Insectes soc. 48 (2001) 57–62
0020-1812/01/010057-06 $ 1.50+0.20/0

Insectes Sociaux

Research article

Comparison of the spatial distribution and reproductive cycle of *Reticulitermes santonensis* Feytaud and *Reticulitermes lucifugus grassei* Clément (Isoptera, Rhinotermitidae) suggests that they represent introduced and native species, respectively

F. Vieau

Laboratoire de Biologie animale, Faculté des Sciences et des Techniques, 2 Rue de la Houssinière, BP 92208, F-44322 Nantes Cedex 3, France, e-mail: vieau.florent@wanadoo.fr

Received 3 August 1999; revised 4 August 2000, accepted 1 December 2000.

Summary. The termites *Reticulitermes santonensis (Rs)* and *R. lucifugus (Rl)* were first regarded as different species (Feytaud, 1924) and then as the same species (Buchli, 1958). In later studies, Clément (1978, 1986) and Bagnères et al. (1990) used morphological and chemical (cuticular hydrocarbons) criteria to show that they actually are two distinct species. Feytaud (1924) and Bagnères et al. (1990) suggested that *Rs* could have originated from a North American population of *R. flavipes* that was introduced into Europe. The present study examined the distribution and reproductive cycle of *Rs* and an *Rl* sup-species, *Rl grassei (Rlg)*, which are partly sympatric in southwest France. In the wild, *Rs* has an aggregated distribution related to human surroundings (past or present), i.e. populations are either dense or entirely absent. Conversely, *Rlg* is distributed randomly (from dense to few) throughout its range. For example, *Rs* is not found in towns within the Landes Forest, an area in southwest France that remained economically depressed and sparsely settled until after 1850 (Sargos, 1997), while *Rlg* is quite common there. Their respective reproductive strategies show that *Rs* populations spread through budding (swarms are scarce and many brachypterous nymphs and neotenics are found), whereas *Rlg* populations reproduce by both swarming and budding. It is likely that *Rs* is an introduced species and *Rlg* a native one that has reached its northern limits in France. A closer examination of North American *Reticulitermes* species, particularly *R. flavipes*, might reveal a relationship with the European species.

Key words: Reticulitermes, France, spatial distribution, reproductive cycle, neotenics.

Introduction

Reticulitermes santonensis Feytaud (*Rs)* and *R. lucifugus grassei (Rlg)* have established colonies in forests that were planted along the French Atlantic coast in the second half of the 19th century to stabilize sand dunes. Since the middle of the 19th century, when termite problems became apparent in France, scientists wondered whether *Rs* was an introduced species and *R. lucifugus* Rossi *(Rl)* an indigenous one. As early as the 1840s, some authors noted that *Rs* was especially urban, while *Rl* was more widely distributed (de Quatrefages, 1843; Lespès, 1856). De Quatrefages (1843) mentioned a termite invasion at the end of the 18th century that seemed to originate around the harbor of Rochefort along the southwestern Atlantic coast and quickly spread over commercial routes to urban areas in adjoining districts. Latreille (1804) had already described wild termite populations in the Landes Forest, which at that times was not highly developed (Sargos, 1997). Given the morphological similarities between *Rs* and *Rl*, it was not until Feytaud (1924) suggested that *Rs* was probably *Reticulitermes flavipes* Kollar that the two were identified as separate species. Buchli (1958), however, questioned this dichotomy and suggested that *Rs* was a northern variety of *Rl*, limited to a single French administrative district (Charente-Maritime; see Fig. 1) bordering the Atlantic Ocean. Although Clément (1986) recorded a similar distribution for *Rs* in southwestern France, his study used morphological and chemical criteria (analysis of cuticular hydrocarbons) to show that *Rs* and *Rl* were distinct species. He divided *Rl* into several sub-species, including *grassei* (southwest, Landes Forest), *banyulensis, lucifugus* (southern and southeastern France, respectively) and *corcisus* (Corsica). Even though populations described as the North American species *R. flavipes* are known to have been introduced into

