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BIOLOGICAL STUDIES ON *TOMICUS DESTRUENS* (WOLLASTON) (COLEOPTERA CURCULIONIDAE SCOLYTINAE): PHENOLOGY, VOLTINISM AND SISTER BROODS

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Lentini A., Coinu M., Luciano P. – Biological studies on *Tomicus destruens* (Wollaston) (Coleoptera Curculionidae Scolytinae): phenology, voltinism and sister broods.

Biology and behaviour of *Tomicus destruens* were investigated in Sardinian pine forest in order to define the seasonal pattern of trunk attacks by breeding adults, the number of annual generations, and the number of sister broods produced per female. The counting of maternal galleries made each month in trap trees shows that *T. destruens* colonizes the pine trunk in all the months between October and May with the highest infestation intensity in autumn followed by decreasing values. Female laid ca. 80 to 95 eggs per gallery in October-November and ca. 70 in the following months. Although the bark beetle's reproductive activity extended for a period of 7-8 months, the flight of new adults was concentrated in a much shorter time period between May and June, when ca. 95% of the emergences occurred. The experiments have ruled out the possibility of a second annual generation. Even the earliest emerging adults, originating from eggs laid in October, were unable to mature their gonads and oviposit before the summer stasis. *Tomicus destruens* females can make up to four sister broods and lay a total of ca. 170 eggs throughout their life, without regeneration feeding in the shoots. The new knowledge provides information useful to calibrate well-timed interventions to control this dangerous pest by means of the trap tree technique.

KEY WORDS: Pine, bark beetle, control, reproductive period, fecundity.

INTRODUCTION

Tomicus destruens (Wollaston) (Coleoptera: Curculionidae) is the main pest of Mediterranean pine forests and can cause severe economic and environmental damage throughout its distribution range (MONLEÓN *et al.*, 1996; KOHLMAYR *et al.,* 2002; GALLEGO *et al.,* 2004). This bark beetle has spread to Spain and southern France, Corsica, Italy, the Anatolian Peninsula, Cyprus, Palestine, North Africa and the Atlantic coast of the Iberian Peninsula as far as the island of Madeira (BALACHOWSKY, 1949; WOOD and BRIGHT, 1992). It has been recorded in Italy in thermophilic pine forests in most of the peninsula (MASUTTI, 1969; NANNI and TIBERI, 1997; FACCOLI *et al.,* 2005) as well as in Sicily and Sardinia (LONGO, 1991; LUCIANO, 2009).

To reach sexual maturity the newly emerged adults attack the pine shoots and cause them to fall; the resulting reduction of the crown weakens the trees and makes them susceptible to attacks on the trunk by breeding adults (LIEUTIER *et al.*, 2015). The larvae then feed on the subcortical tissue, disrupt the lymphatic flow and bring about the death of the trees (RUSSO, 1946). The level of damage caused by this scolytid is affected by the health status of the hosts and, consequently, all the factors that weaken pines support the trunk attack by this dangerous pest. Water stress is considered the main cause of bark beetle outbreaks (LORIO *et al.*, 1995; LOMBARDERO *et al.*, 2000; CHAKALI, 2005; SABBATINI PEVERIERI *et al.*, 2006) although other factors, such as stagnant water, phytophagous infestations, cutting of large branches (CROOKE, 1959; RAISANEN *et al.*, 1986; NANNI and TIBERI, 1997) and fires (FERNANDEZ and COSTA, 1999; BARCLAY *et al.*, 2009) can trigger severe infestations. The damage caused by this insect can involve vast areas. During the restoration of 20,000 hectares of pine forest in the Senalba Chergui massif (Djelfa, Algeria), over 37,000 m³ of timber damaged by *T. destruens* were collected between 1988 and 2000 (CHAKALI, 2003). In south-eastern Spain in the early 1990s, the destruction of about 40,000 ha of *Pinus halepensis* stands, weakened by a period of exceptional drought, was attributed to attacks by this bark beetle (GALLEGO and GALIÁN, 2001).

Despite numerous studies on *T. destruens*, its voltinism has not been completely defined. According to different authors this species can produce one or more generations per year. A single generation has been reported for northern Italy (FACCOLI *et al.*, 2005) and Tunisia (BEN JAMAÀ *et al.*, 2000), with overwintering adults attacking the trunks starting from early spring and the emergence of new adults in June. In Israel, *T. destruens* is considered univoltine with oviposition in autumn and larval development in winter (MENDEL *et al.*, 1985). In southern Italy (RUSSO, 1946; NANNI and TIBERI, 1997), Spain (MONLEÓN *et al.*, 1996) and France (KERDELHUÉ *et al.*, 2002), the bark beetle attacks the trunks in fall and early spring, although it is not clear whether one or more generations per year are completed. Other authors have suggested the presence of two generations per year on the Tyrrhenian coast of central Italy and on the northern Adriatic coast (SANTINI and PRESTININZI, 1991; NANNI and TIBERI, 1997). The extra generations are considered by some authors to be subsequent ovipositions by the same females, which can complete a maternal gallery, re-emerge and lay eggs in new maternal galleries giving rise to sister broods (MONLEÓN *et al.*, 1996), as well known for other bark beetle species (SAUVARD, 1993; JACTEL and LIEUTIER, 1987; WAGNER *et al.*, 1981). The sister broods have

sometime been confused with true generations produced by female offspring, leading to a erroneous number of annual generation (SAUVARD, 2004).

