

The expansion of the Australian tortoise beetle *Trachymela sloanei* (Chrysomelidae: Chrysomelinae, Chrysomelini, Paropsina) in Spain: Using human-made shelters for an early detection strategy

Expansión del escarabajo tortuga australiano *Trachymela sloanei* (Chrysomelidae: Chrysomelinae, Chrysomelini, Paropsina) en España: uso de estructuras artificiales como estrategia de detección temprana

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Abstract

1. Invasive alien species are one main driver of biodiversity loss and can cause severe economic costs. Effective management of such species requires early detection of colonising individuals, but this is often hampered by biological or economic constraints.
2. *Trachymela sloanei* (Blackburn, 1896), an Australian *Eucalyptus* defoliator beetle, is an invasive alien species that has spread to the Americas, Asia and most recently Europe.
3. We analyse the expansion of *T. sloanei* in the Iberian Peninsula both at regional and local scales, study its phenology and relative abundance, and explore the use of nest boxes for early detection.
4. The species is spreading steadily, and the area potentially affected has increased dramatically in a few years. Both adults and larvae were observed between June and November, suggesting the occurrence of several overlapping generations. More than 300 individuals were found aggregated behind single nest boxes.
5. We conclude that the installation and monitoring of nest boxes or similar structures (e.g., cork boards) can facilitate early detection of this species.

KEYWORDS

aggregation behaviour, colonisation, *Eucalyptus*, invasive alien species, life cycle

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INTRODUCTION

Globalisation and intensification of trade and travel have accelerated the rate of biological invasions (IPBES, 2023) that have become a major threat to global biodiversity (Bellard et al., 2016). Best practices aimed at managing invasive alien species are based on prevention, early detection, eradication and control (Hulme, 2006). Early detection of the first occurrence of an alien species is frequently compromised by its low density, the limited power to discern from other closely related native species and the insufficient resources invested in surveillance programmes (Hulme, 2006). It also depends on the inherent features of the target organism such as its behaviour or phenology (Trotter & Hull-Sanders, 2015). Since man-made structures (e.g., railways, floating docks) are frequently exploited by alien species to colonise new areas and spread (Bieńkowski & Orlova-Bienkowskaja, 2018; Gherghel & Tedrow, 2019), or are disproportionately used after settlement, rapid identification of such structures can in some cases facilitate early detection of invasive alien species and their management. When such species become pests, they can have serious economic impacts (Diagne et al., 2021), so effective monitoring of the spread of these organisms and how the expansion process takes place is a priority.

Eucalyptus spp. L'Hér. are recognised among the most important timber species in Europe, providing feedstock for the pulp and paper industries (Brus et al., 2019; Tomé et al., 2021). In Spain, the area occupied by *Eucalyptus* spp. is 0.64 million ha (MAPA, 2019). There are several species such as the shining gum *E. nitens* (Deane & Maiden) and the messmate stringybark *E. obliqua* L'Hér. in the north, and the red gum *E. camaldulensis* Dehn. in the south (Tomé et al., 2021), although the most abundant is the blue gum *E. globulus* Labill. (Tomé et al., 2021). In Portugal, this single species covers 0.84 million ha (ICNF, 2019). Whereas no native insect pest represents a special risk to *Eucalyptus* plantations in Europe, their health status has changed with the arrival of Australian insect pests and pathogens, which has intensified since the 1970s (Tomé et al., 2021).

The Australian tortoise beetle *Trachymela sloanei* (Blackburn, 1896) (Chrysomelidae: Chrysomelinae: Chrysomelini: Paropsina) is endemic to Australia and New Guinea (Reid, 2006). It was accidentally introduced in New Zealand in 1976 (Bain, 2001) and recorded for the first time in California in 1998 (Garrison, 1998). In 2014, it was first recorded in Europe (Southwestern Spain) (Sánchez et al., 2015), and it has recently been established in China (Zhang et al., 2020), South America (Villablanca & Villablanca-Miranda, 2022), Greece (Gastouniotis et al., 2023) and the Canary Islands (Cordero-Rivera & Santolamazza-Carbone, 2023). Recently, the species has widely spread throughout the southern part of the Iberian Peninsula, reaching central Spain as well as Portugal (Pérez-Gómez et al., 2022). This 6–8 mm long beetle feeds mostly on a wide variety of Symphyomyrtales *Eucalyptus* species (Garrison, 1998; Steven & Mulvey, 1977), although it prefers the red gum (Garrison, 1998) and may cause extensive tree damage (i.e., defoliation) on *Eucalyptus* plantations (Steven & Mulvey, 1977). Adults and larvae feed primarily at night, whereas during the day, they typically remain hidden behind the bark or in leaf

