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European gypsy moth (*Lymantria dispar dispar* L.) completes development and defoliates exotic radiata pine plantations in Spain

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Abstract

Background: Like most pines, radiata pine (*Pinus radiata* D.Don) is conventionally considered resistant to defoliation by European gypsy moth, i.e. it is only consumed by late larval stages, or when susceptible species are not available or are scarce. The ability of this moth to complete development on this host in field conditions has never been reported.

Findings: A gypsy moth outbreak in a pure radiata pine plantation was observed in north-western Spain, where this insect is endemic. During the 2 years of outbreak culmination (2012 and 2013), approximately 46 ha of radiata pine were severely defoliated (>75 % of leaf area removed) and no defoliation was evident in nearby stands of susceptible species. Large numbers of egg masses were present on stems of pine trees. Larvae were observed feeding exclusively on radiata pine needles beginning with the first instar and continuing to pupation. Nearly 100 % tree mortality occurred in stands severely defoliated.

Conclusions: The occurrence of a European gypsy moth outbreak in a pure radiata pine plantation contradicts previous observations that larvae of this species cannot complete development in stands comprised entirely of pines. These results suggest that European strains of the gypsy moth hold great potential for damage to commercial radiata pine plantations where this species is utilised in plantation forestry, such as in New Zealand or Australia.

Keywords: *Pinus radiata*, *Lymantria dispar dispar*, Outbreak, Tree defoliation, Tree mortality, NW of Spain

Background

The gypsy moth, *Lymantria dispar* L. (Lepidoptera: Erebidae), is a serious defoliator of forests throughout much of its native range. It has also colonised eastern North America and is one of the most damaging invasive pests in that region (Doane and McManus 1981; Tobin et al. 2009). This species has three recognised subspecies: the European gypsy moth, *Lymantria dispar dispar* L., and the two Asian gypsy moths, *Lymantria dispar asiatica* Vnukovskij and *Lymantria dispar japonica* Motschulsky (Pogue and Schaefer 2007). In most populations of European gypsy moth, females are flightless, but in Asian gypsy moths, females are largely flight capable (Baranchikov 1989; Keena et al. 2008).

All subspecies are polyphagous and can exploit over 300 deciduous and coniferous host species (Liebhold et al. 1995; Tobin and Liebhold 2011). Gypsy moth caterpillars feed on a wide range of host tree species, but hosts vary in their susceptibility to defoliation. According to the system used by Montgomery (1991) and Liebhold et al. (1995), forest tree species can be classified as 'susceptible', 'resistant' or 'immune' to defoliation. Susceptible tree species are described as those that are consumed by all larval stages, and resistant species are consumed by only some larval stages or when susceptible species are not available, while immune species are

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rarely, if ever, consumed by any larval stage. At a stand level, potential for defoliation is closely related to the proportion of basal area comprised of susceptible species (Davidson et al. 2001).

Most pines, including radiata pine, are considered to be resistant to defoliation since they are only consumed by late larval stages or when susceptible species are not available (Miller and Hanson 1989; Liebhold et al. 1995). Moreover, several authors have reported that early instars cannot complete development on non-deciduous conifers (Rossiter 1987; Strom et al. 1996; Tobin and Liebhold 2011). In mixed pine-hardwood stands of eastern North America, defoliation by gypsy moth caterpillars is largely limited to hardwood hosts, and outbreaks generally do not occur in stands in which oaks or other susceptible hosts represent less than 20 % of host basal area (Campbell and Garlo 1982; Davidson et al. 2001).

Radiata pine is native to California but has been widely planted for commercial forestry elsewhere, especially in the southern hemisphere. In the Northern Iberian Peninsula, extensive planting began in the in the 1950s (Mead 2013). Plantations in this region, along with those in other southern European countries, are the only areas where both radiata pine and European gypsy moths overlap, since the insect is absent in the southern hemisphere and radiata pine is virtually absent from gypsy moth's invaded range in North America.

