Evaluation of the potential of control options used in Italy for the management of the oriental chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera; Cynipidae), in the UK

Short Term Scientific Mission carried out as part of COST Action 1301 EuroCoppice

Figure 1. Adult *Dryocosmus kuriphilus*. Photo courtesy of Federico Pedrazzoli (Edmund Mach Foundation of San Michele All'Adige).

Dr. Matthew J Everatt

October 2015

Contents

1. Introduction

1.1. Biology and lifecycle

The oriental chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), is native to China and is a major pest of chestnut trees. Galls produced by the wasp impede shoot and flower development, and lower tree vigour by reducing leaf area, photosynthesis and tree biomass (Kato and Hijii, 1997; Fig. 2). This inevitably reduces nut yield, sometimes by as much as 80% (Battisti *et al.*, 2013), and also impacts negatively on the quality of coppiced timber. The gall wasp has now spread into Japan, Korea, Nepal, USA and Canada, and most of Western Europe (Fig. 3).

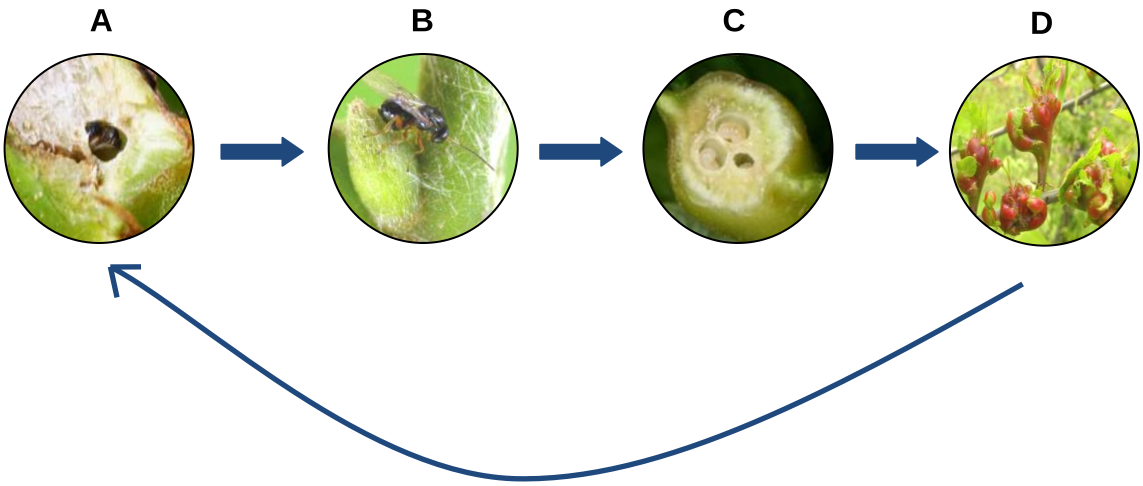

Figure 2. *Dryocosmus kuriphilus* is univoltine and thelytokous. Adults emerge from galls in summer (June to August in Italy) [A] and lay their eggs in chestnut growth buds [B]. Larvae hatch from the eggs and remain dormant in the first instar stage until the spring of the following year, when they feed on the buds [C] and induce gall formation [D]. The larvae pupate and the lifecycle then begins again.

1.2. UK outbreak

The gall wasp was first discovered in the UK by an amateur gall recorder in June 2015, within Farningham wood, Kent. A subsequent survey showed that the gall wasp was widely distributed in the northwest area of the woodland (Fig. 4), and wider surveillance across the UK identified a further six infested chestnut trees, in St Albans, Hertfordshire. Action was taken to coppice infested and neighbouring trees in Farningham wood, mulch the debris, and destroy the infested trees in St Albans. However, these actions are unlikely to eradicate the gall wasp from the UK; mulching is unlikely to destroy 100% of the galls left in debris within Farningham wood and, having been in the UK for at least 2 years, the gall wasp will have had time to spread across the wood, to areas where trees are not being felled, and into other woodlands. There is therefore a need to consider long term management options for *D. kuriphilus*.