rolls of *Eucalyptus* trees. Thus, all life stages can be easily overlooked (Millar et al., 2009). Limited data are available on the reproductive cycle and phenology of this beetle. Females lay 5–40 or more eggs, and larvae are dark green to reddish brown, caterpillar-like (Millar et al., 2009). They pupate under loose bark or in soil around the host tree after four larval instars. In the United States, several generations per year have been recorded from late winter through fall (Millar et al., 2009). We are not aware of any specific studies on the phenology and life cycle of the species in Europe.

The aims of this study are (i) to analyse the expansion of *T. sloanei* in Southwestern Europe, (ii) to provide key information about its life cycle, prevalence and relative abundance in a recently colonised area and (iii) to analyse the use of human-made shelters for its detection.

METHODS

Study area

Fieldwork was carried out in the framework of a long-term project (2005–2022) on the breeding ecology of the European roller *Coracias garrulus* Linnaeus, 1758, a transaharian migratory bird species (classified as Endangered by SEO/BirdLife, 2021) that arrives at southeast Spain in the second fortnight of April.

The study area is located in Campo de Tabernas (Almería, SE Spain, 37°05' N, 2°21' W, 400 m.a.s.l.) (Figure 1) whose climate is thermo-Mediterranean with semi-arid ombrotype (Rivas-Martínez et al., 2004). The average annual rainfall is approximately 230 mm, with high inter- and intra-annual variabilities and a strong water deficit in the summer months (Lázaro et al., 2001). The average annual temperature is 18°C, with significant intra-annual fluctuations (Lázaro et al., 2004). The landscape mainly consists of Badlands and Ramblas (temporary watercourses) with scattered almond (*Prunus dulcis* (Mill) DA Webb) and olive (*Olea europaea* L.) groves. Small patches and isolated *Eucalyptus* trees (mainly *E. camaldulensis*) occur throughout the study area.

Expansion of *Trachymela sloanei*

We estimated the expansion of *T. sloanei* in the Iberian peninsula by using the locations provided by Sánchez et al. (2015) and Pérez-Gómez et al. (2022) and analysing changes in its distribution during 2015–2019 and 2020–2022. The distribution of the beetle was described by estimating its home range (the area in which an animal lives and moves habitually) and core areas (areas intensively used) with the Kernel method (Kernel density estimator [KDE]) (Worton, 1989). This method defines a territory in terms of a probabilistic model and is free of parametric assumptions on the data. Kernel methods are the most statistically efficient non-parametric density estimators (Noonan et al., 2019). The Kernel method calculates iso-lines that delimit the area with the same intensity of use. The smoothing parameter (h) that provided the best fit was established by expert