Preliminary investigations of the biology of *T. destruens* in Sardinia have shown that adults are present inside pine shoots throughout the year although the period of maximum presence is from mid-May to late October. In autumn-winter, most adults reach the trunks of decaying trees to reproduce and the emergence of adults of the new generation begins at the end of March with peaks between early May and mid-June (LUCIANO, 2009). With the aim to define the life cycle of *T. destruens*, we investigated some poorly known aspects of its biology and behaviour in Sardinia, namely (i) the time of year when the females excavate their maternal galleries and oviposit, (ii) the possibility of completing a second generation by the earliest emerging individuals, and (iii) the number of maternal galleries that each female manages to make.

MATERIALS AND METHODS

The study was conducted from September 2010 to July 2013 in a pine forest (*Pinus halepensis* Mill.) of 128 hectares planted in the late 1960s at Prigionette (428956,m East, 4495231 m North, 35 m above sea level) near Alghero (Sardinia, Italy) within the Porto Conte Public Forest. The area is characterized by low rainfall and hot dry summers, with annual precipitation of ca. 600 mm and a mean annual temperature of 16-17°C. The driest month is July (2 mm of rainfall), while the highest precipitations are observed in November (110 mm). In the hottest month (July) the mean temperature is 23.5°C, while the lowest mean monthly temperature is recorded in January (9.9°C) (CHESSA and DELITALA, 1997).

SEASONAL PATTERN OF *T. DESTRUENS* ATTACKS

Trap trees were used to identify the time of year when the females excavate maternal galleries and oviposit. In the central area of the pine forest, three healthy *P. halepensis* trees (diameter at the base 20-25 cm) were felled each month from September 2010 to July 2012 after verification of the absence of adult bark beetle entrance holes. The pines were left on the ground without being cut up or debranched. One month after the felling, a basal portion of the trunk (ca. 40 cm long) was removed. The cut faces of the logs were immediately covered with a thin layer of grafting mastic to prevent rapid drying of the subcortical tissue. The logs were marked and then placed individually in emergence cages located under a specially-built canopy in the pine forest which sheltered the cages from the sun and rain without impeding air flow. The emergence cages were made from PVC pipes (60 cm long, 40 cm diameter) closed at both ends with brass mesh (0.6 mm openings) fixed with screw/band clamps.

An Escort Ilog (model 60D32) data logger was set up beneath the canopy to record the mean daily temperature and relative humidity. *T. destruens* adults emerging from the bark were collected and counted weekly in each cage, the distinction being made between mature and newly emerged individuals. At the end of the emergence period, each log was taken to the laboratory to be measured (length and mean diameter) and examined. After removal of the bark, we noted the number and length of the maternal galleries, recording for each one the total length and the length of the fertile tract, which is the tract interested by egg laying, as well as the number of larval galleries. For each log we determined: the number of maternal galleries per m² of bark; the mean length of the maternal gallery; the mean length of its fertile tract (only in the first year of observations); the mean number of larval galleries per maternal gallery (only in the first year of observations); the number of newly emerged adults per m² of bark; the number of days between colonization of the logs and the peak emergence of new adults.

NUMBER OF GENERATIONS PER YEAR

To investigate whether the earliest emerging *T. destruens* adults had time to mature the eggs and start a second generation before the summer stasis, three *P. halepensis* trees were felled on October 10th, 2012 and left on the ground exposed to bark beetle attack for 30 days. After this period, a portion of the trunk (ca. 40 cm long) was removed from each tree and placed singly in a cubic emergence cage (1 m sides). The cage consisted of a wooden frame with sides made of brass mesh (0.6 mm openings) to prevent the escape of emerged insects and the entrance of bark beetles from the outside. Starting in March, when the first newly emerged adults were observed, potted pine plants, whose shoots provided food for the callow adults, were introduced into the cages. Plants with shoots completely eaten by the bark beetles were regularly replaced. The removed plants were carefully examined and any adults present in the shoots were reinserted in the cage. Each month from the beginning of emergence until the end of June, an uninfested pine log obtained from healthy trees just felled and examined carefully to verify the absence of bark beetle penetration holes was placed in each cage. At the end of June, the three logs per cage, marked to indicate the date of introduction into the cage, were taken to the laboratory to verify the presence of maternal galleries and to count the number of eggs laid.

NUMBER OF SISTER BROODS

The number of maternal galleries excavated by each *T. destruens* female was estimated with an experimental design based on the counting of females that left the log in which they laid the first brood to infest other logs made available to them. On October 10, 2012, three pine trees were felled and left on the ground exposed to bark beetle attacks until November 15. On that date, a portion of the trunk (ca. 40 cm long) was removed from each of the three trees and placed singly inside a cubic emergence cage of the type described above. An uninfested log was set on top of the infested log with spacers placed between the two to ensure the flow of air between the cut surfaces so as to limit mould development. On December 15, after verification of the passage of beetles from the infested log to the uninfested one, the two logs were placed in separate cages and a new uninfested log was placed over each one. This procedure was repeated monthly until June, thus doubling the number of cages at each substitution.

Potted pine plants, whose fresh shoots provided food for the females that abandoned the first maternal gallery, were introduced into the cages. The vegetative state of the pine plants and any movements of the bark beetles from the infested log to the new uninfested log were checked weekly. At the end of June 2013, the logs were taken to the laboratory where we counted the maternal galleries, measured their total length and that of the fertile tract, and counted the number of eggs per gallery. Thirty galleries were analysed for each of the first three broods while it was possible to examine only seven galleries for the fourth brood.

STATISTICAL ANALYSIS

A statistical analysis was performed to compare the data from different months. Three replicates (logs) were tested for each treatment. For each replicate, we calculated the mean values of the breeding data, i.e. the length of the maternal galleries, female fecundity, number of emerging insects, and development time. Thirty individuals of each of the first three broods and seven of the last brood were used to analyse the differences in biological parameters among broods (length of maternal galleries and fecundity). The data were compared by analysis of variance (ANOVA) to determine significant differences among months or broods. When significant differences occurred, the means were separated by Tukey's post-hoc comparison test (P < 0,05; PROC GLIMMIX, SAS Institute 2008). Homogeneity of variance was analysed with Cochran's test and the numerical data were log-transformed when necessary to meet the assumption of homogeneity of variance. However, the data are presented in the tables and figures as mean ± SE to allow comparisons with the results of other studies.