European gypsy moth has previously been reported to feed on *P. radiata* in Europe. For example, Romanyk (1973) and Romanyk and Rupérez (1960) described outbreaks during 1952–1953 in the provinces of Pontevedra (north-western Spain) and Asturias (northern Spain), respectively. In the latter region, Dafauce and Cuevas (1966) reported that 250 ha of *P. radiata* forest were chemically treated with insecticide between 1953 and 1966. In 1991, 290 ha of a 15-year-old plantation of radiata pine located in northwestern Portugal was severely defoliated (Leite 1993). These reports all demonstrate that European gypsy moth may feed on radiata pine and can cause severe defoliation but they do not provide important details regarding the outbreaks. For example, they do not indicate whether early instars completed development on radiata pine nor whether stands contained a component of oak or other susceptible hosts.

In a laboratory trial, Miller and Hanson (1989) demonstrated that European gypsy moth could complete development from egg to adult by feeding solely on radiata pine foliage. However, the ability of this moth to complete development on radiata pine in field conditions has never been described. These reports, although limited, are important since they suggest that radiata pine plantations elsewhere in the world could be at risk from European gypsy moth. However, most previous concern about potential impacts of gypsy moths on radiata pine plantations have focused on the risk associated with invasions by Asian gypsy moth subspecies (Walsh 1993; Matsuki et al. 2001; Withers and Keena 2001; Pitt et al. 2007; Bi et al. 2008; Troncoso 2012).

The purposes of the present report are to: (i) describe a European gypsy moth population that completed development entirely in a pure stand of radiata pine and caused extensive defoliation; and (ii) highlight the potential threat of an accidental introduction of European gypsy moth to other regions of the world where radiata pine is used in planted forests.

Materials and methods

Location of the outbreak

The outbreak was located in the Municipality of Cubillos del Sil, province of León, in north-western Spain (Fig. 1). Elevations in this area are mostly *ca*. 570 m a.s.l. The average annual temperature is approximately 12.7 °C, with a mean daily minimum of 0.9 °C in January and a mean daily maximum of 29.1 °C in July. The average annual rainfall is around 672 mm. Vegetation in the area is dominated by planted radiata pine forests, although stands of native broadleaved species are also present. The most common native species are *Quercus ilex* L., *Q. pyrenaica* Willd., *Castanea sativa* Mill., and *Populus nigra* L. Gypsy moth is endemic in the study area and causes occasional sparse defoliations in native oak species (*Q. ilex*, *Q. pyrenaica*) (Romanyk and Cadahia 1992).

The outbreak described here started in a pure, even-aged stand of 18-year-old radiata pine. The stand had been planted in 1994 using 1-year-old seedlings that were not genetically improved. The site index of the forest was around 20 m (according to the dominant height growth curves of Diéguez-Aranda et al. (2005), which use 20 years as a reference age). Stocking density at establishment was approximately 1600 trees ha^{-1}, which was reduced to approximately 800 trees ha^{-1} by a thinning from below in early 2012. This initial high density of the stand hindered competing vegetation development; at the time of thinning in 2012, competing native broadleaf saplings and shrubs were virtually absent in the understory (Fig. 2a).

Moth assessment and identification

In order to estimate female fecundity, we measured the length and width of a random sample of 23 egg masses in early 2014. Development of the gypsy moth population was visually assessed by a series of field observations over a 6-month period of 2014. Dates of egg hatch, and duration and feeding of larval stages were monitored weekly from egg hatch to the end the larval stage. The presence of flying adults was assessed using two pheromone-baited traps, which were checked once a week from June 5 through October 10, 2014.

