Threshold Temperature for Post-Diapause Development and Degree-Days to Hatching of Winter Eggs of the European Red Mite (Acari: Tetranychidae) in Northern Greece

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ABSTRACT The threshold temperature for postdiapause development in overwintering eggs of the European red mite, *Panonychus ulmi* (Koch), was determined after exposing the eggs to various constant temperatures (5, 10, 15, 20, and 25°C) in the laboratory. The mean number of days to 50% hatch in each temperature was >120, 44.5, 22.0, 14.5, and 8.1 d, respectively. From the regression of postdiapause developmental rate to temperature, it was estimated that 50% of egg hatch coincides with the accumulation of 154.6 degree-days (DD) above the threshold of 7.4°C. In the field (1991 and 1993–1996), 50% egg hatch occurred from the end of March to 20 April after the accumulation of a mean sum of 129.4 ± 4.5 DD above the threshold of 7.4°C from 10 February onward. The starting date of 10 February for heat accumulation was chosen because earlier experiments had shown that diapause is terminated in 50% of the eggs in the first half of February. Using the theoretical sum of 154.6 DD, the predicted dates for 50% egg hatch deviated from field observations by an average of 3.7 ± 0.5 d over the 5 yr of experiments.

KEY WORDS *Panonychus ulmi,* European red mite, degree-days, hatching, winter eggs

THE EUROPEAN RED mite, *Panonychus ulmi* (Koch), is an important foliar pest of apple trees feeding on the palisade and spongy mesophyl cells and causing a reduction in the photosynthetic rates of the plant (Kuenen 1949, Avery and Bringgs 1968, Campbell and Marini 1990, Marini et al. 1994 and references therein). It overwinters as an egg in diapause. Diapause eggs are laid in late summer to early autumn on the twigs and branches of the host plants mainly on roughened areas and close to the buds. In early spring the overwintering eggs hatch and the young larvae move to unfolded leaf clusters and start feeding (Jeppson et al. 1975).

Application of chemical acaricides early in spring against newly hatched larvae is a widely used method for the control of this mite. The development of a degree-day (DD) model for predicting the time of egg hatch in the field could be a useful tool for the proper timing of acaricide applications.

In the current study we determined the threshold temperature for postdiapause development and subsequently using laboratory and field data estimated a sum of degree-days for predicting the time of egg hatch. The accuracy of the degree-day model for predicting egg hatch was validated under field conditions.

Materials and Methods

Threshold Temperature for PostDiapause Development. Twigs bearing diapause-eggs were cut from an apple orchard in the area of Alexandria (Northern Greece) in the second half of February of 1994, and subsequently transferred to the laboratory. The course of diapause termination in the field was determined in earlier experiments by transferring diapause eggs during winter and early spring for 4 yr (1992–1996) from the same and other apple orchards to short days (a photoperiod of 8:16 [L:D] h) at 20°C where percentages of diapause termination were determined (Koveos and Broufas 1999). These experiments have shown that by mid-February diapause development is terminated in 50% of the eggs and by the end of February in almost all the eggs irrespective of the year and location from which the eggs originated. Small pieces of bark bearing 10–40 eggs were cut and glued on pieces of paper fixed on the bottom of open petri dishes. Approximately 100 eggs were kept in each dish. The dishes with the eggs were transferred to a series of constant temperatures (5, 10, 15, 20, and 25°C and constant darkness) in incubators. At the level of the eggs, the relative humidity was ≈70%.

Every third day the percentage of eggs hatch at each temperature was determined. The data were then used to estimate the mean number of days required for 50% egg hatch ($t_{50\%}$) at each temperature. The reciprocal, $1/t_{50\%}$, represented the daily rate of postdiapause embryonic development.

The threshold temperature for postdiapause development was estimated by the x-intercept method (Arnold 1959). According to this method, we assumed that in the range of the temperatures tested, the relationship between the rate of postdiapause development ($1/t_{50\%}$), and temperature is linear. From this linear regression, we determined the value of the x-intercept, below which no postdiapause development occurred, i.e., the threshold temperature for postdiapause development. The reciprocal of the slope of the regression line represented the sum of degree-days required for 50% egg-hatch. The standard error of degree-days for 50% egg hatch was estimated according to Campbell et al. (1974).

Field Hatch of Overwintering Eggs. During spring in 1991 and 1993–1996, hatch of overwintering eggs was monitored in different apple orchards around of Alexandria. For monitoring egg hatch, twigs bearing overwintering eggs of *P. ulmi* were cut from the orchards in early January and transferred to the laboratory. Within the same day, small pieces of bark bearing diapause eggs were cut and fixed on the bottom of a petri dish as described above. The petri dishes with the diapause eggs were transferred back to the orchard and tied on apple trees at a height of ≈1.5 m from the ground. Every week, from the beginning of March to the end of April, the petri dishes were inspected under a stereoscope in the field, and the number of eggs that had hatched was recorded. Each year 800–1,000 ovewintering eggs were monitored.