RESULTS

SEASONAL PATTERN OF *T. DESTRUENS* ATTACKS

Examination of the logs infested in the various months of 2010-2011 showed that females began their reproductive activity in the first week of October and reached their maximum intensity in autumn. The mean number of maternal galleries excavated in October was ca. 65 per m^2 of bark (Tab. 1). Attacks, albeit of lesser magnitude, continued in November and significantly decreased in December when there was a mean of only 7.87 galleries per m^2 ($F_{6,14}$ = 6.1, P = 0.0026). Good egg-laying activity was also recorded in January, March and April, with values ranging from 23 to 41 galleries, while only 6.66 maternal galleries per m^2 were counted in February. The logs exposed in May were attacked by only one bark beetle while no reproductive activity was recorded in the subsequent summer months.

The mean length of the galleries varied from 5.92 to 7.23 cm with no significant differences among the various months ($F_{6,14}$ = 0.64, P = 0.6976). The fertile tracts of the maternal galleries were about 1 cm longer on average in October and November than in the other months of the year. However, the strong variability meant that the differences were not statistically significant ($F_{6,14}$ = 0.94, P = 0.4951). The mean number of larval galleries per reproductive system was also higher in the logs infested in autumn, with significantly higher mean values in November (95.5), whereas in logs infested winter-spring it varied between 68.4 and 74.1 ($F_{6,14}$ = 3.09, P = 0.0384). The mean number of adults emerging from each log was not proportional to the degree of infestation: only 278 adults emerging from logs infested in October, characterized by the highest density of maternal galleries, in contrast to a significantly higher mean number of adults emerging from logs attacked in January (1160), with a much lower density of maternal galleries ($F_{6,14}$ = 3.32, P = 0.0301).

In logs infested in October and November, callow adults began to emerge in mid-April but reached the peak only in mid-May (Fig. I). Infestations in December and January gave rise to new adults starting in the first ten days of May, with peak emergence in the last week of the same month. In logs attacked in February, March and April, emergences began at the end of May and the peak was in early June. The only log infested in May gave rise to a few adults starting in mid-June, with maximum emergence at the end of the same month.

The development of *T. destruens* had an extremely variable duration throughout the year (Fig. II). From logs infested in October, emergences began after just over 200 days and this time gradually decreased during the course of the year to reach a minimum of ca. 47 days for eggs laid in May. Hence there was a 4-fold reduction of the developmental cycle according to the period of the year in which the attacks occurred ($F_{6,14}$ = 817.34, P < 0.0001).

The attacks observed in the second study year were slightly greater in October than in the subsequent months ($F_{6,14}$ = 0.80, P = 0.5848) (Tab. 2), without the sharp declines recorded in December and February of the previous year. There was a progressive decrease in the number of galleries only from February, with a marked decline in May.

The mean length of the maternal galleries ranged from 5.8 to 7.6 cm with no significant differences among the months of infestation ($F_{6,14}$ = 1.89, P = 0.1523). Once again the mean number of adults emerging from each log was

Table 1 – Mean (± SE) morphometric parameters of *Tomicus destruens* reproductive systems observed in bait pine trees felled each month (Alghero, 2010-2011). Values in the same column followed by different letters are significantly different (1 way ANOVA, followed by Tukey's post-hoc comparison test: P<0.05; for each treatment, three logs were used as replicates)

Month of exposure in the field	Egg galleries	Gallery length	Fertile tract length	Larval galleries	Emerging callow adults
	no./m^2 ± SE	cm ± SE	cm ± SE	no. ± SE	no./m^2 ± SE
October	64.38 ± 11.97 a	6.85 ± 0.48 a	5.98 ± 0.81 a	83.52 ± 8.25 ab	278.36 ± 76.16 c
November	27.50 ± 11.47 bc	7.23 ± 0.51 a	5.80 ± 0.41 a	95.53 ± 2.65 a	504.27 ± 155.71 bc
December	7.87 ± 1.33 c	5.99 ± 0.25 a	4.84 ± 0.37 a	68.44 ± 2.16 b	371.89 ± 147.47 c
January	33.82 ± 6.84 b	6.08 ± 0.63 a	4.91 ± 0.38 a	70.04 ± 3.61 b	1160.27 ± 313.23 a
February	6.66 ± 2.33 c	5.92 ± 0.55 a	4.74 ± 0.59 a	71.18 ± 10.46 b	191.31 ± 80.18 c
March	41.15 ± 7.99 ab	6.51 ± 0.31 a	4.83 ± 0.17 a	74.10 ± 2.81 b	1008.54 ± 355.06 ab
April	23.07 ± 8.20 bc	6.28 ± 1.11 a	5.35 ± 0.65 a	68.99 ± 4.33 b	416.83 ± 105.82 bc
May	1.03*	5.0*	3.10*	45*	57.19*

* = not subjected to statistical analysis because calculated on the basis of only one gallery recorded in a single log