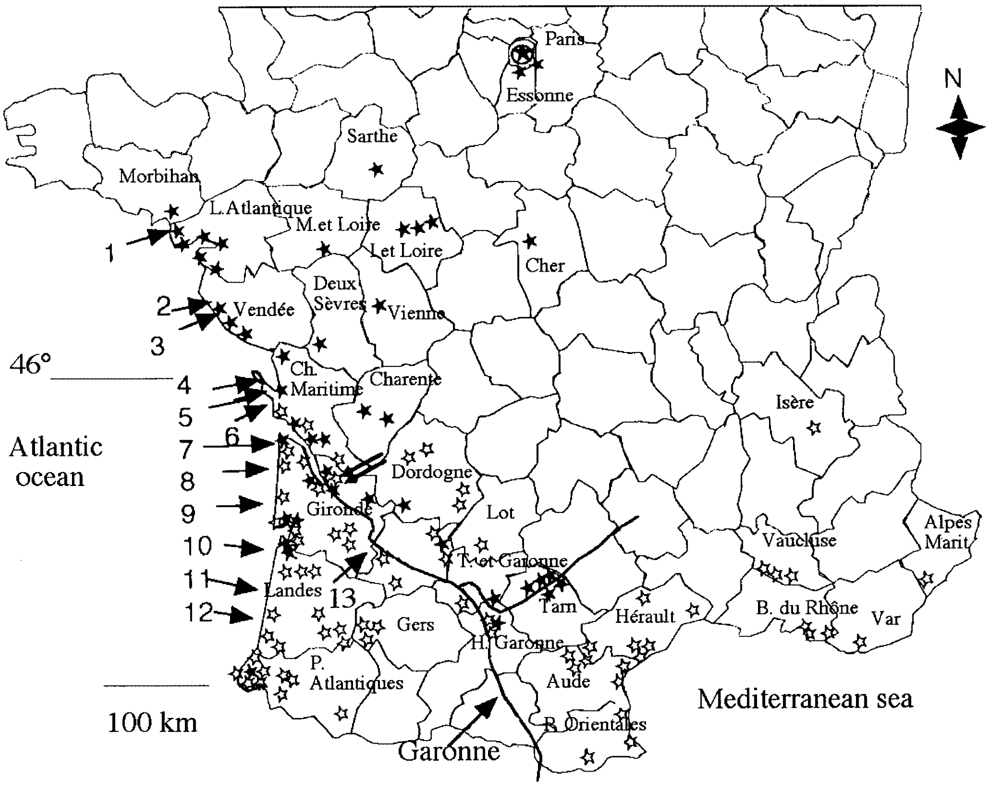

Figure 1. Map of the administrative districts of France showing the distribution of the two species in urban conditions. *R. santonensis* (solid stars; n = 45) and *R. lucifugus grassei* (as a whole) (open stars; n = 61) in administrative districts known to be infested with termites; numbered arrows: sampled forest locations (see: Materials and methods); double arrow: city of Bordeaux.

Europe (Kollar, 1837), the problem of the introduced or indigenous status of *Rs* and *Rl* remains unresolved (Bagnères et al., 1990).

The present study explored the problem of status in terms of the spatial distribution and reproductive strategies of the two species in France. Spatial distributions for introduced species would appear to be limited and rely on human distribution, since most do not migrate far from the introduction site (Gay, 1969). Moreover, Rhinotermitidae introduced into a less favorable climate are known to reproduce mainly through the formation of secondary reproductives (neotenics) rather than by primary reproductives (alates) (Esenther, 1969; Lenz and Barrett, 1982). The reproductive strategies of *Rs* and *Rl* have only been studied under laboratory conditions – by Bulchi in 1958 and more recently by Noirot (1985), who accepted Bulchi's synonymy of the two species. The most recent study of subterranean termite distributions in France was conducted over 20 years ago (Clément, 1977). Because of this and since no field populations were used in the reproductive studies, we decided to investigate the current spatial distribution and reproductive strategies of field populations of *Rs* and *Rlg* in France.

