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The seasonal development of the gonads and fat content of *Tomicus minor* (Coleoptera Scolytidae)*

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Abstract

The seasonal and generational development of the male and female gonads and the fat content of *Tomicus minor* (HARTIG, 1834) throughout their life cycle was observed in a study carried out from 1991 to 1993. Maturation feeding and subsequent overwintering in the shoots of the crowns is reflected in the development and degree of maturity of the reproductive organs, and in the fat content. The beetles were classified as young or mature by observing the size and colour of the gonads. Sperm detected in the female genital tracts provides information on copula and the sperm survival rate.

Keywords : Scolytidae, *Tomicus minor*, seasonal development, gonads, fat content.

Résumé

On a étudié au cours des années 1991, 1992 et 1993 le développement saisonnier des gonades mâles et femelles de *Tomicus minor* (HARTIG, 1834) ainsi que la variation de la quantité de graisse accumulée par les insectes. A partir de l'émergence, les jeunes adultes se nourrissent dans les pousses des pins et ce même pendant l'hiver. Cette alimentation permet un développement et une maturation optimaux des gonades ainsi qu'une réserve graisseuse optimale. Les insectes ont été classés comme adultes immatures ou matures après observation de la taille et de la couleur des gonades. Le critère de fécondation utilisé a été la présence de spermatozoïdes dans les voies génitales femelles. Le taux de survie des spermatozoïdes a aussi été déterminé.

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Introduction

Tomicus minor (HARTIG, 1834) is called the lesser pine shoot beetle due to its feeding behaviour (BEVAN, 1962). This destructive feeding behaviour has been known since the days of Linnaeus (RATZEBURG, 1839). The adult burrows into the pith of pine shoots to become sexually mature and to accumulate lipids to carry out the flight dispersal and to complete its life cycle. Relevant studies like those published by RITCHIE (1917), BAKKE (1968) or LANGSTRÖM (1980, 1983, 1984) have been carried out on the biology and life cycle of *T. minor*.

T. minor is a phloephagous species but the larvae can feed on fungus when the phloem of the tree is not sufficiently nutritional (LEKANDER, 1968; FERNÁNDEZ *et al.*, 1999b). It is considered to be a semi-agressive species because it usually maintains its population at low level, attacking only unhealthy trees. However, when the population level is high, *T. minor* can attack and colonize healthy trees, for example, ROMANYK (1972) detected serious attacks on reforested areas in Spain in the early 70's.

However, the most harmful effect in this species is its capacity to transport the spores of the blue stain fungus, which may result in the death of the tree as its defensive reactions no longer function and favourable conditions for the development of the insect are created (HARRINGTON, 1993). Serious losses in the wood industry in northeastern Spain due to blue stain fungi were recorded in the 70's (AMEZAGA, 1992).

The present paper studies how its feeding behaviour influences the development and maturity of the male and female gonads throughout the life cycle (during the overwintering period, colonization of the logs, regeneration feeding and emergence of the new generation from the brood logs).

The seasonal variation of the fat content in both sexes was studied in young virgins, in repenetrating females and offspring females. The relation of this fat content to the host (*P. sylvestris* or *P. nigra*) was also noticed.

Material and methods

1) Study area

The study was carried out during 1991-1992 and 1993 in a pine reforested area of 130 ha of *Pinus sylvestris* and *P. nigra* sbsp. *salzmanni*, located in La Pola de Gordón, (30TTN84 and 1,225 m.a.s.l., León, NW Spain), in a northern exposure with annual rainfall between 900-1,400 mm. *P. sylvestris* occupies 90% of the area and *P. nigra* only 10%. The average age of the pine trees is 75 years although there are stands of natural regeneration averaging approximately 40 years.

2) Field methods

- *Collection of the burrowed shoots*

All fallen burrowed shoots (1466) were collected on the ground in forest during a prefixed walking itinerary lasting 30 minutes. The dropping candles (47) were collected from the trees in spring and summer, when the feeding galleries were burrowed, in walking itineraries lasting 60 minutes. These samplings were carried out at least once a month throughout the study period (from September 91 to September 93) and the percentage of occupation by the beetles was 35.8% (24,5% in *P. sylvestris* and 47.2% in *Pinus nigra*) (FERNÁNDEZ *et al.*, 1999a).