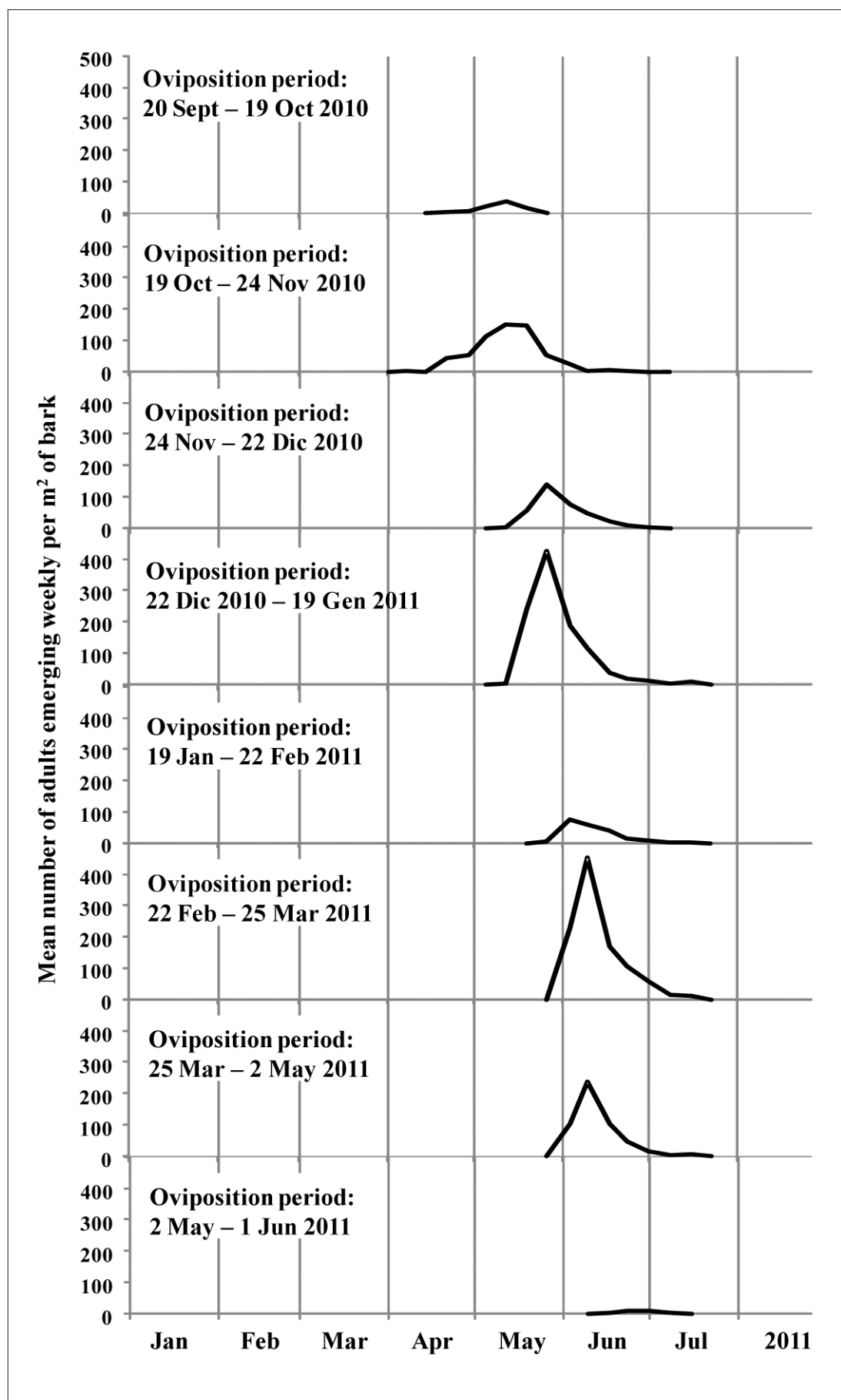Fig. I – Mean number of *Tomicus destruens* adults emerging per m² of bark in logs infested in different periods of the year (Alghero, 2010-2011).
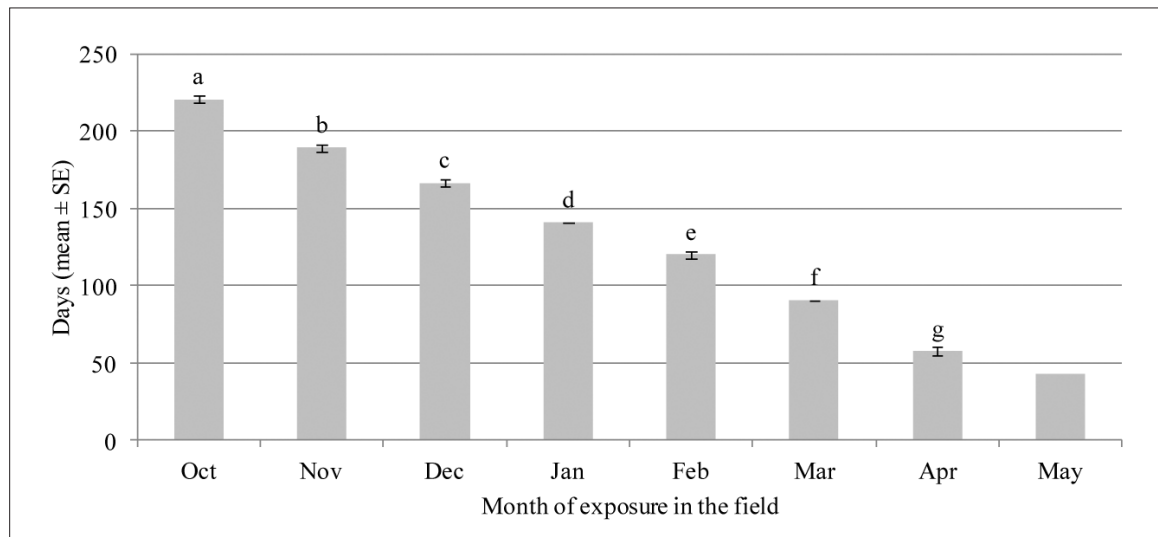
Fig. II – Mean (± SE) number of days between colonization of the logs and the peak emergence of new adults (Alghero 2010-2011). Different letters above bars indicate significant differences among months. Data of May is not subjected to statistical analysis because calculated on the basis of the adults recorded in a single log.