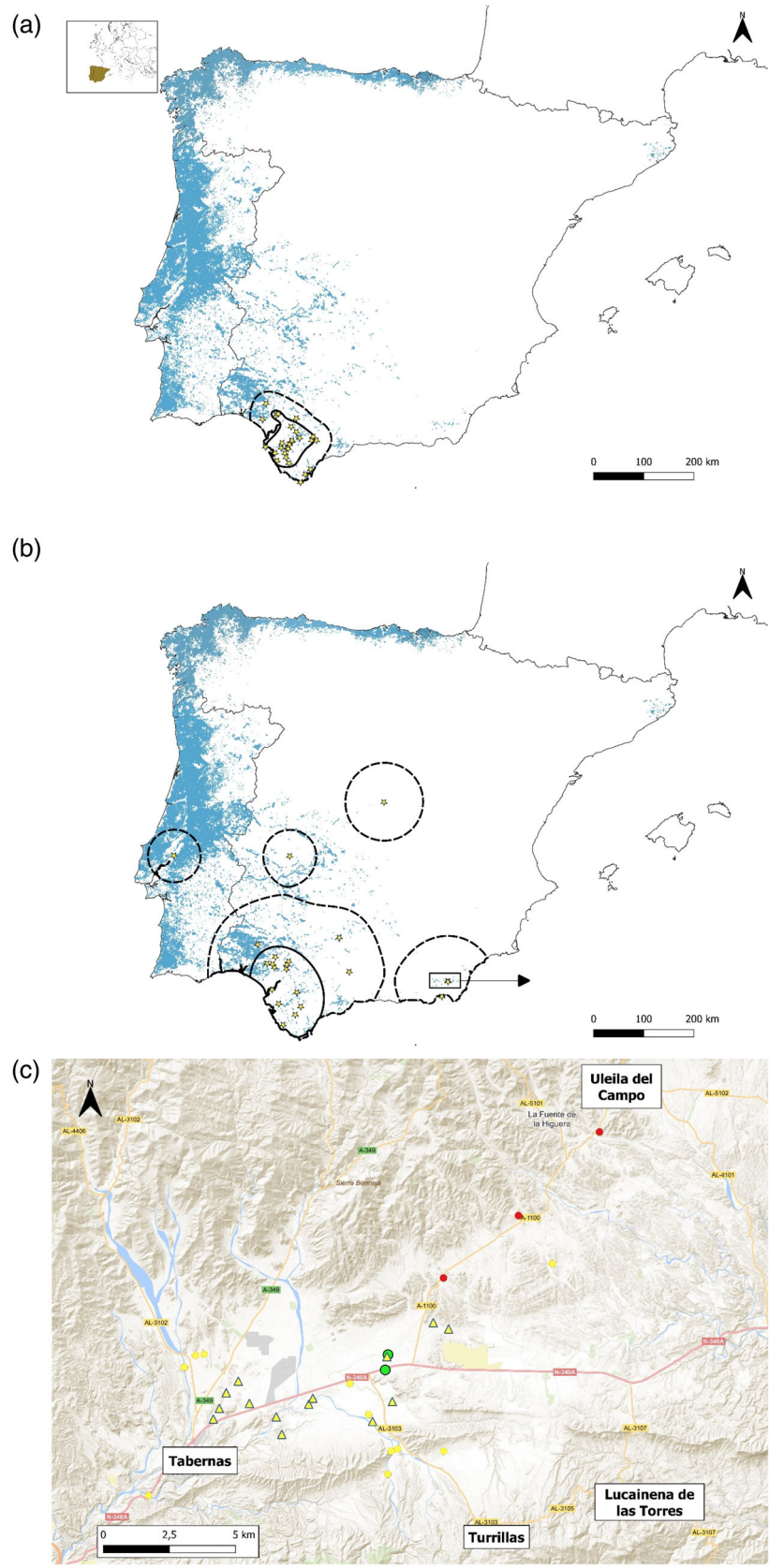


FIGURE 1 Legend on next page.

judgement (in our analysis $h = \text{default}$) (Kie et al., 2010). Two contours representing 95% and 50% probability of space use were estimated. The first (KDE 95%) defines the home range and the second (KDE 50%) the core area (Kie et al., 2010). The analysis was conducted using the ArcGIS 10.5 geographic information system and the Home Range Tools for ArcGIS software (version 2.0; Rodgers et al., 2015).

Then, we estimated the distribution of *T. sloanei* to its preferred habitat by overlapping its core area and home range with the range of *Eucalyptus* trees in Spain and Portugal. Vector information on *Eucalyptus* distribution was obtained for Portugal from the Land Use Database of the Portuguese Government (Direção-Geral do Território, 2018) and for Spain from the Forestry Map of the Ministry of Ecological Transition and Demographic Challenge (MITECO, 2006).