- *Placing the logs*

In 1991, 1992 and 1993, 20 brood logs 1 m long were placed in two areas of the pine forest, in order to obtain adult beetles in different stages of their life cycle and observe the maturity of their gonads and fat content. The adults were removed from the brood galleries of the logs once or twice a month during their reproductive period.

3) Laboratory methods

- *Fat analysis*

To determine the fat content, the method proposed by ANDERBRANT (1989) was used. The procedure involves drying the beetles at 60°C for 24 hours and weighing them individually. Each beetle was extracted twice in 1 ml petroleum ether (60-71°C) for 24 hours at 38°C. After extraction, the beetle was dried at 60°C for 24 hours and their fat-free weight was obtained.

40 adults at the end of their overwintering period (20 males and 20 females), 40 repenetrating females from brood logs (females that have laid once and repenetrated the log to lay again) and 40 F_1 offspring females were studied with the aim of observing seasonal variations.

In the study area there are two potential hosts for *Tomicus minor* : *Pinus sylvestris* and *P. nigra*. FERNÁNDEZ *et al.* (1999a) observed that *T. minor* showed a preference for the *P. nigra* shoots because of their greater nutritional value. This would presumably be seen in the amount of fat accumulated by the insects. For this purpose, overwintering virgin adults (80 of each sex : 40 collected in *P. sylvestris* and 40 in *P. nigra*) removed from the fallen shoots at the end of November were used.

- *Dissection of the gonads*

The general morphology of the male and female gonads is as follows:

- The male has two testes, each of which continues into a vas deferens which dilates into a seminal vesicle. The vesicle is united with a pair of lateral accessory glands. Both seminal vesicles are joined into a common ejaculatory duct, which ends in the aedeagus (Fig. 1).

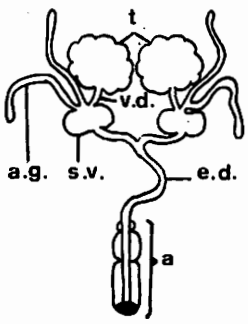

Fig. 1. Male gonads (t : testes, v.d. : vas deferens, a.g. : accessory glands, s.v. : seminal vesicle, e.d. : ejaculatory duct, a : aedeagus).

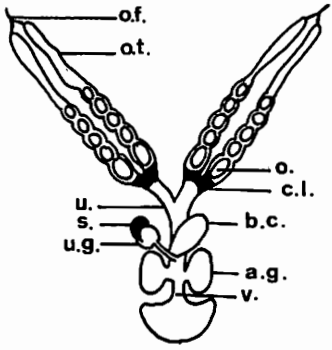

Fig. 2. Female gonads (o.f. : ovarian filament, o.t. : ovarian tube, u. : uterus, b.c. : *bursa copulatrix*, s. : chitinous spermatheca, a.g. : accessory gland, u.g. : unpaired gland, v : vagina, o. : oocyte, c.l. : *corpora lutea*).

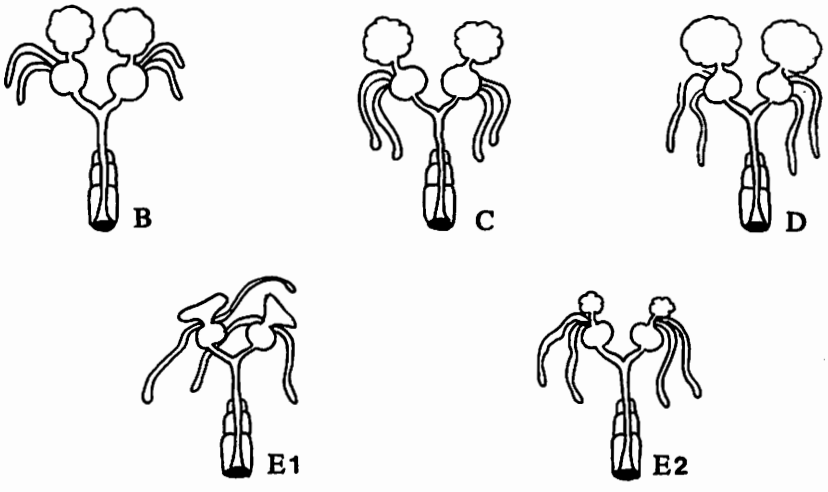

Fig. 3. Types of male gonads. Type B : large, lobular and white testes with large, transparent and empty seminal vesicles and short accessory glands. Type C : large, lobular and white testes with whitish seminal vesicles and large accessory glands. Type D : Larger yellowish testes, small yellow seminal vesicles are full of spermatozoa, elongated accessory glands. Type E_1: yellow triangular shaped testes, small seminal vesicles and large accessory glands. Type E_2: yellow smaller rounded testes, small seminal vesicles and large accessory glands.

