalis	*Dryocosmus kuriphilus*
Andricus callidoma	*Neuroterus sp.*
Andricus conglomeratus	*Neuroterus lanuginosus*
Andricus coriarius	*Neuroterus politus*
Andricus corruptor	*Neuroterus quercusbaccarum*
Andricus corruptrix	*Neuroterus saltans*
Andricus curvator	*Plagiotrochus* sp.
Andricus cydoniae	*Plagiotrochus australis*
Andricus fecundator	*Plagiotrochus quercusilicis*
Andricus galeata	*Synophrus* sp.
Andricus grossulariae	*Trigonaspis brunneicornis*
Andricus hartigi	*Trigonaspis mendesi*
Andricus hispanicus	*Trigonaspis synaspis*
Andricus hungaricus	**Parasitoid hosts**
Andricus infectorius	**Family: Torymidae**
Andricus inflator	*Torymus beneficus*
Andricus lignicolus	Associates
Andricus lucidus	**Family: Cynipidae**
Andricus mayri	*Cynipidae unspecified*
Andricus multiplicatus	**Family: Eupelmidae**
Andricus paradoxus	*Macroneura vesicularis*
Andricus pictus	**Family: Pteromalidae**
Andricus pseudoinflator	*Pteromalus papaveris*
Andricus quercuscalicis	**Plant associates**
Andricus quercusradicis	**Order: not specified**
Andricus quercusramuli	**Family: Fagaceae**
Andricus quercustozae	*Castanea* sp.
Andricus seckendorffi	*Castanea sativa*
Andricus solitarius	*Quercus* sp.
Andricus superfetationis	*Quercus cerris*
Andricus vindobonensis	*Quercus coccifera*
Aphelonyx sp.	*Quercus dentata*
Aphelonyx cerricola	*Quercus faginea*
Biorhiza sp.	*Quercus frainetto*
Biorhiza pallida	*Quercus ilex*
Callirhytis rufescens	*Quercus mongolica*
Cynipidae unspecified	*Quercus pedunculiflora*
Cynips sp.	*Quercus persica*
Cynips amblycera	*Quercus pubescens*
Cynips aries	*Quercus pyrenaica*

Cynips calycis	*Quercus robur*
Cynips conglomerata	*Quercus suber*
Cynips coriaria	**Family: Poaceae**
Cynips coronaria	*Phyllostachys* sp.
Cynips disticha	***Ormyrus pomaceus* Geoffroy, 1785** ***[* Ormyridae]**
Cynips divisa	
Cynips glutinosa	
Cynips longiventris	
Cynips quercus	
Cynips quercusfolii	

Tab. 17- *Sycophila* spp.

Primary hosts	**Plant associates**
Order: Diptera	**Order: not specified**
Family: Cecidomyiidae	**Family: Anacardiaceae**
Cecidomyiidae unspecified	*Pistacia terebinthus*
Order: Hymenoptera	**Family: Fagaceae**
Family: Cynipidae	*Quercus cerris*
Acraspis hirta	*Quercus gambelii*
Andricus quercuscalicis	*Quercus palustris*
Aulacidea macula	**Family: Moraceae**
Barbotinia oraniensis	*Ficus sp.*
Callirhytis cornigera	*Ficus artocarpoides*
Disholcaspis perniciosa	*Ficus microcarpa*
Dryocosmus kuriphilus	*Ficus sur*
Plant hosts	*Ficus vallis-choudae*
Order: not specified	**Family: Oleaceae**
Family: Anacardiaceae	*Jasminum grandiflorum*
Pistacia terebinthus	**Family: Papaveraceae**
Family: Araceae	*Papaver dubium*
Philodendron solimoesense	*Papaver rhoeas*
Family: Fagaceae	**Family: Poaceae**
Quercus prinus	*Ammophila arenaria*
Parasitoid hosts	*Elymus repens*
Order: Hymenoptera	*Festuca rubra*
Family: Eulophidae	*Phyllostachys sp.*
Exurus sp.	***Sycophila Walker, 1871*** ***[Eurytomidae : Eurytominae]***
Family: Eurytomidae	
Tetramesa brevicornis	
Tetramesa eximia	
Tetramesa linearis	
Parasitoids	
Eurytoma sp.	
Family: Ormyridae	

Ormyrus brunneipes	
Family: Pteromalidae	
Gugolzia melengicia	
Associates	
Family: Agaonidae	
Ceratosolen sp.	
Family: Eurytomidae	
Eurytoma sp.	
Family: Formicidae	
Formica obscuripes	
Family: Torymidae	
Megastigmus pistaciae	

