are attracted by stressed *Eucalyptus* trees or freshly cut logs where they mate. Females lay egg batches under loose bark (case of smooth bark) or in bark crevices (case of fissured bark) (CHARARAS, 1969; IVOTY, 1977; SCRIVEN *et al.*, 1986; HANKS *et al.*, 1990; PAINE *et al.*, 1995). Neonate larvae bore into the inner bark and, after passing through bark and phloem feed by boring galleries between the phloem and the sapwood until their maturity. Mature larvae burrow into the sapwood to pupate (SCRIVEN *et al.*, 1986; FRAVAL and HADDAN, 1989; PAINE *et al.*, 2009). In Tunisia, *P. semipunctata* adult emergence is staggered; it begins from April until September (CHARARAS, 1969). Adult flight begins in the evening and continues through the night, at temperatures generally above 16°C (CHARARAS, 1969).

In Tunisia, about 120 *Eucalyptus* species were introduced and planted into various arboreta belonging to various bioclimatic areas (CHARARAS, 1968), among which 117 species were well acclimated (KHOUJA *et al.*, 2001). However, although the two *Phoracantha* species have been reported for many years in that country, their relative distribution is still unknown and very little is known about their preferences and performances in various host species under different climates. We present here a study aiming at clarifying those aspects. We combined field data and laboratory follow-up, in order (i) to define the present distribution and the relative abundance of the two species; (ii) to test the effects of sites and *Eucalyptus* species on attack density, reproductive and developmental performances, and productivity of the insects.

## MATERIALS AND METHODS

#### EXPERIMENTAL LOCALITIES

The study was carried out in *Eucalyptus* plantations of ten localities belonging to four bioclimatic areas of Tunisia (EMBERGER, 1955), ranging from humid to arid (Table 1). Trees were about 15 years old in the humid (Hu), sub-humid (SHu) and semi-arid (SAr) areas, and about 30 year-old in the arid (Ar) area.

#### FIELD EXPERIMENTS

Choice preference and colonization of various selected *Eucalyptus* species by the two insect borers were studied with standing trap trees in all sites and with trap logs in four sites. Table 1 indicates the sites and the tree species used in each case.

#### *Trap trees experiment*

Standing trap trees were prepared by girdling healthy trees in order to create a water stress and therefore plant weakness. Beginning of July 2009, in each site and for each selected Eucalyptus species, three trees, separated by at least 10 m one from another, were striped from their bark and external sapwood until 1 cm deep into the sapwood, on a 10 cm high belt beginning at 20 cm above ground. A total of 75 trees were thus used but, unfortunately, 10 of them were inadvertently removed by farmers during summer, resulting in a total of 65 trees with diameters ranged from 12 to 15 cm. In September 2009, all remaining trap trees were harvested and their heights measured. Their stems were cut into 1-m-long logs, resulting in 501 logs, which were brought to the laboratory separately into insect proof bags with labels where were mentioned locality, species, tree number and level in the stem of each log and further inspected.

#### Trap logs experiment

Trap logs were arranged in each selected site three times in the 2010 growing season, i.e. in May, June and July. Logs were obtained by harvesting three trees of each selected Eucalyptus species (see Table 1) and cutting each of them into four 1-m-long logs beginning from the stem base. Tree diameters ranged from 12 to 15 cm. In one of the 4 sites (Mouaden), three E. gomphocephala sprouts were used instead of stems, to be added to the experimental device. They originated from an old local tree and were at least 6 m long, with diameters of 10 to 13 cm. In each site, logs from a same tree were separated into two groups so that in each group, the first and the third log from a tree were combined with the second and the fourth log from another tree, the logs of the third tree being equally distributed between the two groups. Two sets of logs were then built with one group of each tree species. 18 logs in Mouaden, 12 logs in Dar Chichou, 18 logs in Aïn Jammala and 18 logs in Bouflije in each set were randomly arranged on forest ground so that every two neighboring logs were separated by 1 m (HANKS et al., 1993). The two sets of logs were separated by about 70 m. A total of 132 logs were thus used each month of the tests. At each experimental period and for each study site, ten days after logs exposure, two logs of each Eucalyptus species from the same set were randomly selected. In total 66 logs were selected and called "A" trap log in the followings, the other logs (330 logs) of that experiment being called "B" trap logs. In the field, oviposition places of Phoracantha females were externally recognized on the "A" trap logs by nutrition traces of young larvae which bore on the bark surface before boring in, or by frass extruded from the bark when boring in, and counted. At each oviposition place careful debarking was then locally performed in order to count the young larval galleries without disturbing the larvae. This allowed to calculate the average number of young larval galleries per attack (= "Gallatt" parameter). Then all the logs ("A" and "B") were conserved in insect proof bags with labels where were mentioned locality, species, tree number and level in the stem of each log. After two months from the day of their exposition to Phoracantha attacks, all trap logs ("A" and "B") were brought to the laboratory.

#### LABORATORY OBSERVATIONS

Bark area of each log was calculated. Logs were then kept separately outside in mesh bags until adult emergence. Bags were examined once or twice a week. Emerging adults were collected and sorted by species and sex.

# Distribution and abundance of adults of Phoracantha species

Percentages of the two beetle species emerging from all logs of each experiment were calculated for each locality. These percentages were used to determine the distribution and the abundance of the two beetle species.

Data of emergence of adults/logs (from all logs of each experiment) were used to calculate the density of emerging *Phoracantha* species (number of emerging *Phoracantha* species /  $m^2$  of bark) by locality and by *Eucalyptus* species.

## *Bioclimate, locality and* Eucalyptus *species effects on insect colonization parameters*

Once the emergence had ceased, all logs from the trap trees experiment and the "A" logs from the trap logs experiment were debarked and the following additional parameters were measured:

 Attack density (number of female oviposition places / m<sup>2</sup> of bark= "Densatt" parameter);

*Table 1* – Study sites and *Eucalyptus* species selected for the trap tree and the trap log experiments. (\*)=sites of trap logs experiment. Species without asterisk were used to attract beetles with trap trees. \*=species used in the trap trees experiment and in the trap log experiment. \*\*= species used only in the trap logs experiment. GOM = *E. gomphocephala*, CAM = *E. camaldulensis*, CIN = *E. cinerea*, SAL = *E. saligna*, AST = *E. astrengins*, LEU = *E. leucoxylon*, OCC = *E. occidentalis*, MIC = *E. microtheca*.

