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A North American invasive seed pest, *Megastigmus spermotrophus* (Wachtl) (Hymenoptera: Torymidae): Its populations and parasitoids in a European introduction zone

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Abstract

Megastigmus spermotrophus (Hymenoptera: Torymidae) is a seed pest of Douglas-fir (*Pseudotsuga menziesii*) in its natural range of North America. Along with seeds, this invading species was accidentally introduced to Europe where it became the major pest in Douglas-fir seed orchards and stands. The high rates of seed parasitism observed in Europe were first ascribed to a reduced control by natural enemies which apparently did not followed *M. spermotrophus* from their native American range.

However, our survey showed that the parasitoid censuses carried out so far were incomplete. Several other chalcid parasitoid species were present in the stands surveyed in Belgium. Four parasitoid species emerged from Douglas-fir seeds infested by the Douglas-fir seed chalcid. Two pteromalids of North American origin, *Mesopolobus spermotrophus* and *Mesopolobus americus*, were dominant whereas a few specimens of an eupelmid, *Brasema urozonus*, and of another pteromalid, *Anogmus hohenheimensis*, were also observed. Our results strongly suggest that the previous evaluations of parasitoids populations were biased by an inadequate timing in the censuses.

Keywords: *Megastigmus spermotrophus*; Douglas-fir; Seeds; *Mesopolobus spermotrophus*; *Brasema urozonus*; *Anogmus hohenheimensis*; *Mesopolobus americus*

1. Introduction

Megastigmus spermotrophus Wachtl (Hymenoptera: Torymidae) originates from North America and has probably been introduced in Europe together with its host seeds during the last part of 19th century (Roques and Skrzypczynska, 2003). In Europe, it is the major pest in Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seed orchards and stands (Roques, 1981, 1983). Previous studies mention seed damage ranging up to 100% of the seed crop in Europe (Roques, 1983) while the damage is significantly lower (usually <10%) in its native range where the pest spectrum is largely dominated by other cone pests (Rappaport and Roques, 1991). In Belgium, literature data indicated seed damage rates ranging from 5.2% to 30% (Jamblinne de Meux and Nanson, 1969). This extended impact may result in a significant seed deficit, especially in case of low seed crop.

Several biological and ecological characteristics favour the spread and establishment of the seed chalcid (Roques et al., 2004). (1) It is specialized in exploiting the seeds of Douglas-fir, *Pseudotsuga* spp.; (2) Its ovo-larval development takes place completely in the same seed; (3) It is

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capable of doing arrhenotokous parthenogenesis; (4) It is capable of developing in unpollinated, unfertilised seeds, which allows it to survive periods of low pollination (Rappaport et al., 1993; Rouault et al., 2004; von Aderkas et al., 2005a,b); (5) Chalcid larval diapause in seeds can be extended to up to 5 years, which permits to bypass years with no seed crop; (6) There are no or very few native competitors in the invaded areas because of the absence of native *Pseudotsuga species* (Roques et al., 2006); (7) Only very few natural enemies are thought to have followed *M. spermotrophus* from their native American range (Rappaport and Roques, 1991). Indeed, due to the limited impact of *Megastigmus spermotrophus* in its native range, its parasitoid complex has not been widely surveyed. In the European introduction range, ca. 850,000 chalcid-infested seeds were reared from commercial collections in France in 1980–1995 without showing any evidence of parasitization (Roques et al., 2004). Nevertheless, since the 1950s a pteromalid species of North American origin, *Mesopolobus spermotrophus* Hussey (Hymenoptera: Pteromalidae), has been recorded from infested seeds fallen to the ground (Hussey, 1955; Kristek, 1967; Roversi, 1995; Schnaider, 1970).

The near-absence of natural enemies and competitors has been assumed a key factor explaining why the Douglas-fir seed chalcid tends to occupy the entire seed niche in introduction areas (Rappaport and Roques, 1991). However, preliminary, unpublished studies suggested that the parasitoid populations may have been largely underestimated in Europe because of irrelevant sampling method. Thus, in 2003 and 2004, we collected opened mature cones in spring on the ground of a number of Douglas-fir stands all over Wallonia in order to evaluate the amount of seeds destroyed by *Megastigmus* and to survey the possible parasitoid populations. Here, we present the results of this survey. We also compare the efficiency of sampling timing for the census of parasitoids in a perspective of biological control.

