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SEASONAL PATTERNS OF OVIPOSITION AND EGG HATCHING RATE OF *AEDES ALBOPICTUS* IN ROME

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ABSTRACT. Since its introduction in Italy in 1990, *Aedes albopictus* has spread quickly across the northern and central regions of the country. The Italian populations of the species probably originated from temperate areas and are able to survive the cold season through the production of diapausing eggs. In 1997, *Ae. albopictus* was detected in Rome, where it seems to have found ideal environmental conditions for proliferating and extending its season of activity. In 2000, we carried out a study to evaluate the length of the season favorable to the species in Rome and the factors that might induce production of diapausing eggs. Adults of the species were reported to be active from February–March to December, peaking in August–September. Female *Ae. albopictus* produced different numbers of summer eggs throughout the study. The peak of egg hatching occurred at the 35th week of the year (end of August). Starting from the 36th week, when the photoperiod dropped below 14:10 h light:dark, a rapid decrease in summer egg production was recorded; the minimum value (17%) was reached during the 1st week of October. A new rise in egg hatching was recorded in October–November, probably because of a polymorphism in the diapause response of the population of *Ae. albopictus* and to the persistence of mild temperatures up to the end of November. Eggs completely ceased to hatch in mid-December.

KEY WORDS *Aedes albopictus,* overwintering eggs, diapause, Rome, Italy

INTRODUCTION

The presence of *Aedes albopictus* (Skuse) in Italy has been known since 1990 (Sabatini et al. 1990, Dalla Pozza and Majori 1992). This species apparently was introduced in used tires (Dalla Pozza et al. 1994), and then spread rapidly across the country (Romi 2001).

Aedes albopictus was 1st detected in Rome in August 1997 (Romi et al. 1999). Within 3 years, the species had spread to the entire city and its outskirts (Di Luca et al. 2001). In Rome, *Ae. albopictus* found optimal environmental conditions for proliferation, and its presence represents the 1st example of extensive colonization of an urban area in Italy.

As in the USA (Hawley et al. 1987), Italy has been colonized by populations of *Ae. albopictus* able to overwinter through egg diapause (Romi 1995). Diapause seems to be induced mainly by a combination of photoperiod and temperature, and is adaptative in nature (Hawley 1988). Longer days and higher temperatures favor hatching of nondiapausing summer eggs, whereas shorter days (<13–14 h of daylight) and lower temperatures encourage the production of overwintering eggs (Mori and Oda 1981, Hanson and Craig 1995). Moreover, the photoperiodic response of *Ae. albopictus* from temperate areas seems to vary with latitude (Pumpuni et al. 1992). In northern Italy, the season favorable to the development of the species lasts from April–May to October–November, and eggs hatch when minimum temperatures are not lower than 10°C and the day length is more than 13 h of light (Romi 1995). The population of *Ae. albopictus* established in Rome presumably was introduced into northern Italy in shipments of used tires (Romi and Majori 1998), then adapted to the mild climate of the city, extending its season of activity.

This study, as part of a 3-year program for monitoring the presence and distribution of *Ae. albopictus* in Rome, was funded by the City Council. The study's primary objective was to evaluate the length of the season favorable to the species in Rome and to determine the factors inducing egg diapause.

MATERIALS AND METHODS

Monitoring of Ae. albopictus *in Rome:* The study was carried out between March and December 2000 (41 wk) and covered the entire urban area of the city (about 350 km^2). The presence and abundance of *Ae. albopictus* were monitored by a network of 500 ovitraps. Rome (41.9°N, 12.4°E) was divided into 20 sectors, corresponding to as many administrative units. Twenty-five ovitraps per sector were placed in selected areas most likely to support populations of the species. Black plastic flower pots (500-ml capacity) were employed as ovitraps. A strip of Masonite® (3 × 15 cm) was suspended vertically in the middle of the pots to provide a suitable surface for oviposition. Pots were filled with 350 ml of water. Every week, pots were rinsed and refilled and strips were changed and checked for egg presence. Eggs were counted by observing the strips under a dissecting microscope. Ovitraps were removed 2 wk after the last egg-positive record. To evaluate the distribution and the abundance of the species in the study area, 2 parameters were considered: the number of positive ovitraps of the total ovitraps operating, and the mean number of eggs in the total ovitraps operating. Data were collected weekly, entered in a database (Microsoft Access; Microsoft Corp., Redmond, WA), and analyzed with Arcview 3.1 GIS software (Environmental System Research Institute, Redlands, CA).

