Introduction

The Mediterranean corn borer, *Sesamia nonagrioides* Lefèbvre, is a major maize pest around the Mediterranean Basin, where it can complete two to four generations, depending mostly on the latitude (Anglade, 1972). Females insert eggs between the stem and the lower leaf sheaths of maize plants, and soon after hatching the larvae bore quickly into the stem, where they develop until the adult stage. Because of this endophytic behaviour the larvae are well protec-
ted against insecticides, so other control methods have been tested.

In the last few years, the efficacy of synthetic sex pheromone sprays for disrupting the mating of the Mediterranean corn borer has been evaluated in Spain, Greece and France with variable but promising results. To achieve good control of a pest by means of mating disruption techniques, at least three domains of knowledge should be investigated: proper pheromone characterisation, the key biological traits of the pest, and the development of a slow-release formulation (Campion et al., 1989). The sex pheromone of the Mediterranean corn borer was initially identified in a French population by Sreng et al. (1985), as a mixture of two components: (Z)-11-hexadecenal-1-yl acetate (Z11-16:Ac, I from here onwards) and (Z)-11-hexadecen-1-ol (Z11-16:OH, II) in a 90:10 ratio. Later, Mazomenos (1989a) found two minor components, (Z)-11-hexadecenal (Z11-16:Al, III) and dodecyl acetate (12:Ac, IV), and determined the mixture ratio of 69:8:8:15 (I:II:III:IV) as the best performer for catching males in Greece. In Spain, Sans et al. (1997) found that the ratio 70:10:10:5 caused more males to respond in electroantennogram trials in the laboratory and in pheromone-baited traps in the field, but they were unable to detect the minor components in the female glands. Mazomenos (1992a) and Babilis and Mazomenos (1992) studied the calling behaviour of females, and found that they mated from the second night of emergence. However, females of Spanish populations of *S. nonagrioides* were repeatedly observed to mate also the first night after emergence. Bues et al. (1996) found differences in enzymatic and ecophysiological patterns of close populations, indicating that *S. nonagrioides* is a sedentary species. These data lead us to consider the possibility that, as has been pointed out for other sedentary species (Frérot and Foster, 1991), *S. nonagrioides* could have evolved different phenotypes in Spain and Greece; geographic differences in the optimal component ratio of the pheromone blend may lead to failures in both monitoring with pheromone-baited traps and mating disruption with synthetic pheromones when a common mixture is used everywhere.

To optimise the pheromone identification (extraction and analysis) we decided to study first the mating behaviour of local populations, taking into account not only the factors studied by Mazomenos (1989a), such as the day and time of calling and mating, but others that may have great importance in the application of pheromones for control purposes. To determine whether the calling behaviour changes were elicited in response to the other sex, we compared the behaviour of individual males and females with the behaviour of couples. Another goal for applying pheromones in the field is to determine whether *S. nonagrioides* females approach to males by visual stimuli (as has been reported in *Agrotis ipsilon* by Swier et al., 1976) or respond to male pheromones (as has been reported in several noctuid moths (Lenczewski and Landolt, 1991; Landolt and Heath, 1989)). Moreover, we wanted to determine whether the calling behaviour varies in the presence of maize plants, because Lopez et al. (1999) indicated that *S. nonagrioides* mated without maize plants in overwintering fields and in the laboratory. As pointed out by Cardé and Minks (1995), several pest biological traits related to the population dynamics and mating system may constrain the successful application of mating disruption. Firstly, the prevalence of migration of mated females from outside the area treated with disruptant may mask the efficacy of the technique, particularly when it is evaluated in small experimental plots. Very few data on the dispersal capacity of *S. nonagrioides* are available. It seems that females that have emerged from overwintered populations are able to disperse to several kilometres to colonise a newly emerged maize field (Larue, 1984), although it is not known whether they fly before or after mating. Secondly, interaction between population density and mating disruption efficacy must also be considered. At comparatively high pest densities mate location can be augmented by the use of non-pheromonal cues (Cardé and Minks 1995). As the mating disruption technique to control the Mediterranean corn borer is experimentally applied against adults of the second flight, when densities are comparatively higher than earlier in the season, occurrence of high population densities could diminish the efficacy of the technique.

The research reported in this paper aimed: (i) to characterise the mating behaviour of both males and females in a Spanish population of *S. nonagrioides*; and (ii) to determine whether adult density influences mating rate. This research was being conducted in the framework of a larger project in which potential factors affecting the efficacy of mating disruption technique were investigated.