Table 2 – Mean (± SE) morphometric parameters of *Tomicus destruens* egg galleries observed in bait pine trees felled each month (Alghero, 2011-2012). Values in the same column followed by different letters are significantly different (1 way ANOVA, followed by Tukey's post-hoc comparison test: P<0.05; for each treatment, three logs were used as replicates).

Month of exposure in the field	Egg galleries no./m² ± SE	Gallery length cm ± SE	Emerging callow adults no./m² ± SE
October	83.54 ± 14.87 a	6.29 ± 0.47 a	238.79 ± 73.29 a
November	68.05 ± 3.56 a	7.51 ± 061 a	591.23 ± 227.76 a
December	62.20 ± 22.45 a	7.59 ± 0.89 a	863.85 ± 159.53 a
January	77.20 ± 34.71 a	7.15 ± 0.52 a	903.16 ± 161.12 a
February	55.65 ± 21.11 a	6.83 ± 0.17 a	588.79 ± 265.61 a
March	38.39 ± 27.99 a	5.81 ± 0.39 a	503.28 ± 363.56 a
April	31.73 ± 5.95 a	5.80 ± 0.53 a	321.61 ± 70.69 a
May	10.60*	6.13*	77.71*

* = not subjected to statistical analysis because calculated on the basis of only one gallery recorded in a single log

not closely related to the degree of initial infestation: only 238 adults emerging from the logs infested in October in contrast to 900 adults emerging from the logs attacked in January. Even though they had a slightly lower density of maternal galleries (Tab. 2), the differences were not statistically significant ($F_{6,14} = 1.38$, P = 0.2893). The emergence trend largely confirmed the results of the previous year as callow adults emerged very early from the logs infested in October, with some individuals observed outside the bark already in January and February (Fig. III). However, there was a constant increase of emergences only from mid-March, with the peak in the first week of May. In logs infested in November, callow adults began to emerge in mid-April and reached the peak in mid-May. The attacks in December generated new adults starting from early May, with the peak in the second ten-day period of the same month. The emergences originating from ovipositions in January had a similar pattern to that observed in the logs infested in December but postponed by one week. Subsequently the length of

the emergence period gradually decreased, with peak emergences on June 2, 9 and 16 from the logs infested in February, March and April respectively. The limited ovipositions in May generated new adults in the first 20 days of July.