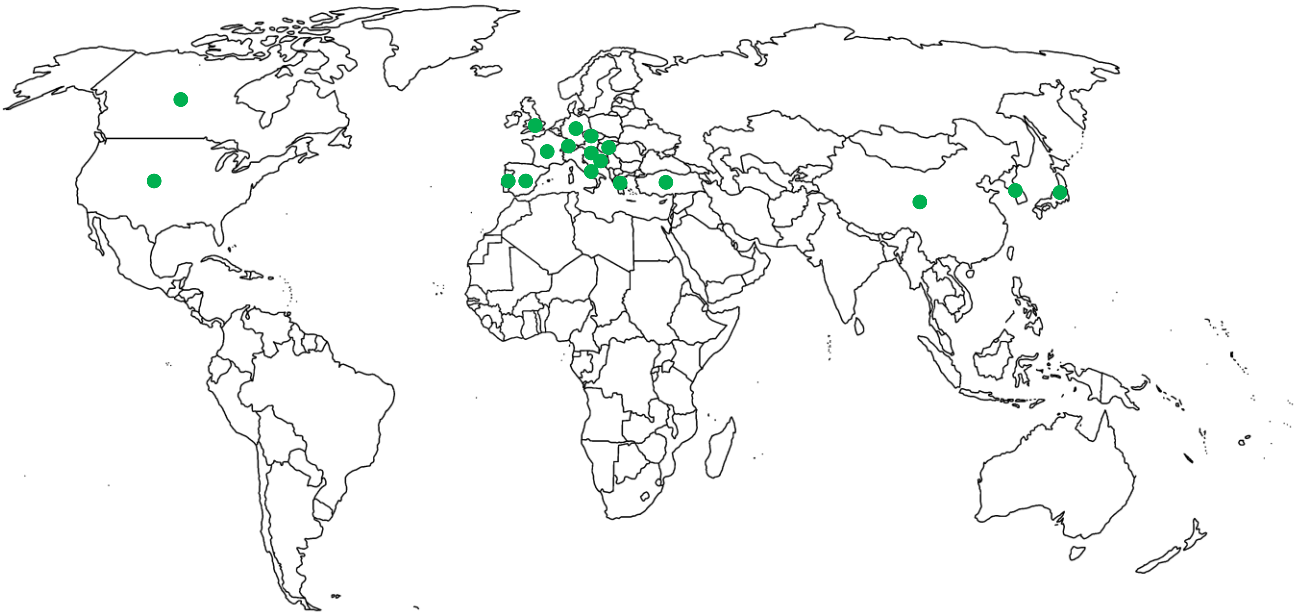

Figure 3. *Dryocosmus kuriphilus* is distributed in China, Japan (1941), Korea (1958), USA (1974), Nepal (1999), Italy (2002), France (2005), Slovenia (2005), Switzerland (2009), Hungary (2009), Croatia (2010), Spain (2010), Canada (2012), the Czech Republic (2012), Germany (2012), Portugal (2014), Greece (2014), Turkey (2014), and the UK (2015). Green dots indicate presence.