**Material and methods**

Experimental individuals of *S. nonagrioides* were obtained from a colony reared in the laboratory. The
colony was renewed every 3 or 4 generations with larvae or pupae collected in the field in the Lleida area (north-eastern Spain, 42ºN). The rearing method and materials are described in Eizaguirre and Albajes (1992).

The pupae were disinfected by submerging them for a few seconds in a 5% solution of sodium hypochlorite. Afterwards, they were sexed and the two sexes were separated into two transparent plastic boxes 25 cm in diameter. Recently emerged adults were fed on a 5% sugar solution. The experimental conditions were: photoperiod of 16L:8D, 60±10% R.H. and 25±1°C temperature.

Behavioural sequence of courtship and mating: effect of age and maize plant

Just before the beginning of the scotophase, pairs of one- or two-day-old virgin females and males were placed in a 0.5×0.5×0.5 m³ transparent metachrylate cage together with one potted maize plant or without any plant. During the eight hours of the scotophase, male and female behaviour was observed continuously by means of a red bulb (4 lux), and the onset time of each behavioural step was recorded. Only one couple were introduced into one cage during the same scotophase. The individual behaviour of caged males and females was also observed and compared with that of the insect couples. The cages were cleaned with hexane at the end of each scotophase after removing the individuals.

Mutual attractant capacity of males and females

To investigate female-to-male and male-to-female attraction, two-day-old virgin males or females were individually caged with tethered individuals of the other sex and their behaviour throughout the scotophase was observed. There were no maize plants to avoid disturbing visual stimuli. The tethering set-up consisted of a vertical stake to which one end of a thread of 15 cm long was tied; the other end was tied around the moth abdomen just behind the forewing insertion. It had been checked previously that the tethered adults were able to fly. Cages and experimental conditions were as described for the previous experiment.

Influence of the density of adults on mating rate

The influence of adult density on mating rate was determined in laboratory and field experiments. In the laboratory, one vs. two newly emerged (<48 h) adults were caged for 48 hours and then the females were dissected to check for the presence of spermatophores in the bursa copulatrix.

For the field experiment, cages of 0.6×0.7×2 m³ with a wooden frame covered with a plastic screen (2×2 mm mesh) were placed in maize fields so that they contained three maize plants each. The field cages were separated from each other by at least ten metres. A variable number (1, 2, 4 or 8) of virgin adult pairs were then released into cages and left there for 48 hours, after which the females were recovered and taken to the laboratory for dissection. Two fields were used for this experiment. One of them was submitted to mating disruption conditions one week prior to adult release by placing pheromone blend dispensers every 10×10 m² to ensure a treatment dose of 80 g ha⁻¹. The blend was a mixture of components I, II, III and IV (see introduction) at a proportion of 69:8:8:15. The other field was left untreated. In each field, a complete randomised block design with ten replications was used. A few females released in the cages could not be recovered due to predation.

Statistical analyses

As the two-way ANOVA (age and presence vs. absence of plant) performed to determine the influence of the two factors on the onset time of mating showed a significant (p≤0.05) interaction effect, the two factors were analysed separately by means of Student’s T-tests. Differences in the onset time and duration of mating between tethered and free females were also analysed by a Student’s T-test. The influence of these factors on the percentage of mating was analysed by a χ² test. To analyse the influence of adult density on the mating rate, the number of mated and unmated females at each couple density was compared by a χ² test.

Results

Behavioural sequence of courtship and mating: effect of age and maize plant

A sequence of steps was consistently observed in courtship and mating behaviour in S. nonagri-
males and females and they are described in Table 1.

Females showed a very passive behaviour. They started to call showing firstly some bouts, but they soon adopted the calling posture with the pheromone glands continuously extruded. Wing fluttering was never observed in females. Flying males always showed the hair pencils extruded but no apparent females response to this male behaviour was observed.

To copulate, the males landed on the wall about 5 cm from the female, walked toward her laterally and by turning his abdomen mated with her, head-down. This last sequence, which included landing, approaching the female and mating, lasted only few seconds. Copulation always took place in the vertical posture on cage walls except in one case (< 2%), in which it occurred on the plant stem, with the female above and the male below head-down.

After copulation both the female and male normally remained there in a resting posture for the rest of the night, but some males flew away from the copulation site and quickly landed in other resting places. Repetition of courtship or mating postures was never recorded in resting mated adults. Females never laid eggs on the night that they mated. The courtship behaviour of unpaired males and females did not differ from the first three steps described in Table 1 for paired adults (resting, starting activity and flight) either in behavioural sequence or in onset times.

