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A Population Survey of the Italian Subterranean Termite *Reticulitermes lucifugus lucifugus* Rossi in Bagnacavallo (Ravenna, Italy), Using the Triple Mark Recapture Technique (TMR)

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ABSTRACT—The present paper presents an application of the Triple Mark Recapture technique on two Italian populations of the subterranean termite (*Reticulitermes lucifugus*). Two large infested areas in the historical center of the town Bagnacavallo (Ravenna) were chosen. Two dye markers were utilized, Nile Blue A or Neutral Red respectively for the two sites. The first population was estimated at 1,085,000 ± 92,000 insects, foraging on a surface of 1500 m^2; the second, smaller, population has been estimated to be 640,000 ± 33,000 insects, foraging on an area of 675 m^2. Maximum linear distance observed in both sites was 45-50 m. Population dynamics look different in the two sites: in the first site the spread distribution with a few insects at each feeding point probably indicates an old, well established, population which grows slowly in all directions during the hot season. In the second site the expansion was much more rapid and it is possible to hypothesise that the termite colony settled in this area more recently and that it is a relatively young, growing colony. The gathered data appear of great utility in extending the knowledge about biology, ecology and behaviour of the Italian subterranean termite.

INTRODUCTION

The subspecies *R. lucifugus lucifugus* (Clément *et al*., 1982) belongs to one of the 2 Italian species of termites (other is *Kalotermes flavicollis*). This termite lives in subterranean environment and is destructive for wood structures in urban environment, causing serious damages to historical buildings all over Italy. Despite its economical importance little is known about its ecology, behaviour and populations size.

We were interested in the case of urban infestation in Bagnacavallo because it is the only example to date in Italy of systematic infestation extending over an entire old town (Campadelli, 1987, 1988; Lozzia, 1990; Marini and Ferrari, 1993).

Bagnacavallo is a lowland town of about 10,000 inhabitants. It lies between Bologna and Ravenna in the Po river basin.

For the last ten years the Italian termite *R. lucifugus lucifugus* has been recorded in large numbers in the old part of the town. The termites caused considerable damage to historical buildings of architectural and artistic interest.

This is the second report of infestation by Italian subterranean termites in the Emilia Romagna region, the first was reported in Salsomaggiore in the province of Parma (Springhetti, 1965).

In Bagnacavallo, termites attacked several parts of the old town. The infested portion encompassed an area of ca. 800 m in diameter and is made up of two-three stored buildings, built between the 17th and the 20th century. The buildings are joined, being aligned along the roads and are provided with contiguous internal gardens.

Many aspects of the buildings make them favourable sites for the development of termite colonies: unplastered brickwork; crumbling mortar; rising damp; structural wood in the walls and ground; floors directly laying on the ground. The buildings have walls and foundations in common and can in no way be isolated. This easily enables the termites to move from one building to another making it difficult to carry out control in a single building.

The absence of any systematic study of the infestation has meant that, while the termite problem was fought sporadically, there has been no effective solution. Therefore we set up a study in Bagnacavallo with the aim of understanding the actual area infested by termites and possibly exploring suitable methods of control.

The study started with a careful monitoring of the area using wood stakes which were placed within the area where

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termite attacks on buildings had been reported, and in their surroundings. We were thus able to draw up a map of the zone where termites were present. The zone turned out to be almost circular, some 400 m in diameter (Ferrari *et al*., 1996) mainly located in the North West part of the old town.

In order to obtain data of *R. lucifugus lucifugus* in Bagnacavallo-population size, and foraging territory; capacity for movement-we adopted and modified the Triple Mark Recapture Technique (TMR), developed by Su and Scheffrahn (1986, 1988).

Given that the infested area is circular in shape we hypothesised that the centre was the point of origin of the infestation and we decided to conduct TMR in two separate areas, one in the centre and the other on the periphery, in order to relate the results obtained to the geographical position.

The first site was the Monastery and the church of S. Giovanni, the internal cloisters and some of the nearby buildings. (Monastery site). The second site was an area of private houses and public roads (Via Fiera site) on the edge of the infested area. The two areas were about 200 m apart.

The study began in March and ended in September 1995.

We obtained full co-operation from the residents who allowed access to the buildings and did not interfered with the monitoring equipment.

MATERIALS AND METHODS

Between the end of March and the beginning of April 1995, 64 stakes were placed at the Monastery site and 68 at the Via Fiera site. These were used in order to detect and locate termite foraging.

Only in the garden of the Monastery we found suitable conditions for placing the stakes in a grid pattern.