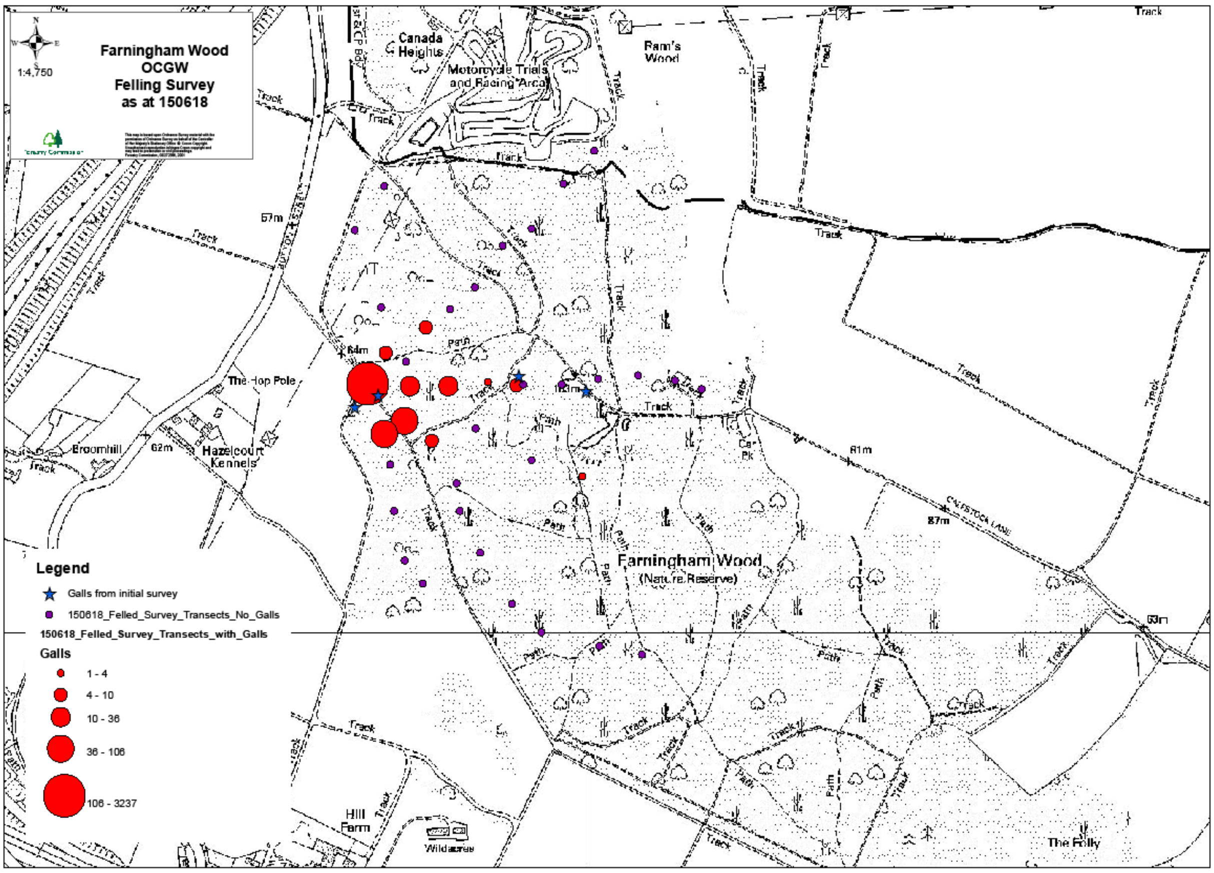

Figure 4. Distribution of *D. kuriphilus* in Farningham wood, following a transect survey.

1.3. Management options

Control of the gall wasp using pesticides has previously been difficult. Because of the structure of the galls, most pesticides have been unable to penetrate the galls and reach the larvae (Bosio *et al.*, 2009). While some success has been gained when targeting adult emergence, this was only on small plants and would not be suitable for large trees (Bosio *et al.*, 2009). Selecting resistant varieties of chestnut tree is also unlikely to be an option; there is currently only one resistant variety (Bouche de Bétizac; Sartor *et al.*, 2007) and using just this would reduce the richness of chestnut trees in any one area. Planting this variety would also make no difference to the chestnut trees already present (Quachia *et al.*, 2008). This leaves the use of biological control, using native parasitoids and/or the non-native parasitoid, *Torymus sinensis*. Native parasitoids generally give low levels of parasitisation; in Italy, parasitisation rates have usually been 0.6-1.6%, and this may be explained by poor synchronisation between the parasitoids and *D. kuriphilus* (Aebi *et al.*, 2006). *Torymus sinensis*, on the other hand, is very well synchronised with *D. kuriphilus* and has been very successful in reducing numbers of *D. kuriphilus* in Japan, USA and Italy, and has been released in many other European countries. The non-native parasitoid therefore looks to be the most promising option for the UK.

2. Purpose

By travelling to Italy and meeting with a number of researchers, who have been investigating the control of *D. kuriphilus* with *T. sinensis*, I hoped to gain a better understanding of *T. sinensis* and how effective and appropriate it might be for release in the UK. Four areas were focussed on: 1) establishment and spread, 2) environmental and ecological risks, 3) efficacy, and 4) methodology for rearing and release. There was also the opportunity to find out more about the potential of native parasitoids and cultural methods for controlling *D. kuriphilus*.

3. Activities

I visited Italy between the 18th and 25th October 2015. Over the course of the week, I was able to do the following:

- Meet with Chiara Ferracini (University of Turin) and Emilio Guerrieri (Institute for sustainable plant production) to:
    - Discuss the *T. sinensis* biocontrol programme in Italy and how applicable the programme would be to the UK outbreak of *D. kuriphilus*
- Meet with Giorgio Maresi and his team (Edmund Mach Foundation of San Michele All’Adige) to:
    - Take a tour of the gall wasp storage and rearing facilities
    - Visit five chestnut stands around the Trentino area where *T. sinensis* has been released

    - Discuss the release process

- Meet with Alberto Maltoni (University of Florence) to:
    - Discuss pruning and other cultural techniques for controlling *D. kuriphilus*

- Meet with Tiziana Panzavolta and her team (University of Florence) to:
    - Discuss the efficacy of native parasitoids and *T. sinensis*

- Visit a nursery where *T. sinensis* is being “propagated” for release into chestnut orchards and coppices (Garfagnana)

- Visit chestnut stands (orchards and coppices) where *T. sinensis* is being released (Garfagnana)

- Visit chestnut trees that were previously susceptible, but have become resistant to the gall wasp

4. *Torymus sinensis*

4.1. Establishment and spread

Classical (or inoculative) control involves the collection of a natural enemy from the origin of the pest and releasing it into a new environment in which the pest has invaded. The natural enemy is then allowed to build up its numbers and suppress the pest over long periods of time (Bale *et al.*, 2008). If *T. sinensis* is to be an effective classical control in the UK, therefore, the parasitoid must be first able to establish. There are currently no studies on the thermal tolerance of *T. sinensis*, but based on climate matching, the parasitoid is highly likely to survive and develop in the UK. The climates of the Trentino and Cuneo regions in Italy, where *T. sinensis* is thriving, are similar to that of the UK, and may even be more severe in some cases; for example, temperatures between -10 and -15°C are not uncommon in north west Italy (Fig. 5).