Bioclimatic area	Locality	Locality code	Latitude	Longitude	Altitude	Selected Eucalyptus species
Humid (Hu)	Sidi Bader	SidiB	36°56'39.06"N	8°48'51.23"E	89 m	GOM CAM
	Kef Bouras	KefB	36°55'30.01"N	9°06'12.11"E	119 m	CAM CIN SAL
	Mouaden	Mou (*)	37°09'49.36"N	9°16'03.39"E	84 m	GOM** CAM* AST*
	Ragoubet Essid	RgE	37°03'37.16"N	9°15'52.95"E	183 m	CAM
Sub-humid (SHu)	Dar Chichou	DarC (*)	36°57'45.59"N	10°59'25.07"E	38m	GOM* CAM*
Semi-arid (SAr)	Aïn Jammala	AïnJ (*)	36°30'18.94"N	9°18'44.44"E	226m	GOM* LEU* OCC*
Arid (Ar)	Bouflije	Bouf (*)	35°21'47.35"N	10°36'36.64"E	128 m	GOM* OCC* MIC*
	Tlil El Ejla	TlE	35°02'00.26"N	10°13'49.50"E	106 m	GOM OCC MIC
	Orbata	Orb	34°26'24.08''N	8°49'26.59''E	344 m	GOM OCC MIC
	Zrig	Zrig	33°43'56.25"N	10°09'22.67''E	46 m	GOM OCC MIC

- Number of young larvae galleries per attack ("Gallatt" parameter). The number of young galleries represents the number of young larvae passing through the bark;
- Larval survivorship (number of mature larvae / number of young larvae = "SurvL" parameter). The number of mature larvae was determined by the number of holes of galleries bored into the sapwood by the mature larvae in order to pupate.
- Density of emergence (number of emerging beetles / m<sup>2</sup> of bark = "Densem" parameter)
- Adult success (number of emerging beetles / number of mature larvae = "SuccAd" parameter);
- Offspring productivity (ratio densem / densatt = "Product" parameter).

For both experiments these insect parameters were measured in order to determine the effects of bioclimates, localities, and *Eucalyptus* species on the colonization of insects. For the trap trees tree zones effect was also studied.

The differences between the number of mature larvae and the number of emerging beetles corresponded to closed larval galleries. To understand the reasons for this, the "A" trap logs were split into many pieces. Dead and alive larvae, pupae and adults were collected from galleries or pupal chamber in the sapwood and counted.

## STATISTICAL ANALYSIS

We performed a multivariate analyze MANOVA of insect colonization parameters: Denatt, Gallatt, SurvL, Densem, SuccAd and Product as dependent variables by the factors: bioclimate, locality and *Eucalyptus* species as independent variables. This analyze was performed at different level; first by a multivariate test (Pillai's trace) of the effect of interaction between factors then by the between-subjects effects test. Then we tested separately the factors when difference was not significant. Comparisons between mean number of emerging adults of *Phoracantha* species per m<sup>2</sup> of bark, by tree species and localities and comparisons between means of insect colonization parameters by the tree zones were performed by one way analyses of variance (GLM procedure). All tests were followed by a post-hoc comparison (Scheffe test, p<0.05). Pearson's and Spearman's correlations for insect colonization parameters were calculated. All statistical treatments were performed with the SPSS software (IBM SPSS 20.0, 2011).

## RESULTS

DISTRIBUTION AND ABUNDANCE OF ADULTS OF PHORACANTHA SPECIES

In the trap tree experiment, there was no locality effect on CAM (p=0.6420) and OCC (p=0.0901) regarding the percentage of *P. recurva* among the emerging beetles when the tests were performed per tree species while percentage of *P. recurva* differ significantly on GOM (p=0.0244). When tested per locality, a tree species effect was detected only in the trees of "KefB" (p < 0.0001) with 100% of *P. recurva* on CAM, 97.6% on SAL and 70.7% on CIN.

Combining all tree species in the other localities revealed that *P. recurva* was significantly more abundant than *P. semipunctata* in all localities of the humid (SidiB, RgE and Mou), sub-humid (DarC) and arid (TIE) bioclimatic areas (p < 0.001) (Fig. I). In "AïnJ" (semi-arid area), "Bouf" and "Orb" (arid area), the two species were equally represented, whereas there was more emerging *P. semipunctata* in "Zrig" (arid area) (p < 0.0001).



Fig. I – Percentage *P. semipunctata* and *P. recurva* adults emerging from logs of trap trees experiment (tree species gathered). Bars represent the standard errors. Means ( $\pm$ SE) marked with asterisk differ significantly (*p*<0.05, Scheffe means separation test).

The density of *P. recurva* adults emerging from logs coming from attacked trees (Fig. II) differed significantly among the localities of origin for GOM ( $F_{6,40}$ =16.06; p < 0.0001), CAM ( $F_{4,48} = 7.27$ ; p < 0.0001) and OCC ( $F_{4,41}$ =4.23; p=0.006) but in that latter case the Scheffe test did not separate the localities. On GOM (Fig. II, 1), more P. recurva adults emerged from logs originating from trees attacked in "AïnJ" than from those coming from most other localities except "Orb". No adult emerged from those of "Zrig". The localities other than "AïnJ" and "Zrig" did not differ among each other's. On CAM (Fig. II, 2), more P. recurva adults emerged from "SidiB" than from "DarC", "RgE" and "KefB". For P. semipunctata, the density of emerging adults differed significantly on GOM ( $F_{6,33}$ =5.05; p=0.0010), CAM ( $F_{4,12}=4.10$ ; p=0.025) (but in that case, the Scheffe test did not separate the localities) and OCC (F<sub>4.41</sub>=4.23; p<0.0001). On GOM, more P. semipunctata emerged from "Orb" than from "SidiB", "AïnJ" and "Zrig" (Fig. II, 1). On OCC, more P. semipunctata emerged from "Orb" than from "AïnJ", "Bouf" and "TIE" (Fig. II, 3).