The stakes were checked one month later, in May. Termite feeding activity was found in 10 stakes at the Via Fiera site and 11 stakes in the Monastery site. Each attacked stake was then replaced by a monitoring station, similar to that described by Su and Scheffrahn (1986)-soft wood (*Picea* sp.) block surrounded by an underground plastic collar and covered by soil. The blocks were made of wooden boards separated by 2 mm wood sticks and nailed together to form a solid structure ($12 \times 10 \times 8$ cm).

All stations were checked one month later and for each site the most active station was selected to collect termites. The infested block from this monitoring station only was collected and replaced with a new block. Then infested blocks were individually placed in a sealed bucket in order to avoid exposure to direct sunlight or high temperatures.

In the laboratory the blocks were opened and termites separated from the debris using the method developed by Tamashiro *et al*. (1973). The mean body weight of the termite workers from each station was determined by weighing 5 groups of 20 individuals and the number of workers collected was determined by dividing the total weight by the mean weight of a worker.

To test whether there was a connection between the two sites, termites were marked by forced feeding with coloured paper. We used two different vital colours. Based on the results of a previous study on marking techniques on *R. lucifugus* we decided to mark termites with Nile Blue A and Neutral Red. Both dyes were reported suitable for staining termites by Su *et al*. (1991). For termites from Via Fiera we used Whatman filter paper with Nile Blue A 0.2% wt/wt, and for termites from the Monastery site we used a similar procedure with a solution of Neutral Red, with the same concentration: 0.2% wt/wt.

The termites were placed in staining units at a temperature of 27-28°C, in high humidity and complete darkness for 5 days.

After dying, termites were visibly blue-green for Nile Blue A and pinkish-red for Neutral Red. The weighing procedure described above was again used to determine the final number of termites released.

We released the marked termites into the monitoring station from which they were originally collected, and one week after release we collected blocks from all monitoring stations under the study which had been attacked. These were replaced with new wood blocks. All stakes were checked.

In the laboratory the wood blocks were opened and the termites separated from the debris. The termites were then weighed and their number calculated. Each station was processed separately. The number of marked termites collected at each monitoring station was calculated. Each station containing marked termites was considered interconnected with the original releasing site.

The termites collected from each monitoring station containing marked termites were then marked for the second cycle. Termites from each station were processed separately.

The marked termites were then released for a second time into the monitoring station from which they had been collected.

The same procedure was adopted for a third cycle which we repeated two weeks later in both sites (Tables 1 and 2).

The total foraging population size (N) and the associated standard error (SE) were estimated using a weighted mean model (Begon, 1979):

$$N = (\sum M_i \times n_i)/[(\sum m_i) + 1]$$

$$SE = N/\{[1/(\sum m_i + 1)] + [2/(\sum m_i + 1)^2] + [6/(\sum m_i + 1)^3]\}^{1/2}$$

where for each ith cycle n_i is the number of captured termites, m_i is the number of marked individuals among captured termites, and M_i is the total number of marked individuals up to the ith cycle.

We decided to continue to follow the sites after the third recapture until September. At the end of the summer we carried a further cycle of marking release and recapture. This was a purely qualitative survey to see whether there had been any variation in the size of the foraging territory. The territory was taken as being the area with interconnected monitoring stations, which was defined by the presence of marked individuals (Su and Scheffrahn, 1986).

RESULTS

The data gathered at the two sites during the three cycles of marking, release and recapture is given in Tables 1 and 2, and were used to calculate the number of foraging termites (N) and standard error (SE).

At the end of each cycle the size of the foraging territory was calculated. Figures 1 and 2 show the increase of the range. Elaboration of the data enabled us to show that the foraging territories of the two termite colonies spreaded outward radially over a short period of time.

From the number of foraging termites and the size of the foraging territory after the third recapture we were able to calculate the density (n° termites/m^2) in the two sites in the study. The results were similar (989 and 702 for Fiera and Monastery respectively, Table 3).

The maximum linear movement of the marked termites in ca. one month was taken as the linear distance between the two most distant interconnected stations (Table 3). Termites marked with Nile Blue A were never found in the same station as termites marked with Neutral Red.