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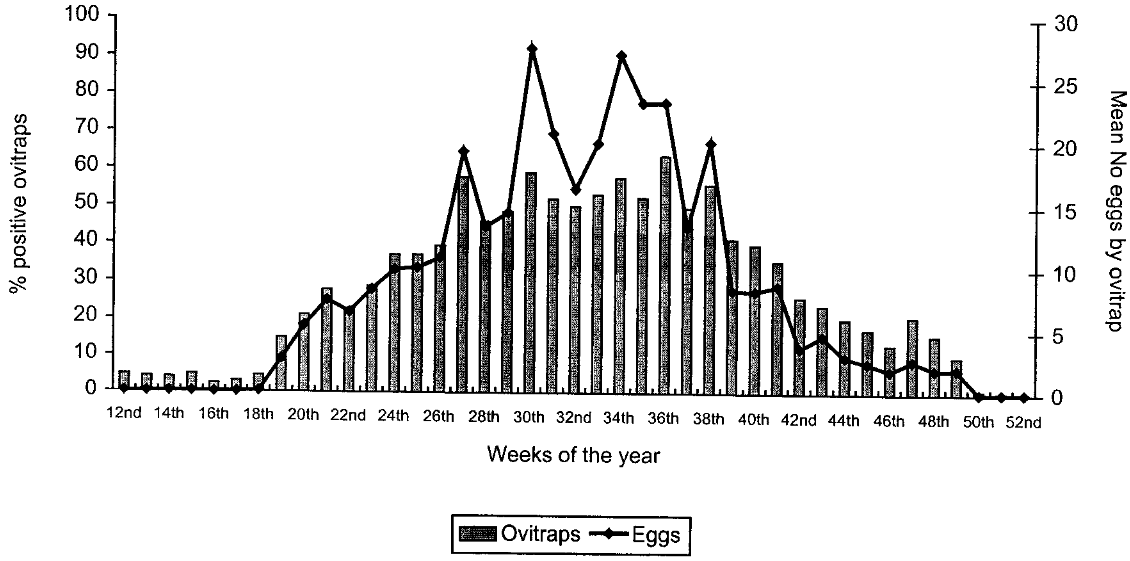

Fig. 1. *Aedes albopictus* in Rome, March–December 2000. Bars represent the mean rate of positive ovitraps; the line represents the mean number of eggs per ovitrap.

Egg hatching study: Between the 2nd half of June 2000 (25th week of the year) and the end of November, 2000 (47th week), 10 Masonite strips from ovitraps were selected weekly to evaluate the mean rate of egg hatching. Only strips with more than 50 eggs were selected from June to October; only strips with more than 10 eggs were selected from October to November. The selected strips were left to dry at room temperature for 3 days, then placed individually into plastic trays (15 × 10 × 6 cm) containing 350 ml of dechlorinated tap water. Trays were kept outdoors for 2 days in a sheltered place, after which, the number of 1st-stage larvae was counted and strips were left to dry again. This alternating wet and dry procedure was repeated twice. A total of 17,312 eggs was used for the present study. The study of egg hatching lasted about 6 months, and ended when the planned numbers of strips and eggs were no longer available. Meteorological data were provided by the Ufficio Centrale di Ecologia Agraria (UCEA) (2000).

Statistical analysis: To evaluate the relationships between the egg hatching rate and the factors being investigated (mean temperature, rainfall, and photoperiod), a linear regression analysis was carried out by adopting a stepwise procedure. The percentage of variability explained by the regression model (R^2) and its significance (F- and P-values) are reported. The correlation among experimental variables was evaluated by the Pearson's correlation coefficient (r) and its significance. All statistical tests were considered significant at the $\alpha = 0.05$ probability level. Statistical analyses were by BMDP software (Dixon 1988).

RESULTS

Figure 1 shows the trend of infestation by *Ae. albopictus* in Rome in 2000. The 1st egg-positive ovitrap was recorded in the 12th week of the year (end of March). The rate of positive ovitraps increased regularly, reaching the highest values between July and September (maximum value 63.5% at the 36th week), as did the mean number of eggs per ovitrap, which peaked in August and September (maximum value 27.6 eggs/ovitrap at the 30th week). Both parameters then decreased regularly up to the 52nd week (end of December), when the last positive ovitrap was recorded.