Degree-Days Under Field Conditions. Daily air temperatures were recorded at 1.5 m above ground, by a meteorological station located ≈7 km away from the experimental orchards. Both the station and the orchards were at sea level, and no hills or large bodies of water (lakes), which might affect the microclimate existed in the region. Starting 10 February, degree-day summation above the threshold temperature (7.4°C) was calculated by fitting a sine wave to the daily air temperature minima and maxima (Allen 1976). The starting date of 10 February for the accumulation of degree-days was chosen because as explained above earlier experiments had shown that diapause termination in 50% of the eggs in that area occurred in the first half of February (Koveos and Broufas 1999). For each year the sum of degree-days required for 50% egg-hatch was estimated. Percentages of egg-hatch were plotted against the respective sum of degree-days using the 5-yr data.

Voucher specimens of *P. ulmi* were deposited in the Museum of Natural History, London.

Results and Discussion

Effect of Temperature on PostDiapause Development. The effect of temperature on the number of days required for 50% egg hatch and the rate of postdiapause development is shown in Fig. 1. The regression of the rate of postdiapause development to temperature was significant ($y = 0.0064x - 0.0483$; $r^2 = 0.956$) ($F = 38.32$; df = 1, 3; $P < 0.05$). The threshold temperature estimated from this regression was 7.4°C. The reciprocal of the slope of the regression line was 154.6 (SE = 27.7), which represents the theoretical sum of the degree-days required for 50% egg-hatch (Campbell et al. 1974).

The threshold temperature for postdiapause development of *P. ulmi* from other countries such as Canada (Herbert and McRae 1982), Japan (Tsugawa et al. 1966), and England (Lees 1953; Cranham 1971, 1972) varied from 5.5 to 7°C. Our estimation of the threshold temperature is near the upper end of the reported values, which may indicate a low interpopulation variability in the threshold temperature for postdiapause development of *P. ulmi*.

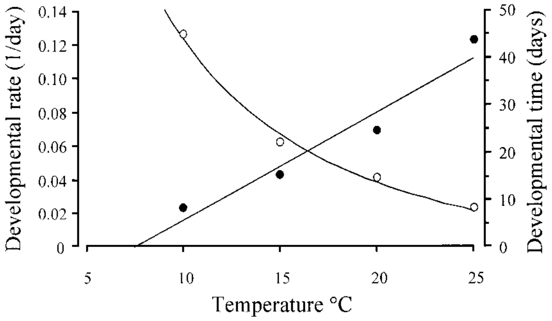

Fig. 1. Temperature-dependent developmental time (○) ($y = 601.17/x - 16.28$; $r^2 = 0.994$) and developmental rate (●) ($y = 0.0064\,x - 0.0483$; $r^2 = 0.956$) for postdiapause overwintering eggs of *P. ulmi*.

The Course of Egg Hatch in the Field. Cumulative percentages of egg-hatch in different dates during the spring for 5 yr are shown in Fig. 2A. The hatching period varied on the different experimental years. Fifty percent of egg hatch occurred in late March to early April in 1994 and 1995, ≈10 d later (9–12 April) in 1991 and 1993 and even later (19–21 April) in 1996. The first hatch occurred ≈1–2 wk before 50% egg hatch. Fig. 2B shows the cumulative percentages of

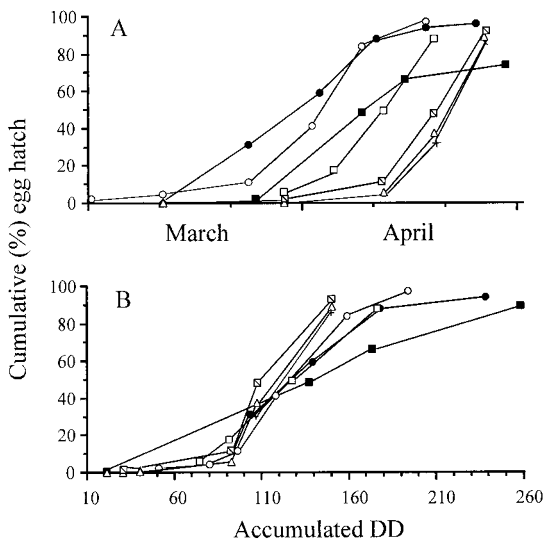

Fig. 2. Cumulative percentage of overwintering egg hatch of *P.ulmi* in the field against calendar dates (A) and degree-days (B). Egg hatch was monitored in different apple orchards in the area of Alexandria (northern Greece) for 5 yr (1991: (-○-), 1993: (-●-), 1994: (-□-), 1995: (-■-), 1996: orchard 1(-△-), orchard 2(-X-), orchard 3(-⊠-).

egg-hatch and the respective degree-days accumulated from 10 February above a threshold temperature of 7.4°C for each year of observation. The initiation of egg hatch in all the years occurred ≈80 DD.