Phenology, life cycle and relative abundance of *T. sloanei*

Our study of the European roller involves placing wooden nest boxes before their arrival and removing them in autumn. Thus, nest boxes were placed in their usual locations in early March 2021. Nest boxes (height \times length \times width: 310 \times 232 \times 230 mm, entrance diameter: 60 mm, with a removable upper lid to allow monitoring; Figure 2a) were installed on *Eucalyptus* trees ($n = 34$, height range from the ground ca. 3–7 m), other tree species ($n = 5$, height range from the ground ca. 3–7 m) and sandstone cliffs and farms ($n = 25$, height range from the ground: 2.5–6 m). In general, nest boxes leave a narrow gap between their back and the supporting surface, which, in the case of *Eucalyptus* trees, ranges from a few millimetre to a maximum of 4 cm (Figure 2a). This scheme has proved useful for accurately dating the occurrence of *T. sloanei* in our study area (see below).

The nest boxes were scanned regularly (every 4–6 days) from 14 June 2021 to 1 August 2021 to record roller breeding. Once *T. sloanei* was detected (mid-June), we started recording its occurrence and abundance behind the boxes. Thus, we monitored 10–20 nest boxes per week during this first period. When the breeding season of the roller ended, we randomly selected 14 nest boxes on *Eucalyptus* trees (43% isolated, 57% in patches) and monitored them weekly from mid-August to the end of November 2021. In the sampling of both seasons, we counted the number of larvae and adults and the occurrence of mating pairs by removing the nest box while taking care to pick up any individuals that might fall out. After counting, the nest box and the individuals were left in place. This information enabled us to calculate the prevalence (i.e., occurrence) of adults and larvae with respect to the number of nest boxes examined. The occurrence of mating pairs was also calculated as the percentage of

cases (i.e., nest boxes) where pairs were observed in relation to the total number of nest boxes examined.

Use of human-made shelters to detect *T. sloanei*

To explore the extent to which monitoring just behind the nest boxes could provide accurate information on the presence of the beetle, pieces of bark (approximately 1–2 m above the ground) were removed from 11 trees selected at random in search of adults and larvae. This sampling was carried out at the same time as the one behind the nest box. As this sampling took place about 5–6 m below the nest box, we assume it did not affect the detection of the beetles during the following sampling sessions.

We calculated the probability of detection (in %) of *T. sloanei* (number of visits where it was detected divided by the total number of visits) behind both nest boxes and pieces of bark in those trees where at least five consecutive surveys had been conducted at both sites ($n = 11$ trees). We tested for differences in detection probability between the two sites using the Wilcoxon matched-pairs test. A Spearman correlation test was also performed to check whether sampling at both sites yielded similar results. Finally, Fleiss' generalised kappa tests of concordance were run to check the consistency of detection of *T. sloanei* behind both nest boxes and bark.

Analyses were conducted under R (R Core Team, 2022) and the packages irrCac and dplyr (Gwet, 2019; Wickham et al., 2023).

RESULTS

Expansion of *T. sloanei* in Southwestern Europe

During 2015–2019, both the home range and the core area of the species remained almost exclusively in the southwest of the Iberian Peninsula (province of Cádiz and neighbouring provinces of Seville and Huelva) (Figure 1a). The only exception is a record in Málaga about 100 km east of the locality where the species was first recorded (Pérez-Gómez et al., 2022). Since then, and in a short period (2020–2022), there has been a remarkable expansion, so that the current core area has practically overlapped with the previous home range and the home range has extended in several directions (Figure 1b).

Calculation of the overlap between the estimated distribution of the species and the area occupied by *Eucalyptus* within such area shows that between the two periods, the area potentially affected has increased by seven-fold (core area) and nine-fold (home range) and that there could currently be between ca. 30,000 and 336,000 ha of *Eucalyptus* affected (range considering the core area and the home range) (Table 1, Figure 1a,b).

FIGURE 1 Expansion of *Trachymela sloanei* in the Iberian Peninsula. Locations (yellow stars), core area (black continuous line) and home range (black intermittent line) of the species during (a) 2015–2019, and (b) 2020–2022. The distribution of *Eucalyptus* trees (blue) is also shown. (c) Negative (red circles) and positive (yellow symbols, triangles: trees monitored the whole study period, circles: trees monitored during June–July) records of *T. sloanei* on *Eucalyptus* trees in Campo de Tabernas (Almería), whose location is marked by a rectangle in (1b). Green circles are positive records reported in Pérez-Gómez et al. (2022). See Methods section for further details.