Phoracantha species emergence density differed also among tree species (Fig. III). In the following comparisons, MIC was excluded because it was never attacked in this experiment. "RgE" was also excluded because only one species was present in this locality. For P. recurva, differences among tree species were noted in logs coming from trees attacked in "SidiB" ( $F_{1,10}$ =7.39; p=0.0220), "KefB" ( $F_{2,12}$ =11.30; p=0.0020), "Mou" ( $F_{1,18}$ =20.31; p<0.0001), "AïnJ" ( $F_{2,21}$ =41.87; p<0.0001), "Zrig"  $(F_{1.5}=11.30; p=0.0230)$  and "Orb"  $(F_{1.6}=60.54; p<0.0001)$ , but not in "DarC", "Bouf" and "TIE". Depending on localities, when a significant difference existed, the tree species giving the higher density of emerging beetles were CAM, SAL, GOM and OCC, without allowing to define a general tendency. The density of emerging P. semipunctata differed among tree species only in "SidiB" ( $F_{1,8}$ =11.60; p=0.009), "KefB" (F<sub>2,12</sub>=28.46; p<0.0001) and "Bouf"  $(F_{16}=10.51; p=0.0180)$ . In all these localities however, differences were significant only when a tree species did



Fig. II – Comparisons between localities of the densities of adults *Phoracantha* species emerging from logs of the trap tree experiment with the following *Eucalyptus* species: (1) *E. gomphocephala*, (2) *E. camaldulensis*, (3) *E. occidentalis*. Bars represent the standard errors. Means ( $\pm$ SE) marked with different letters are significantly different (*p*<0.05, Scheffe means separation test).

not give any emerging beetle, and no general tendency was either visible.

In the trap log experiment, there was no locality effect on the percentage of emerging *P. recurva* when tested per tree species: CAM (p=0.1776), GOM (p=0.1229) and OCC (p=0.3563). When tested per locality, a tree species effect was observed in the logs from "Mou" ( $F_{2,91}$ =14.85; p<0.0001), "DarC" ( $F_{1,68}$ =11.83; p=0.0010) and "AïnJ" ( $F_{2,100}$ =4.47; p=0.0010). In "Mou", the percentage of *P. recurva* was higher on CAM (96.5±1.1%) and AST (88.6 ±3.2%) than on GOM (69.8±5.1%). In "DarC", it was higher on CAM (93.9±1.5%) than on GOM (78.6±4.2%). In "AïnJ", it was higher on LEU (94.6±1.4%) than on OCC (79.3±5.5%) and GOM (73.9 ±5.5%). Combining all tree species in each locality, *P. recurva* was significantly more abundant in all the four tested localities (p < 0.0001), including "AïnJ" and "Bouf", than its congener *P. semipunctata* (Fig. IV).

As for the trap tree experiment, comparisons of the densities of emerging beetles were also carried out among



Fig. III – Comparisons, between *Eucalyptus* species, of the densities of emerging adults *Phoracantha* species from logs coming from trees attacked in the localities of the trap tree experiment. Bars represent the standard errors. Means ( $\pm$ SE) marked with different letters are significantly different (p<0.05, Scheffe means separation test).



Fig. IV – Percentage *P. semipunctata* and *P. recurva* adults emerging from trap logs experiment (tree species gathered). Bars represent the standard errors. Means ( $\pm$ SE) marked with asterisk differ significantly (*p*<0.05, Scheffe means separation test).

localities and among tree species for each *Phoracantha* species in the trap log experiment. The density of *P. recurva* emerging adults differed significantly among localities (Fig. V) on GOM ( $F_{3,140}$ =8.95; p<0.0001) and OCC ( $F_{1,70}$ =5.46; p=0.0220), but not in CAM ( $F_{1,70}$ =2.87; p=0.0950). On GOM (Fig. V, 1), there were more emerging *P. recurva* from "Bouf" than from "Mou" and "AïnJ". On OCC (Fig. V, 3), they were more abundant from "Bouf" than from "AïnJ". The density of emerging *P. semipunctata* never differed among localities (Fig. V, 1, 2 and 3).

Comparisons of the densities of emerging beetles by tree species for the two *Phoracantha* species were shown in Figure VI. The density of *P. recurva* emerging adults differed significantly among tree species in "Mou" ( $F_{2,105}$ =16.32; p<0.0001), "AïnJ" ( $F_{2,105}$ =37.11; p<0.0001) and "Bou" ( $F_{2,105}$ =27.99; p<0.0001), but not in "DarC" ( $F_{1,70}$ =0.90; p=0.3440). That of *P. semipunctata* emerging adults differed significantly among tree species in all localities ( $F_{2,105}$ = 15.98 with p<0.0001 in "Mou";  $F_{1,70}$ =14.54 with p<0.0001 in "DarC";  $F_{2,105}$ = 9.01 with p<0.0001 in "AïnJ";  $F_{2,105}$ = 4.49 with p=0.0130 in Bouf. In "Mou" (Fig. VI, 1), more *P. recurva* emerged from GOM and CAM than from AST, whereas more *P. semipunctata* 



Fig. V – Comparison between localities, of the densities of adults *Phoracantha* species emerging from logs of various *Eucalyptus* species attacked in the field, in the trap log experiment: (1) *E. gomphocephala*, (2) *E. camaldulensis*, and (3) *E. occidentalis*. Bars represent the standard errors. Means ( $\pm$ SE) marked with different letters differ significantly (p<0.05, Scheffe means separation test).



Fig. VI – Comparisons, between tree species, of the densities of adults *Phoracantha* species emerging from logs attacked in the various localities of the trap log experiment: (1) Mouaden, (2) Dar chichou, (3) Aïn Jammala, (4) Bouflije. Error bars represent the standard errors. Means ( $\pm$ SE) marked with different letters are significantly different (p<0.05, Scheffe means separation test).

emerged from GOM than from the other two tree species. In "DarC" (Fig. VI, 2), more *P. semipunctata* emerged from GOM than from CAM. In "AïnJ" (Fig. VI, 3), there was higher density of emerging *P. recurva* from LEU than from GOM and higher from this latter species than from OCC, whereas density of emerging *P. semipunctata* was higher on GOM than the other two species. In "Bouf" (Fig. VI, 4), emergence density of *P. recurva* were higher on GOM than OCC and MIC and that of *P. semipunctata* was higher on GOM than MIC.