Figure 2 shows the weekly mean hatching rate (MHR) of summer eggs, with regard to the mean temperature, rainfall, and photoperiod. In the 1st week of the study (25th week of the year, mid-June), an MHR of 65% was recorded (mean temperature [T] = 24.2°C; 15.9:8.1 h light:dark [L:D]). In the following 10 wk, an irregular increase of the MHR, as well as an increase in the mean temperature, was recorded. The highest MHR (>90%) was recorded at the 35th week (late August–early September). Starting from the 36th week (mean T = 20.6°C; 13.6:10.4 h L:D), a nearly constant decrease of the MHR was recorded. This trend reached the minimum value (17%) at the 40th week (early October), with a photoperiod of about 12.2:11.8 h L:D and a mean temperature of 18.5°C. From the 41st week up to the 45th week (1st week of November: mean T = 15.9°C; 10.5:13.5 h L:D), a new increase in the MHR was recorded. A new decrease in the MHR occurred in the last 2 wk of the study, which was interrupted at the 47th week with an MHR of 21.1% (mean T = 12.3°C; 10:14 h L:D). In the following weeks, our monitoring system reported a rapid reduction of both rate of positive ovitraps and the mean number of eggs per ovitrap (Fig. 1). Very few eggs still hatched at the 48th

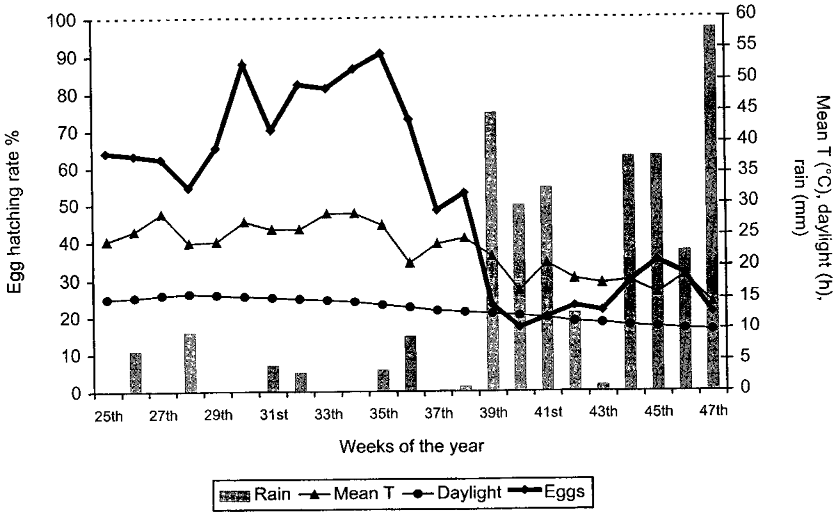

Fig. 2. Mean hatching rate of eggs of *Aedes albopictus* in Rome, June–November 2000, with regard to mean temperature, rainfall, and photoperiod.

and 49th weeks and eggs completely ceased to hatch at the 50th week, when the temperature dropped down below 10°C (data not reported).

The linear regression analysis showed a significant correlation ($R^2 = 0.73$; $F = 58.18$; $P < 0.0001$) between MHR and mean temperature (Fig. 3), whereas no significant relation was found with rainfall or photoperiod. A strict correlation between mean temperature and the other 2 variables was found by the Pearson's test ($r = 0.88$, $P = 0.0001$ and $r = -0.73$, $P = 0.0001$ for rainfall and photoperiod, respectively).

DISCUSSION

In 2000, the 1st record of eggs of *Ae. albopictus* in an ovitrap was reported in the last week of March (12th week of the year). Considering that development time from egg hatch until pupation may be as long as 3 wk at temperatures from 14 to 18°C (Hawley 1988), and that development may cease at temperatures below 11°C (Hawley 1988), the assumption can be made that the 1st overwintering eggs in Rome hatched between the end of February and the beginning of March 2000, when day length was 11–11.5 h of light and the mean temperature was 10–11°C. Both the parameters considered for monitoring the presence and the relative abundance of the species, rate of positive ovitraps and mean number of eggs per ovitrap, followed a quite regular trend during the season, and peaked between August and September (Fig. 1), which correspondened to or immediately followed

