Separation of photoperiodic and circadian effects on the termination of diapause in the spider mite *Tetranychus urticae*

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Summary. The same critical nightlength was found for both photoperiodic induction and termination of diapause in *Tetranychus urticae*, indicating that the same clock mechanism operates during both induction and termination of diapause in these mites. Resonance experiments revealed the involvement of the circadian system in the photoperiodic termination of diapause. The period of the circadian oscillation involved in diapause termination was about 1.5 h shorter, however, than that of the oscillation involved in diapause induction. Moreover, the time course of the appearance of the first peak of diapause termination appeared to be different from that of the subsequent peaks in the resonance experiment, suggesting that the mechanism of diapause termination in regimes with nights shorter than the critical nightlength is different from that with nights longer than the critical nightlength. These results lead to the conclusion that the circadian system does not operate as the photoperiodic clock in these mites, as suggested already by experiments on diapause induction.

Key words. Photoperiodism; diapause; circadian rhythms; resonance effect; diapause termination; spider mites; *Tetranychus urticae.*

Involvement of the circadian system in photoperiodic phenomena has been demonstrated for various organisms, although rhythmic effects on photoperiodic responses are far from universal[1-4]. The most powerful technique to reveal these effects is the so-called resonance experiment, in which a photophase of constant length is coupled with a scotophase of variable duration, e.g. from 4 up to 80 h or more. Rhythmic variations observed in the photoperiodic reaction indicate an influence of the circadian system on the photoperiodic response concerned. Although these rhythmic responses have generally been interpreted as evidence that photoperiodic time measurement is a function of the circadian system, this interpretation has recently been challenged[5,6]. An alternative explanation of the rhythmic effects which have been observed in resonance experiments is given by the so-called non-clock hypothesis, in which the influence exerted by the circadian system on the photoperiodic response is interpreted as an indirect or 'resonance' effect. According to this hypothesis the photoperiodic clock, at least in insects and mites, is a non-rhythmic 'hourglass' timer measuring nightlength, whereas the influence of the circadian system is restricted to a modifying effect on the expression of the photoperiodic response, to be seen only in certain light-dark cycles with very long periods, such as those used in resonance experiments[4,7], and under conditions of temperature which are much more restricted than those for the photoperiodic response proper[2,8,9]. Evidence in favor of the 'non-clock' hypothesis has recently been found in experiments on diapause induction with the spider mite *Tetranychus urticae*[10] and the large white butterfly *Pieris brassicae*[9]. Here we present further evidence, resulting from an experiment in which the time course of the photoperiodic termination of diapause in *T. urticae* is measured in a resonance experiment.

Material and methods

The experiments were done with a Russian strain of *T. urticae*, originating from Leningrad (60 °N). History of the strain and maintenance of the mites in the laboratory have been described before[7]. For the experiments the mites were kept on detached leaf cultures of bean (*Phaseolus vulgaris*). Eggs, differing no more than 24 h in age, were maintained for four days under rearing conditions (LD 17:7, 25 ± 1 °C); they were then ready to hatch. Subsequently the cultures were transferred to the diapause inducing short-day regime of LD 10:14, 19 ± 1 °C, where they remained for the entire post-embryonic development of the mites. Diapause incidence under these conditions is practically 100%. Diapausing females were stored for 3 weeks at 4 ± 1 °C, after which they were transferred onto fresh bean leaves and kept under various light-dark regimes, as indicated in the section 'Results and discussion', at 19 ± 1 °C in a number of lightproof cabinets in a climatic room. Air of constant temperature and humidity was forced through the cabinets. Each cabinet was equipped with one fluorescent tube of 8 W, separated from the working space of the cabinet by a plexiglass screen, and controlled by an electronic timer capable of automatically generating all diel and non-diel light-dark cycles required. Light intensity at the level of the mites varied from 600 to 1000 lux. Approximately 100 females were subjected to each photoperiodic treatment. The mite cultures were examined at regular intervals of 3–4 days and the number of females which had terminated diapause was determined, according to the criteria for diapause termination as described before for a Dutch strain of *T. urticae*[11]. In case the examination had to take place during the scotophase of one of the test cycles a weak dark red light was used which had been found to be without effect on the photoperiodic response (Veerman, unpublished results).