Torymus sinensis also has a very high spread potential. While the spread of the parasitoid during the first years of release in Japan and Italy was slow (< 1 km/year), the parasitoid travelled at a rate of 60 km/year in Japan 7 years after the first release (Moriya *et al.*, 2003), and spread over most of Tuscany in 2008-2009. These figures should be viewed with caution, however, as *T. sinensis* was readily available in Italy in 2008, and possibly also in Japan, and could have been released by private owners with no record taken. The spread may therefore not be due to natural dispersal.

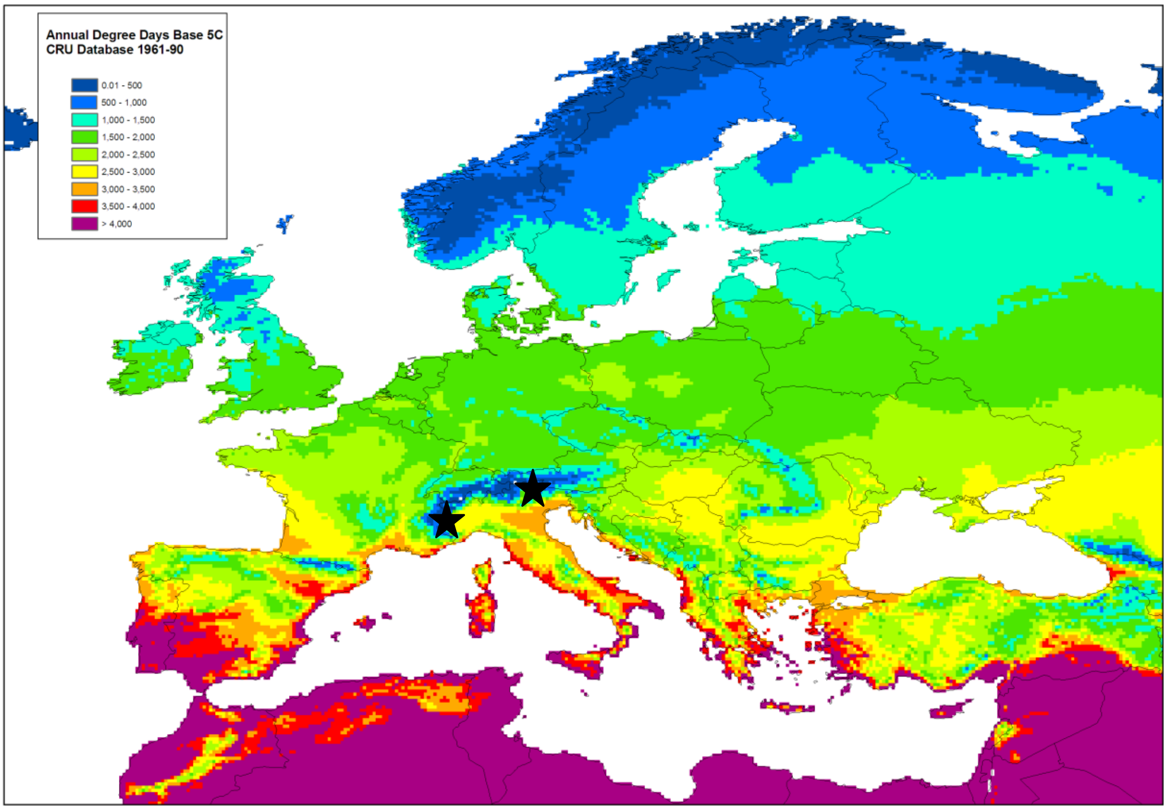

Figure 5. Annual degree days across Europe based on an invertebrate development threshold of 5°C. The black stars indicate regions in Italy that are climatically similar to the UK and where *T. sinensis* is present.