2. Materials and methods

In March of 2003 and 2004, one-year-old Douglas cones lying on the ground were collected namely 2 months before adult emergence of *Megastigmus spermotrophus*. Collections were carried out in 51 different Douglas-fir stands located in various environments in the southern part of Belgium (Wallonia). For each stand, we recorded latitude, longitude, elevation, year of plantating, and size of plantation. The selected stands were rather old (from 50 to 120 years) as older trees are more likely to produce cones and thus to contain parasites and parasitoids. Cones were randomly picked up during about two hours in the chosen sites. The average number of seeds collected per site was 5300 in 2003 and 9800 in 2004. Cones were brought back to laboratory and dried for about four days at ambient temperature (about 20 °C). Remaining seeds were extracted manually. In order to be sure to rear only seed insects and their parasitoids, all vegetive parts (eg. scales) were removed. Seeds were placed in clear polystyrene boxes and surveyed until insect emergence. The boxes were placed in outdoor insectaria under semi-natural climatic conditions. Adults of *Megastigmus spermotrophus* and their parasitoids that emerged from seeds were recorded daily, identified and counted. In November, when the annual emergence was completed, the respective number of larvae remaining in prolonged diapause and dead ones were assessed by dissecting seeds.

A total of 273477 and 137835 seeds were, respectively, examined in 2003, and 2004. The populations of seed chalcids and parasitoids were estimated as follows. For *Megastigmus spermotrophus*, we calculated (i) the occurrence ratio = the number of sites where the chalcid was present/the total number of visited sites; and (ii) the infested seeds rate (ISR) = the number of chalcid-infested seeds/the total number of seeds per site. We included the unpollinated seeds in the calculation of ISR because *M. spermotrophus* can develop in unpollinated seeds as well as in pollinated ones (Rappaport et al., 1993).

For the parasitoid species, we calculated (i) the occurrence ratio similarly as above; and (ii) the parasitism rate = the number of parasitoids/the number of *Megastigmus* and parasitoids. Parasitism rate was calculated for each parasitoid species and for sites where *Megastigmus* was present. The emergence period of each species was also assessed.

Data were analyzed using GraphPad Instat version 3.05 for Win95/NT (GraphPad Software 1998, Inc., San Diego, California, USA). To state the possible development of the insect populations, we compared the occurrence ratios with Fisher test. Infested seed rates and parasitism rates were compared with Mann–Withney test.

3. Results

3.1. Seed damage by Megastigmus spermotrophus

Megastigmus spermotrophus was found in almost all the stands we sampled in Wallonia, with an occurrence ratio of 0.98 in 2003 and 1.00 in 2004 (Table 1). Infested seed rates (ISR) averaged to 0.16 in 2003 and 0.05 in 2004 (Table 2). ISR values were linearly correlated from one year to the next ($ISR_{2004} = 0.17 * ISR_{2003} + 0.03$, $R^2 = 0.31$, df = 50, $P < 0.005$). ISR increased with elevation (*m*) in both years (best-fit relationships: $ISR_{2003} = 0.01 * e^{0.005(\text{elevation})}$, $R^2 = 0.35$, df = 50, $P < 0.001$; $ISR_{2004} = 0.01 * e^{0.004(\text{elevation})}$, $R^2 = 0.24$, df = 50, $P < 0.005$). Exponents of data regressions were not statistically different (*t*-test: t = 0.09, df = 100, $P > 0.2$). Hence, both ISRs were related to elevation in the same quantitative way.

Under semi-natural climatic conditions (outdoor insectaria), emergence of *Megastigmus spermotrophus* began at the end of April, peaked at mid-May and ended at the end of May (Fig. 1).