Results and discussion

Previous work had already shown that termination of diapause in *T. urticae* can be achieved by long daylengths ('photoperiodic activation'[12]) after a short cold rest of 2–3 weeks[11,13]. In figure 1 the response curve for the photoperiodic termination of diapause in the Leningrad strain of *T. urticae* is presented, together with the response curve for the induction of diapause in the same strain. Both curves not only show exactly the same critical nightlength of 7.75 h, but the transition of the response around the critical nightlength is equally sharp for both diapause induction and diapause termination. These facts taken together form a strong indication that the same photoperiodic clock is functioning in both processes. Similar results have been found for a number of insect species[14–16], although with other insects more or less pronounced differences were observed between the two response curves[17–21]. However, for the European corn borer, *Ostrinia nubilalis*, it has been shown that whether or not the same critical nightlength is found for both diapause induction and diapause termination may depend on the temperature at which the experiments have been done[16].

Next, the termination of diapause was determined in a resonance experiment with a constant photophase of 12 h and a scotophase which was varied from 4 to 84 h, in steps of 4 h (fig. 2). A clear resonance pattern was observed after 34 days, with 5 peaks of diapause termination at regular intervals of 16 h (fig. 2h). In previous work a similar resonance experiment for the induction of diapause in the same strain of mites, performed at the same temperature, had also produced a clear rhythmic response, with peaks 17.5 h apart[7]. Although at the time 17.5 h was considered to be an extremely low value for the period of a circadian oscillation and possibly the lowest encountered in any animal, the value of 16 h found here in the resonance rhythm for the termination of diapause in the same strain of mites is still considerably lower and is equalled only by the period of the oscillation observed in a resonance experiment on diapause induction in the linden bug, *Pyrrhocoris apterus*[22]. It may even raise some doubts about the nature or entrainability of the rhythm concerned. The τ-value of this rhythm appears to be far outside the range of the τ-values (17.5–21.5 h) determined for the induction of diapause in resonance experiments with 10 geographic strains of *T. urticae* from Western and Central Europe[22].

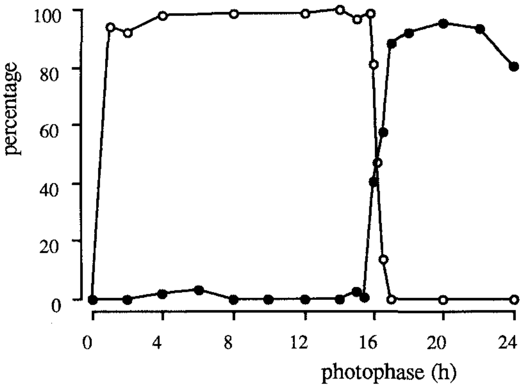

Figure 1. Photoperiodic response curves for the induction of diapause (open circles) and the termination of diapause (closed circles) in a Russian strain of the spider mite *Tetranychus urticae*.

The difference of 1.5 h found between the periods of the rhythms involved in diapause induction and termination in one and the same strain of mites, determined at the same temperature, makes it very unlikely that the same circadian oscillator is involved in both processes. Thus, since the experiment shown in figure 1 presented strong evidence that the same clock mechanism is involved in both photoperiodic induction and termination of diapause, it becomes very unlikely that the oscillators involved function as the photoperiodic clock.

Similar experiments with two insect species, *O. nubilalis*[21,24] and *P. brassicae*[25], produced no evidence for any involvement of the circadian system in diapause termination, which led the authors to conclude that the photoperiodic clock involved in diapause termination is based on an hourglass mechanism. Only in one instance, in a resonance experiment of rather limited extent with the crane fly *Tipula simplex*, was some indication of the involvement of the circadian system in the photoperiodic termination of diapause obtained, but comparison with the results of other authors is difficult because in the experiment with *T. simplex* the dark phase was held constant (at 6 h) and the light phase was varied between 6 and 42 h, in steps of 12 h[26].