4.2. Environmental and ecological risks

4.2.1. Taxonomic confusion

Torymus sinensis is taxonomically similar to a number of other species, including *Torymus beneficus*. To differentiate between *T. sinensis* and *T. beneficus* previously, differences in the ratio of ovipositor sheath length and thorax length was used. However, this has since been shown to be insufficient, raising concern that the specimens being distributed from Japan into Italy and beyond were in fact not just *T. sinensis*, but a mixture of *Torymus* species. DNA sequencing of parasitoids emerging from *D. kuriphilus* galls and comparison with sequences in the National Center for Biotechnology Information (NCBI) are now being carried out, and Chiara Ferracini and Emilio Guerrieri are confident that the parasitoids which have been released have been *T. sinensis*.

4.2.2. Host shift

Torymus sinensis is considered to be specific to *D. kuriphilus*. However, this is an unusual trait among torymids; as they are ectoparasitoids (feed from the outside of gall wasp larvae), they do not need to adapt to, and tolerate, their host's immune system and so they are able to utilise a wide range of hosts (Emilio Guerrieri personal communication). *Torymus sinensis* may therefore not be as specific as has previously been recorded. A recent study by Ferracini *et al.* (2015) indicates that this might be the case. In no choice oviposition trials, oviposition by *T. sinensis* was recorded once on *Andricus curvator*, and

Torymus sinensis adults also emerged from three *Biorhiza pallida* galls in 2013. These results should be viewed with caution, however. The oviposition trials force encounters between parasitoids and potential hosts, producing a situation that would not necessarily occur in nature, and may therefore give rise to false positives. While, the adults emerging from *B. pallida* galls were males and *T. sinensis* adults are known to be less selective over where they oviposit this sex; these three oviposition events may thus be on a less preferred host and are likely to be only very occasional (Chiara Ferracini personal communication). A mass collection of *B. pallida* galls in 2014 also did not show any further *T. sinensis* emergence. Further galls have been collected in 2015, but the data has not yet been analysed.

4.2.3. Hybridization

In Italy, hybridization has not been observed between *T. sinensis* and native parasitoids (Quacchia *et al.*, 2014), and so far, hybridization has only been recorded between *T. sinensis* and *T. beneficus* in Japan (Yara *et al.*, 2000, 2012). To better understand the risk of hybridization to the UK, a study could be conducted between *T. sinensis* and UK native parasitoids that mate during the same or similar time period (Emilio Guerrieri personal communication).

4.3. Efficacy

4.3.1. Italian situation

Prior to the release of *T. sinensis* in Italy, infested chestnut trees were often covered with thousands of galls ("cherry trees"), exhibited bare foliage and produced very few nuts. Ten years after the release of the parasitoid, chestnut trees are now very healthy in north-west Italy, with parasitisation rates of *D. kuriphilus* galls by *T. sinensis* often greater than 90% (Fig. 6). Trentino is seeing similar results, though the pace at which chestnut trees have recovered has been accelerated (within 5 years of release). Control of the gall wasp with *T. sinensis* was also achieved within a similar time frame, within 6-18 years of release, in Japan (Moriya *et al.*, 2003). However, *T. sinensis* may not be the only reason for the recovery of chestnut trees. In Tuscany, chestnut trees have begun to recover after only 3 years following the release of *T. sinensis*. Alberto Maltoni thinks that this is too quick for the biological control to suppress *D. kuriphilus* populations and suggests that the climate might have a bigger role to play; the cold 2014 summer followed by the hot 2015 summer may have been unfavourable for *D. kuriphilus*. Still, parasitisation by *T. sinensis* has been high, particularly in the northern regions, and has likely contributed to the recovery of chestnut trees, even if this recovery has also been aided by recent climatic conditions.

4.3.2. Hyperparasitism

Hyperparasitism of *T. sinensis* has previously been observed in the USA, by *Ormyrus labotus*, and Cooper and Rieske (2011) suggest that the high abundance of *O. labotus* at the site in Bowling Green is the reason for the low numbers of *T. sinensis* there. In Italy, Tiziana Panzavolta and colleagues have now also found another hyperparasitoid (*Eupelmus urozonus*) in association with *T. sinensis*, though the effect it is having on *T. sinensis* in the wider environment is unknown. It is also not clear whether *E. urozonus* was feeding on *T. sinensis* or *D. kuriphilus*, as *E. urozonus* can feed on both. In Kent, *Eurytoma brunniventris* has been observed to attack *Torymus* spp.

Figure 6. Chestnut production has been high in 2015 (Trentino, Italy).

4.4. Methodology for rearing and release

4.4.1. Multiplication sites

Between 2004 and 2006, Italy received 28,010 galls from Japan. Adult *T. sinensis* were reared from these galls and released onto infested trees (Quacchia *et al.*, 2008). Since 2006, Italy has been able to rely on galls collected from its own infested sites, due to the growth in the *T. sinensis* population. Multiplication sites, where populations of *T. sinensis* are built up, were set up in different regions of Italy to increase the efficiency of gall collection (Fig. 7). These sites are created by planting a small number of infested trees or by planting uninfested trees that are close by to an infested site. Sometimes, populations of *D. kuriphilus* are artificially increased by introducing infested galls. Trees in these sites are generally kept small to make gall collection easier.