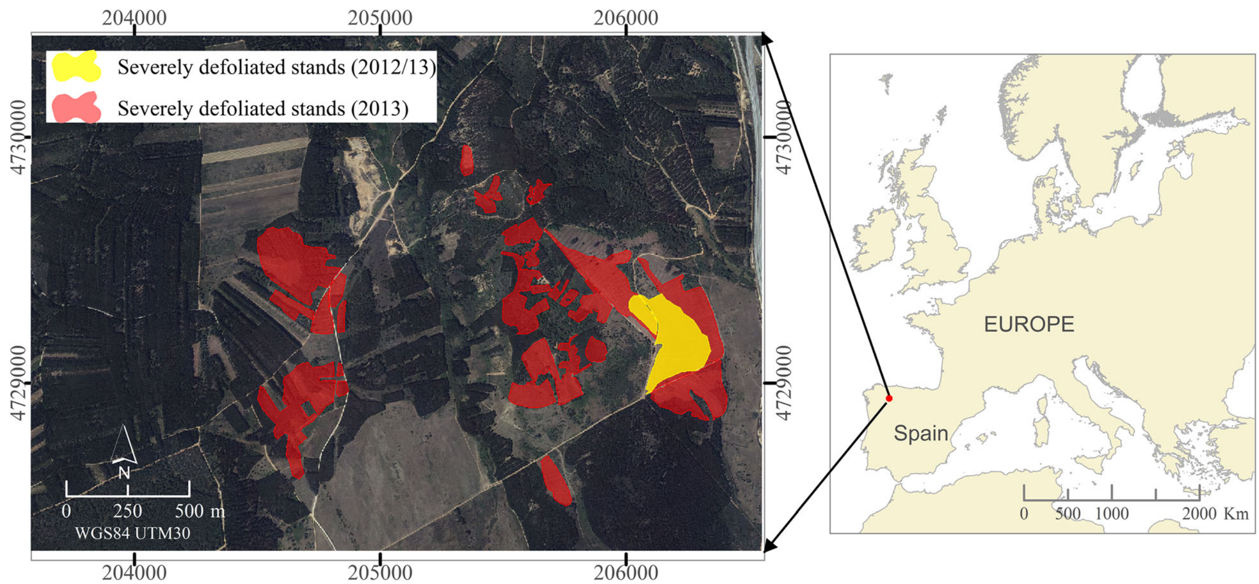

Fig. 1 Location of the study area in Spain and distribution of the *Pinus radiata* stands severely defoliated by the gypsy moth in 2012 and 2013

To confirm that the outbreak population was the European strain of the gypsy moth and not an introduced Asian strain, 40 male moths were collected in the field in July 2014. Their DNA was analysed using a Multiplex Real-Time PCR Assay (Islam et al. 2015) at the USDA-APHIS Center for Plant Health Science and Technology, Otis Laboratory, Buzzards Bay, MA, USA.

Radiata pine defoliation and growth and mortality assessment

Defoliation was mapped at the stand level using data obtained from the Landsat 8 satellite and from operation of an unmanned aerial vehicle (UAV). Regarding the satellite imagery, four multispectral Landsat OLI scenes (203/30) were used (years 2013 and 2014). The images were radiometrically corrected and calibrated to at-sensor reflectance. Regarding the UAV, two flights were carried out using an in-house built fixed-wing drone in April 2014 (GSD 15 cm), using a Canon Powershot S100 12 MPix standard camera (4000 × 3000 pixels), with a standard RGB sensor for the first flight and a NIR filter for the second flight. The aerial photographs were geometrically corrected using ground control points, and an orthoimage of the study area was created (GSD 15 cm). The remote sensing data were collected during 2013 and 2014, and a supervised classification technique was used to map defoliation. Three defoliation classes were defined in the field at stand level: non-defoliated (<25 % loss of leaf area); moderate defoliation (25–75 % loss of leaf area); and severe defoliation (>75 % loss of leaf area). Image classifications were validated using 85 field plots, achieving an overall accuracy higher than 97 %. For further details, see Alvarez-Taboada et al. (2014).

To assess the effect of defoliation on tree growth and mortality, 18 plots were established in April 2014. Six rectangular plots, each comprised of 30 trees, were established in severely defoliated stands, three in moderately defoliated stands and nine in non-defoliated stands. Diameter at breast height and total tree height was measured for each tree at the time of the plot establishment and again in October 2014. Additional variables were also recorded for each tree, e.g. if they were alive or dead, their defoliation class, and the number of egg masses on the stem.

Findings

Outbreak populations were first detected in the radiata pine plantation in 2011, peaking in 2012 and 2013 and suddenly decreasing in 2014. During the 2 years of outbreak culmination, approximately 6 ha were severely defoliated during two consecutive years (2012 and 2013), and *ca.* 40 ha were severely defoliated in 2013 (Fig. 1). Moderate defoliation was observed in surrounding radiata pine stands (around 15 ha).