Table 1. Numbers of collected termites (workers) calculated from mean body weight determined for each wood block in each phase, during Triple Mark Recapture program in Via Fiera site–Bagnacavallo (Italy)

TMR phase	Date 1995	Monitoring station n°	Marked released	Collected	Marked recaptured
1st Release	29 May	5	8984		
1st Recapture	5 June	1		836	7
		2		3851	47
		5		1092	188
2nd Release	12 June	2 (5-1 dead)	3844		
2nd Recapture	26 June	1		240	6
		2		1389	27
		3		4570	32
		5		1272	21
3rd Release	3 July	2	1295		
		3	3841		
		5	1200		
3rd Recapture	17 July	1		86	1
		2		82	1
		3		19	0
		4		3148	16
		5		1373	17
		7 (1 dead)		20	4
4th Release (n°not determined)	21 July	1-2-3-4-5-7	released		
4th Recapture (n°not determined)	14 Sept.	1		active	1
		2		active	2
		3		not active	0
		4		not active	0
		5		active	2
		6		active	1
		7		not active	0
		9		active	1
		10		active	4
		1 stake		active	1

CONCLUSIONS

1) The number of foraging termites, was estimated using the method of marking and recapture on the Italian colonies of *R. lucifugus lucifugus*, at a maximum of a million individuals at the Monastery site. Data also showed that the Bagnacavallo termites form colonies which extend for several hundreds of square metres.

The magnitude of the *R. lucifugus* population from our results falls within the ranges recorded for other *Reticulitermes* in urban residential areas: *R. flavipes* in Florida (0.1-2.8 million; max. linear distance 70 m) (Su *et al*., 1993) and in Toronto (0.7-0.9 million; max. linear distance 46 m) (Grace, 1990), *R. santonensis* in Paris (1.2 million; max. linear distance 65 m) (Paulmier *et al*., 1997). Similar values are reported for *Coptotermes gestroi* in Bangkok (1.1-2.7 million) (Sornnuwat *et al*., 1996) and higher values are reported for *Coptotermes formosanus* (1.4-6.8 million; max. linear distance 115 m) (Su and Scheffrahn, 1988).

Our previous observations showed that foraging territory of *R. lucifugus* colonies expanded quickly in the hot season and reduced in size during winter.

2) The potential for movement of the termites, as observed in the study, is considerable: the maximum linear foraging distance observed was 50 m and termites appear to move easily under gardens, buildings and paved roads.

Given the fact that blue and red individuals which were from different colonies, were never found at the same station we hypothesised that the distance travelled over a period of two months must, in any case, be lower than the 200 m, the distance between the two sites (Via Fiera and Monastery) or, likely, the two colonies are not interconnected.

3) The two sites showed different dynamics of expansion.

In the Monastery site the population was, at first, limited to the internal cloister area (I-II recapture) and only after the

Table 2. Numbers of collected termites (workers) calculated from mean body weight determined for each wood block in each phase, during Triple Mark Recapture program in Monastery site–Bagnacavallo (Italy)

TMR phase	Date 1995	Monitoring station n°	Marked released	Collected	Marked recaptured
1st Release	12 June	10	602		
1st Recapture	19 June	10		3039	19
		11		3298	1
2nd Release	26 June	10	2758		
		11	2605		
2nd Recapture	3 July	10		1219	74
		11		1959	23
3rd Release	10 July	10	985		
		11	1705		
3rd Recapture	24 July	2		1434	2
		3		6441	6
		4		550	1
		9		2964	2
		10		1157	1
		11		2821	12
4th Release (n° not determined)	28 July	2-3-4 9-10-11	released		
4th Recapture (n° not determined)	15 Sept.	2		active	1
		3		active	1
		4		active	1
		7		active	1
		8		active	3
		9		active	2
		10		active	1
		11		active	2
		1 stake		active	2

Table 3. TMR results. Foraging populations, territory areas, density of individuals, linear foraging distances and mean worker body weight in the study sites at Bagnacavallo.

Site	Via Fiera	Monastery
Foraging population		
3rd recapture	638,600	1,083,892
Standard error	± 33,380	± 91,809
Foraging territory		
3rd recapture	675 m^2	1525 m^2
(4th recapture after 50 days)	(2125 m^2)	(1950 m^2)
Termite density	989 termite/m^2	702 termite/m^2
Max linear distance	50 m	45 m
Mean worker body weight	2.742 mg	2.789 mg

III recapture a wider extension of it was observed.

This trend is probably due to the fact that at the beginning of the TMR programme many of the monitoring stations were active but the number of termites was low: only a few hundreds, so that only a small number of marked termites were released during the first cycle and that the probability of finding these back in a nearby station was lower.

The spread distribution with a few insects at each feeding point probably indicates an old, well established, population which grows slowly in all directions during the hot season.

At the Via Fiera site, on the other hand, large numbers of termites were observed at some foraging points with a peak of 10,000 termites concentrated in one station in May.