4.4.2. Collection and rearing

Galls are collected in February and March. Any earlier and *T. sinensis* under artificial conditions loses its synchrony with *D. kuriphilus*; any later and *T. sinensis* has already emerged from the galls. Collected galls are cleaned to remove contaminants (e.g., fungi) and are stored in cardboard boxes that have two plastic tubes attached (see Fig. 8). These cardboard boxes are placed under suitable climatic conditions, usually in a sheltered location (e.g., an open wooden hut), to allow *T. sinensis* to synchronise its lifecycle with that of *D. kuriphilus*.

Figure 7. *Torymus sinensis* multiplication site on a Tuscan nursery.

Figure 8. Cardboard box used for storing collected galls.

Emerging adults are attracted to light and enter the plastic tubes. The emergence of adults is checked frequently (e.g., two times per day). Once a sufficient number of adults has emerged, the tubes are taken into the laboratory, where adults are coupled (using a pooter), in a ratio of 2:1 females to males, into new plastic containers. Adults are fed on honey drops that are placed on top of a plastic slip (Fig. 9). The containers are subsequently placed under suitable climatic conditions until release. In Trentino, this was within an incubator (16°C day, 10°C night), and in Tuscany, this was within a fridge (4-6°C). The honey needs to be replaced every 2 weeks in the incubator, and every 2 days in the fridge (due to the crystallization of the honey at low temperatures).

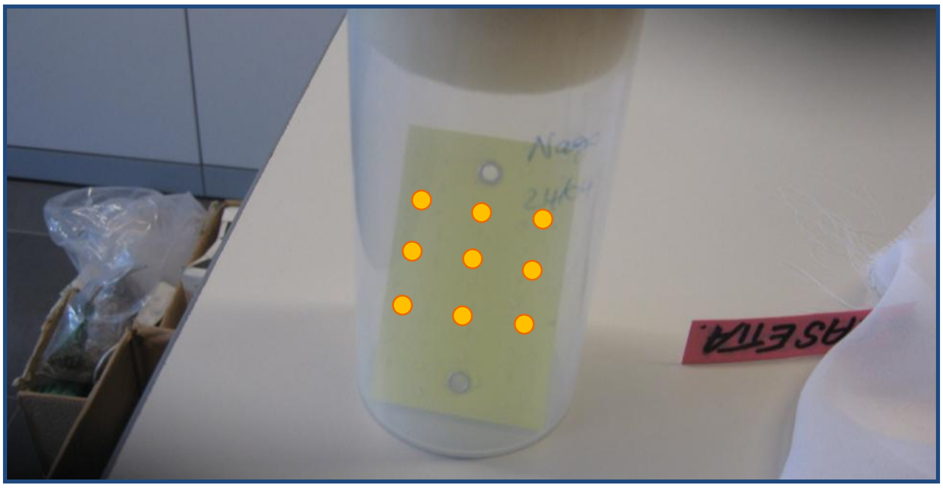

Figure 9. Plastic tube, containing a plastic slip covered in honey drops (yellow dots).

4.4.3. Release

During the transfer of coupled *T. sinensis* to a release site, adults are kept in a climatic box to maintain their synchronicity with *D. kuriphilus*. Adults are subsequently released in a concentrated manner onto one or two infested branches. Trials have shown that releasing adults in separate spots is less likely to result in establishment and spread (Emilio Guerrieri personal communication). The release of adults is timed with the initial development of galls on the tree.

A new method is being trialled by the multiplication site in Tuscany. Because they know roughly how many cells there are in a gall and the parasitisation rate of *T. sinensis* in the regions where galls are being collected, they are able to estimate the number of galls it would take to give a ratio of 100 females to 50 males (the quantity required for a single release). These galls are placed into a net, stored until release, and then tied onto an infested tree in the release site (Fig. 10).

It should be noted that multiplication sites are finite and will eventually be exhausted as the *D. kuriphilus* population decreases. Newly infested field sites then become the new source of galls. In Italy, *T. sinensis* has now spread across the whole country, making controlled releases less important.

Figure 10. Net containing parasitized galls.