Of the 23 egg masses sampled, the mean egg mass length was 2.93 cm (S.D. = 0.94 cm) and mean width was 1.61 cm (S.D. = 0.49 cm). Applying the regression equation of Moore and Jones (1987), these dimensions correspond to a mean fecundity of 422 eggs per mass. Such fecundity levels are typical of outbreak populations of the European strain of the gypsy moth in North America (Campbell 1967).

Virtually all-noticeable defoliation was limited to radiata pine. Isolated *Pinus pinaster* Ait. (maritime pine) trees within radiata pine stands were not affected (Fig. 2b) indicating a difference in susceptibility between the two

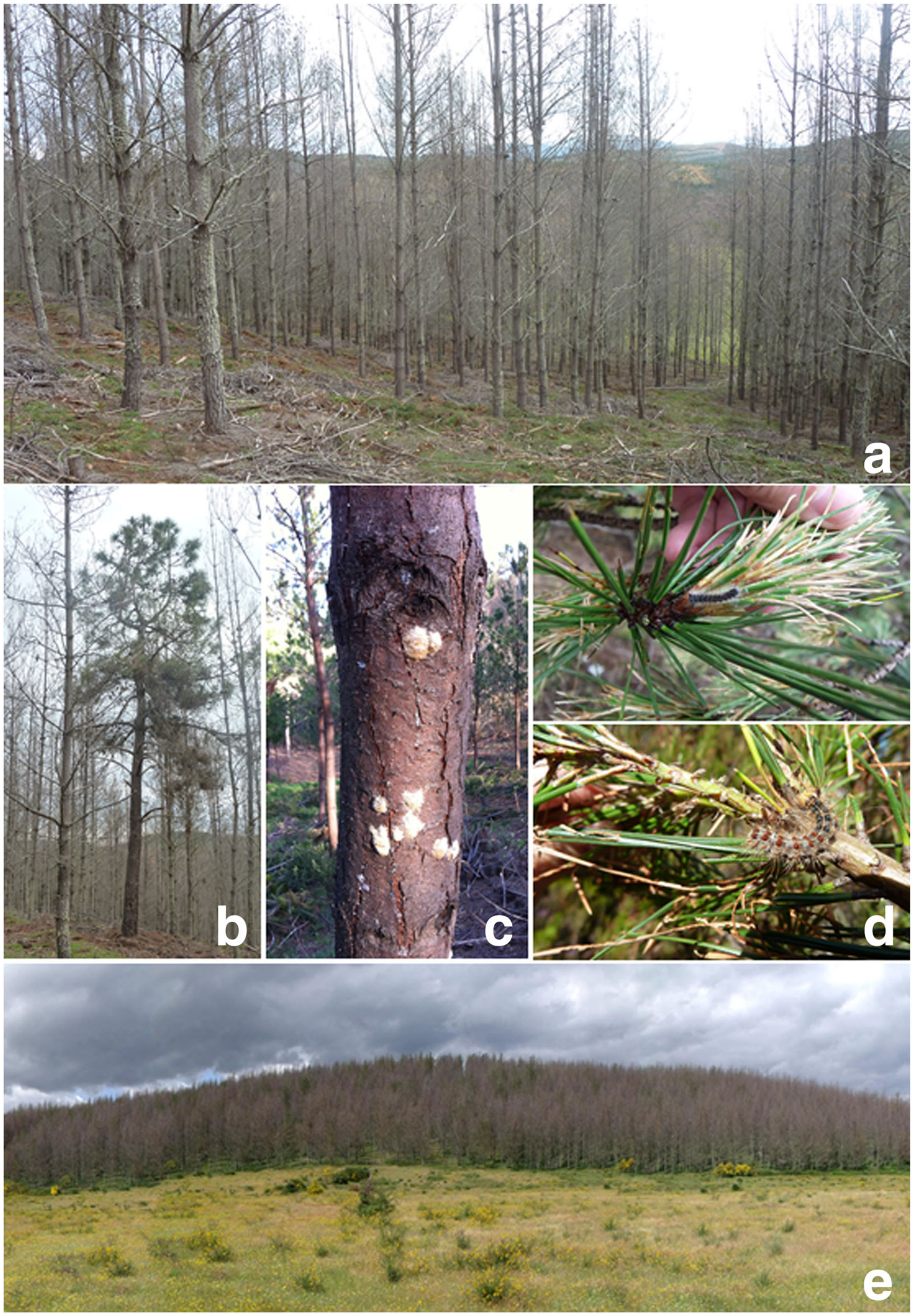

Fig. 2 a Condition of a severely defoliated radiata pine stand (note the absence of broadleaf saplings and shrubs in the understorey). **b** *Pinus pinaster* Ait. (maritime pine) tree unaffected by defoliation within a severely defoliated stand of radiata pine. **c** Egg masses of *Lymantria dispar dispar* on the lower bole of a radiata pine tree. **d** Larva of second instar (*above*) and fourth instar (*below*) of *Lymantria dispar dispar* feeding on radiata pine needles. **e** Severely defoliated radiata pine stand, with almost total tree mortality species. Defoliation was also not evident in stands of *Quercus ilex* (a priori, a preferred host) located within 3 km of the radiata pine plantation. Large numbers of egg masses (mean = 21.8 and 4.1; S.D. = 13.0 and 4.1, egg masses per tree for moderately and severely defoliated plots, respectively) were present on stems of radiata pine trees (Fig. 2c). Larvae were observed feeding exclusively on radiata pine needles beginning with the first instar and continuing to pupation (Fig. 2d). In other parts of the world, early instars are observed feeding on susceptible hosts (e.g. *Quercus* spp.) and then moving to resistant hosts (e.g. *Pinus* spp.) in late instars (Lance and Barbosa 1982). However, this was not the case here since defoliation occurred in pure radiata pine stands and susceptible hosts were not present in the understorey.

Results of DNA analysis from the sampled males were similar to other populations of European gypsy moth, which ruled out the possibility of an Asian gypsy moth introduction. The monitoring of the biological cycle showed that egg hatch occurred from mid-April to the end of May and that larvae fed until the end of August. According to captures in the lure traps, flying adults were present through early July to early October, with a peak at the end of August.

