Boll. Zool. agr. Bachic.
Ser. II, 22 (1) (1990): 31-41

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Diapause in *Amblyseius andersoni* Chant (= *A. potentillae* Garman) (Acarina Phytoseiidae) in the climate of the Po Valley

Diapause in phytoseiid mites has been and is being studied by various authors, all of whom have noted in the various species a «facultative diapause» which appears during winter among inseminated females and is induced by certain photoperiod and temperature conditions that work upon the early stages of development by means of an anticipatory mechanism. Consequently the response to the ecological conditions that induce diapause in different geographic populations of the same species varies according to the latitude. Beck (1968) affirms that the «critical photophase» for the induction of diapause in insects tends to increase at the rate of 1.0 to 1.5 hours for every 5° increase in latitude. In like manner Croft (1971) noted similar differences in the critical photophase of diapause among populations of the phytoseiid *Typhlodromus occidentalis* Nesbitt collected at different latitudes.

Recent research has shown that *Amblyseius andersoni* Chant and *A. potentillae* Garman are synonymous (McMurtry, 1977; Messing & Croft, in press); Chant & Yoshida-Shaul (1990), on the basis of the type material and Principal Component Analysis based on 21 morphometric characters, conclude that «the two forms are conspecific and that *A. potentillae* is a junior synonym of *A. andersoni*».

A. andersoni Chant is a valid predator of phytophagous mites and is widespread in many regions of North Italy. This species has interesting biological and ecological characters and quite easily segregates OP-resistant populations (Ivancich Gambaro, 1986).

Familiarity with the hibernations of this phytoseiid, and knowing whether or not it goes into diapause, are fundamental to a knowlegde of its phenology and of the dynamics of its populations, and aid us in ascertaining the possibility and means of transporting and spreading this species in winter.

Diapause in *A. andersoni* was studied by McMurtry et al. (1976); a «race» from the Netherlands living in short-daylight conditions was compared to another «race» from Fondi (Italy), that does not go into diapause in winter. The aim of this research was to find a relationship between the hibernation conditions and the different latitudes between the Netherlands (52° N) and Fondi (Italy) (41° N).

Diapause in *A. potentillae*, studied in laboratory research by Dutch authors, takes place in the Netherlands with a critical photophase of 14 hours at 18 °C (Van Zon et al., 1988). Recent research has revealed the importance of the thermophase in the induction of diapause of this species, in particular the temperature of the «scotophase», which can be considered a determining factor (Van Houten et al., 1988).

Given the above, I thought it would be quite interesting and useful to study the hibernation conditions and the possible existence of diapause in *A. andersoni* in the Po Valley climate in Verona (45° 23'N), the latitude of which is about 7° lower than that of the Netherlands and 4° higher than that in Fondi.

MATERIALS AND METHODS

The research was made on overwintering females collected from peach trees in the Verona area by means of band traps that were tied to the plant (in the second and third 10-day periods of September). The females were then taken to the laboratory with a photoperiod of 8 hours, a temperature of 20 °C, 80-90% RH, and were fed *Mesenbrianthemum* pollen.

The mites were put in small Petri dishes (diameter 5 cm) sealed with birdlime, set in plastic boxes and placed on foam-rubber floating in water; everything was then wrapped in plastic bags so as to maintain the RH level.

The temperature was automatically programmed and lighting came from a 20W cool light fluorescent bulb.

The females were put in the Petri dishes in groups of 20-25; during the research those that appeared to be gravid were gradually isolated so as to be observed individually. Observation was effected every 24 hours, always during the photoperiod, and lasted 10-15 minutes. Each experiment was repeated two or more times.

As has already been noted (Hoy & Flaherty, 1970), the 8-hour photoperiod does not exist at temperate zone latitudes, but it clearly reveals the occurrence of diapause. On the other hand, the temperature, RH and photoperiod conditons I used were chosen so as to be consistent with those used by McMurtry in his comparative study of the Netherlands and Fondi, Italy populations.

My research was carried out in the winter of 1988-89. The overwintering females were collected in four periods of the winter season, from October to February, and the evaluation of the results took into consideration the following: 1) the percentage of females in diapause; 2) the period of time that elapsed from the collection of the females to the resumption of feeding; 3) resistance to lack of food.

The criterion adopted to identify the females in diapause was the absence of ovideposition in a 25-day period.

No cannibalism was noted, not even in those females kept without food.

The loss of females for various reasons during this research was roughly 8-9%, and reached 15% in Febraury.

The ascertainment of diapause in these populations was also effected by means of laboratory research beginning with the egg stage. The eggs were obtained from females kept at a temperature of 25 °C and 80-90% RH. Once again, the experiment was carried out in an 8-hour photoperiod, at 20 °C and at RH of 80-90%, and the females were fed partly with prey (*Tetranychus urticae* Koch) and partly with *Mesembrianthemum* pollen. The reaction and activity of the populations were kept under daily observation.