Materials and methods

Territorial distribution in wild and urban habitats

Termite distribution along the west coast of France, a region previously studied by Clément (1977) and Vieau (1993), has been classified into 3 categories (Clément, 1977):

- northern forests where only *Rs* is found: La Baule, St. Jean de Monts, Olonne, and Oléron Island (Saumonard Forest) (Fig. 1, numbers 1–4, respectively);
- mid-Atlantic coastal forests where both species are found: La Coubre and Combots d'Ansoine (Fig. 1, numbers 5 and 6, respectively);
- southern forests in which only *Rlg* is found: Hourtin, Carcans, Lacanau, La Teste, Mimizan, Vielle St. Girons, and Bazadais (Fig. 1, numbers 7–13, respectively.

Termites were sampled from maritime pine forests (mainly *Pinus pinaster* plantations) using 100 × 100-m plots determined according to known areas (Clément, 1977; Vieau, 1993) and transections perpendicular to the coast. All stumps and branches within a given plot were considered potential feeding sites for subterranean termites. Twenty stumps or branches at least 10 cm in diameter were randomly selected for sampling throughout each plot. The wood was cleft with an axe, and the termites were collected in flasks containing 70° alcohol. Samples analyzed in the laboratory included some soldiers. A mandibular morphological criterion (Vieau, 1993), in accordance with analysis by cuticular hydrocarbons (Clément, personal communication), allowed us to distinguish between the two species. When infested pieces of wood were found at the edge of a plot, this point was considered the center of a new plot. A contamination coefficient (C) was attributed to each plot according to the number of termite-infested samples found: C = 0, no termites; C = 1, one to four infested samples; C = 2, five to nine infested samples; and C = 3, ten to twenty infested samples. The mean of C (m) compared to its variance (v) enabled us to classify allopatric and sympatric colonies into three categories according to Frontier and Pichod-Viale (1991): $v > m$ (over-dispersion), $v = m$ (random dispersion), and $v < m$ (under-dispersion).

In urban areas, the distribution of both species (*Rl* species as a whole) was studied (without determining C) from 118 samples collected in all French districts known to harbor termites, as indicated by the map of the "Centre Technique du Bois et de l'Ameublement" (= Technical Center for Wood and Furniture). This organization conducts periodic censuses of infested urban areas in France, but does not differentiate between *Rs* and *Rl*. Companies specialized in termite control used a bait system (Senticron) to collect the termites. The termite samples with soldiers were then sent to the laboratory in 70° alcohol.

Reproductive strategies

The post-embryonic stages in Rhinotermitidae diverge along two different pathways after two larval stages: the neuter or worker line and the sexual or nymphal line. The later starts at post-embryonic stage 3 (N3), which is considered to be the first nymphal stage (Noirot, 1985). The succession of stages was established in the nymphal line by measuring the head-capsule width and the length of the third tibia on wingbud-bearing nymphs (*Rs*, n = 193 and *Rlg*, n = 132) collected from different plots sampled for an entire year. The same measurements were taken on secondary reproductives of the nymphal line (brachypterous neotenics) from different sampling sites.

Results

Spatial distribution

None of the sampled plots showed a mixture of the two species in the wild. Table 1 indicates that all but one plot of the *Rs* samples had a contamination coefficient (C) of 3, that one plot had a score of C = 1. This area, which had been identified by Clément (personal communication) as highly exploited, contained many colonizable stumps, although only one small colony in poor condition was actually found. All four coefficients applied to *Rlg* colonies.

For *Rs* in allopatric conditions (Table 1A), 39 plots had C = 0 and 13 had C = 3. The mean of C for the 52 plots was 0.75 (v = 1.721). The mean was 0.74 (v = 0.849) for *Rlg* in allopatric conditions (Table 1C; 50 plots). In the sympatric area (Table 1B; 65 plots), *RS* had a mean of 0.292 (v = 0.773), while *Rlg* had 1.323 (v = 1.378).

As the mean of C for *Rs* was low compared to the variance, the distribution showed over-dispersion. In contrast, the mean of C for *Rlg* was near the variance so that the distribution was close to random dispersion.