Tab. 18- ***Sycophila variegata***

Primary hosts	**Plant associates**
Order: Hymenoptera	**Order: not specified**
Family: Cynipidae	**Family: Anacardiaceae**
Andricus sp.	*Pistacia lentiscus*
Andricus coriarius	**Family: Fagaceae**
Andricus corruptrix	*Castanea* sp.
Andricus fecundator	*Castanea sativa*
Andricus grossulariae	*Quercus* sp.
Andricus hispanicus	*Quercus brantii*
Andricus kollari	*Quercus cerris*
Andricus lignicolus	*Quercus coccifera*
Andricus pseudoinflator	*Quercus faginea*
Andricus quercustozae	*Quercus frainetto*
Andricus vindobonensis	*Quercus ilex*
Biorhiza pallida	*Quercus lanuginosa*
Callirhytis rufescens	*Quercus pedunculiflora*
Chilaspis israeli	*Quercus pubescens*
Cynips sp.	*Quercus pyrenaica*
Diplolepis sp.	*Quercus robur*
Dryocosmus kuriphilus	*Quercus suber*
Neuroterus sp.	
Neuroterus glandiformis	
Neuroterus lanuginosus	
Neuroterus saliens	
Plagiotrochus sp.	***Sycophila variegata* Curtis, 1831**
Plagiotrochus australis	**[Eurytomidae : Eurytominae]**
Plagiotrochus kiefferianus	
Plagiotrochus panteli	
Plagiotrochus quercusilicis	
Synergus umbraculus	
Trichagalma serrata	
Trigonaspis brunneicornis	
Parasitoid hosts	
Synergus umbraculus	

Tab. 19- *Sycophila biguttata*

Primary hosts	**Plant hosts**
Order: Hymenoptera	**Order: not specified**
Family: Cynipidae	**Family: Fagaceae**
Andricus sp.	*Quercus* sp.
Andricus aestivalis	**Parasitoid hosts**
Andricus aries	**Order: Hymenoptera**
Andricus callidoma	**Family: Eulophidae**
Andricus caputmedusae	*Aulogymnus aceris*
Andricus conglomeratus	**Parasitoids**
Andricus coriarius	**Family: Torymidae**
Andricus corruptrix	*Torymus nigricornis*
Andricus gallaetincticorne	**Associates**
Andricus grossulariae	**Family: Eulophidae**
Andricus hungaricus	*Aulogymnus trilineatus*
Andricus inflator	**Plant associates**
Andricus kollari	**Order: not specified**
Andricus lignicolus	**Family: Anacardiaceae**
Andricus lucidus	*Pistacia* sp.
Andricus multiplicatus	**Family: Fagaceae**
Andricus panteli	*Quercus* sp.
Andricus quercuscalicis	*Quercus brantii*
Andricus quercusfolii	*Quercus cerrioides*
Andricus quercusramuli	*Quercus cerris*
Andricus quercustozae	*Quercus coccifera*
Andricus seckendorffi	*Quercus faginea*
Andricus testaceipes	*Quercus ilex*
Andricus trotteri	*Quercus pedunculiflora*
Andricus vindobonensis	*Quercus petraea*
Aphelonyx cerricola	*Quercus pubescens*
Aylax sp.	*Quercus pyrenaica*
Biorhiza sp.	*Quercus robur*
Biorhiza pallida	*Quercus suber*
Callirhytis glandium	**Family: Rosaceae**
Chilaspis israeli	*Rosa* sp.
Chilaspis nitida	*Rosa canina*
Cynipidae unspecified	
Cynips sp.	
Cynips divisa	
Cynips longiventris	***Sycophila biguttata* (Swederus, 1795)**
Cynips quercus	
Cynips quercusfolii	[**Eurytomidae** : Eurytominae]
Diplolepis sp.	
Diplolepis fructuum	
Diplolepis mayri	

Diplolepis rosae	
Neuroterus albipes	
Neuroterus glandiformis	
Neuroterus lanuginosus	
Neuroterus numismalis	
Neuroterus quercusbaccarum	
Neuroterus tricolor	
Phanacis sp.	
Plagiotrochus sp.	
Plagiotrochus australis	
Rhodites sp.	
Synophrus politus	
Trigonaspis mendesi	
Family: Torymidae	
Megastigmus pistaciae	

Tab. 20- ***Eurytoma brunniventris***

Primary hosts	**Parasitoid hosts**
Order: Hymenoptera	**Family: Cynipidae**
Family: Cynipidae	*Synergus* sp.
Andricus sp.	*Synergus albipes*
Andricus albopunctatus	*Synergus gallaepomiformis*
Andricus argenteus	*Synergus nervosus*
Andricus caliciformis	*Synergus pallicornis*
Andricus callidoma	*Synergus reinhardi*
Andricus caputmedusae	*Synergus umbraculus*
Andricus conglomeratus	**Family: Eulophidae**
Andricus coriarius	*Olynx arsames*
Andricus coronatus	**Family: Eurytomidae**
Andricus corruptrix	*Eudecatoma biguttata*
Andricus curvator	*Eurytoma brunniventris*
Andricus fecundator	**Family: Pteromalidae**
Andricus galeata	*Caenacis divisa*
Andricus glutinosus	*Cecidostiba semifascia*
Andricus grossulariae	*Mesopolobus sp.*
Andricus hispanicus	*Mesopolobus tibialis*
Andricus hungaricus	**Family: Torymidae**
Andricus infectorius	*Syntomaspis cyanea*
Andricus inflator	*Torymus* sp.
Andricus kollari	*Torymus cingulatus*
Andricus legitimus	*Torymus cyaneus*
Andricus lignicolus	**Parasitoids**
Andricus mitratus	**Family: Pteromalidae**
Andricus multiplicatus	*Mesopolobus fasciiventris*
Andricus nudus	**Family: Torymidae**
Andricus ostreus	*Torymus auratus*
Andricus paradoxus	*Torymus cingulatus*
Andricus quercuscalicis	*Torymus nigricornis*
Andricus quercusradicis	**Associates**
Andricus quercusramuli	**Family: Cynipidae**
Andricus quercustozae	Cynipidae unspecified
Andricus seminationis	**Plant associates**
Andricus seminatorius	**Order: not specified**
Andricus solitarius	**Family: Fagaceae**
Andricus stefani	*Castanea* sp.
Andricus testaceipes	*Castanea crenata*
Andricus vindobonensis	*Castanea sativa*
Aphelonyx cerricola	*Quercus* sp.
Biorhiza sp.	*Quercus cerris*
Biorhiza pallida	*Quercus coccifera*
Callirhytis erythrocephala	*Quercus faginea*