# BIOCLIMATE, LOCALITY AND EUCALYPTUS SPECIES EFFECTS ON INSECT COLONIZATION PARAMETERS

In the followings, as it was not possible to identify the beetle species before their adult stage, the results refer to both species gathered.

## *Trap trees experiment*

#### Correlations among variables

Statistics of correlations among variables are given in Table 2. The number of larval galleries per attack was positively correlated with attack density p=(0.0397). The density of emerging beetles was positively correlated with attack density (p<0.0001), the number of young larval galleries per attack (p=0.0003), larval survival (p=0.0009), and adult success (p=0.0315), whereas offspring productivity was positively correlated with the number of young larval galleries per attack (p=0.0015), whereas offspring productivity was positively correlated with the number of young larval galleries per attack (p=0.0018), larval survival (p<0.0001), adult success (p=0.0053) and the density of emerging beetles (p<0.0001). The percentage of *P. recurva* among the emerging beetles was positively correlated with

attack density (p=0.0408) and the number of young larval galleries per attack (P=0.0068) whereas its sex-ratio was negatively correlated with larval survival (p=0.0395). No insect parameter was significantly correlated with tree height.

## Relations between attack localization on trap trees and insect parameters

Thirty trap trees were not attacked at all and thus discarded from the statistical analyses. In addition the upper zone (zone 4) was not attacked in several attacked trees. As a result, few trees (Table 3) and data were available to test the tree zone effect, which led us to gather trees from the different localities in that test, while separating tree species. Tests could be performed only for GOM, CAM, OCC and LEU since the other species were present in only one locality with one tree or were not attacked. Zones 1, 2 and 3 never differed from each other's for any of the colonization parameters on host plants (Table 3), except for "Densatt" in GOM where in zone 3 the host plant was less attacked than in zones 1 and 2 (see statistics Table 3). Moreover, when a significant localization effect was detected between tree zones, zone 4 always appeared having high or low value compared to the other three zones; "Densatt" in the four species was lower in zone 4. In CAM, "SurvL" and "Product" were higher in zone 4 whereas "Densem" was lower. In GOM, "SurvL" was higher in zone 4 whereas "Densem" and "SuccAd" were lower (Table 3).

As consequence of these results, in all other tests with trap trees, data from zone 4 were discarded and data from zones 1, 2 and 3 in a same tree were gathered.

Densatt		Х							
Gallatt		<i>r</i> =0.3493 <i>p</i> =0.0397(p)	Х						
SurvL				Х					
Densem		<i>r</i> =0.6263 <i>p</i> <0.0001(s)	r=0.5804 p=0.0003(s)	<i>r</i> =0.5381 <i>p</i> =0.0009(s)	Х				
SuccAd				<i>r</i> =0.3883 <i>p</i> =0.0233(s)	r=0.3694 p=0.0315(p)	Х			
Product			r=0.5087 p=0.0018(s)	<i>r</i> =0.7762 <i>p</i> <0.0001(s)	<i>r</i> =0.8869 <i>p</i> <0.0001(p)	r=0.4676 p=0.0053(p)	Х		
Pcrec		<i>r</i> =0.3527 <i>p</i> =0.0408(p)	r=0.4554 P=0.0068(s)					X	
Sexrec				<i>r</i> =-0.379 <i>p</i> =0.0395(p)					Х
Sexsem									
	TreH	Densatt	Gallatt	SurvL	Densem	SuccAd	Product	Pcrec	Sexrec

Table 2 – Correlation coefficients (r) among insect variables (Trap tree experiment). Only the highest of Pearson's (p) and Spearman's (s) correlation coefficient is given and only the significant correlations are presented. TreH=tree height.

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*Table 3* – Means ( $\pm$  SE) of each colonization insect parameters in each of the four tree zones for various *Eucalyptus* species (trap trees experiments, all localities gathered). Within a same column (but separately between plant hosts), means with different letters are significantly different (p<0.05, Scheffe means separation test).