Rs sites were peripheral to a forested habitat and adjacent to inhabited areas, with a maximal spread into the forest of about 2 hectares (a few hundred meters from urbanized areas along a 200-m front). *Rs* forest sites were limited to areas characterized by present or past human activity (e.g. homes of foresters). The sites classified by Clément (1977) in the Coubre Forest (5, Fig. 1) correspond to the exact location of a railway terminal that has now disappeared (document of the Office National des Forêts = National Forestry Bureau). Similarly, a plot in the Combots d'Ansoine Forest (6, Fig. 1) was identified by old residents as a village where a sawmill had been located some decades before.

In urban areas, no districts to the north of Charente-Maritime (Fig. 1) were found to be infested with *Rlg*. All infestations (21 towns) were due to *Rs*. *Rs* infestations also occurred in more southern towns on the eastern side of the Garonne River (n = 11) and along its tributary, the Tarn. Towns on the western side of the river were infested with *Rs* only along the Atlantic coast (n = 7) peripheral to the Landes Forest, notably around the Bay of Arcachon (Fig. 1, between arrows 9 and 10). Conversely, 34 towns and villages in this forest (between the Garonne River and the Atlantic Ocean) were infested with *Rlg*. The city of Bordeaux (see map, Fig. 1), situated to the west at the upper limits of the Garonne estuary, showed a mix of both species. *Rs* was not found in southern towns around the Mediterranean Sea (22 samples).

Table 1. Forestry samples from north to south (for location, see arrows Fig. 1): total sampled plots (Ts p) equals total sampled plots in each forest; C values = coefficient (see text) measuring the number of contaminated pieces of wood in each plot; species: *Rs = R. santonensis: Rlg = R. lucifugus grassei.* T1: total results in forests (A) where only *Rs* was found; T2: total results with the two termite species in sympatric conditions (B); T3: total results in forests (C) where only *Rlg* was found.

Sampled forests		Ts p	C = 0	*Sp*	C = 1	C = 2	C = 3
A	La Baule	6	4	*Rs*	0	0	2
	St Jean de Monts	6	5	*Rs*	0	0	1
	Olonne	15	12	*Rs*	0	0	3
	Saumonards (Oléron)	25	18	*Rs*	0	0	7
T1		**52**	**39**	***Rs***	**0**	**0**	**13**
B	La Coubre	32	9	*Rs+Rlg*	1+5	0+10	5+2
	Combots d'Ansoine	33	7	*Rs+Rlg*	0+7	0+6	1+12
T2		**65**	**16**	***Rs+Rlg***	**1+12**	**0+16**	**1+12**
C	Hourtin	4	2	*Rlg*	1	1	0
	Carcans	12	7	*Rlg*	4	0	1
	Lacanau	10	5	*Rlg*	4	1	0
	La Teste	3	2	*Rlg*	1	0	0
	Mimizan	10	5	*Rlg*	4	0	1
	Vielle St Girons	6	0	*Rlg*	2	2	2
	Landes Bazadais	5	4	*Rlg*	1	0	0
T3		**50**	**25**	***Rlg***	**17**	**4**	**4**

Reproductive cycles: nymphal stages (Fig. 2 A, B)

The *Rlg* nymphal line (Fig. 2A) included 5 stages of brachypterous nymphs with short wing buds jutting slightly out from the thorax and one stage with long wing buds (lwbn) up to the middle of the abdomen. The latter preceded the imaginal molt or the alate swarming stage. The first four brachypterous stages formed a sequence from immature stage 3 (N3; the first nymphs) to stage 6 (N6). A dichotomy appeared in nymphal development subsequent to the N6 stage. In the wild, N6 populations appear in August and develop into either an additional brachypterous stage or the lwbn stage. The lwbn continue to develop into alates (primary reproductives) during the winter and spring, and the additional brachypterous stage develops into brachypterous neotenics (secondary reproductives; designated her as bneo). Thus, the additional brachypterous stage was considered to be a deviant pre-neotenic brachypterous stage (designated as pbneo) and not the N7 stage established by Buchli (1958), which in fact would correspond to lwbn.