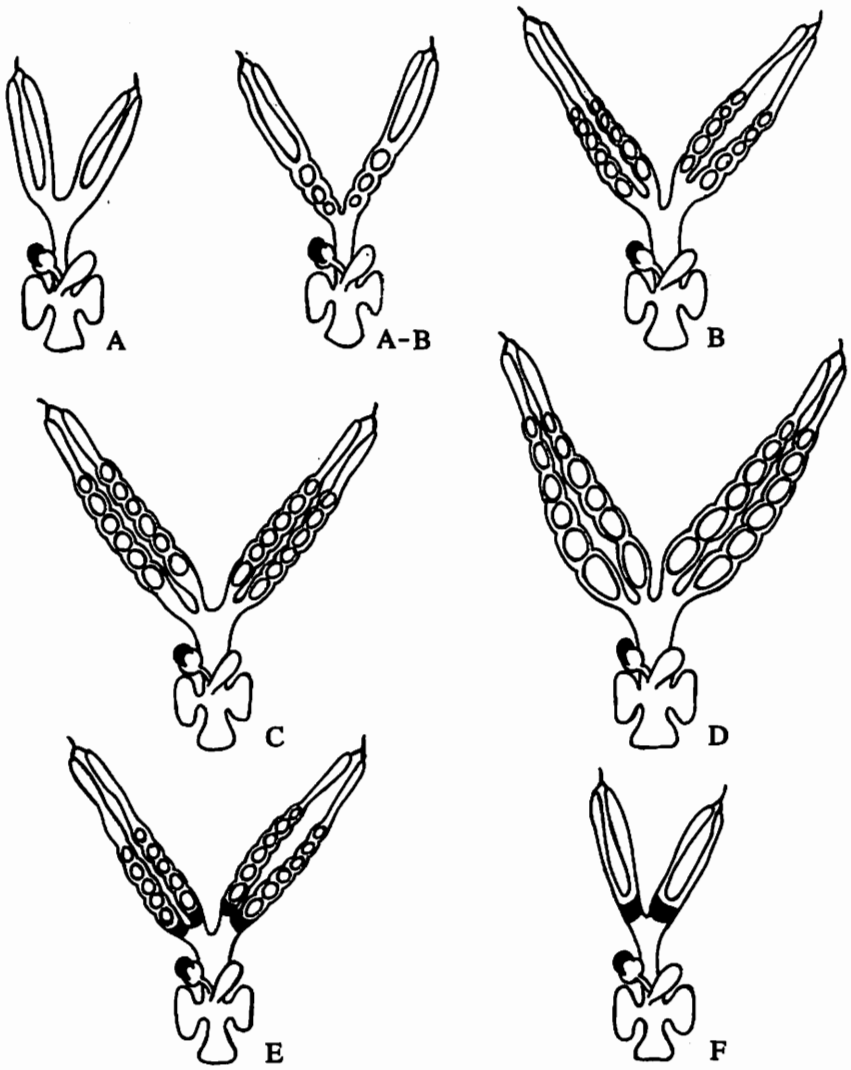

Fig. 4. Types of female gonads. Type A : short oviducts not yet divided into ovarian chambers and no oocytes; the bursa copulatrix and the accessory glands are whitish and no spermatozoa were detected. Type B : wider oviducts with oocytes and larger ovarioles. Type A-B : few oocytes and medium length ovarioles. Type C : large number of oocytes and larger ovarioles. Type D : larger ovarioles with great number of oocytes; spermatheca turgid and rounded with a large number of mobile spermatozoa and accessory glands swollen. *Corpora lutea* begining to appear. Type E : ovarioles shorter with some oocytes and *corpora lutea* very distinct. Mobile sperm. Type F : general characteristics of type A but with the presence of *corpora lutea*. No oocytes, oviducts reduced in length and sperm not mobile. Spermatheca and accessory glands flat and the *corpora lutea* were very noticeable, indicating that laying had already finished.