Tree mortality (87 %) occurred during at least one year in plots suffering severe defoliation (Fig. 2a, e); much lower levels of tree mortality (around 5 %) occurred in plots with moderate defoliation, where the main effect was the reduction of tree growth. In the 2014 growth season, the radial and height growth of individual trees was, on average, 45 and 40 % lower, respectively, than that observed in non-defoliated trees (Lago-Parra 2014). Additionally, trees that had been weakened by defoliation were observed to be attacked by borers, especially bark beetles (Coleoptera: Scolytinae), from April–May of 2014. The major species involved was *Ips sexdentatus* (Boern.), but other secondary species present were *Hylaster ater* (Payk.), *Hylurgus ligniperda* (F.), and *Orthotomicus erosus* (Woll.). Most of the stands experiencing severe defoliation were clear-cut in July–August, 2014, to avoid expansion of bark beetle outbreaks.

Conclusions

The occurrence of a European gypsy moth outbreak in a pure radiata pine plantation contradicts previous observations that larvae of this moth species cannot complete development in stands comprised entirely of radiata pine. These results also have implications concerning the potential for damage that European gypsy moth may inflict where radiata pine is widely utilised in plantation forestry in New Zealand, Australia and elsewhere in the world. The potential for damage caused by invading Asian strains of the gypsy moth has been recognised and biosecurity management of imported automobiles and other pathways used by these strains are currently given high priority (Walsh 1993; Armstrong et al. 2003). However, this study suggests that European strains of the gypsy moth also hold great potential for damage to commercial radiata pine plantations and that continued vigilance in biosecurity efforts targeting this strain are appropriate.

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Authors' contributions

FCD wrote the manuscript and carried out the field monitoring. GLP carried out the field monitoring, mapped the defoliated area and revised the text. MJL and AML contributed to research design and provided critical revisions of the manuscript. MFAT coordinated the research contract, carried out the field monitoring, mapped the defoliated area and revised the text. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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