When the experiment was performed with maize plants, female age did not significantly (P>0.05) influence the onset time of mating (Table 2), but when there were not maize plants 2-days-old females mated before 1-day-old ones (P ≤ 0.05). The first mating occurred at 2 h 3 min and the last one at 3 h 41 min for free females.

The percentage of mating did not depend on the age or the presence of plant (P>0.05). The 1-day-old females mated as 2-days-old females with or without maize plant (Table 3).
Mutual attractant capacity of males and females

When males were coupled with tethered females, the courtship and mating behaviour described above for unmated females was the same, and the percentage of mated females did not differ significantly (P>0.05) from that recorded for free females (Table 3). The onset time of mating of tethered females was later than the onset time of free females (Table 2). In contrast, when the sex tethered was the male, he stayed in the same place throughout the scotophase and did not respond to the call of the female, which displayed an identical calling sequence to that described above; the female never approached the male and the resulting percentage of mating was 0 (Table 3). The duration of mating of tethered females was longer than that of free females.

Influence of adult density on mating

When adult density in laboratory cages was doubled, without pheromone treatment, no significant increase (P>0.05) in the mating rate was found (Fig. 1A). This result is consistent with that obtained with field

Table 2. Influence of adult age, presence of maize plant and free vs. tethered females on the onset time of mating. Means followed by the same letter within each cell are not significantly (p<0.05) different. N indicates the number of couples used for calculating the means

<table>
<thead>
<tr>
<th>Age</th>
<th>With plant</th>
<th>Without plant</th>
<th>With plant</th>
<th>Without plant</th>
<th>With plant</th>
<th>Without plant</th>
<th>With plant</th>
<th>Without plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>With plant</td>
<td>1 day</td>
<td>2 days</td>
<td>1 day</td>
<td>2 days</td>
<td>1 day</td>
<td>2 days</td>
<td>1 day</td>
<td>2 days</td>
</tr>
<tr>
<td>N</td>
<td>31</td>
<td>16</td>
<td>17</td>
<td>35</td>
<td>31</td>
<td>17</td>
<td>31</td>
<td>35</td>
</tr>
<tr>
<td>Mean (min)±sd</td>
<td>F=1.88</td>
<td>P=0.1773</td>
<td>F=5.58</td>
<td>P=0.0222</td>
<td>F=0.00</td>
<td>F=8.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>176±34 a</td>
<td>190±31 a</td>
<td>176±34 a</td>
<td>154±31 b</td>
<td>176±34 a</td>
<td>161.34 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>181±33 a</td>
<td></td>
<td>165±35 a</td>
<td></td>
<td>165±35 a</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T-test</td>
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</tbody>
</table>

Table 3. Influence of adult age, presence of maize plant and free vs. tethered females on the percentage of mating. Percentages followed by the same letter within each cell are not significantly (p<0.05) different. N indicates the number of couples used for calculating the percentage

<table>
<thead>
<tr>
<th>Age</th>
<th>With plant</th>
<th>Without plant (free ♀)</th>
<th>Without plant (tethered ♀)</th>
<th>With plant</th>
<th>Without plant (free ♂)</th>
<th>Without plant (tethered ♂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>43</td>
<td>45</td>
<td>16</td>
<td>23</td>
<td>45</td>
<td>16</td>
</tr>
<tr>
<td>% mating</td>
<td>72.1 a</td>
<td>77.8 a</td>
<td>69.6 a</td>
<td>68.0 a</td>
<td>77.8 a</td>
<td>69.6 a</td>
</tr>
<tr>
<td>χ²</td>
<td>0.0079</td>
<td>0.1207</td>
<td>0.9292</td>
<td>0.1207</td>
<td>0.8817</td>
<td>0.7780</td>
</tr>
<tr>
<td>% mating</td>
<td>71.2 a</td>
<td>74.3 a</td>
<td>74.3 a</td>
<td>70.6 a</td>
<td>75.0 a</td>
<td>75.0 a</td>
</tr>
<tr>
<td>χ²</td>
<td>0.0255</td>
<td>0.8731</td>
<td>0.8731</td>
<td>0.0562</td>
<td>0.8186</td>
<td>0.8186</td>
</tr>
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</table>
cages placed in pheromone-untreated fields where, although adult density was increased by eight times, no significant increase (P>0.05) in the mating rate was recorded (Fig. 1B). In contrast, adult density did significantly influence (P ≤ 0.05) mating rates in cages placed in the field treated with pheromone (Fig. 1B).