Table 1
Occurrence ratio of the Douglas-fir seed chalcid, *Megastigmus spermotrophus*, and of its parasitoids in 51 Wallonian sites in 2003 and 2004

Species	Occurrence ratio		Fisher test 2003 vs. 2004
	2003	2004	
Megastigmus spermotrophus	0.98	1.00	$D = 0.02$, $P = 1.000$
Mesopolobus spermotrophus	0.90	0.65	$D = 0.25$, $P < 0.005$
Mesopolobus americus	0.52	0.31	$D = 0.21$, $P < 0.05$
Eupelmus urozonus	0.04	0.10	$D = 0.06$, $P = 0.44$
Anogmus hohenheimensis	0.18	0.04	$D = 0.14$, $P < 0.05$
Total parasitoids	0.94	0.79	$D = 0.19$, $P < 0.005$

Occurrence ratio is defined as the number of sites where the insect was present over the total number of visited sites. Occurrence ratios of 2003 and 2004 are compared using Fisher tests. Values of P and results of the statistic test ($\alpha = 0.05$) are given in the last column for each parameter.

Table 2
Infested seeds rate (the number of chalcid- infested seeds/the number of seeds per site) and parasitism rate (the number of parasitoids/number of Megastigmus + parasitoids) in 51 Wallonian sites in 2003 and 2004

Infested seed rates and parasitism rate	2003	2004	Mann–Withney test
Infested seed rates	0.16 ± 0.15	0.05 ± 0.04	$P < 0.0001, U = 645$
Mesopolobus spermotrophus parasitism rate	0.14 ± 0.13	0.12 ± 0.17	$P < 0.05, U = 981$
Mesopolobus americus parasitism rate	0.01 ± 0.01	0.05 ± 0.06	$P = 0.51, U = 1155$
Brasema urozonus parasitism rate	0.003 ± 0.004	0.03 ± 0.05	$P = 0.57, U = 1172$
Anogmus hohenheimensis parasitism rate	0.002 ± 0.001	0.01 ± 0.02	$P = 0.23, U = 1083$
Total parasitism rate	0.15 ± 0.13	0.14 ± 0.18	$P = 0.19$, $U = 1082.5$

The mean ± SD of the rates are compared using Mann–Withney tests. Values of P and results of the statistic test ($\alpha = 0.05$) are given in the last column for each parameter.

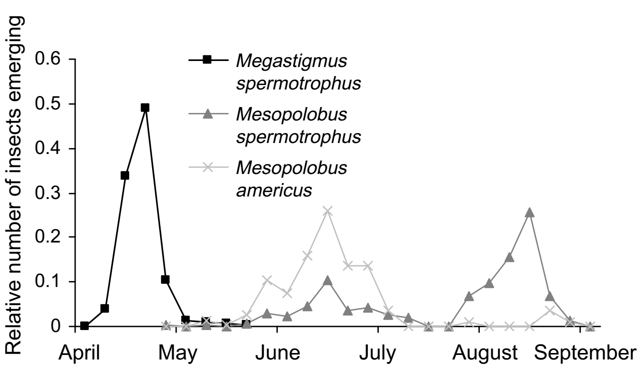

Fig. 1. Emergence period of *Megastigmus spermotrophus* and its two main Mesopolobus parasitoids observed in outdoor insectaria.

3.2. Parasitoids of *Megastigmus spermotrophus*

Parasitoids were present in most of the prospected sites in both 2003 and 2004 (Table 1). The total parasitism rates were 0.15 in 2003 and 0.14 in 2004 (Table 2). Parasitism was mostly due to two pteromalid species of the genus *Mesopolobus*, *Mes. spermotrophus* and *Mes. americus* sp. n. (under description by Dzhakomn and Roques). A few specimens of *Brasema* (Eupelmus) *urozonus* Dalman (Hymenoptera: Eupelmidae) and *Anogmus hohenheimensis* Ratzb. (Hymenoptera: Pteromalidae) were also found.

3.2.1. *Mesopolobus spermotrophus (Ms)*

Mesopolobus spermotrophus (Ms) was the predominant parasitoid. During the study period, it was nearly present at all sites (Table 1). Ms parasitism rate was not correlated with ISR (Table 2; 2003: $R^2 = 0.04$, df = 45, NS, 2004: $R^2 = 0.06$, df = 32, NS). In outdoor insectaria, Ms emergence began by late May, about three weeks following the onset of host emergence, peaked twice and ended by early September (Fig. 1).