Chilaspis nitida	*Quercus frainetto*
Cynipidae unspecified	*Quercus ilex*
Cynips sp.	*Quercus pedunculiflora*
Cynips agama	*Quercus pubescens*
Cynips disticha	*Quercus pyrenaica*
Cynips divisa	*Quercus robur*
Cynips longiventris	***Eurytoma brunniventris* Ratzeburg, 1852 [Fam.Eurytomidae : Eurytominae]**
Cynips quercus	
Cynips quercusfolii	
Dryocosmus kuriphilus	
Neuroterus sp.	
Neuroterus albipes	
Neuroterus anthracinus	
Neuroterus glandiformis	
Neuroterus lanuginosus	
Neuroterus numismalis	
Neuroterus quercusbaccarum	
Neuroterus saltans	
Neuroterus tricolor	
Plagiotrochus australis	
Plagiotrochus burnayi	
Plagiotrochus quercusilicis	
Trigonaspis synaspis	
Family: Pteromalidae	
Caenacis divisa	

Tab. 21- ***Eupelmus annulatus***

Primary hosts	**Parasitoid hosts**
Order: Coleoptera	**Order: Hymenoptera**
Family: Buprestidae	**Family: Braconidae**
Agrilus planipennis	*Apanteles* sp.
Family: Curculionidae	*Apanteles melanoscelus*
Pissodes sitchenisis	*Apanteles solitarius*
Pissodes strobi	*Bracon piger*
Family: Scolytidae	*Cotesia melanoscela*
Scolytus ratzeburgi	*Macrocentrus* sp.
Order: Diptera	*Macrocentrus ancylivorus*
Family: Tephritidae	*Macrocentrus delicatus*
Urophora cardui	*Meteorus versicolor*
Order: Hymenoptera	**Family: Cynipidae**
Family: Cynipidae	*Synergus pallicornis*
Andricus aestivalis	*Synergus umbraculus*
Andricus caputmedusae	**Family: Ichneumonidae**
Andricus coriarius	*Cremastus minor*
Andricus fecundator	*Ephialtes caudatus*
Andricus glutinosus	*Glypta* sp.
Andricus grossulariae	*Glypta rufiscutellaris*
Andricus hispanicus	*Liotryphon caudatus*
Andricus hungaricus	*Omorgus borealis*
Andricus kollari	**Parasitoids**
Andricus legitimus	**Family: Eupelmidae**
Andricus lignicolus	*Eupelmella vesicularis*
Andricus multiplicatus	**Associates**
Andricus pictus	**Order: Coleoptera**
Andricus polycerus	**Family: Scolytidae**
Andricus quercustozae	*Tomicus piniperda*
Aphelonyx cerricola	**Plant associates**
Cynipidae unspecified	**Order: not specified**
Cynips flosculi	**Family: Fagaceae**
Cynips kollari	*Quercus* sp.
Cynips lignicola	*Quercus cerris*
Cynips polycera	*Quercus coccifera*
Cynips quercusfolii	*Quercus faginea*
Neuroterus glandiformis	*Quercus pyrenaica*
Neuroterus saltans	*Quercus suber*
Synergus pallicornis	**Family: Pinaceae**
Synergus umbraculus	*Pinus contorta*
Synophrus politus	*Pinus halepensis*
Family: Diprionidae	*Pinus strobus*

Diprion simile	*Pinus sylvestris*
Order: Lepidoptera	**Family: Rosaceae**
Family: Gelechiidae	*Malus pumila*
Exoteleia dodecella	***Eupelmus annulatus* Nees, 1834** **[Eupelmidae : Eupelminae]**
Family: Lymantriidae	
Leucoma salicis	
Lymantria dispar	
Nygmia phaeorrhoea	
Porthetria dispar	
Stilpnotia salicis	
Family: Momphidae	
Chrysoclista lariella	
Family: Psychidae	
Apterona crenulella	
Luffia lapidella	
Family: Pyralidae	
Etiella zinckenella	
Family: Tortricidae	
Carpocapsa pomonella	
Grapholitha molesta	
Rhyacionia buoliana	

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