FIGURE 2 (a) Nest box hanged on a *Eucalyptus* tree showing the space where *Trachymela sloanei* usually aggregates; (b) an aggregation of 282 beetles and 61 larvae on the back of a nest box just taken from a red gum *Eucalyptus* tree. Additionally, an indeterminate number of beetles and larvae fell down from the box while removing it.

TABLE 1 Overlap (hectares) between the core area and the home range of *T. sloanei* and the area occupied by *Eucalyptus* woods.

	2015–2019		2020–2022	
	Spain	Portugal	Spain	Portugal
Core area (50%)	4088	-	30,365	
Home range (95%)	34,986	-	193,710	142,486

Our study, carried out in a recently colonised area, Campo de Tabernas, revealed that *T. sloanei* was widespread throughout the study area, covering at least 18 km from east to west (Figure 1c). The species has also been found on the southern slope of Sierra Alhamilla (ca. 1000 m a.s.l.), which separates Campo de Tabernas (north of the Sierra) from the coastal plain (minimum distance between the nearest records: 9300 m).

Phenology, life cycle and relative abundance of *T. sloanei*

On our first visit (14 June 2021), both adult and larval stages and a batch of eggs were observed, suggesting that the species was present earlier.

Adults were present during the whole study period, but their prevalence had two peaks, one in the second half of July and the other in the first 3 weeks of October. The greatest decline in adult presence and abundance occurred during the driest and hottest period (Figure 3).

The number of beetles using the nest boxes in Campo de Tabernas was highly variable (highest median in the period mid-August to end-November: 16, lower and upper quartiles: 5 and 68, respectively), and, in some nests, large aggregations (>350 beetles and larvae in about 625 cm²) occurred (Figure 2b).

Larvae were observed behind nest boxes throughout the study except for one sampling period during summertime (at the end of August and the beginning of September) (Figure 3). However, some larvae could be observed behind the bark of two trees during this period, and small larvae were detected behind nest boxes at the end of July. The prevalence of larvae peaked in mid-June, decreased steadily during summertime and increased slightly during autumn until they were no longer observed from mid-November onwards (Figure 3).

Matings were observed from mid-July until the third week of August. Then, they ceased, except for a brief period in late September (Figure 3).