Species	Zone	Densatt	Gallatt	SurvL	Densem	SuccAd	Product
	1	24.63±4.82ª	13.48±2.3ª	29.78±5.02 <sup>b</sup>	65±20.54ª	50.32±8.01ª	2.27±0.65b
CAM	2	25.72±3.09ª	15.40±2.75ª	30.07±5.54 <sup>b</sup>	85.39±20.4ª	65.18±7.48ª	2.91±0.58ab
(n=8)	3	16.62±3.46ª	12.21±1.61ª	42.63±6.75 <sup>b</sup>	56.17±16.27 <sup>ab</sup>	61.22±6.4ª	3.38±0.80 <sup>ab</sup>
(11 - 8) -	4	2.53±1.34 <sup>b</sup>	7.58±1.46ª	93.75±1.88ª	13.84±6.84 <sup>b</sup>	85.22±4.9ª	6.22±1.64ª
F values		$F_{3,28} = 9.82$	F <sub>3,22</sub> =1.22	$F_{3,22} = 14.92$	$F_{3,28} = 3.19$	F <sub>3,22</sub> =2.38	F <sub>3,22</sub> =2.99
P values		0.0001	0.3249	< 0.0001	0.0388	0.0970	0.0530
	1	22.44±3.02ª	9.41±0.81ª	45.12±5.9 <sup>b</sup>	79.11±15.77ª	75.75±4.7ª	3.13±0.51ª
GOM	2	20.57±2.46ª	8.96±0.78ª	48.00±6.8 <sup>ab</sup>	78.35±15.67ª	76.35±4.1ª	3.33±0.58ª
(n = 12)	3	8.09±2.97 <sup>b</sup>	7.71±0.59ª	64.69±10.8 <sup>ab</sup>	30.32±10.65 <sup>ab</sup>	75.42±5.97ª	4.22±0.98ª
(n - 12)	4	1.46±1.1 <sup>b</sup>	10.38±1.62ª	$91.55{\pm}~0.11^{\rm a}$	4.76±3.41 <sup>b</sup>	36.93±0.56 <sup>b</sup>	3.50±0.5ª
F values		$F_{3,44} = 16.08$	F <sub>3,29</sub> =0.89	$F_{3,29} = 3.09$	$F_{3,44} = 8.76$	$F_{3,27} = 4.40$	F <sub>3,29</sub> =0.44
P values		< 0.0001	0.4580	0.0427	0.0001	0.0121	0.7239
	1	19.87±2.75ª	7.98±0.95ª	38.98±8.53ª	49.27±15.11ª	79.46±4.13ª	2.66±0.79ª
OCC	2	19.95±3.68ª	7.56±0.67ª	36.15±7.4ª	55.96±22.23ª	78.70±4.14ª	2.35±0.63ª
(n = 11)	3	10.16±3.38 <sup>ab</sup>	9.35±0.93ª	44.49±12.97ª	23.06±6.83ª	82.17±4.8ª	3.39±1.12ª
(11 11) -	4	4.62±2.39 <sup>b</sup>	9.36±1.31ª	28.44±9.83ª	10.70±5.72ª	97.92±2.1ª	2.46±0.66ª
F values		$F_{3,40} = 6.00$	F <sub>3,29</sub> =0.90	F <sub>3,29</sub> =0.31	F <sub>3,40</sub> =2.29	F <sub>3,29</sub> =2.43	F <sub>3,29</sub> =0.28
P values		0.0018	0.4529	0.8200	0.0934	0.0852	0.8408
	1	18.70±4.0ª	9.50±1.69ª	46.27±2.4ª	68.00±23.89ª	80.74±1.78ª	3.52±0.52ª
LEU	2	14.84±2.45 <sup>ab</sup>	9.40±2.12ª	67.72±14.63ª	75.15±14.97ª	82.92±1.96ª	5.04±0.18ª
(n = 2)	3	10.64±3.59 <sup>ab</sup>	10.67±1.6ª	77.81±8.67ª	65.76±2.23ª	80.58±3.79ª	6.89±2.11ª
	4	0.00 <sup>b</sup>	-	-	-	-	-
F values		$F_{3,4} = 7.47$	F <sub>2,3</sub> =0.15	F <sub>2,3</sub> =2.63	F <sub>2,3</sub> =6.14	F <sub>2,3</sub> =0.24	F <sub>2,3</sub> =1.79
P values		0.0408	0.8693	0.2185	0.0560	0.8012	0.3080

t- = not determined because Densatt=0

#### Bioclimate, locality and Eucalyptus species effects

MIC (present in all localities of the arid area) was never attacked, as well as AST present only in "Mou" (humid area) and thus these two species were discarded from the statistical analyses.

In the trap trees experiment, bioclimate effect (F=1.568; df=18; p=0.1008) and species effect (F=0.804; df=18; p=0.6879) on insect colonization parameters were not significant while locality effect (F=2.226; df=48; p<0.0001) was significant. Interaction bioclimate\*species effect (F=1.058; df=6; p=0.4241) and interaction locality\*species effect (F=0.903; df=12; p=0.5573) were not significant.

The between-subjects effects test showed that interaction bioclimate\*species effect was not significant for all parameters. Interaction locality\*species effect was significant for Gallatt (F=3.903; df=2; p=0.0416) and not significant for Densatt (F=1.807; df=2; p=0.1961), SurvL (F=0.062; df=2; p=0.9401), Densem (F=0.253; df=2; p=0.7797), SuccAd (F=0.550; df=2; p=0.5874) and Product (F=1.053; df=2; p=0.3717). This test showed that bioclimate effect was significant for Gallatt (F=5.244; df=3; p=0.0069), Densem (F=3.636; df=3; p=0.0286) and SuccAd (F=3.417; df=3; p=0.0351) while it was not significant for Densatt (F=0.984; df=3; p=0.4183), SurvL (F=0.467; df=3; p=0.7084) and Product (F=2.619; df=3; p=0.0763). Locality effect was significant for Densatt (F=3.447; df=8; p=0.0168), Gallatt (F=17.077; df=8; p<0.0001), Densem (F=3.689; df=8; p=0.0126) and SuccAd (F=6.666; df=8; p=0.0006) while it was not significant for SurvL (F=0.891; df=8; p=0.4555) and Product (F=2.361; df=8; p=0.0683). Finally, species effect was significant for Gallatt (F=3.562; df=3; p=0.0380).

Compared between biclimates, Gallatt was the highest in humid bioclimate than in subhumid, arid and semiarid bioclimates. Product was higher in semiarid bioclimate than in subhumid bioclimate (Table 3).

Compared between localities (Table 4) Gallatt was the highest in "Mou". Densem was higher in "Mou" than in "Orb" whereas SuccAd was higher in "Mou", "DarC", "AïnJ", "Bouf", "TIE", "Zrig" and "Orb" than in "KefB".

On *Eucalyptus* species, Gallatt was higher on CAM than on OCC while SuccAd was higher on LEU and lower on CAM (Table 4).

## Trap logs experiment

Correlations among variables

"SurvL" was negatively correlated with "Densatt" (p<0.0001) and the "Gallatt" (p=0.002), these latter two parameters being correlated with each other (p=0.018) (Table 5). "Densem" and "Product" were positively correlated with each other (p<0.0001) and with "SurvL" (p<0.0001) and "SuccAd" (p=0.002). "Pcrec" was also positively correlated with "Densem" (p=0.009), "SuccAd" (p<0.0001) and "Product" (p=0.020). "Product" was negatively correlated with "Densatt" (p=0.002). The sex ratio of *P. recurva* was also negatively correlated with emergence density.

#### Locality and Eucalyptus species effects

In the trap log experiment, locality effect (F=1.937; p=0.0165) and species effect (F= 2.640; p<0.0001) on insect parameters were significant, whereas interaction effect was not significant (F=1.727; p=0.0715).