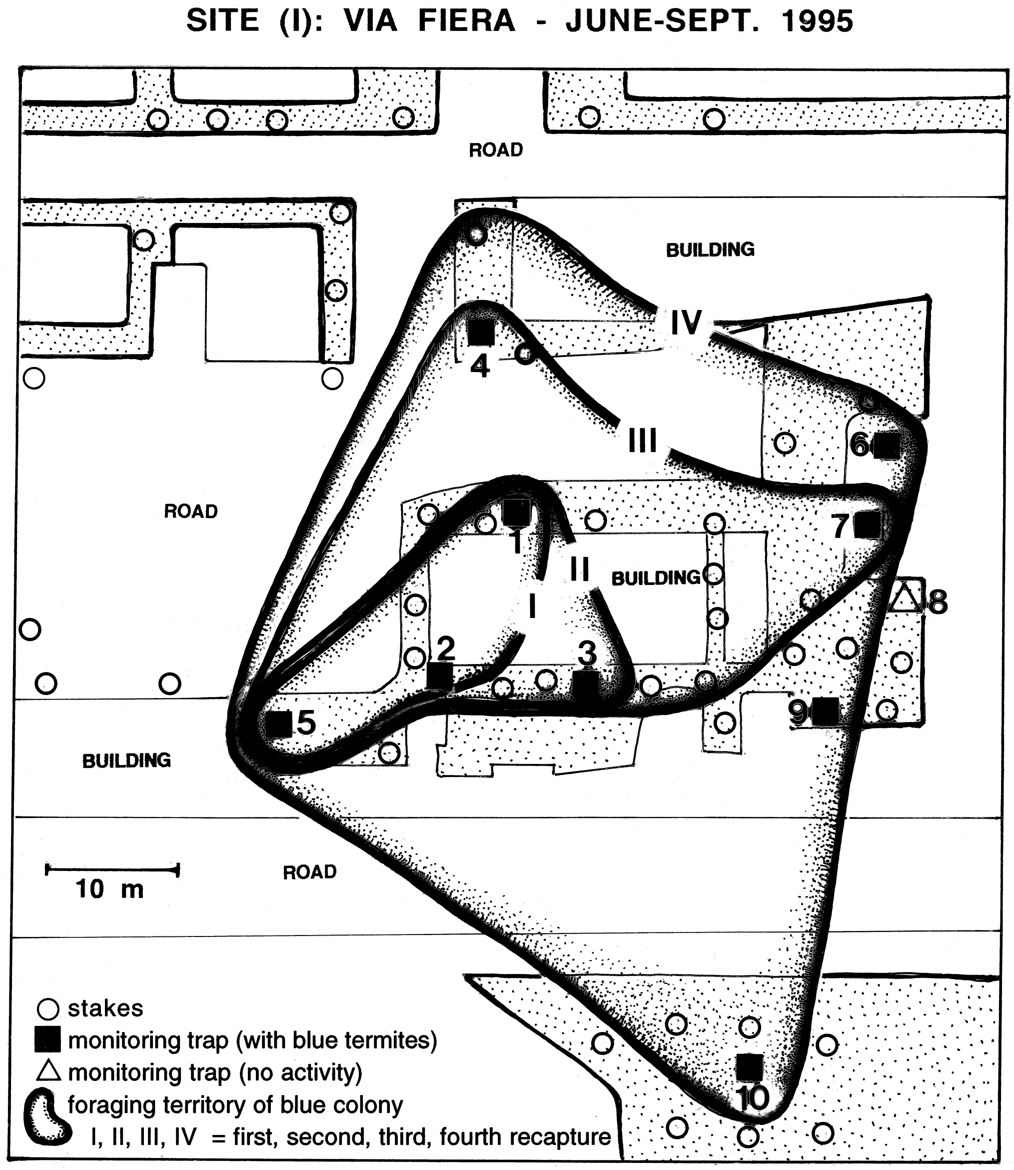

Fig. 1. Foraging territories of termite colonies at the study site Via Fiera for each phase of the TMR process. ○ denotes a survey stake; the solid ■ (with identification number) denotes an underground monitoring station; survey stakes or monitoring stations without termite activity were excluded from the map.

At this site expansion was much more rapid and it is possible to hypothesise that the termite colony settled in this area more recently and that it is a relatively young, growing colony.

4) The foraging territories are in progressive and continual expansion during the summer months until September and marked termites were found in almost all infested stations. The periphery of the colony cannot be taken as its border: given time it would be possible to find marked termites beyond it. The edge of the colony shape must be taken as the minimum feeding range of the termite population.