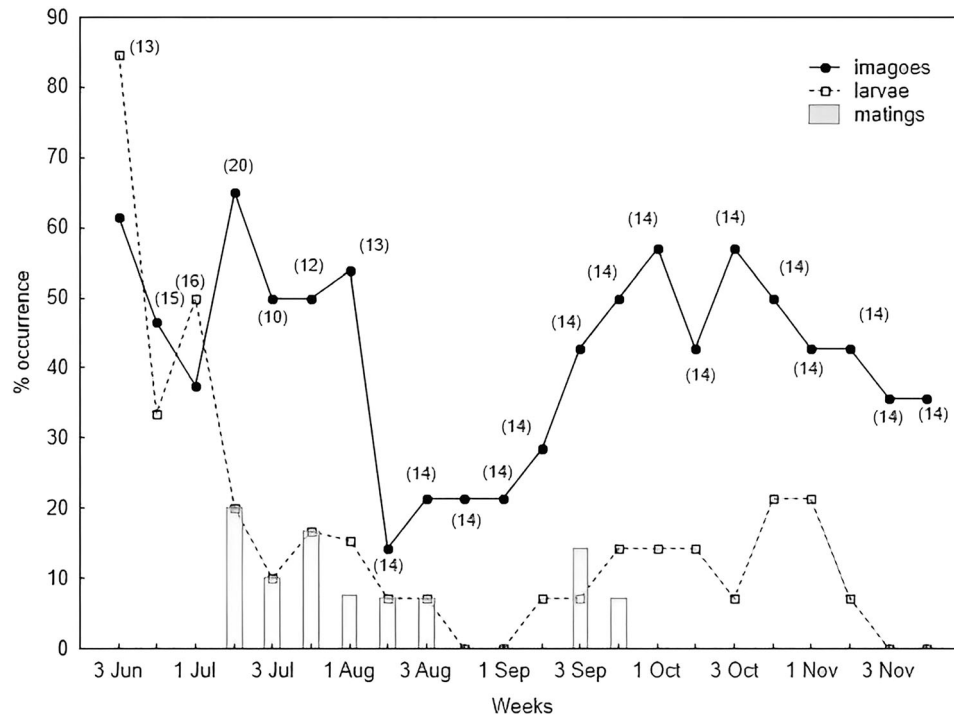


FIGURE 3 Percent occurrence of imagoes and larvae of *Trachymela sloanei* and of mating pairs observed behind nest boxes from mid-June 2021 (first sampling date: 14 June) to the end of November 2021 (weekly data). Sample sizes (i.e., number of nest boxes monitored) for each sampling date are shown in brackets.

The species was never found behind nest boxes on cliffs, farmhouses or non-*Eucalyptus* trees, and no pupa was ever observed behind or into the nest boxes.

Use of human-made shelters to detect *T. sloanei*

The probability of detection of *T. sloanei* behind nest boxes and bark pieces did not differ significantly (mean \pm SE = 45.2 \pm 12.0 and 40.5 \pm 8.80, respectively, Wilcoxon test, $z = 0.89$, $p = 0.92$, $n = 11$ trees). Furthermore, there was no correlation between the probabilities of detection in both sampling locations (Spearman correlation, $r_s = 0.30$, $p = 0.36$, $n = 11$).

Consecutive samplings behind bark pieces (presence/absence) had a low agreement, which is not significantly different from the value expected by chance (Kappa = 0.23; 95% confidence interval = -0.168 to 0.64, $p = 0.22$, $n = 11$ trees). However, records from nest boxes had a higher agreement significantly different from the value expected by chance (Kappa = 0.55; 95% confidence interval = 0.171 to 0.99, $p = 0.009$, $n = 11$ trees).

DISCUSSION

Here, we explore the spread of *T. sloanei* from its first location on the European continent (Cádiz, Southwestern Spain) in 2015 (Sánchez et al., 2015). During 2015–2019, most records came from

neighbouring provinces (Pérez-Gómez et al., 2022). Since then, there has been a remarkable expansion both to the north (Cáceres and Madrid), east (Almería), northeast (Córdoba) and northwest (Santarem, Portugal) (Pérez-Gómez et al., 2022). The expansion may have been a consequence of natural dispersal movements and/or may be associated with human activity. Yet, the fact that the expansion occurred in several directions and in a short time suggests that *T. sloanei* might have travelled as a hitchhiker in means of transport and/or during the transport of logs/timber trees for trade (see Bieńkowski & Orlova-Bienkowskaja, 2018). These authors pointed out that quick spread after establishment outside their native ranges is a common feature of leaf beetles. Considering the records summarised by Pérez-Gómez et al. (2022), the most presumable route of expansion of the species to Almería is via Málaga (i.e., Montecorto, about 330 km from Tabernas). If so, it would have dispersed ca. 400 km in 5 years. Kay (in Tribe & Cillié, 1997) reported that the dispersal rate of *T. sloanei* in New Zealand was 30–40 km in 8 years, much slower than that described for *T. tincticollis* (Blackburn), another Australian *Eucalyptus* defoliating tortoise beetle, which dispersed 1330 km in 4 years.

Regardless of the mechanism and speed of dispersal, our data suggest that the distribution of *T. sloanei* may already overlap to a considerable extent with that of *Eucalyptus* plantations, which could be even greater if the area between the various colonised cores is occupied by the species. Moreover, our results suggest a rapid population growth in a recently colonised area in a very short time. The first records in Almería were taken in October 2020 and in our study area (Pérez-Gómez et al., 2022). Precisely, in that area, we had

installed and checked several dozens of nest boxes in *Eucalyptus* trees in the spring–summer of 2020 without finding the beetle. The review of the nest boxes in the same trees in 2021 revealed that colonisation occurred after the summer of 2020 and that, within a few months, *T. sloanei* reached high prevalences (above 70%) and aggregations of up to several hundred individuals in some trees.