RESULTS AND DISCUSSION

Diapause in overwintering females

The results of this research, seen in Table 1, demonstrate that *A. andersoni* has imaginal reproductive diapause and that the percentage of diapause differs in the different periods of the winter season. In fact, whereas in October 13% of the females resumed ovipositing after a two-week period (under experimental laboratory conditions), in November 100% of the overwintering females were in diapause; in January diapause had already ended for some females, since 25% of them began ovipositing after 15-18 days; in February diapause had ended for 95% of the females. A certain variability in reaction was also noted; this obviously depended on the age of the females and when diapause began.

The above leads to the conclusion that practically after the first ten days of January part of the overwintering females would be able to resume reproductive actvity and are prevented from doing so only by the low temperature; and this occurs a long time (about 3 months) before these females can normally oviposit on the host plant. Such potential precocious reproductive activity has also been noted in other species of both phytoseiids and insects.

These findings also show that for *A. andersoni*, as well as for other species of phytoseiids, the cessation of diapause does not depend upon being exposed to a cold period and that, as Beck (1980) observed in the case of certain insects, the evolution of diapause is accompanied by delayed maturation of the ovaries.

The gradual resumption of activity in overwintering females in diapause is also quite clearly manifested in the more or less marked delay in resuming their

Table 1 - Percentage of overwintering females in diapause at different periods of winter (photop. 8h, temp. 20 °C, RH 80-90%).

Date	n.	% in diap.	% dead
2nd 10-day period, Oct.	130	88	9
2nd 10-day period, Nov.	145	100	7
3rd 10-day period, Jan.	100	75	7
2nd 10-day period, Feb.	100	5	15

feeding habits when they are brought back to laboratory conditions (20 °C, RH 80-90%). The behaviour of *A. andersoni* is contrary to that of *Typhlodromus* (=*Metaseiulus*) *occidentalis* Nesbitt (Hoy & Flaherty, 1975): during diapause the former remain in a lethargic state, gather together in groups even when food is available, and resume eating a few days later, but only temporarily and in varying percentages that depend upon the period of the season in which they were collected. This may be considered a sign of the females' physiological condition; that is to say, in November they eat after 15 days (75%), in January after 7 days (100%), and in February after 4 days (100%). Afer a short feeding period the females in diapause once again take on their characteristic pumice-white color and become lethargic and gregarious.

In those studies in which the reaction was observed in the same temperature and RH conditions right from the egg stage, the females, once they matured and mated, looked like gravid females; but a few days later they gradually stopped feeding and manifested all the characteristics typical of diapause, remaining in this state for over a month, at which time the experiment ended.

Resistance to lack of food

According to Croft (1971), the fact that overwintering females in temperature and RH conditions of 70 °F and 70±20 respectively are able to survive for 20 days without food, is a confirmation of diapause; and in the case of *T. occidentalis* he noted a mortality rate of less than 5% after this period.

On the basis of these observations I carried out some research on *A. andersoni* females (with 2-3 repetitions) collected in three periods in winter.

The results of this research (Table 2) demonstrate that these females, kept at a temperature of 20 °C and 80-90% RH, resist hunger for more than 25 days, and some survive for more than 40 days. The percentage of the females resisting a lack of food varies in the different periods of winter, but this variation

Table 2 - Overwintering females' resistance to lack of food in different periods of winter (photop. 8h, temp. 20 °C, RH 80-90%).

Date	n.	% alive after 25 days	% alive after 40 days
09 Nov.	120	95	—
11 Jan.	110	89	27
19 Feb.	120	80	20

is not so marked as to indicate (in this species) a characteristic of females in diapause, since even after diapause ended some females manifested a remarkable resistance to the lack of food.

This comes as no surprise if we stop to consider that in the natural state overwintering females live without food from October to the end of April. In fact, whereas Hoy (1971) notes that before mites appear on the host plant *M. occidentalis* feeds on tarsonemids, tydeids and eriophyids, this does not apply to *A. andersoni*, which has no tarsonemids and eriophyids available and does not feed upon tydeids, even though these latter live together with *A. andersoni* on the band traps; and *A. andersoni* does not attack the tydeids even in laboratory conditions (at a temperature of 20-25 °C) without food. I feel on the other hand that the remarkable resistance to hunger on the part of these females at the end of winter may be due to other factors: 1) during this period the metabolism is always quite reduced in those females still in a state of lethargy in their winter refuges; 2) the *A. andersoni* females have a natural marked resistance to a lack of food, as was shown in preliminary researches carried out in the summer when certain individuals resisted hunger for a maximum of 15-18 days at a temperature of 21-22 °C and 80-90% RH.