The between-subjects effects test showed that interaction effect was significant for SurvL (F= 3.6; df=2; p=0.0339) but not for Densatt (F=1.950; df=2; p=0.1519), Gallatt (F=3.267; df=2; p=0.8201), Densem (F=0.270; df=2; p=0.7646), SuccAd (F=0.101; df=2; p=0.9038) and Product

Table 4 – Means ( $\pm$ SD) of insect parameters from trap trees experiment compared by bioclimates, localities and *Eucalyptus* species. Means with different letters are significantly different (p<0.05, Sheffe means separation test).

		G 11	с. т.	5	<u> </u>	D 1 /				
	Densatt	Gallatt	SurvL	Densem	SuccAd	Product				
	Bioclimate									
Hu	24.31±4.20ª	16.39±6.96ª	43.53±19.32ª	93.40±24.35ª	63.36±6.12ª	$3.95{\pm}0.8^{ab}$				
SHu	$17.41{\pm}10.09^{a}$	8.71±1.73 <sup>b</sup>	33.75±7.76ª	32.14±30.15ª	57.74±18.72ª	$1.77 \pm 0.69^{b}$				
SAr	18.43±5.61ª	11.28±2.38 <sup>b</sup>	53.75±10.57ª	91.50±34.78ª	83±1.94ª	4.89±0.41ª				
Ar	17.56±9.04ª	8.23±2.26b	41.07±24.33 <sup>a</sup>	52.24±43.92ª	76.55±20.58ª	$2.78{\pm}2.08^{ab}$				
	Locality									
SidiB	$20.82{\pm}0.97^{a}$	$10.44 \pm 1.78^{b}$	$60.27 \pm 0.37^{a}$	$83.96 \pm 32.69^{ab}$	62.67±11.7 <sup>ab</sup>	$4{\pm}1.38^{a}$				
KefB	19.26±10.88ª	8.87±0.59 <sup>b</sup>	32.34±7.52ª	$11.08 \pm 9.6^{ab}$	50.72±19.3 <sup>b</sup>	1.58±0.91ª				
Mou	28.48±20.94ª	22.35±0.32ª	26.79±0.04ª	111.31±14.01ª	65.19±0.45ª	3.9±0.09ª				
DarC	20.22±11.45ª	8.55±2.56 <sup>b</sup>	35.17±8.88ª	$42.67 \pm 32.25^{ab}$	$68.16 \pm 8.46^{a}$	1.96±0.41ª				
AïnJ	18.43±5.61ª	11.23±2.38 <sup>b</sup>	53.75±10.57ª	$91.50{\pm}34.78^{ab}$	83±1.94ª	4.89±0.41ª				
Bouf	22.15±6.98ª	$8.79{\pm}2.08^{b}$	48.76±17.24ª	71.60±41.1 <sup>ab</sup>	$78.40 \pm 8.06^{a}$	$3.41{\pm}1.89^{a}$				
TIE	20.63±5.07ª	8.30±1.85 <sup>b</sup>	48.08±20.16ª	$68.85{\pm}37.84^{ab}$	81.95±4.58ª	3.59±2.21ª				
Zrig	14.57±13.59ª	9.14±2.12 <sup>b</sup>	9.28±9.00ª	$15.49{\pm}0.31^{ab}$	48.55±42.17ª	$0.54{\pm}0.53^{a}$				
Orb	$9.1 \pm 7.89^{a}$	6.60±3.04 <sup>b</sup>	44.63±32.16ª	7.85±6.57 <sup>b</sup>	87.96±8.1ª	$2.50{\pm}2.18^{a}$				
Eucalyptus species										
GOM	19.06±5.89ª	$8.89{\pm}2.44^{ab}$	49.52±9.21ª	66.64±35.01ª	$75.29{\pm}12.05^{ab}$	$3.4{\pm}1.37^{a}$				
CAM	22.91±9.09ª	13.33±6.41ª	32.88±13.16 <sup>a</sup>	69.16±46.48ª	60.07±17.93 <sup>b</sup>	2.7±1.62ª				
OCC	17.25±9.62ª	7.85±2.49 <sup>b</sup>	38.34±26.93ª	44.35±48.68ª	79.76±6.4 <sup>ab</sup>	2.51±2.27ª				
LEU	15.28±4.67ª	9.58±1.51 <sup>ab</sup>	59.36±11.46ª	69.70±21.50ª	81.65±0.56ª	4.56±0.13ª				

Х	_
	Х
Sexrec	Sexsem
	X

*Table 5* – Correlation coefficients (r) between studied variables from trap logs experiments. Only the highest of Pearson's (p) and Spearman's (s) correlation coefficient is given and only the significant correlations are presented.

(F= 1.585; df=2; p=0.2140). Locality effect was not significant for Gallatt (F=2.372; df=3; p=0.0802), Densem (F=1.567; df=3; p=0.2076), SuccAd (F=1.730; df=3; p=0.1714) and Product (F=1.644; df=3; p=0.1897) but was significant for Densatt (F=5.849; df=3; p=0.0015) and SurvL (F=3.221; df=3; p=0.0295). Species effect was not significant for Densatt (F= 1.951; df=5; p=0.1519) and SuccAd (F=0.101; df=5; p=0.9038), it was significant for Densem (F=0.270; df=5; p=0.7646), Gallatt (F=0.199; df=5; p=0.8201), SurvL (F=3.6; df=5; p=0.0339) and Product (F=1.585; df=5; p=0.2140).

Tested by localities Densatt was higher in "DarC" and lower in "Mou" whereas, SuccAd was higher in "Mou" and lower in "AïnJ" (Table 6). On *Eucalyptus* species, SurvL, Densem and Product were higher on LEU and lower on AST while SuccAd was higher on AST and lower on OCC (Table 6).