5. Native parasitoids

As mentioned at the beginning of this report, native parastioids generally give low levels of parasitisation (0.6-1.6% in Italy, Aebi *et al.*, 2006). However, a parasitisation rate of over 30% by the native parasitoid, *Torymus flavipes*, was observed in 2011 (Santi and Maini, 2011 – but not confirmed by sequencing), and Emilio Guerrieri and Alberto Maltoni also know of instances where parasitisation rates by native parasitoids have exceeded 60% in other sites. These instances are peculiar, though, and seem to occur under very specific conditions (warm, with chestnut trees surrounded by oak). Native parasitoids are therefore unlikely to provide a consistently effective control. Instead, the impact of native parasitoids may be greater on their regular oak hosts. Because *D. kuriphilus* galls are found in much higher numbers than oak galls, native parasitoids that have shifted onto chestnut may be reproducing at a higher rate. In turn, native parastioids may inflict greater rates of parasitisation on their oak hosts, particularly when the level of *D. kuriphilus* galls is reduced, and cause a crash in oak gall wasp populations (Emilio Guerrieri personal communication). Emilio Guerrieri and colleagues will be looking into this hypothesis.

6. Additional control measures

6.1. Elongation of the growth period

Alberto Maltoni has shown that in vigorous (healthy) chestnut trees, damage by *D. kuriphilus* increases from the apex of a shoot or branch to the base, and in the top three nodes, no severe damage is seen (Maltoni *et al.*, 2012a; fig. 11). This is reasoned to be due to the buds on these nodes forming after the end, or towards the end, of the flight period of the gall wasp. In non-vigorous plants, growth of the shoots finishes before the end of the flight period and the shoots sometimes show severe damage. Therefore, damage can be minimised by elongating the growth period of the plant, which can be achieved through irrigation, fertilisation and pruning. This may also increase the resources overall, allowing the plant to better resist attack from the gall wasp in the basal nodes.

6.2. Green pruning

Pruning of actively growing shoots forces new shoots to grow in the same season that would otherwise be delayed until next year. Pruning that occurred in July produced healthy new shoots, as they were grown after the end of the flight period. However, this also did not give the tree time to produce many more buds. There is therefore a trade-off in the health of the shoots and the number of buds depending on when the trees are pruned. It should be noted that pruning in this way would have to be done relatively frequently (every 2 years) and would therefore only be suitable for small plants. See Maltoni *et al.* (2012b) for more details.

Figure 11. Healthy apical shoots of a chestnut tree.

6.3. Rotation of coppice

With every year, the length of new branches/shoots on a chestnut tree shortens. The reduced vigour of older trees means that the growth of new shoots may finish before the end of the flight period of the gall wasp and shoots may become more susceptible to attack. Shorter rotation times for coppice stands are therefore preferred.

6.4. Development of resistance

An unusual situation has developed on a person’s land in Tuscany, Italy. Five or six chestnut trees have become resistant to the gall wasp over the last two years. These trees show a slightly different phenology in that the leaves stay on the tree longer. However, it is not understood how the trees have developed resistance.

7. Conclusion and recommendations

Torymus sinensis has been able to establish and spread quickly across Italy, including in the northern regions, where the climate is similar to, and sometimes more severe than, the UK. *Torymus sinensis* has also been effective in Italy, parasitizing the gall wasp at rates of greater than 90% in some regions. The parasitoid therefore seems to be a good candidate for release into the UK.

However, there are also potential risks associated with *T. sinensis*: its ability to shift onto new hosts and its ability to hybridize with native parasitoids. Hybridization has not been observed in Italy, and has previously only been recorded in Japan with *T. beneficus*. And even if hybridization was observed with UK native parasitoids (and hybrids did not move onto oak), it is unlikely to impact heavily on native parasitoids, as they would still be able to persist where chestnut trees, and therefore *T. sinensis*, are not present. The danger arises if *T. sinensis* is also able to shift onto new hosts and spread over a wider region. While *T. sinensis* has been observed to parasitise *B. pallida*, such instances have been very rare. On current evidence, the risk of *T. sinensis* shifting onto new hosts and causing any significant impact is small.

Because of the value of nut production in Italy, the decision to release *T. sinensis* was easy. The negative economic impact far outweighed any risks to native gall wasps and parasitoids. In contrast, the economic impact for the UK would be far lower. Chestnut trees are far less abundant (28,500 ha), with only 18,000 ha used for coppice and few trees used for nut production. The environmental and ecological value of the trees is also relatively small. Therefore, whether the benefits outweigh the risks in the UK is less clear cut.

If the UK decides to release the parasitoid, the researchers in Italy recommend that *T. sinensis* is released as early as possible to avoid the build-up of *D. kuriphilus*. As the gall wasp is currently at a low level within Farningham wood, a single release of *T. sinensis* (~150 adults) is advised initially, which could be supplemented depending on the progress of the parasitoid. In conjunction, the vigour of the chestnut trees could be improved through irrigation, fertilisation, and shortened coppice rotations.