Although the peaks of diapause termination in the Leningrad strain of *T. urticae* show a tendency to appear one by one, those at shorter nightlengths first and those at longer nightlengths later in time, there is a striking difference in the kinetics of the appearance of the first peak (at the 'short' nightlength of 4 h) and the subsequent peaks at the 'long' nightlengths of 20, 36 and 52 h (fig. 2). Whereas the first peak, after a slow start between days 7 and 10, increases very rapidly over a few days time, almost reaching saturation at day 14 after which the increase slows down to attain complete saturation at day 22, the increase of the peaks at longer nightlengths is not only much slower but also more gradual and more-or-less linear with time (fig. 3). This leads us to conclude that the first peak of diapause termination is governed by another mechanism than that which governs the second and subsequent peaks: the first peak represents the photoperiodic reaction proper, i.e. the termination of dia-

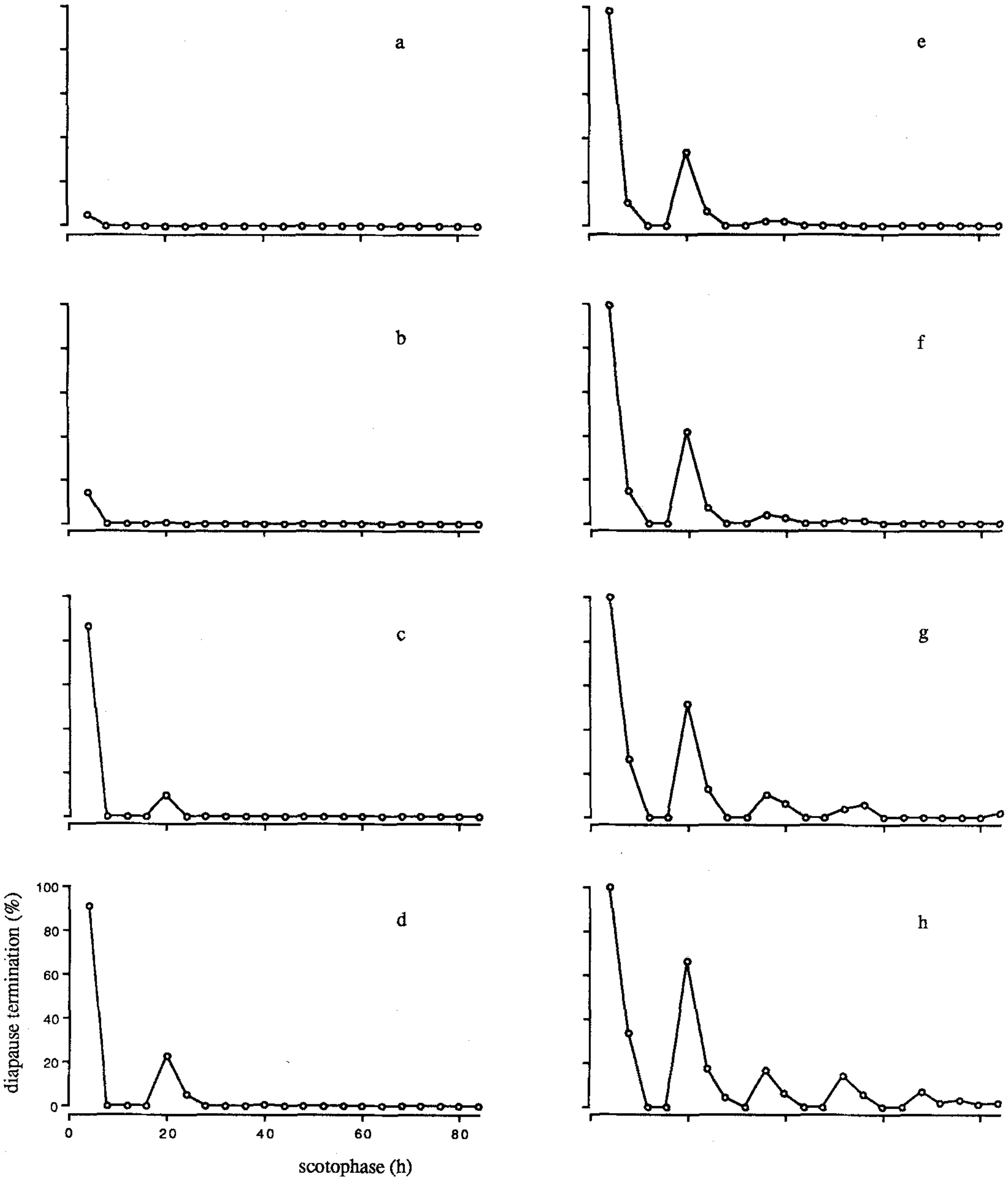

Figure 2. Termination of diapause in a Russian strain of the spider mite *Tetranychus urticae* in a resonance experiment with a constant light phase of 12 h and a dark phase which was varied from 4 to 84 h, in steps of 4 h. The experiment started with the transfer of the mites from cold storage (3 weeks at 4 ± 1 °C) to 19 ± 1 °C (day 0). The number of females that had terminated diapause, expressed as a percentage of total females present, was determined after 7 days (a), 10 days (b), 14 days (c), 18 days (d), 22 days (e), 26 days (f), 30 days (g) and 34 days (h).

pause in response to the measurement of a short night, whereas the peaks of diapause termination occurring at nightlengths longer than the critical nightlength, at 20, 36 and 52 h, are 'resonance' peaks, caused by some action of the circadian system on the photoperiodic mechanism. The results presented in figure 2 cannot be explained on the basis of 'classical' coincidence models for the photoperiodic clock, as in these models the subsequent resonance peaks are considered as true long-day (or short-night) effects, caused by either the coincidence of the 'photoinducible phase' ϕ_i with light ('external coincidence'[2]) or of certain phase relationships between the constituting oscillators characteristic of long-day measurement ('internal coincidence'[3,8]). As shown above, however, the second and subsequent peaks of diapause termination are not explicable as the result of 'normal' short-night (long-day) measurement. They