- The female has two pairs of ovarian tubes joined at the end by an ovarian filament. The oviducts are at the base of the ovarian tubes and end in the uterus which, in turn, ends in the vagina. On both sides of the vagina there is a pair of accessory glands. The *bursa copulatrix* and the chitinous spermatheca are joined to another unpaired gland that mixes its secretion with the sperm (Fig. 2).

A total of 388 beetles were dissected : 188 females in 1992, 99 females and 101 males in 1993, to study the condition of their gonads. Methyl blue was used to obtain contrast in the structures. They were collected when burrowing in the brood logs or inside the shoots. Sperm was detected using the binocular, opening the seminal receptacle and observing its mobility.

Figures 3 and 4 show the different types of gonads in males (types B to E) and females (types A to F). In some cases organs, which were an intermediate stage between two consecutive types, appeared. For this reason, sub-indexes indicating evolution from one stage to the next were used.

- Pronotal width

The maximum pronotal width of the overwintering adults was measured in 156 overwintering beetles (44 males and 41 females removed from *P. sylvestris* and 41 males and 30 females removed from *P. nigra*).

Results

1) Development of the gonads

The male gonads

The size and colour of the testes and the seminal vesicles were observed, although the information obtained is not as complete as in the case of the females (Table 2). According to RITCHIE (1917) and LANGSTRÖM (1983) two main types of male gonads were detected : immature males with long, lobular, whitish testes, short accessory gland and a large seminal vesicle with no sperm inside (types B, C and D) and mature males (types E_1 and E_2) with yellowish testes, elongated accessory glands and small seminal vesicles full of spermatozoa (Fig. 3).

- Overwintering

Most of the males collected in the fallen shoots during the second overwintering period (Sept-92-Feb 93) had type B gonads, with large, white, lobular testes and large empty seminal vesicles (Table 2). Some had type C organs, though somewhat more mature than type B. This indicates that all the males examined belonged to the first generation and none belonged to the previous one (Fig. 1).

Table 1. Mean values and standard deviation of the fat content in males and females in both pine hosts.

	males		females	
	mean value (mg)	standard deviation	mean value (mg)	standard deviation
P. nigra	$0.6\ 10^{-3}$ mg	$0.222\ 10^{-3}$	$0.9\ 10^{-3}$ mg	$0.220\ 10^{-3}$
P. sylvestris	$0.7\ 10^{-3}$ mg	$0.252\ 10^{-3}$	$0.9\ 10^{-3}$ mg	$0.305\ 10^{-3}$

- *Colonization of the logs*

At the start of colonization of the logs (March 1993) the males collected from the brood logs presented type C organs, with large testes and whitish seminal vesicles. Type D beetles with large testes and small yellow seminal vesicles were observed in April. In May and during the development of the larvae, the testes gradually reduce in size, becoming smaller than the seminal vesicle and deeper yellow in colour. Two types were observed at this time (Fig. 3) : E_1 with triangular shaped testes and E_2 with smaller, rounded testes. Only one E_1 type male was found in June since most of them had already re-emerged from the egg galleries to carry out regeneration feeding in the shoots of the pine crowns.

- *Regeneration feeding*

In May 92 the parent males left the logs and flew to the shoots of the crowns. One month later they returned to the brood logs to inseminate the females again. Although no adults were located inside the shoots collected from the crowns and the ground in May, the galleries burrowed in the shoots indicated that regeneration feeding had taken place. These males presumably remained inside the shoots for about a month to regenerate their gonads because in June they were found again in the egg galleries to carry out mating with the repenetrating females.

No burrowed galleries were found inside the shoots of the crowns, or inside the fallen shoots in the samplings corresponding to April and May 93. Neither were there any re-emerging males inside the shoots.

Those males collected from the logs and dissected in May and June presented E_1 and E_2 type gonads (Fig. 3). They remained inside the logs until the first week in June. It would appear that shoot feeding did not occur, or was not necessary. No indication of shoot feeding was observed in the fat content of the insects examined from the logs. The males did not apparently leave the logs in 1993 since they were continuously found inside them in April, May and the beginning of June. The difference observed with regard to the previous year could be because of the prolonged colonization flight period in spring 1992, which was interrupted for almost 4 weeks, due to snow.