Given that this is the first time this technique has been used to study such extensive termite attacks in Italy, the data on the number of foraging individuals may be subject to greater error than that using the weighted mean model used for closed colonies (Su and Scheffrahn, 1986, 1988; Su *et al.*, 1993; Thorne *et al.*, 1996), where superimposing of colonies and migration between colonies is not a factor.

The data gathered are of great use in extending knowledge of the biology, ecology and behaviour of the Italian subterranean termite. Foraging activities are extensive and are closely related to the seasons and the position of the site.

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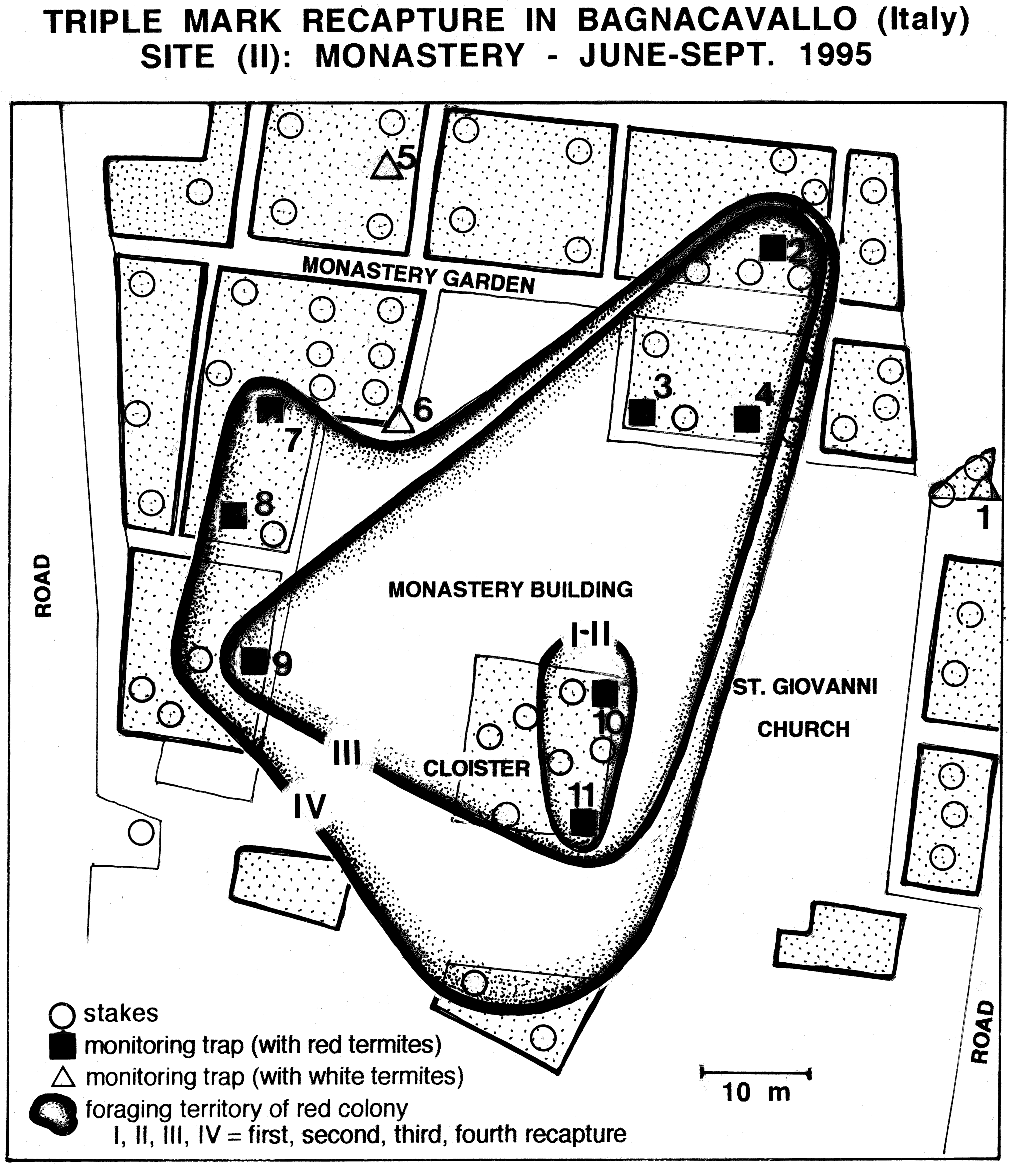

Fig. 2. Foraging territories of termite colonies at the study site Monastery for each phase of the TMR process. ○ denotes a survey stake; the solid ■ (with identification number) denotes an underground monitoring station; survey stakes or monitoring stations without termite activity were excluded from the map.

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