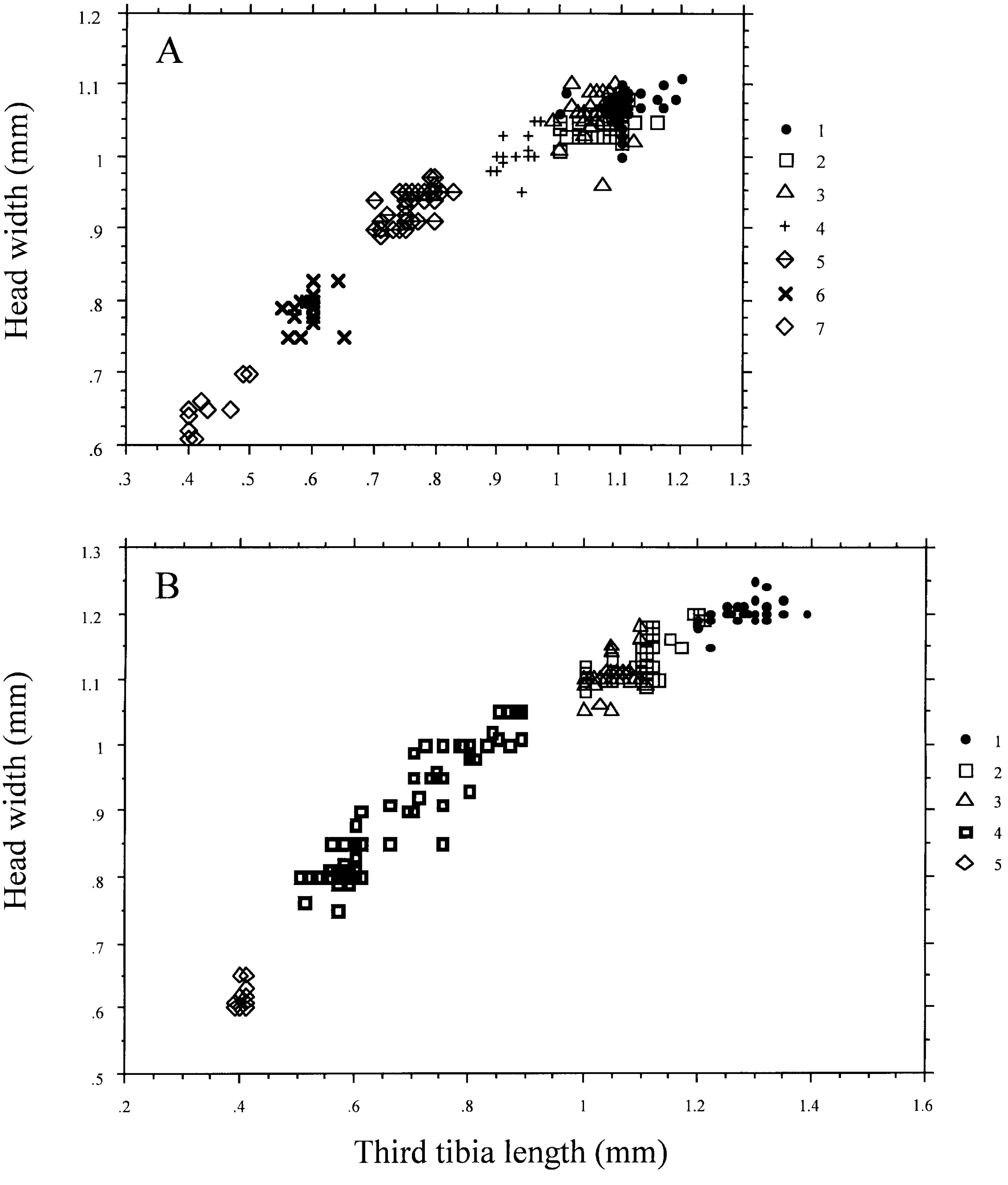

Figure 2. **A** Stages of the *R. lucifugus grassei* nymphal line showing the origin of secondary reproductives (brachypterous neotenics): 1. brachypterous neotenic (bneo); 2. prebrachypterous neotenic (pbneo); 3. nymphal stage 7 (nymphs with long wing buds: lwbn); 4 to 7. nymphal stages 6, 5, 4, 3. **B** *R. santonensis* nymphal line stages. For 1, 2, 3: see Fig. 2 A; 4: 4th, 5th, 6th nymphal stages as a whole; 5. nymphal stage 3.