The time between the infestation and the emergence of new adults decreased during the course of the year ($F_{6,14} = 92.88$, P < 0.0001) (Fig. IV). Only 184 days elapsed between the ovipositions in October and the emergence of new adults, with a decrease of more than a month compared to the previous year. Smaller decreases were observed for the development time of the individuals originating from eggs laid from November 2011 to March 2012. This may be due to the fact that in the second study year the mean monthly temperatures in October, November and December were about 1-2°C higher than in the previous year. In the subsequent months the mean temperatures were similar in the two years, with the exception of February when the mean temperature in the second year was about 2.5°C lower (Fig. V). However, this
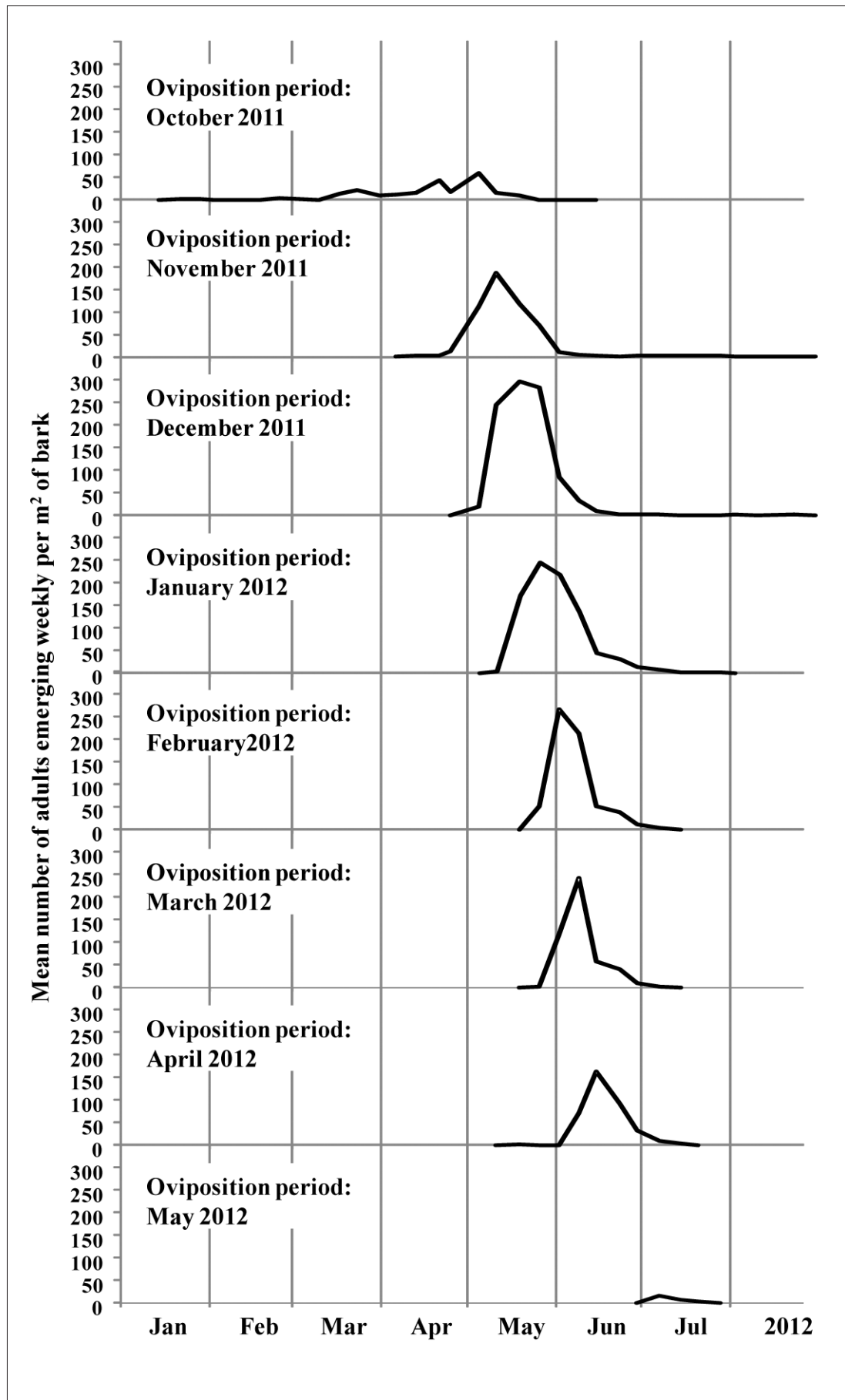
Fig. III - Mean number of *Tomicus destruens* adults emerging per m² of bark in logs infested in different periods of the year (Alghero, 2011-2012).
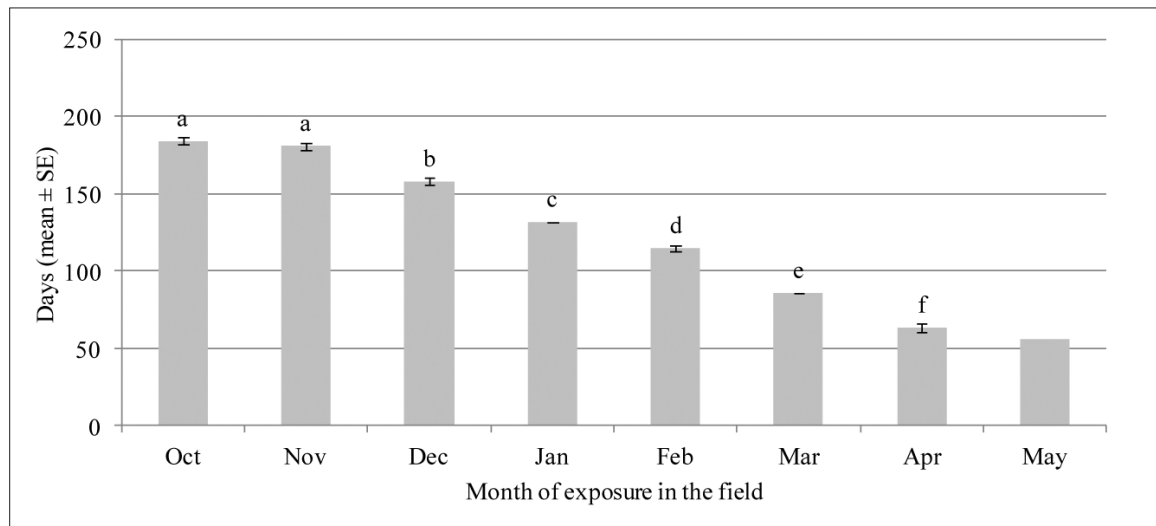
Fig. IV – Mean (± SE) number of days between colonization of the logs and the peak emergence of new adults (Alghero 2011-2012). Different letters above bars indicate significant differences among months. Data of May is not subjected to statistical analysis because calculated on the basis of the adults recorded in a single log.

reduction does not appear to have affected the rate of development of the bark beetle which was slightly higher than that recorded in the previous year.

NUMBER OF GENERATIONS PER YEAR

In the trials carried out to determine the number of generations per year, the emergence of callow adults began in the first ten days of March 2013 and reached peak levels in the first week of May. A mean (± SE) of 95.7 ± 14.3 males and 114.0 ± 14.2 females emerged from each log. The newly emerged adults rapidly infested the shoots of the potted pines and also excavated small galleries in the uninfested logs periodically placed in the cages. In the latter case, the bark beetles penetrated under the bark from the cut surfaces of the logs, excavating short individual galleries or small niches that sheltered several individuals (Fig. VI). From the beginning of April, some of the beetles penetrated the bark and opened small irregular galleries underneath it (mean length ca. 2.8 cm) that were generally occupied by a single individual; a pair that had

still not oviposited was found in only two cases. Adults were found in this type of gallery until May and they opened a mean of 35.0 ± 3.2 galleries per cage. Eggs were never observed in these galleries, a finding indirectly confirmed by the dissection of 30 females which were all found to have no mature eggs in their ovaries. From June, adults were observed only in the pine shoots.