8. Acknowledgements

I would like to thank the following people for the effort and time they put into this short term scientific mission: Debbie Bartlett, Chiara Ferracini, Emilio Guerrieri, Giorgio Maresi, Federico Pedrazzoli, Alberto Maltoni, and Tiziana Panzavolta and her team.

9. References

Aebi, A., Schönrogge, K., Melika, G., Alma, A., Bosio, G., Quacchia, A., Picciau, L, Abe, Y., Moriya, S., Yara, K., Seljak, G. and Stone, G. N. (2006) Parasitoid recruitment of the globally invasive chestnut gall wasp *Dryocosmus kuriphilus*. In: K. Ozaki, J. Yukawa, T. Ohgushi and P. W. Price (eds.). Galling Arthropods and their Associates. Japan: Springer, pp. 103-121.

Bale, J. S., van Lenteren, J. C. and Bigler, F. (2008) Biological control and sustainable food production. Philosophical Transactions of the Royal Society B. 363, 761-776.

Battisti, A., Benvegnù, I., Colombari, F. and Haack, R. A. (2013) Invasion by the chestnut gall wasp in Italy causes significant yield loss in *Castanea sativa* nut production. Agricultural and Forest Entomology. 16, 75-79.

Bosio, G., Gerbaudo, C. and Piazza, E. (2009) *Dryocosmus kuriphilus* Yasumatsu: An outline seven years after the first report in Piedmont (Italy). Acta Horticulturae. 866, 341-348.

Cooper, W. R. and Rieske, L. K. (2011) A native and an introduced parasitoid utilize an exotic gall-maker host. Biocontrol. 56, 725-734.

Ferracini, C., Ferrari, E., Saladini, M. A., Pontini, M., Corradetti, M. and Alma, A. (2015) Non-target host risk assessment for the parasitoid *Torymus sinensis*. Biocontrol. 60, 583-594.

Kato, K. and Hijii, N. (1997) Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) on the growth of chestnut trees. Journal of Applied Entomology. 121, 9-15.

Maltoni, A., Mariotti, B. and Tani, A. (2012a) Case study of a new method for the classification and analysis of *Dryocosmus kuriphilus* Yasumatsu damage to young chestnut sprouts. iForest. 5, 50-59.

Maltoni, A., Mariotti, B., Jacobs, D. F. and Tani, A. (2012b) Pruning methods to restore *Castanea sativa* stands attacked by *Dryocosmus kuriphilus*. New Forests. 43, 869-885.

Moriya, S., Shiga, M. and Adachi, I. (2003) Classical biological control of the chestnut gall wasp in Japan. In: R. G. van Driesche (ed.). Proceedings of the 1st international symposium on biological control of arthropods. USDA Forest Service, Washington, pp. 407-415.

Quacchia, A., Moriya, S., Bosio, G., Scapin, I. and Alma, A. (2008) Rearing, release and settlement prospect in Italy of *Torymus sinensis*, the biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*. Biocontrol. 53, 829-839.

Quacchia, A., Moriya, S., Askew, R. and Schönrogge, K. (2014) *Torymus sinensis*: Biology, host range and hybridization. In: L. Radócz et al. (eds.). Proceedings of the 2nd European Congress on chestnut. Acta Horticulturae, pp. 105-112.

Santi, F. and Maini, S. (2011) New association between Dryocosmus kuriphilus and Torymus flavipes in chestnut trees in the Bologna area (Italy): first results. Bulletin of Insectology. 64, 275-278.

Sartor, C., Mellano, M. G., Quacchia, A., Alma, A. and Botta, R. (2007) Cinipide galligeno del castagno: prospettive di impiego di strategie da affiancare alla lotta biologica. Riassunto dei lavori, VIII giornate scientifiche SOI. Italus Hortus. 14, 130-133.

Yara, K., Yano, E., Sasawaki, T. and Shiga, M. (2000) Detection of hybrids between introduced *Torymus sinensis* and native *T. beneficus* (Hymenoptera: Torymidae) in central Japan, using malic enzyme. Applied Entomology and Zoology. 35, 201-206.

Yara, K., Matsuo, K., Sasawaki, T., Shimoda, T. Moriya, S. (2012) Influence of the introduced parasitoid *Torymus sinensis* (Hymenoptera: Torymidae) on *T. koreanus* and *T. beneficus* as indigenous parasitoids of the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) on chestnut trees in Nagano Prefecture, Japan. Applied Entomology and Zoology. 47, 55-60.