Table 2. Brachypterous neotenics: *R. santonensis* (n = 40) and *R. lucifugus grassei* (n = 33). Comparison of the mean of the 3 measures, head width (HW), prothorax width (Pth W), tibia 3 length (T3 L). The difference is significant ($p < 0.0001$) for each pair of parameters.

	HW	Pth W	T3 L
Rs	1.203 ± 0.016	1.221 ± 0.050	1.289 ± 0.040
Rlg	1.068 ± 0.023	0.976 ± 0.093	1.107 ± 0.042
t	29.709	14.46	18.964

N3 stages were distinguishable in *Rs* (Fig. 2B), whereas N4, 5, and 6 stages could not be differentiated. The other stages of *Rs* were similar to those of *Rlg*, although pbneo, which was differentiated about one month earlier, descended from the oldest nymphs of the N4–6 stages, which have a third tibia ranging from 0.8–0.9 mm in length.

A comparison of the two nymphal lines showed that N3–N6 measurements did not differ (e.g. tibia measurements ranged from 0.6 to 1.1 mm), whereas *Rs* bneo were larger than *Rlg* bneo (Table 2) and thus easily distinguished (Fig. 2B).

Expected percentage of alates one month before swarming

Pbneo and lwbn gather near the top of stumps one to two months before swarming (Vieau, 1994). As noted above, pbneo molt into brachypterous neotenics (bneo) and lwbn into alates (Al) while at these locations. One hundred insects in the pbneo+bneo+lwbn+Al population (after removal of workers and soldiers) were collected from the stumps prior to the alate flight season. This allowed us to calculate the expected percentage of alates (%Al) produced per colony in relation to the total reproductive potential (primary + secondary) at the end of the nymphal line. The samples were statistically representative of the whole population (Vieau, 1994). This technique appears to show the total reproductive potential of these colonies, as apterous neotenics obtained with workers under experimental conditions (Buchli, 1958) were not found in the field. The average potential alate percentage among *Rs* (22.88 ± 22.83) (Table 3) was low in comparison to *Rlg* (81.53 ± 17.85) (Table 3). The standard deviations show the variability among our *Rs* estimates, which was indicative of the physical location of the stump (sunny or shaded exposure). Conversely, *Rlg* estimates were more consistent ($t = 8.751$; $p < 0.0001$) and less subject to environmental influences.

Discussion

The spatial distribution and the reproductive cycle of the two termite species were different. *Rs* showed a limited spatial distribution that included many colonies very close to one another and a reproductive cycle that produced many neotenics. Comparison with other known characteristics of introduced Rhinotermitidae (Gay, 1969; Lenz and Barrett, 1982) suggests that this species might be an introduced one. Unlike *Rlg* distributions, *Rs* populations are related to habitats affected by human activities, are uncommon in the wild, and do not display agonism (Clément, 1986). These conditions seem to be attributable to the low alate potential, the great distance between *Rs* populations reported here, and the imbalance of the alate sex ratio in favor of the male (Vieau, 1996). These factors would be conductive to an inefficient scattering of the alate flight and an absence of outbreeding.