Hibernation in a natural environment

A. andersoni hibernates in the inseminated adult female stage. It takes shelter in the shallow cracks in the bark of the host plant or under the webs of small Lepidoptera or in some other refuge, in groups of 20-30. The overwintering females gradually abandon the foliage at end of September or in October (Ivancich Gambaro, 1986).

In autumn females of different ages and generations coexist on the host plants, be they in diapause or not, given the exceptional longevity of this species, which is greater thn that of all other known species of phytoseiids (Amano-Chant, 1977). It is not possible to distinguish those females in

Table 3 - Photoperiod and minimum 10-day temperature in August and in the first 10 days of September in the Po Valley climate.*

Date	Approx.* day length (h)	minimum 10-day period temp.		
		1985	1986	1987
1st 10-day period, Aug.	14.44	13.6	17.1	11.6
2nd 10-day period, Aug.	14.6	15.4	15.9	16.4
3rd 10-day period, Aug.	13.13	13.3	13.7	13.8
1st 10-day period, Sept.	12.36	11.3	11.8	14.2

* From sunrise to sunset, not including twilight (Beck, 1968).

diapause, but in a natural environment this can be done during unfavourable winter conditions, when only the females in diapause survive.

Bur in what period of the season do diapause induction conditions exist in the climate of the Po Valley?

According to the Dutch authors, in the Netherlands (52° N) the photoperiod that induces diapause in this species is 14 hours at 18 °C; consequently, along with Beck (1968) we must presume that at the latitude in Verona (45° N) the «critical photophase» lasts roughly 13 hours, and this photophase occurs in the first ten days of September (Beck, 1980) (Table 3). But we also know that temperature plays a major role in diapause induction (Sapozhnikova, 1964; Rock et al., 1971; Hoy, 1975; Beck, 1980), in particular the scotophase temperature which, according to researches carried out by Van Houten et al. (1988), is more important than the photophase temperature.

Table 4 - Photoperiod in three localities in which comparative research was made: Fondi (Italy) 41° N; Verona (Italy) 45° N; Amsterdam (Netherlands) 52° N.*

Date	Day length (h)		
	Fondi 41° N	Verona 45° N	Amsterdam 52° N
01 Aug.	14.18	14.44	15.17
15 Aug.	13.46	14.60	14.30
01 Sept.	13.30	13.13	13.29
15 Sept.	12.31	12.36	12.42
01 Oct.	11.44	12.41	11.38
15 Oct.	11.14	11.40	10.51

* From sunrise to sunset, not including twilight (Beck, 1968).

Table 3 shows that in the last ten days of August and the first ten days of September the photophase in Verona lasts approximately 13 hours and the average scotophase temperature rarely goes over 14 °C. It therefore seems that during this period the photoperiod and night temperature conditions are such as to induce diapause in the populations on the host plant, or at least among those populations that are on the plant during the early stages of development which are sensitive to diapause-inducing ecological conditions (Putnam, 1962; Wysoki, 1974; Hoy, 1975; Overmeer, 1985).

My long-term observation of *A. andersoni* has in fact revealed that in the Po Valley climate the presence of eggs and newborn larvae on the host plant is still considerable both in the last ten days of August (40-60% of the population) and in the first ten days of September (10-15% of the population) (Ivancich Gambaro, 1988).

We may therefore well presume that the females in diapause come from the oviposition of the third (but also partly from the second) ten-day period in August and the first ten-day period in September (1). And studies made on preliminary laboratory research seem to confirm the response of *A. andersoni* to these photoperiod and temperature conditions.

CONCLUSIONS

The reply to the query that gave rise to this research is summed up in Table 1, which shows that in the climate in Verona (Po Valley, Italy, 45° 26' N) 100% of the *A. andersoni* females are in reproductive diapause in the month of November, this percentage gradually decreasing up to February. The Po Valley populations are therefore subject to a halt in their reproductive activity during winter, as is the case with the populations in the Netherlands (52° N), with a difference in the «critical photophase» that depends on the difference in latitude (7°).

These results differ from those obtained by McMurtry et al. (1976) in the comparison made between the «Italian race» and the «Dutch race». In fact these authors state that the «Italian race from citrus does not enter diapause in the winter». However we must bear in mind that the environmental conditions in which McMurtry et al. carried out their researches are quite different from

(1) On the other hand, according to recent researches carried out by Van Houten (1989) photoperiodic sensitivity in *A. potentillae* is not restricted to the juvenile stages, but extends over the whole adult life span.

those in the Po Valley, and they do not depend solely upon the difference in latitude.

The researches made by Croft (1971) on populations of *T. occidentalis* Nesbitt collected at latitudes varying as much as 13°, reveal differences in the response to the photoperiod, but not in the presence or absence of diapause. Therefore if in the climate of South Italy *A. andersoni* has no winter diapause. I believe the reason for this is not so much to be sought in the difference in latitude and hence in the photoperiod (see Table 4), but in other factors such as the night temperature (of the scotophase) and the difference between the host plants, which certainly plays an important role.