Table 2. Stages of gonadal development observed in 1992 and 1993 for males and females.

	91-92				
	FEMALES				
	shoots		brood		
months	crown	fallen	logs	months	crown
sept-91				sept-92	
oct-91		A-B(8)		oct-92	
nov-91		A(15)		nov-92	
dec-91		A(3)		dec-92	
jan-92		A-B(24)		jan-93	
févr-92	A-B(1)	A(1), B(14)		févr-93	
mars-92	B(2)	B(8), C(1)		mars-93	
apr-92		C(1)	C1(10) C2(7), F(1)	apr-93	
mai-92			C(26), E2(34), E1-E2(9), E1(2)	mai-93	
juin-92			F(21)	juin-93	
juil-92				juil-93	A(1)
aug-92				aug-93	
sept-92				sept-93	
total	3	75	110		1

- *Emerging males from the F_1 generation*

All the males that emerged from the logs (generation F_1) in July and August were virgins with type B gonads. The testes were large, white and lobular and the seminal vesicle was large. They all flew to the shoots of the crown to carry out maturation feeding. A proportion of more mature males was found in the shoots in July. These were the first to emerge from the brood logs, and they had already carried out a certain degree of feeding because they presented D type organs with large yellowish testes. One male belonging to type B was also found in the shoots of the crowns in August (Table 2).

Female gonads

The maturity of the female gonads is also characterised by the yellowish colour of all the structures, the presence of distinct chambers in the ovarian tubes and accessory glands full of secretion. The immature organs are whitish, the ovarian chambers are not well defined, the ovarian tubes are short and transparent, the accessory gland is empty and there is no sperm in the spermatheca nor in the *bursa copulatrix*; finally all the structures are smaller and less developed (Fig. 4).

92-93					
FEMALES			MALES		
shoots	brood		shoots		brood
fallen	logs	months	crown	fallen	logs
		sept-92		B(4)	
B(6)		oct-92		B(12)	
		nov-92			
		dec-92			
A-B(8), F(3)		jan-93		B(22)	
B(13), F(1)		févr-93		B(23), C(5)	
	F(3), B(21)	mars-93			C(4)
	D1(9), C(1)	apr-93			D(10)
	D2(14), E(1)	mai-93			E1(1), E2(10)
	D2(1), F(2)	juin-93			1(1)
	A(10)	juil-93	B(1), D(7)		
	E1(5)	aug-93	B(1)		
		sept-93			
31	67		9	66	26

- *Overwintering*

During the 92-93 overwintering period, the females dissected in October, November, December, January and February presented type A gonads with short oviducts not yet divided into ovarian chambers. The *bursa copulatrix* and the accessory glands were whitish and empty. As the overwintering period progressed, type B gonads were detected, with wider oviducts and the presence of oocytes. Gonads in an intermediate stage between A-B were clearly seen, with few oocytes and medium length ovarian tubes.

Types B and C were detected at the end of overwintering in the shoots (March-April 92). The effects of maturation feeding were observed, indicating that feeding continued in winter, or began again at the end of the same period. The oviducts were wide and longer, the ovarian chambers were full of oocytes and the accessory glands were whitish. No spermatozoa were detected in the spermatheca, indicating that mating did not occur during overwintering.

Females captured inside the shoots during the second overwintering period (January-February 93) had types A, B and intermediate stage gonads. The first female was found in a shoot in October 1992. In January and February 1993, 12% females that had already overwintered once were found inside the shoots. They were at least one year old since they had type F gonads and the general characteristics of type A, but with one significant difference; the presence of

corpora lutea indicating that they had already laid eggs. Three females with type F organs were located in the brood logs in March, whilst in April 92 only one female with type F organs was found (Table 2).

An abundance of colonizable material in spring results in a lower number of surviving parents the following winter period (SCHROEDER & RISBERG, 1989). In contrast, the lack of colonizable material in the pine forest (92-93 period) determines a larger number of overwintering parents the following winter. The survival of those parents will be the only way of the population surviving because the absence of colonizable material in spring determines a low number of young emerging beetles from the logs.