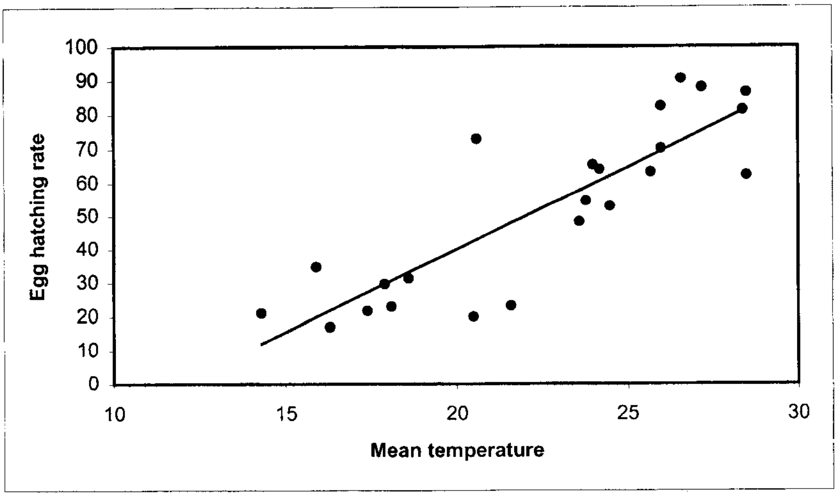

Fig. 3. Linear relation between the mean hatching rate of eggs of *Aedes albopictus* and mean temperature in Rome, June–November 2000 ($R^2 = 0.73$, $P < 0.001$).

the highest mean temperatures of the summer of 2000. Adult females of the last seasonal generation survived up to the end of December (mean T = 8.5°C, 9:15 h L:D), and the last eggs were collected in 2 ovitraps at the 52nd week of the year (December 27, 2000).

Female *Ae. albopictus* produced different numbers of summer eggs throughout the study (Fig. 2). The rate of egg hatching apparently was correlated with the mean temperature. Although photoperiod and precipitation did not correlate with egg hatching ($P > 0.05$), the critical photoperiod, at which the production of summer eggs quickly starts decreasing, may be recorded at the 36th to 37th weeks (mid-September) when day length drops below 14 h of light.

However, examination of data collected in the last (47th) week of the study showed that almost one fifth of the eggs (21.1%) still hatched in late November, at less than 10 h of daylight but with a mean temperature higher than 10°C. Production of summer eggs ceased completely when the mean temperature dropped below 10°C. In Rome, the mean monthly temperatures of November and December usually are almost 3°C lower than those reported in 2000 (UCEA 2000).

Geographical variations in critical photoperiod among strains of *Ae. albopictus* have been reported in the existing literature (Focks et al. 1994, Hawley 1988). The photoperiodic response is polymorphic, and varies with longitude (Hawley et al. 1987) and latitude (Hawley et al. 1989). Hatching also is influenced by temperature (Pumpuni et al. 1992). The dramatic decline in egg hatching rate that occurred in our study between the 36th and 39th weeks suggests that photoperiod is the primary cue for inducing the production of winter eggs. Nevertheless, the reduced but sustained egg hatching recorded between the 39th and 47th weeks suggests that the population of *Ae. albopictus* in Rome is polymorphic for the photoperiodic response. The mild temperatures recorded in the autumn of 2000 could have allowed a small number of mosquitoes that require a shorter photoperiod for diapause induction to lay summer eggs up to late November. Also, the experimental hatching stimulus possibly has been stronger than that experienced by natural populations, even if we tried to reproduce natural conditions. Although our study on egg hatching stopped at the end of November, examination of the data collected by the monitoring nets of ovitraps indicates that below the mean temperature of 10°C (December), even these short-diapausing eggs cease hatching.

We conclude that *Ae. albopictus* is active in Rome for up to 10 months of the year, from March to December, and that prolonged mild temperatures also may allow a fraction of the mosquito population to lay summer eggs in spite of a short day length (<10 h of light). Predicted global climate changes and the rapid adaptation of Italian populations of *Ae. albopictus* to the local latitude could further extend the length of the season favorable to this species and enhance its performance if precipitation remains dependable (Alto and Juliano 2001).

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