NUMBER OF SISTER GENERATIONS

The three logs removed from the pine trees felled in early October 2012 and exposed to *T. destruens* attacks until mid-November accumulated a total of 284 maternal galleries. Inside the emergence cages, 79 females re-emerged from these logs between November 15 and December 15 and another 20 between mid-December and mid-January and they excavated second maternal galleries in the nearby uninfested logs. Only 38 females, all belonging to the first group of 79, moved on after the second oviposition to new logs to make a third maternal gallery. This third colonization took place over a period of
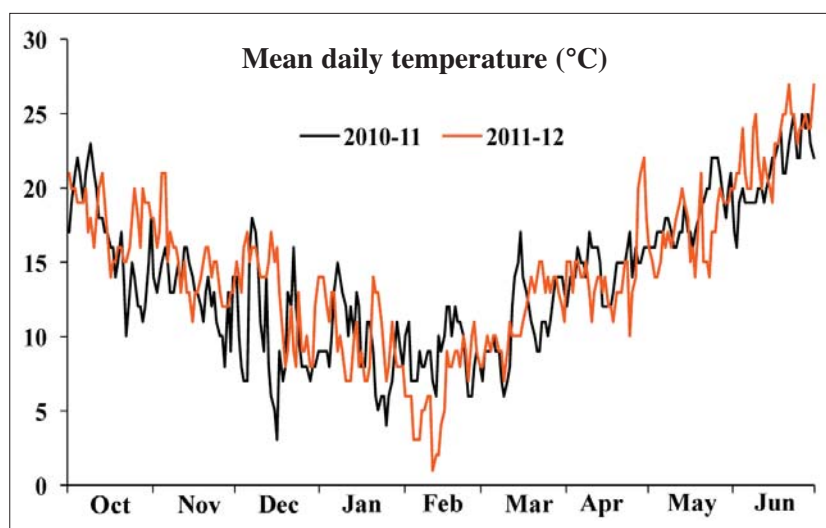

Fig. V – Mean daily temperature under the canopy in the pine forest during the study period (Alghero 2010-2012).

Fig. VI – Log attacked by newly-emerged *Tomicus destruens* adults (1); Detail of entrance holes (2); Galleries excavated by newly-emerged adults (3); Log with galleries without oviposition (4); Detail of a gallery excavated by a callow adult (5).

four months, but almost all the new galleries were excavated in the first two months (34 between mid-December and mid-February and only 4 between mid-February and mid-April). Finally, only seven females made a fourth maternal gallery in April.

The mean density of galleries in the logs involved in the first oviposition was very high, with more than 200 galleries per m² of bark, but it progressively decreased in subsequent colonizations ($F_{3,10}$ = 18.05, P = 0.0002) (Tab. 3). The maternal galleries of the first oviposition, as well as their fertile tract, had a significantly shorter mean length than those opened for the subsequent broods, among which there were no significant differences in length (gallery lenght $F_{3,93}$ = 3.74, P = 0.0137; fertile tract lenght $F_{3,93}$ = 3.13, P = 0.0292). In the first two broods, the females laid ca. 50 eggs per gallery while in the third and fourth broods the number remained around 35; however, significant differences were found only between the third and the first two broods ($F_{3,93}$ = 6.55, P = 0.0005).

DISCUSSION AND CONCLUSIONS

The results of our three-year study have provided important information to define the biological cycle of *T. destruens*. The bark beetle's reproductive phase, with colonization of the logs and the beginning of oviposition, always started in the first week of October and continued uninterrupted until early May. However, the attacks were not constant over time, with the maximum intensity in autumn. Female fecundity showed the same trend with mean values of ca. 80 to 95 eggs per gallery in October-November and ca. 70 in the following months. Similar fecundity values were observed by FACCOLI (2007) in breeding trials involving this bark beetle on the host *P. halepensis*. However, the fecundity of *T. destruens* is influenced by the density of attack, as shown by FACCOLI (2009) who reported ca. 90 eggs per gallery with a density of 25 ovipositing females per m² of bark and ca. 50 eggs with a density equal to or greater than 75 females.

Table 3 – Parameters of *Tomicus destruens* egg galleries excavated by females for successive broods. Values in the same column followed by different letters are significantly different (1 way ANOVA, followed by Tukey's post-hoc comparison test: P<0.05; for the analysis of the density of galleries the logs were used as replicates while for the other analyses the galleries themselves were used).

	Egg galleries	Gallery length	Fertile tract length	No. eggs/gallery
	no./m² ± SE	cm ± SE	cm ± SE	no. ± SE
I brood	218.62 ± 10.35 a	5.46 ± 0.25 b	3.31 ± 0.25 b	47.20 ± 2.70 a
II brood	83.83 ± 21.84 ab	6.84 ± 0.49 ab	4.76 ± 0.41 ab	54.53 ± 4.64 a
III brood	26.17 ± 5.60 bc	7.31 ± 0.47 a	4.85 ± 0.40 a	34.80 ± 3.04 b
IV brood	9.13 ± 5.22 c	6.15 ± 0.62 ab	3.72 ± 0.38 ab	35.14 ± 4.81 ab

It is known that the preimaginal development of this scolytid has a variable duration depending on the temperature and that the larvae hatching from eggs laid in autumn reach the adult stage in 6 months while those hatching in early spring need only 2-3 months (SABBATINI PEVERIERI *et al.*, 2008). Our observations largely confirm those findings, even though we did not calculate the duration of preimaginal development but only determined the period between each month of oviposition and the relative peak of emergences. In fact the time between the attack on the logs and the peak of emergences was approximately 200 days when the eggs were laid in October and decreased progressively to reach ca. 50 days for the ovipositions in May. However, the preimaginal development of individuals originating from the October egg-laying was actually much shorter, since they reached the adult stage already from January to February but generally remained in the bark and did not emerge until the air temperature reached a mean of about 14°C. Our results show that although the bark beetle's reproductive activity extended for a period of 7-8 months the emergence of new adults was concentrated in a much shorter time period between May and June, when ca. 95% of the emergences occurred.