## Debarking and splitting "A" trap logs

All tree species gathered, the closed sapwood galleries represented 21.94±1.87% of the total number of sapwood

galleries, significantly less than the opened galleries ( $F_{1,128}$ =451.85; p<0.0001). Significantly more larvae than pupae and adults were found in the closed galleries ( $F_{2,177}$ =437,65; p<0.001) (Fig. VII, 1). Among these insects, 94.5±2.5% of the larvae and 97.7±2.3% of the pupae were dead. Adults of both beetle species were present equally. *P. recurva* represented 58.1±7.59% of the adults which did not differ from the percentage of *P. semipunctata* ( $F_{1,68}$ =2.27; p=0.1360). Its sex-ratio was 1.19, and 94.79±4.25% were dead (equally males and females ( $F_{1,44}$ =0.221; p=0.6410)). The sex-ratio of *P. semipunctata* adults was 0.35 and 88.89±6.46 % of adults were dead (Fig. VII, 2) with mainly females ( $F_{1,32}$ =5.49; p=0.0250) (Fig. VII, 3).

## DISCUSSION

Assuming that mortality of immature stages was comparable in the two *Phoracantha* species, we hypothesize that a high density of emerging beetles results from a high density of attacks. Tree species differed among

*Table 6* – Means ( $\pm$ SD) of insect parameters from trap trees experiment compared by bioclimates, localities and *Eucalyptus* species. Means with different letters are significantly different (p<0.05, Sheffe means separation test).

	Densatt	Gallatt	SurvL	Densem	SuccAd	Product			
			Locality						
Mou	28.04±12.13 <sup>b</sup>	10.58±3.62ª	35.65±25.87ª	62.73±47.71ª	80.63±14.49ª	2.66±2.31ª			
DarC	42.74±8.38ª	11.52±4.29ª	29.32±12.57ª	92.90±38.03ª	$71.81{\pm}12.07^{ab}$	2.23±0.92ª			
AïnJ	38.28±16.06 <sup>ab</sup>	10.92±3.00ª	36.87±29.29ª	86.37±62.71ª	62.92±22.87 <sup>b</sup>	2.71±2.31ª			
Bouf	$40.44{\pm}19.18^{ab}$	10.56±3.61ª	28.02±12.36ª	76.56±54.13ª	$70.86{\pm}16.67^{ab}$	$2.03{\pm}1.26^{a}$			
	Eucalyptus Species								
AST	$38.31{\pm}14.29^{a}$	12.60±2.82ª	6.64±4.08°	27.04±18.30 <sup>b</sup>	90.61±14.09ª	$0.68 {\pm} 0.35^{b}$			
CAM	33.46±13.45ª	$12.80{\pm}4.08^{a}$	$35.54{\pm}19.24^{abc}$	$89.52{\pm}44.67^{ab}$	$71.41{\pm}13.28^{ab}$	$3.20{\pm}2.32^{ab}$			
GOM	38.20±18.79ª	9.79±3.00ª	$41.07{\pm}20.21^{ab}$	$96.10{\pm}51.48^{ab}$	$73.37{\pm}12.63^{ab}$	$2.84{\pm}1.63^{ab}$			
LEU	41.80±16.73ª	$11.82 \pm 3.98^{a}$	48.60±31.13ª	137.55±52.93ª	$73.29{\pm}15.32^{ab}$	3.87±2.19ª			
MIC	33.13±12.12 <sup>a</sup>	9.56±4.52ª	$31.24{\pm}15.42^{abc}$	$54.37{\pm}27.08^{b}$	$68.17 \pm 11.54^{ab}$	$1.67{\pm}0.89^{ab}$			
OCC	36.40±14.46ª	10.23±2.79ª	$19.19 \pm 13.92^{bc}$	40.19±30.65 <sup>b</sup>	59.35±28.66 <sup>b</sup>	$1.32{\pm}1.17^{ab}$			



Fig. VII – Results of debarking and splitting "A" trap logs. (1) Developmental stages of *Phoracantha* sp. found in the sapwood; (2) Relative importance and mortality of *Phoracantha* adults according to species; (3) Mortality of *Phoracantha* adults in the sapwood, according to sex. Error bars represent the standard error of the means. Means ( $\pm$ SE) marked with different letters or asterisk are significantly different (p<0.05, Scheffe means separation test).

localities, but all localities contained at least one of the two species *E. gomphocephala* and *E. camaldulensis*, both being well appreciated by the two *Phoracantha* species. In the semi-arid and the arid areas, *E. camaldulensis* was replaced by *E. occidentalis* which also allowed emergence of the two beetle species. Based on the beetle emergence from these three tree species, and considering results from trap trees and trap logs together, it is possible to conclude that the two *Phoracantha* species are present in all considered bioclimates, and probably in all sites of these bioclimates.

*P. semipunctata* was not captured in Ragoubet Essid but it certainly also exists there since it was captured in Mouaden and Kef Bouras (humid area) which were far from this locality with less than 20 km.

P. recurva was largely dominant over P. semipunctata everywhere, even in Aïn Jammala and Bouflije, as indicated by the trap log experiment. An exception may be the two southern localities in the arid zone, where *P. semipunctata* densities were equal to or higher than those of P. recurva. These observations however, do not allow concluding that P. semipunctata copes better than P. recurva with the driest localities. Indeed, in the trap log experiment, the density of emerging P. semipunctata was the same in all logs whatever their origin but that of P. recurva was higher in logs that had been attacked in the arid area than in those attacked in semiarid and humid areas. This dominance of P. recurva over P. semipunctata in Tunisia is in agreement with observations from other regions in the world where the two species have been introduced. In Gharb Maamora region (Morocco), P. semipunctata and P. recurva cohabit in Eucalyptus plantations, where P. semipunctata represents 3.5% of the population (HADDAN and LIEUTIER, 2005). In Southern California, P. recurva populations have increased and P. semipunctata decreased in their shared habitats. P. recurva in space of 4 years has almost completely replaced P. semipunctata (HANKS et al., 1997; PAINE and MILLAR, 2005). In fact, P. recurva populations emerging from naturally infested Eucalyptus increased from 1.4% in 1996 to 74% in 1997 and in 1998 were >95% (ByBEE et al., 2004a).