Population growth and the possibility of pest outbreaks depend on factors such as the number of generations per year (Skendžić et al., 2021). Millar et al. (2009) recorded several generations of *T. sloanei* per year from late winter to autumn in California. Some facts suggest that several generations may also occur in the semi-arid southeast of the Iberian Peninsula: (i) the length of the life cycle is seemingly short. Millar et al. (2009) found that the egg-to-adult development took 5 weeks in California, and we observed that the pupation period of two larvae collected when descending from the trunk to pupate (see Tribe & Cillió, 1997) was 8 and 11 days (unpubl. data); (ii) we recorded imagoes during at least 6 months and Pérez-Gómez et al. (2022) found them all year round in south Spain; (iii) larvae were found during at least 5 months and small ones were observed at the end of July, which suggests a new cohort; and (iv) matings were recorded during 2 months. Thus, assuming in our area a developmental period similar to that reported by Millar et al. (2009) would result in at least four generations per year. Many invasive insects that have expanded their range show latitudinal gradients in voltinism (Skendžić et al., 2021). For species with flexible voltinism, changes in temperature (due to geographic expansion or climatic change) will define the number of generations per year and, thus, the population growth. More information is needed on the factors regulating voltinism in this species to clarify the likelihood of it becoming a pest.

Overall, the prevalence of *T. sloanei* is at least 75% (considering all 34 nest boxes monitored during the study period), as the absence of the species behind the nest boxes does not mean that the tree is not infested. We recorded lower prevalence and abundance during periods with harsh weather (summertime and the start of winter). This could be explained by two non-mutually exclusive reasons: (i) mortality due to stressing environmental conditions (Tribe & Cillió, 1997) and (ii) a preference for hiding behind bark rather than behind nest boxes during unfavourable periods.

Our study shows that a new microhabitat created by nest boxes hung on trunks is readily exploited by both larvae and adults and in both individual *Eucalyptus* trees and those grouped in small patches. However, the species was not found behind nest boxes placed elsewhere (other tree species, cliffs or farmhouses) (but see Pérez-Gómez et al., 2022), nor pupae were ever observed behind or in the nest boxes. We found a similar probability of detection of the species behind trees and bark, although future studies with a larger sample size would be desirable. Moreover, the results obtained with both methods did not agree, so sampling under the bark in trees with nest boxes resulted sometimes in the detection of larvae and/or beetles that were not found behind the nest boxes. The opposite was also true. However, the consistency of detection over time was greater behind nest boxes than behind bark. Nest boxes used continuously by

T. sloanei throughout the study period were those closest to the trunk, thus probably offering more protection from inclement weather. Variation in this seemingly important feature can also explain the high variability in relative abundance found among nest boxes as well as the disagreement in the results obtained from nest boxes and bark. Both the scarce number of individuals found in natural hides and the large aggregations behind the nest boxes indicate that suitable, natural hiding places for this species can be limited (the bark of some *Eucalyptus* species naturally peels off in small pieces).

Even though we did not find evidence of severe impact on trees (e.g., defoliation) at this early stage of the invasion process, it is known that *T. sloanei* can cause extensive defoliation and tree damage out of its range or in cultivated stands (Millar et al., 2009; Steven & Mulvey, 1977). Given the economic importance of *Eucalyptus* spp., monitoring the expansion and population growth of *T. sloanei* is necessary. Future research should focus on a better knowledge of the distribution and abundance of the species in the Iberian Peninsula, its mode of expansion (natural and/or human-mediated) and the abundance and viability of recently colonised sites. Considering that gregariousness increases the probability of colonisation and establishment (Lodge, 1993), we suggest taking advantage of this behaviour to facilitate early detection of this species and changes in its distribution, by placing wooden or, preferably, cork boards (with a rougher surface) or similar structures (Millar et al., 1999; Tribe & Cillió, 1985; Tribe & Cillió, 1997) closely fixed to the trunks of *Eucalyptus* plantations.

AUTHOR CONTRIBUTIONS

Francisco Valera: Conceptualization; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Jesús Veiga:** Conceptualization; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **Eulalia Moreno:** Funding acquisition; investigation; project administration; resources; supervision; writing – original draft; writing – review and editing. **Luis Bolonio:** Formal analysis; investigation; methodology; software; writing – original draft; writing – review and editing. **Teresa Martínez:** Investigation; methodology; visualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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