3.2.2. *Mesopolobus americus (Ma)*

Mesopolobus americus (Ma) was the second most frequent and abundant parasitoid species. During the two years of study, it was present on around one-third of the sites (Table 1). There was no correlation between Ma parasitism rates and ISR (Table 2; 2003: $R^2 = 0.06$, df = 15, NS, 2004: $R^2 = 0.001$, df = 25, NS). Ms and Ma parasitism rates were not correlated ($P > 0.05$ for all R^2 in 2002 and 2003). In outdoor insectaria, Ma emergence also began by late May, peaked at mid-June and ended by early September (Fig. 1).

3.2.3. *Brasema urozonus (Bu) and Anogmus hohenheimensis (Ah)*

Brasema urozonus (Bu) and *Anogmus hohenheimensis* (Ah) were present on 14% and 20% of the sites visited during the two years, respectively (Table 1). These two species were relatively scarce (Table 2). No correlations were found between the parasitism rates and ISR. The imagos of the two species emerged sporadically in June and July.

4. Discussion

Our study shows that *Megastigmus spermotrophus* is widely distributed in Wallonia. Its seed damage intensity seems to increase with the stand elevation. The increase of seed damage with altitude has been already shown on other conifers (Dormont and Roques, 1999; Trosset and Roques, 1986) but the underlying mechanisms of such a phenomenon remain unknown. This could be taken in account when making out a schedule of implantation of new orchards.

Parasitoids of *Megastigmus spermotrophus* are widely distributed in Wallonia, including three more chalcid species, two of which being pteromalids and one being an eupelmid. Our study shows that *Mes. spermotrophus* iss the dominant parasitoid, and is present in nearly all the surveyed sites. An additional species of *Mesopolobus*, *M. americus*, is rather widespread. This species has just been identified as a parasitoid of *Megastigmus spermotrophus* in North America, where it is present all over the native range of Douglas-fir from British Columbia to California (Roques, in preparation). Unlike *Mes. spermotrophus*, it has not yet been observed in the countries surrounding Belgium, especially north-eastern France where populations of *Megastigmus spermotrophus* were rather high in the last years (Roques, unpublished observations). Thus, we assume that *M. americus* was introduced rather recently in Belgium together with Douglas-fir seeds. The status of the minor parasitoids, *Brasema urozonus* and *Anogmus hohenheimensis* (i.e., primary parasitoids, hyperparasitoids, facultative or not) as well as their native area are still unclear. Further studies are needed to determine if their presence is common or only incidental.

Parasitoids eliminate roughly 15% of the larvae of *Megastigmus spermotrophus* during their development in cones. Thus, it seems unlikely that parasitoids currently will be able to effectively control the pest. However, this possibility can not be excluded for the future because the arrival date of both *Mesopolobus* species in Belgium is still not precisely known yet. *Mes. spermotrophus* could potentially be an effective control agent as this species is a well-known specialist of the Douglas-fir seed chalcid and was relatively abundant in Walloon Douglas stands in our study. It is possible that *Mes. spermotrophus* could be constrained by its early emergence, desynchronized with cone opening. During our study, we observed that at least 70% of *Mes. spermotrophus* emerged before September, i.e. during a period when Douglas-fir cones are still immature and their scales closed. Under these conditions, the female parasitoid is likely constrained by its short ovipositor preventing her to reach a chalcid larva within a seed. Thus, most imagos of *Mes. spermotrophus* which emerge prior to cone opening should only have access to the diapausing larvae of *Megastigmus* to be found either in overmature, 1-year-old cones or in infested seeds shed on the ground during the previous years. However, adults of *Mes. spermotrophus* are also known to be able to survive over 2 months (Hussey, 1955), and most of the early-emerging insects may thus be able to wait for cone opening.

The biological limit imposed by the ovipositor length, which also applies to the three other species of parasitoids, could explain why parasitoid populations were previously underestimated. Previous censuses were made from commercial harvests which always occur when cones are ripe but still closed, in order to maximize seed collections. The results of the preceding censuses were thus biased because harvests took place before parasitoid egg-laying period. Our results emphasize the importance of an adequate timing in censuses of both pest and parasitoid populations in order to get a reliable survey.

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