**Discussion**

Some noctuid moths require several days to reach sexual maturity and to mate (Howlader and Gerber, 1986) while others do not require a pre-reproductive period (McNeil, 1991). The *S. nonagrioides* population studied here belongs to the latter group. Most females start calling and mate during the first scotophase after emergence unlike the findings of Babilis and Mazomenos (1992) reported for a Greek population. The pre-reproductive period in insects is governed by genetic and epigenetic factors such as temperature, photoperiod and the presence of plant host (Thornhill and Alcock, 1983). The experimental temperature and daylength were the same in both studies, and although the adults from Greece (Mazomenos, 1989a) were not provided with host plants, we have discarded the influence of maize plants on the mating rate in *S. nonagrioides*. López *et al.* (1999) found that *S. nonagrioides* females mated, in spring, in overwintering fields without maize. Differences found in the pre-reproductive period between Spanish and Greek populations, almost the two extremes of the distribution area of the Mediterranean corn borer, may thus indicate genetic differences in these two geographic strains. Differences in the pre-reproductive period among strains of differing geographical origin have also been reported in the noctuid *Helicoverpa armigera* (Hübner) (Colvin *et al.*, 1994), one of the insects in which the regulation of the pre-reproductive period has a genetic component (Colvin and Gatehouse, 1993).

In addition to age at mating, the onset of calling in females from Greece and north-eastern Spain was also different: the latter started to call 2.5 hours earlier. Differentiation of calling time is one of the ways to achieve reproductive isolation between sympatrically occurring insects in which sex location is mediated by pheromones (Saunders, 1982). *S. nonagrioides* share major pheromone components with other noctuid species such as *Mythimna unipuncta* (Haworth) (McDonough *et al.*, 1980), *Discestra trifolii* (Hufnagel) (Struble and Swailes, 1975) and *Lacanobia oleracea* (L.) (Descoins *et al.*, 1978), and these species are frequently captured when traps baited with pheromone are used to monitor *S. nonagrioides* populations (Sans *et al.*, 1997). Although these three noctuid moths are ubiquitous around the Mediterranean basin (Carrol 1972), *D. trifolii* is not caught in Greece in traps baited with the *S. nonagrioides* pheromone blend (Mazomenos 1989b), indicating that the species does not usually occur alongside *S. nonagrioides* on maize fields in Greece. Although we have no reported data describing the onset of calling time in *D. trifolii*, the sympatric occurrence of this species could cause *S. nonagrioides* to differentiate calling time in north-eastern Spain. The time of female calling may also be affected by age, the presence of conspecific pheromone, daylength, light intensity and temperature (McNeil 1991). All these conditions were identical or very close in Spanish and Greek experiments. Females from Greek populations, however, came from a region with warmer nights than those from north-eastern Spain, which has a more continental climate, and this could cause them to call later in the night. The influence of a fluctuating temperature regime on calling periodicity has been recorded in several other noctuid moths (McNeil, 1991).

The differences in the onset of calling time and age at first mating, in addition to the different male response to pheromone component ratios found in Greek and north-eastern Spanish populations (Sans *et al.*, 1997), may indicate some degree of reproductive isolation.
between the two populations. *S. nonagrioides* is sedentary, and there are clear genetic and ecophysiological differences between geographically close populations (Bués et al., 1996). These differences in courtship and mating behaviour should be considered when pheromones are used for baiting monitoring traps or for mating disruption purposes, or when local populations are investigated to elucidate pheromone composition from excised female glands or in a wind tunnel, as is the case of results reported on *S. nonagrioides* (Sreng et al., 1985, Mazomenos, 1989a; Sans et al., 1997).

*S. nonagrioides* females do not display any overt response to the males; they remain mostly stationary during the courtship and never orient themselves towards the male. Similar stationary female behaviour has also been reported in other noctuid moths such as *Phlogophora meticulosa* (L.) (Birch, 1970) and *M. brassicae* (Tóth 1982; Birch et al., 1989), whereas in others, such as *Agrotis ipsilon* (Hufnagel), females approach males when these do not respond to a calling female (Swier et al., 1976). In *S. nonagrioides*, when a calling female is not approached by a male, she continues to call until the end of the scotophase but never moves; short flights are only observed when the female rejects a male for mating. Lack of movement in unmated females recorded in other noctuid Lepidoptera (Latheef et al., 1991; Bailey et al., 1985; Birch, 1970) would explain why we rarely captured unmated females in light traps (Lopez et al., 1999).