The distribution of *Rs* in southwestern France appears to be related to urban development. The species is found on both sides of the Garonne River and its tributary, the Tarn, where urban development is greatest. The absence of *Rs* from the region southwest of the Garonne to the coast within the Landes Forest may be related to past economic conditions. The Landes Forest was developed during the 1850s and, except in the Arcachon basin (Sargos, 1997) where many *Rs* colonies were found, was slowly opened to economic activi-

Table 3. Expected percentages of primary reproductives (alates) (%Al) at the swarming time in comparison with the total expected reproductives (primary + secondary), in *R. santonensis (Rs)* (n = 31 colonies) and *R. lucifugus grassei (Rlg)* (n = 23 colonies); sampled forests (for location see Fig. 1); S.c.: numbers of sampled colonies in each forest; %Al: potential alates (%) in each sampled wood; T.S.c.: total of sampled colonies in each species; Al aver.%: Average % alates.

Species	*Rs*					*Rlg*		
Forests	**1**	**2, 3**	**4**	**5**	**5.6**	**5.6**	**7, 8, 9**	**11, 12, 13**
S.c.	**7**	**3**	**7**	**8**	**6**	**7**	**8**	**8**
% Al	5.9	14.32	10.19	3.09	0	65.59	99.21	89.82
	11.54	27.94	11.86	68.72	3.05	84.73	40.68	43.8
	9.64	2.13	28.91	4.74	6.06	89.28	90.47	90
	71.43		24.29	0	10	100	90	92.59
	62.26		9.38	8.54	12	100	94.64	95
	73.86		29	17.53	32.14	62.12	89.82	75.82
	14.04		26	47.48		79.86	89.8	86.54
				63.31			96.15	60.25
T.S.c.			**31**				**23**	
Al aver.%			**22.88 ± 22.83 %**				**81.53 ± 17.84 %**	

ty: *Rs* is also not found along the Mediterranean coast, perhaps because budding in the soil is more difficult in this dry climate (Clément, 1986).

Rlg appears to be limited to urban areas as well as wild habitats south of the latitude of Oléron Island (arrow number 4, Fig. 1), whereas *Rs* is found along the Atlantic coast north of Bordeaux and widespread in urban areas north of Oléron Island. It would be interesting to study areas occupied by *Rs* in the wild, notably the Coubre Forest which no longer shows any traces of human activity. The infestation there probably originated in the 1870s, a period coinciding with the planting of the forest (Vasselot de Regné, 1878) and the arrival of the railway (now gone). That *Rs* population has not spread since it was originally discovered more then 20 years ago by Clément (1977). It might be supposed that this infestation site would be more heavily contaminated, compared to the first census (in 1977), because of the reproductive cycle proposed for this region (through budding rather than alate flights). It is possible that the spread of *Rs* is limited by ecological or genetic factors.

It is difficult to differentiate nymphal line stages in *Rs*, possibly because of heterochronia. It has been shown that the post-embryonic development of ovaries and testicles is more advanced in pbneo than in lwbn (Vieau, 1994). This deviant developmental pathway may have resulted from inadequate climatic conditions, leading to high neoteny and a corresponding probability that budding would be the only viable reproductive strategy. Conversely, *Rlg* produces far more alates (close to 80% potential alates), and fewer and smaller neotenics than *Rs*. Although Feytaud (1955) collected 21 physogastric *Rlg* (primary reproductives), there have been no similar reports for *Rs*. Therefore, the suggestion by Buchli (1958) that alates are not involved in the propagation of *Reticulitermes* under natural conditions in France is questionable. Actually, this author grouped all French *Reticulitermes* together, whereas our data suggest that *Rlg* propagation involves alates. Likewise, the supposition of Clément (1977) that *Rs* nymphs spend the winter with short wing buds and *Rlg* nymphs with long wing buds may be incorrect. This discrepancy with our data may be due to the small sample used by Clément (1977).

In conclusion, the hypothesis of an introduced origin for *Rs* cannot be ruled out. The data presented here and in more recent reports (Clément, 1977, 1978; Bagnères et al., 1990; Vieau, 1993), as well as in the much earlier reports by de Quatrefages (1843), indicate that a termite invasion occurred in southwestern French port cities. Nevertheless, further research is needed to verify the introduced or native status of *Rs*, with a specific comparison to *R. flavipes* regarding morphometric features, chemotaxonomic traits, and genetic analysis.