The variability in the dates for egg to hatch in different years may be due, among other reasons, to differences in field temperatures in spring. For example, in most days of March 1996, the mean daily temperature was below 7.4°C, which may explain the observed delay in the initiation of egg hatch in that particular year.

In all the experimental years, the period between the initiation and the completion of egg-hatch was ≈2 wk. This relative short period of egg hatch may be the result of the fact that although diapause is terminated from the beginning to the end of February (Koveos and Broufas 1999), postdiapause development is retarded by the low field temperatures until March when temperatures rise and probably synchronize egg hatch.

The period of egg hatch in the field varies widely in different regions of the world. It was 6 wk in England (Light et al. 1968), 48–53 d in New Zealand, 1–2 wk in Canada (Herbert and McRae 1982 and references therein), and 10 d in Japan (Tsugawa et al. 1966). The above differences in the egg hatch period could be caused among other reasons by the genetic variation in diapause intensity of the different populations. Cranham (1973) supposed that the relative timing of egg-hatch must be based on inherited variation in the heat sum required for the completion of diapause development, whereas the heat sum required for postdiapause morphogenesis probably shows very little variation within the species, as in other species studied (Danilevski 1961). This geographical variation in egg-hatch is probably the result of different populations that have adapted to local climates.

The short period of egg-hatch allowed us to develop a predictive degree-days model that may be used in Greece to predict accurately the proper time of acaricide applications against the susceptible and exposed young larvae. In England, such an attempt failed earlier because of the long period of hatch and the high variation in heat unit requirements for egg-hatch among different orchards or sucessive years (Light et al. 1968, Cranham 1972). However, Cranham (1973) suggested that forecasting the egg hatch in the spring may be possible for individual British orchards.

Field Validation. To test whether the estimated values of degree-days can predict egg hatch in the field, we use the theoretical sum of 154.6 DD and determined the expected dates for 50% egg hatch from 10 February. During the 5-yr experiments, the mean sum of DD above 7.4 from 10 February until 50% egg hatch was 129.4 ± 4.5 (mean ± SE), which is lower than the 154.6 ± 27.7 (Table 1). The predicted dates deviated from the observed ones by 3.7 ± 0.5 d for 5 yr of experimentation (Table 1). In all the years, bias in prediction of 50% egg hatch was positive, which could be caused, to some extent, by residual diapause in the groups of eggs used in the laboratory for the construction of the model or to a microclimate effect

Table 1. Observed and predicted dates for 50% egg-hatch of *P. ulmi* and degree-days accumulated from 10 February, above the threshold temperature 7.4°C

Year	Observed		Predicted dates for 50% egg-hatch	Predicted-observed, d
	Dates for 50% egg-hatch	DD		
1991	9 April	151.2	10 April	1
1993	12 April	131.5	15 April	3
1994	30 March	130.8	4 April	4
1995	3 April	133.9	7 April	4
1996				
Orchard 1	19 April	112.4	25 April	6
Orchard 2	21 April	123.3	25 April	4
Orchard 3	21 April	123.3	25 April	4
Mean ± SE		129.4 ± 4.5		3.7 ± 0.5

Predicted dates for 50% egg-hatch were determined using the theoretical sum of 154.6 DD.

because inside tree canopy temperature could be slightly higher. Based on our previous experiments (Koveos and Broufas 1999), eggs were transferred from the field to different constant temperatures in the laboratory in the second half of February and diapause in these eggs was terminated by that time. However, because there is no apparent morphological change, indicating the transition from diapause to postdiapause development, we could not exclude the possibility that in some of the eggs diapause was not terminated and a higher sum of degree-days was required for hatch.

Our degree-day model can predict the time of 50% overwintering egg hatch with an acceptable accuracy and may be used for the proper timing of acaricides application. Degree-day accumulation for all the 5 yr was combined, and a nonlinear regression procedure was used to fit a logistic model (Fig. 3). This model will provide an even more accurate prediction of overwintering egg hatch. From the regression line, we can estimate the number of degree-days required for different percentages of egg-hatch and predict their respective dates.

Optimal timing of spray applications against winter eggs of *P. ulmi* based on the phenology of the pest could reduce the over all number of spray applications during the growing season and thus reduce the cost of

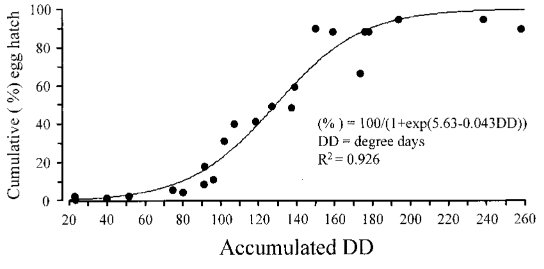

Fig. 3. Cumulative percentages (%) of overwintering egg hatch of *P. ulmi* and degree-days accumulated from 10 February for 5 yr (1991, 1993–1996) (base temperature 7.4°C) in the area of Alexandria, northern Greece.

apple production and risk for the environment. From that end, our degree-day model could be very useful for a more effective control of the European red mite in Greek apple orchards.

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