Very little is known about the role of the male in courtship and mating in *S. nonagrioides*. In their description of mating behaviour of *S. nonagrioides*, Bablis and Mazomenos (1992) rarely refer to male behaviour. The behavioural pattern described above for the male has traits that are common with other Lepidoptera. The precopulatory flight is similar to that described for *P. meticulosa* (Birch, 1970) and *M. brassicae* (Birch et al., 1989). The «cat and mouse» behaviour described by Birch et al. (1989) is not observed in any case. Apparently, the female of *S. nonagrioides* mates with the first male that tries and does not perform sexual selection as Cardé and Haganan (1984) described for *Lymnantria dispar* (L.).

Such a passive behaviour by the female rules out some of the functions attributed to the abdominal hair pencils present in all mating males. Hence, these do not seem to stimulate the calling behaviour of the female (Szentesi et al., 1975), since the female normally called when the males were tethered and did not display their hair pencils or when they were caged without the males.

Male hair pencils do not seem to have a female-attractant capacity because when the male lands close to her, the female always remains stationary and she never moves towards the male. As proposed by Phelan and Baker (1987) for several Lepidoptera, the presence of hair pencil volatiles in *S. nonagrioides* may increase its reproductive isolation from other sympatrically occurring noctuid moths with common pheromone components. These include *P. unipuncta*, in which hair pencils have also been observed (Fitzpatrick and McNeil, 1988), and which are captured in traps baited with *S. nonagrioides* virgin females in the study area (Guerreiro et al., 1986). Independently of the exact role of male hair pencils in courtship and mating, it does not seem that the male is able to attract the female, even when the female can see it. This would mean that disrupting the male’s orientation to the female by permeating the air with pheromone could prevent mating.

Visual cues do not seem to play an important role in the mating success of *S. nonagrioides*, as no statistically significant differences in mating rates were found when adult density varied in laboratory and field cages. However, when adult density increased in pheromone-permeated field cages, mating rates also increased. This could indicate that visual cues could be more decisive for female location and mating, as suggested by Farkas et al. (1974) for *Pectinophora gossypiella* (Saunders) and by Charlton and Cardé (1990) for *Lymnantria dispar* (L.). Loss of efficacy of the pheromone blend to prevent *S. nonagrioides* mating under high densities could cause mating disruption to be ineffective to control the pest; 2-4 females per 0.42 square metres seems to be the threshold above which efficacy of the pheromone blend to disrupt mating is greatly reduced. This density would correspond to a mean infestation of 2 larvae per plant in the first pest generation (if a 1:1 sex ratio in larval population is assumed). This is a quite unusually high infestation in the area according to the author’s records, but may occur in several spots of the field, considering that *S. nonagrioides* larvae distribute aggregately over the field and emerged females do not disperse greatly before mating.

In summary, several consequences for investigating the composition and role of the *S. nonagrioides* pheromone and using it in population monitoring and control arise from the results reported here.

Firstly, the optimal time in the scotophase for extraction of pheromone from female glands varies with the geographical origin of the population, and this should be taken into account when the pheromone titre
and composition is investigated by gland excision or by collecting gland effluents.

Secondly, as recently emerged females do not fly before mating, they probably mate near where they emerged. This has particularly important consequences for females emerging from overwintered individuals. These can colonise a new maize field only after mating, and therefore the application of pheromones during the first flight for mating disruption may be ineffective. On the other hand, the females that emerge in a maize field during the season probably mate within the same field. Thus, permeation of the field air with a synthetic pheromone blend may prevent mating, although it would be inefficient to prevent oviposition by fertile females coming from neighbouring fields or non-agricultural host plants.

Thirdly, as transgenic corn varieties that incorporate the insecticidal capacity of Bacillus thuringiensis Berliner (Bt) are being deployed in Spain, low mobility of unmated females may affect the efficacy of establishing untreated refuges to delay resistance to Bt in *S. nonagrioides* populations.

Fourthly, since females tethered in the present study did not show differences in mating rates in comparison with non-tethered females, this technique may be used for evaluating the efficacy of the application of pheromone blend for mating disruption as it is used in other Lepidoptera (Rauscher and Arn, 1979).

Fifthly, females were found to be attractive to males as early as the first scotophase after emergence, and thus recently emerged females can be used as controls in field-testing synthetic pheromones. Since a period of 2-3 days of protandry may be expected in spring or summer field conditions, females will be ready to mate soon after emergence and mating disruption techniques should be applied when the first males are caught in pheromone traps.

Sixth and finally, air-permeation with two components of the pheromone blend of *S. nonagrioides* at a dose of 80 g per ha is able to disrupt mating at low pest densities but may be inefficient at high pest densities. In high pest situations, the efficacy of higher doses should be evaluated.

**Acknowledgments**

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