References

Bagnères, A.G., J.L. Clément, M.S. Blum, R.F. Severson, C. Joulie and C. Lange, 1990. Cuticular hydrocarbons and defensive compounds of *Reticulitermes flavipes* (Kollar) and *R. santonensis*: Polymorphism and chemotaxonomy. *J. Chem. Ecol. 16:* 3213–3224.

Buchli, H.H., 1958. L'origine des castes et les potentialités ontogénétiques des termites européens du genre *Reticulitermes. Ann. Sci. Nat. Zool. Biol. Animale 20:* 263–429.

Clément, J.L., 1977. Ecologie des *Reticulitermes* (Holmgren) français (Isoptères). Position systématique des populations. *Bull. Soc. Zool. Fr. 102:* 169–185.

Clément, J.L., 1978. Nouveaux critères taxonomiques dans le genre *Reticulitermes* (Holmgren) (Isoptera). Description de nouveaux taxons français. *Annls Soc. Ent. Fr. (N.S.) 14:* 131–139.

Clément, J.L. 1986. Open and closed societies in *Reticulitermes* termites (Isoptera, Rhinotermitidae). Geographical and seasonal variation. *Sociobiology 11:* 311–323.

Esenther, G.R. 1969. Termites in Wisconsin. *Ann. ent. Soc. Am. 62:* 1274–1284.

Feytaud, J., 1924. Le Termite de Saintonge. *C.R. Acad. Sci. 171:* 203–205.

Feytaud, J., 1955. L'extension du péril des termites en France. *Naturalia 19:* 32–38.

Frontier, S. et D. Pichod-Viale, 1991. *Ecosystèmes: Structures, Fonctionnement, Evolution.* Masson, Paris. 392 pp.

Gay, F.J., 1969. Species introduced by man. In: *Biology of Termites* (K. Krishna and F.M. Weesner, Eds.) Vol. 1, 459–494.

Kollar, V., 1837. Naturgeschichte der Schädlichen Insekten. *Verh. Landw. Gesl. Wien, n.s., 5:* 411 (*Termes flavipes*).

Latreille, L.P., 1804. Dictionnaire d'histoire naturelle (Déterville), t. XXII: p. 49.

Lespès, Ch., 1856. Recherches sur l'organisation et les moeurs du termite lucifuge. *Annls. Sci. Zool. 5:* 227–282.

Lenz, M. and R.A. Barrett, 1982. Neotenic formation in field colonies of *Coptotermes lacteus* (Frogatt) in Australia, with comments on the role of neotenics in the genus *Coptotermes* (Isoptera Rhinotermitidae). *Sociobiology 7:* 47–59.

Noirot, C., 1985. Pathways of caste development in the lower termites. In: *Caste Differentiation in Social Insects* (J.A.L. Watson, B.M. Okot-Kotber and C. Noirot, Eds.), Pergamon Press, Oxford, Vol. 1, pp. 41–57.

Quatrefages, A. de, 1843. Note sur les termites de La Rochelle. *Ann Sc. Zool., 30:* 16.

Sargos J., 1997. Histoire de la forêt landaise. Du désert à l'age d'or. L'Horizon chimérique, Bordeaux, Vol. 1, 559 pp.

Vasselot de Régné, M., 1878. Notice sur les dunes de La Coubre (Charente inférieure), B.N., Vol. 1, Paris, 78 pp.

Vieau, F., 1993. Le termite de Saintonge *Reticulitermes santonensis* Feytaud: Termite urbain. *Soc. Zool. Fr. 118:* 125–133.

Vieau, F., 1994. Les stades nymphaux 7 et 8 chez *Reticulitermes santonensis* Feytaud (Isoptera, Rhinotermitidae). *Actes Coll. Insectes Soc. 9:* 61–66.

Vieau, F., 1996. Les variations saisonnières des castes chez le termite *Reticulitermes santonensis* Feytaud (Isoptera: Rhinotermitidae) dans un biotope de l'ouest de la France. *Ann. Soc. Entomol. Fr. (N.S.) 32:* 207–216.