If, as these authors affirm, the absence of diapause occurs even in laboratory studies, we are bound to admit that the faculty of response to diapause induction conditions is a hereditary factor (Beck, 1980).

All these hypotheses could thus be tested and proved only by means of an investigation into the *A. andersoni* populations in natural conditions in the climate of South Italy on deciduous and evergreen plants; only then would it be possible to ascertain the response of these phytoseiids to the true ecological conditions responsible for diapause induction.

Diapause in Phytoseiids

In looking over the numerous researches made on the aspects and nature of this phenomenon in various species of insects and mites, we note that the authors' evalutations, definitions and classifications differ. If from among these we take into consideration «diapause» (the most evolved form of arrested development due to its higher degree of biochemical changes), we must conclude that the so-called diapause in phytoseiids does not have all its characteristics: for example, in «diapause» the cessation of reproductive activity during winter is induced by ecological factors that act upon the early stages of development with a certain anticipatory mechanism that depends upon hormonal factors (De Wilde, 1962). On the other hand the evolution of diapause among these mites in a natural environment takes place gradually and does not require a period of exposure to cold. And this is not all; it may end more or less rapidly by exposing the overwintering females to higher or lower temperatures in any period of winter, and it may even be avoided by keeping the females at a temperature of 23-25 °C.

It therefore seems that we may conclude that the determining factor in phytoseiids is the temperature, as De Wilde (1962) noted in the diapause of certain insects.

Yet the anticipatory mechanism which under natural conditions is induced by the concomitant action of the photoperiod and the temperature, remains a complex and marvellous phenomenon thats is a characteristic of great ecological value for phytoseiids.

SUMMARY

Overwintering *Amblyseius andersoni* females were collected from peach trees in different periods of winter and were kept in the laboratory with a photoperiod of 8 hours, a temperature of 20 °C and 80-90% RH, with the aim of finding out whether these females enter diapause. The results were as follows:

1) There is imaginal reproductive diapause in 100% of the *A. andersoni* females during the month of November, this percentage decreasing gradually up to Febraury.

2) In a natural environment at the beginning of winter the immature forms and males disappear, and only the females in diapause survive.

3) After the first ten days of January some of the females are able to oviposit and are prevented from oviposition only by the low temperature; this condition continues for about three months (up to the last ten days of April) in a natural environment.

4) Keeping the overwintering females totally without food at a temperature of 20 °C and 80-90% RH revealed a resistance to hunger that is greater among the females in diapause, but is considerable even after diapause. This is therefore to be considered a valid character of this species.

5) Diapause is induced by ecological conditions (duration of daylight and scotophase temperature) which in the Po Valley climate obtain in the last ten days of August and the first ten days of September; the females in diapause therefore come from eggs laid during this period.

The caracteristics and nature of diapause are then discussed.

RIASSUNTO

La diapausa in Amblyseius andersoni *Chant nella Pianura Padana.*

Femmine svernanti sono state raccolte da piante di pesco in periodi diversi dell'inverno e mantenute in laboratorio a 8 h di luce, 20 °C, 80-90% U.R. per conoscere se esista o meno una condizione di diapausa.

I risultati hanno messo in evidenza che:

1) *A. andersoni* presenta una diapausa imaginale riproduttiva che interessa il 100% delle femmine nel mese di novembre e si riduce in seguito gradualmente fino a febbraio.

2) In natura all'inizio dell'inverno tutte le forme immature e i maschi scompaiono e sopravvivono soltanto le femmine in diapausa.

3) Dopo la 1ª decade di gennaio parte delle femmine è in grado di ovideporre e ne è impedita soltanto dalle basse temperature; situazione che in natura si mantiene per circa 3 mesi (fino alla 3ª decade di aprile).

4) Il mantenimento di femmine svernanti a totale digiuno, a una temperatura di

20 °C e 80-90% U.R., ha rilevato una resistenza che è maggiore nelle femmine in diapausa, ma è notevole anche dopo la conclusione della diapausa, rilevando con ciò ancora una valida caratteristica di questa specie.

5) La diapausa è indotta da condizioni ecologiche (lunghezza del giorno e temperatura della scotofase) che nel clima della Valle Padana si verificano nella 3ª decade di agosto e 1ª decade di settembre. Le femmine in diapausa pertanto provengono da uova deposte in questo periodo. Le caratteristiche e la natura della diapausa vengono discusse.

Key words: diapause, *Amblyseius andersoni*, photoperiod, Po Valley.

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Ricevuto il 15 aprile 1990; pubblicato il 30 luglio 1990.