are more likely to be caused by some deregulating effect of the circadian system on the long-night response, occurring at some cycle lengths, but not at others ('resonance' effect[5,6]). It should be kept in mind that the function of the photoperiodic response investigated in this paper is most probably the maintenance of diapause in autumn under the influence of long nights[11,13]. The photoperiodic termi-

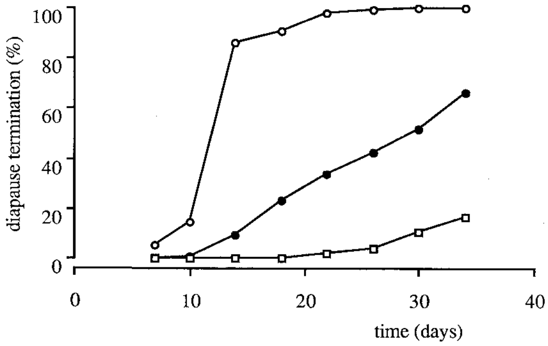

Figure 3. Time course of diapause termination at the first maximum at LD 12:4 (open circles), the second maximum at LD 12:20 (closed circles), and the third maximum at LD 12:36 (open squares) in the resonance experiment presented in figure 2.

nation of diapause or 'activation' by short-night cycles is clearly an artificial effect; photoperiod is not the factor responsible for diapause termination in nature in these mites, since the sensitivity to photoperiod in diapausing females of *T. urticae* has already disappeared before midwinter[11, 13]. The appearance of the resonance peaks in figure 2 may therefore be seen as a circadian disturbance of diapause maintenance in these mites.

The occurrence of the first peak of diapause termination in figure 2 seems to be a threshold phenomenon, whereas that of the other peaks is not (cf. fig. 3). About 12 short-night cycles appear to be necessary to obtain 50% diapause termination. This might be seen as an indication that some kind of 'counter' mechanism is involved in the photoperiodic termination of diapause, like that which has previously been shown to form part of the induction mechanism[6,10]. However, further experiments will be required to analyze the kinetics of this supposed photoperiodic counter for diapause termination in *T. urticae*.

Evidence in favor of the non-clock hypothesis mentioned in the introduction, based on various experiments concerning the induction of diapause in insects and mites, has been presented in previous studies[9, 10]. The outcome of the experiments on diapause termination in *T. urticae* presented here again leads to the conclusion that, although also in diapause termination an influence of the circadian system on the photoperiodic reaction is clearly present, the circadian system apparently does not function in photoperiodic time measurement in these mites but exerts only an indirect effect on the photoperiodic mechanism, which is best interpreted as a disturbance of the normal photoperiodic response in certain non-diel regimes.

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