Combining observations from the two experiments, both beetle species were able to emerge from all tree species tested in our experiments, including E. astringens and E. microtheca not attacked at all in the trap tree experiment, showing that all tested tree species allow complete development of the two beetle species. The reason for no emergence from *E. astringens* and *E. microtheca* in the trap tree experiment may be that girdling was not sufficient to create a water stress leading to a significant decrease of trees natural defenses in these species, contrary to logs without defense to attacks. HANKS et al. (1993) indicate that killed or felled trees are highly receptive to larvae colonization due to loss of their defensive system. Moreover, species that are not attacked in a country can be attacked in another, certainly depending on local environmental conditions. For example in Morocco, E. microtheca trees were attacked by P. semipunctata (EL YOUSFI, 1992). Eucalypt susceptibility to Phoracantha attacks is mainly influenced by bark moisture content (HANKS et al., 1991; CALDEIRA et al., 2002; HADDAN et al., 2010). Generally, water-stressed standing trees are highly attractive, particularly under typical Mediterranean climate conditions of prolonged summer droughts (HANKS et al., 1998). Some species however, among which E. astringens, are more resistant than others to both water stress and Phoracantha attacks, even under arid climate (CHARARAS 1968, 1969; CHARARAS et al., 1969).

The correlations between insect colonization parameters and tree height show that this latter did not affect insect parameters. However, insect characteristics and performances depended on attack localization on tree stem. In all tree species where a comparison could be made, attack densities were higher on the lowest parts than on the upper parts of the trees. Since basal stem zone have generally a larger diameter than the upper stem zone, insects colonization parameters are depending on the trunk section size. Similarly with *Phoracantha solida* in Australia, HELEN et al. (2014) reported that 63 % of attacks occurred in the lower 0.1-0.5m of the bole of eucalypt tree and 83 % within 1m of the base. Our observations suggest that such a situation can result in a strong intraspecific competition among larvae in the basal parts of the trees. This is attested by a lower larval survival in these zones, finally resulting in lower offspring productivity although emerging densities were higher, as visible in *E. camaldulensis*.

Supposing that high attack densities result from high population levels, the observation that the number of young larval galleries per attack was positively related to attack density in both experiments could mean that female fecundity was correlated with population level. Such a correlation could result in fast population increase, and the positive relation between the percentage of P. recurva and attack density in the trap tree experiment may indicate that this species was particularly concerned with population increase. However, in spite of a strong tendency in favor of P. recurva, HADDAN AND LIEUTIER (2005) did not find significant difference in fecundity between the two species in laboratory studies. BYBEE et al. (2004b) supposed that the replacement of *P. semipunctata* populations in southern California by P. recurva can be due to its earlier and higher emergence, giving it possibility to colonize the nutritional resources before P. semipunctata attack. In the trap log experiment, the negative correlations between larval survival and both attack density and number of young larval galleries per attack indicates a larval intraspecific competition, which would thus moderate population increase. Lethal effects of intraspecific competition among Phoracantha larvae have already been reported (MENDEL, 1985; HADDAN and FRAVAL, 1988; GONZALEZ-TIRADO, 1987). This negative effect of high attack densities and high fecundity on larval survival was not observed in the trap tree experiment, certainly because attack densities were much lower, as visible on E. gomphocephala in Dar Chichou, Aïn Jammala and Bouflije, localities where both experiments took place (Tables 4 and 6). As the trap tree experiment was carried out one year before the trap log experiment, it is possible that the beetle populations increased from one year to the other. The difference in attack density may however also result from less favorable oviposition substrate in living trees than logs.

It is perfectly coherent that the density of emerging beetles correlated positively with attack density, number of young larval galleries, larval survival and adult success in the trap tree experiment. However, it did not correlate with attack density and the number of young larval galleries in the trap log experiment, certainly a consequence of larval competition in such a situation.

In both experiments nevertheless, high larval survival and adult success resulted in high offspring productivity, thus counteracting effects of larval competition in the trap log experiment. A rather important part of sapwood galleries (22%) did not give emerging beetles which was mainly due to death of old larvae, confirming the decisive role of larval mortality (both young and old larvae) in offspring productivity. Larval survival in trap trees and density of emerging beetles in trap logs correlated negatively with sex ratio of *P. recurva*. However, adult mortality concerned equally males and females of *P. recurva* (Fig. VII, 3). Consequently, intraspecific competition probably affected more particularly larvae with male potentiality than larvae with female potentiality.

In both the trap tree and the trap log experiment, the locality had effect on insect parameters, especially the intensity of infestation (attack density or number of galleries per attack), larval survival. However, whatever the tree species concerned, these differences were not related to climatic areas. Significant differences even existed between localities belonging to the same climatic area, as visible for adult success and mean number of galleries per attack between Kef Bouras and Mouaden (Table 3).

Both E. gomphocephala and E. camaldulensis, largely planted in Tunisia, and E. leucoxylon, planted in semi arid area, seem very favorable to both beetle species. Compared with E. cladocalyx, E. camaldulensis was also demonstrated to be more attractive to P. semipunctata and more favorable to larval development (HANKS et al., 1993). After artificial infestations on healthy trees however, HADDAN et al. (2010) found that larval survival in the phloem was low in both E. gomphocelala and E. camaldulensis. In Morocco, KISSAYI (2011) showed that E. gomphocephala was more susceptible to P. semipunctata attacks than E. camaldulensis and E. occidentalis. In all localities, E. gomphocephala and E. camaldulensis when it was present were the tree species providing the highest densities of emerging adults for both P. recurva and P. semipunctata, one or the other being dominant depending on localities (Fig. III and VI). Because these species were not tested together in all localities, it is however not possible to conclude in a better adequacy to beetles of one or the other of these two species in a particular locality or area.

#### CONCLUSIONS

Results from the trap tree experiment and those from the trap log experiment often completed each other, giving information on the geographic distribution of P. recurva and P. semipunctata in Tunisia, and allowing better understanding of between Eucalyptus - Phoracantha relationships. It appeared that both beetle species were present in all localities, the populations of P. recurva being by far the most abundant. Propositions have been made regarding interactions between population level, beetle mortality and larval intraspecific competition. However, no consistent results have been obtained regarding a possible effect of climate and tree species on the various insect parameters. This is certainly due mainly to a strong interaction between localities and Eucalyptus species. However, considering a lower number of bioclimatic areas (for example only the two extreme situations), a lower number of tree species, and a higher number of trees in each situation would possibly give more reliable results.

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