The absence of sperm in the seminal receptacle of one-year-old females indicated that mating had to be repeated, as the sperm could not survive long enough without deteriorating. Thus, the older females were inseminated again in the brood logs. The lack of sperm in the overwintering females indicated that early mating did not take place in the shoots during winter, or if it did, the percentage was very low, and therefore not detected.

- *Colonization of the brood logs*

Mainly type B and C gonads with large number of oocytes in the oviducts were observed in the colonization of the brood logs (March-April) in both overwintering periods. The *corpora lutea* became more and more distinct throughout the oviposition period (May-June) until perfectly observed in type D. The spermatheca were turgid and rounded, and when dissected a large number of mobile spermatozoa appeared. The accessory gland was swollen indicating that mating had taken place. Several subtypes identified by subindexes were established. These indicated a more visible *corpora lutea*, larger oviducts and a greater number of oocytes (Table 2). In April 92, a one-year-old type F female that had just arrived at the brood log was noticed and another 3, one-year-old, were seen in the same place in March 93. Type C beetles continued to be seen throughout the laying period (May), which transformed into type D with a more and more distinct *corpora lutea.*

By the end of the laying period (June), most of the females had type F gonads, without oocytes; the oviducts were reduced in length, the sperm was non-mobile, the accessory gland and spermatheca were flat, and the *corpora lutea* were very noticeable indicating that laying had already finished. In April, May and August repenetrating females with type E gonads were detected.

- *Regeneration feeding*

As in the case of the males, regeneration feeding in shoots was studied in females. However, only repenetrating and not re-emerging females were observed. The repenetrating females are the ones that burrow several galleries without previously carrying out regeneration feeding in the pith of the shoots. Thus, repenetrating females were located in the logs from the end of May to the end of June 1991 representing 3.2% of the whole population however no females were found inside the fallen shoots or the crowns.

In 1992 the first colonization females (in March) presented C and D type gonads. Some more mature females that had already burrowed long galleries, presented type E gonads. In May, only 15% females that had just started burrowing tunnels presented type E organs with a large number of oocytes and very distinct *corpora lutea*. The lack of fat content in these females indicated that previous feeding in the shoots of the crowns had not taken place, and that these females were thus repenetrating and not re-emerging. In May 1993 females that had burrowed galleries 2-3 cm in length were noticed whilst the majority of the galleries of the females that had laid at the beginning of March, measured about 10 cm in length.

In August the repenetrating females were removed from the egg galleries and presented type E_1 gonads, with perfectly mobile sperm indicating recent mating. As observed in the previous year, they had not accumulated fat, which would indicate shoot feeding. Neither was any female found in the shoots of the crowns, nor the fallen shoots from May to August. If regeneration feeding took place it had to be to a very limited extent. The percentage of detected repenetrating females for this year 1993 was 33%.

- *Offspring females belonging to the F_1 generation*

The immature, virgin females emerged from the logs in July-August and flew to the pine crowns to burrow maturation tunnels inside the shoots. These shoots fell to the ground in autumn and winter months. The females remained inside the shoots until the following spring. Most of them had type A gonads with short oviducts, no oocytes and a whitish structure. No spermatozoa were detected, so presumably mating did not occur prior to emergence from the brood logs. In October 92, the females captured inside the fallen shoots had intermediate A-B organs as a result of maturation feeding (Table 2).

2) Evolution of the fat content

- *Seasonal changes*

Table 3 shows the mean values of fat content, typical standard deviation and variation range in overwintering beetles (males and females), as well as in repenetrating and F_1 offspring females. A χ^2 test showed significant differences between both sexes: the females presented a greater accumulation of fat during the winter period with a mean value of 0.8 10^{-3} mg compared to 0.6 10^{-3} mg for the males. The fat content decrease during the laying period and the females presented a lower fat content, reduced by about 40% (mean value=0.5 10^{-3} mg). Finally, the offspring beetles had no fat content at all when they emerged from the logs. This makes shoot feeding absolutely fundamental because this way the beetles can accumulate fat and carry out gonadal maturation.

Significant differences between the maximum pronotal witdh between sexes (60 males and 60 females) were recorded (χ^2=0.00235, α=0.05). The females had wider pronotum and higher fat content (Table 3). The correlation between

size and fat content was low and consequently the width of the pronotum accounted for only 20% of the variation observed in the fat content.