Therefore, the life cycle of *T. destruens* in Sardinia is very similar to that described in Tuscany (SABBATINI PEVERIERI *et al.*, 2008), Algeria (CHAKALI, 2005) and Catalonia (MONLEÓN *et al.*, 1996) where the colonization of pines takes place in autumn and continues for several months while the adults of the new generation appear only in the following spring. This species exhibits a very long oviposition phase, which has been interpreted in different manners. RUSSO (1946), SANTINI and PRESTININZI (1989) and NANNI and TIBERI (1997) stated that *T. destruens* has two partly overlapping generations per year, while for other authors it is univoltine although a single female can excavate several maternal galleries giving rise to sister broods (MONLEÓN *et al.*,1996; CHAKALI, 2005; FACCOLI *et al.*, 2005; SABBATINI PEVERIERI *et al.*, 2008). The long reproductive phase has also been explained by the presence of subpopulations that reach sexual maturity at different times of the year (MASUTTI, 1969). Our experiments have ruled out the possibility of a second annual generation. Even the earliest emerging adults, originating from eggs laid in October, were unable to mature their gonads and oviposit before the summer reproductive stasis. They fed by penetrating the pine shoots and in part also took refuge in the bait logs where they dug small galleries and sheltered without laying eggs. Similar behaviour has been described in pine woods along the Veneto coast: before the reproductive phase the adults were found inside the shoots and in short irregular maturation tunnels excavated in the pine trunks with a total absence of oviposition (MASUTTI, 1969).

The ability of females to make multiple maternal galleries, as hypothesized for *T. destruens*, has been well documented for other bark beetle species, such as *T. piniperda* (SAUVARD, 1993), *Ips sexdentatus* (JACTEL and LIEUTIER, 1987) and *Dendroctonus frontalis* (WAGNER *et al.*, 1981). The study by SAUVARD (1993) in France showed that *T. piniperda* can carry out four different ovipositions in the wild, with female fecundity of ca. 200 eggs (ca. 50 per laying). Our observations in Sardinia demonstrated that *T. destruens* females can make up to four maternal galleries and lay a total of ca. 170 eggs throughout their life, thus exhibiting behaviour and fecundity not unlike that observed in *T. piniperda*. It cannot be excluded, however, that the fecundity observed in Sardinia was influenced by the strong level of initial infestation of the logs used in the experiment. On the other hand the reduced number of eggs found in the third and fourth broods could be explained either by the experimental conditions, which did not exactly reproduce the natural conditions, or by inadequate nutrition of females which, although they had available a large quantity of pine plants within the experimental cage, never fed on the shoots. The latter observation could also indicate that the females do not require "regeneration feeding" between one oviposition and the next or that this type of nutrition is achieved directly by feeding on the subcortical tissue during excavation of the maternal galleries. Accordingly with our observation on *T. destruens*, females of *Tomicus minor* (Hartig) do not feed on the shoots between successive ovipositions but they accumulate lypids by eating the phloem during laying (FERNADEZ FERNANDEZ *et al.*, 1999).

About 35% of the females that completed the first oviposition made a second maternal gallery, while 13% made a third and only 2% a fourth. The rapid decline in the number of females in the successive oviposition waves, also observed in the study of *T. piniperda* (SAUVARD, 1993), could be attributed to their progressive mortality. They may have been killed by several factors, including the many arthropod predators found in the emergence cages which certainly had an impact even though they were always promptly removed. Our experimental design did not allow us to assess the number of supernumerary galleries excavated by females which, having completed the first maternal gallery, made another in the same log instead of colonizing a new one. This phenomenon, described by SAUVARD (1993) for *T. piniperda*, could have

led to an under-estimation of the percentage of females that made multiple galleries. Nonetheless it does not invalidate the main result of our observations, i.e. the experimental demonstration of the ability of *T. destruens* females to make several maternal galleries during their life.

In the trials on the number of broods, the females abandoned the first infested log to make a second maternal gallery between mid-November and mid-January. The re-emergence after the second brood was even more gradual, involving a period between January and April, although the greatest activity was recorded in January-February. Finally, the females that completed the third brood and excavated their fourth maternal gallery were found in April. This pattern corresponds to the dynamics of attacks observed in the field by the counting of maternal galleries made each month, which showed that *T. destruens* colonized the pine logs in all the months between October and May with the highest infestation intensity in autumn followed by decreasing values. The field data did not allow determination of whether the winter and spring attacks were made exclusively by females that completed the first brood in the fall and then started other broods or whether there was a partial participation of virgin females that were late in reaching sexual maturity. Our observations allowed us to conclude, however, that the attacks on the logs by females that still had to lay the first brood certainly lasted for at least two months (October and November).

In Sardinia, the beginning of the reproductive phase was very early and the first ovipositions were recorded in the first ten days of October, similar to what was recorded in central Italy (SABBATINI PEVERIERI *et al.*, 2008) and Catalonia (MONLEÓN *et al.*, 1996). Such an early attack and the absence of a second generation by new adults emerging in March-April support the hypothesis that *T. destruens* has only one generation per year throughout its distribution range, with a variable period of oviposition in the different climatic zones (LIEUTIER *et al.*, 2015). This phenology has also been well demonstrated in Turkey where the period in which this bark beetle enters the reproductive phase varies according to the altitude of the pine stands: under 300 m asl, egg-laying is recorded at the end of October, between 300 and 600 m asl in mid-December and above 600 m in February (SARIKAYA and AVCI, 2010).

The new knowledge acquired during our three-year study on the biology and behaviour of *T. destruens* in a pine forest in the coastal zone of northern Sardinia provides information useful to calibrate well-timed interventions to control this dangerous pest by means of the trap tree technique (SANTINI and PRESTININZI, 1991). In particular, the setting out of bait logs should be advanced to mid-September, since adults begin to excavate maternal galleries in early October. Given this bark beetle's reproductive behaviour, the use of bait logs can probably be limited to the period from October to November when most of the females produce the first brood. We believe that the yearly use of this control strategy could remove a significant portion of the population, progressively reducing its abundance and at the same time limiting the possibility of successive broods not only in the bait logs but also in weakened trees growing nearby which otherwise could become new breeding sites, thus negating some of the positive results achieved.

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