Table 3. Mean fat content value, standard deviation and deviation range corresponding to overwintering, re-emerging and offspring beetles.

	Overwintering adults (at the end of the overwintering period)		Repenetrating	Offspring
	males	females	females	females
Mean fat content value (mg)	$0.6\ 10^{-3}$	$0.8\ 10^{-3}$	$0.45\ 10^{-3}$	$0.1\ 10^{-4}$
Standard deviation	$0.18\ 10^{-3}$	$0.23\ 10^{-3}$	$0.52\ 10^{-3}$	$0.3\ 10^{-4}$
Deviation range	$0.4\ 10^{-3}$-$0.1\ 10^{-3}$	$0.4\ 10^{-3}$-$0.12\ 10^{-3}$	$0.1\ 10^{-3}$-$0.19\ 10^{-2}$	0-$0.1\ 10^{-4}$
Pronotal width (mm)	1.47	1.53		

- *Fat content in relation to the host*

After applying a χ^2 test, the values obtained showed that there were no significant differences between the hosts. However, significant differences were observed between the two sexes, independent of the chosen hosts (Table 1).

Discussion

Tomicus minor remains in the shoots approximately 8 months from the emergence from the brood logs in June-July till the end of the overwintering period (approximately the following March). During this time, the young virgin beetles from the F_1 generation carry out gonadal maturation feeding and they accumulate lipids as a fat reserve.

15% of the males observed in this study carried out regeneration feeding in the shoots in May 92. This was not detected in 91 or 93 because the parent males remained inside the logs the whole time. In contrast, the females did not leave the logs at any time to carry out regeneration feeding because their gonads gradually mature when they eat the phloem during laying.

12% females with type F gonads were detected in the shoots during the second overwintering period. Their distinct *corpora lutea* indicated that these females were one year old and had already survived a second winter. These observations coincide with LANGSTRÖM (1980) and SCHROEDER & RISBERG (1989), who discovered a proportion of parent beetles capable of surviving one year and reproducing again. According to the latter two authors, this process guarantees the survival of the entire population in years when the reproductive performance is low due to unfavourable climatic conditions, or to lack of available colonization material.

The absence of sperm in the seminal receptacle of one-year-old females indicated that mating had to be repeated, as the sperm could not survive long enough without deteriorating. Thus, the older females were inseminated again

in the brood logs. The lack of sperm in the overwintering females indicated that early mating did not take place in the shoots during winter, or if it did, the percentage was very low, and therefore not detected. Certain authors, for example JANIN & LIEUTIER (1988) have observed the opposite in *Tomicus piniperda*. They found 15% females inseminated during the shoot feeding period, which precedes overwintering. They believe that copula occurs when the beetles move from one shoot to another. CHARARAS (1962), MCCAMBRIDGE (1969) y WITANACHCHI (1989) observed early mating in other species of bark beetles. KIRKENDALL (1990) observed the same in *Ips typographus* populations, whose sex-ratio clearly inclines towards the females. In this case, the sperm is a limiting factor, and consequently, those females inseminated before undertaking the dispersal flight are more likely to reproduce as there is little chance of coming across a male in the brood logs. In a monogamous species like *Tomicus minor* the sex-ratio is 1:1 and competition for the sperm during colonization should be mitigated; the pioneering beetles are the female and it is the male who competes for the females.

The fat content is directly related to the degree of survival of the insect, as well as to its capacity to carry out the dispersal flight during which the energy from the accumulated lipids is consumed. THOMPSON & BENNET (1971) observed an initial decrease from 55% to 5% in the fat content in *Drendoctonus pseudotsugae*, after a five-hour dispersal flight. Other authors have also related fat content to response capacity and pheromone production, as well as the ability to respond positively to the volatiles emitted by the host. Fat content is also generally related to the size and weight of the insect. BOTTERWERG (1983) and ANDERBRANT et al. (1985) observed in *Ips typographus* that the males (pioneering sex in this species) are heavier than the females and have a wider pronotum. The fat content was greater in the *T. minor* females (the pioneering sex) (Table 3) and have also a wider pronotum. The fat content in the overwintering males and females was independent of the host used to carry out feeding (*Pinus sylvestris* or *P. nigra*).

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