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Selection for non-diapause in *Amblyseius cucumeris* and *Amblyseius barkeri* and exploration of the effectiveness of selected strains for thrips control

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**Key words:** biological control, greenhouse crops, sweet pepper, western flower thrips, *Frankliniella occidentalis*, predatory mites, *Amblyseius barkeri, Amblyseius cucumeris*, diapause, genetic variation

**Abstract**

In Europe and North America the western flower thrips, *Frankliniella occidentalis*, is an important pest in various greenhouse crops, such as sweet pepper and cucumber. Two species of predatory mite are commercially applied for biological control of this pest: *Amblyseius cucumeris* and *A. barkeri*. Thrips control is generally successful from March onwards. During winter, however, thrips control by these predatory mites is less effective. An important reason for this is that the commercially applied strains of both mite species enter reproductive diapause under short-day photoperiods, whereas the western flower thrips does not enter diapause. In this paper we report on selection experiments for non-diapause in strains of both mite species, aimed at obtaining predators that do not enter diapause under light- and temperature conditions prevailing in winter. Additional experiments were done to estimate the potential of the selected lines as control agents of *F. occidentalis*. Selection for non-diapause proved highly successful in both predatory mite species. In a New Zealand strain of *A. cucumeris* diapause incidence decreased from 41% to 0% in about ten generations; in a Dutch strain of *A. barkeri* diapause incidence decreased from 67% to 0% in about six generations. Furthermore, selection for non-diapause had no influence on predator performance, measured as predation rate and oviposition rate on a diet of first instar thrips larvae. Rates of predation and oviposition were the same for selected and unselected lines in both species; rates of predation and oviposition were higher for *A. cucumeris* than for *A. barkeri*. After 18 months under non-diapause conditions, no less than 92% of a sample of the selected non-diapause line of *A. cucumeris* did not enter diapause when tested under diapause-inducing conditions. This indicates that 'non-diapause' is a stable trait in these predatory mites. Finally, a small-scale greenhouse experiment in a sweet pepper crop showed that the selected non-diapause line of *A. cucumeris* established successfully under diapause-inducing short-day conditions.

**Introduction**

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), was accidentally introduced into Europe around 1983 and has since become a major pest in several greenhouse crops. At present, the predatory mite *Amblyseius cucumeris* (Oudemans) (Acarina: Phytoseiidae), previously used against onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (de Klerk & Ramakers, 1986; Ramakers, 1988), is commercially produced for biological control of *F. occidentalis*. For example, in sweet pepper the predator is successfully released from March onwards, as it persists in the crop during the rest of the growing season. Its maintenance is observed even in absence of thrips, which may be attributed to the continuous presence of pollen as an alternative food source (Ramakers, 1990; van Rijn & Sabelis, 1993). During winter, however, thrips control by *A. cucumeris* is less effective. One reason for this is that female predators of the commercial strain enter a reproductive diapause when daylength
shortens (Morewood & Gilkeson, 1991; van Houten & van Stratum, 1993). \textit{Frankliniella occidentalis} does not enter diapause under short-day conditions (Y. M. van Houten, personal observations; Brødsgaard, 1994) and thus can cause damage to greenhouse crops during winter. Therefore, a non-diapausing thrips predator is needed to improve biological control during this period.

\textit{Amblyseius barkeri} (Hughes) is a second species of predatory mite that has been used for control of thrips, mainly in cucumber crops (Ramakers et al., 1989, Brødsgaard & Hansen, 1992; Steeghs et al., 1993). The commercially applied strain of this predator also enters reproductive diapause under winter greenhouse conditions. In sweet pepper greenhouses in The Netherlands, however, \textit{A. barkeri} occasionally occurs spontaneously during the winter. Van Houten (1991) demonstrated a relatively low incidence of diapause (c. 50%) in a strain of such mites, collected from a sweet pepper greenhouse in winter. Comparative studies on a sweet pepper crop indicate that \textit{A. barkeri} is less successful in controlling thrips than \textit{A. cucumeris} (Ramakers, 1988, 1990), under conditions that did not induce diapause. However, a strain of \textit{A. barkeri} that is less sensitive to diapause-inducing conditions than \textit{A. cucumeris} could do better as a control agent of thrips during the winter.

Morewood & Gilkeson (1991) have selected for non-diapause in a commercial strain of \textit{A. cucumeris} in Canada. Under their selection regime (light phases (L) of 8 h, at 22°C, and dark phases (D) of 16 h, at 17°C), they obtained a reduction of diapause incidence from 88% in the parental generation to 33% in the F14-generation. It is uncertain whether this selected line would also exhibit reduced diapause under the less extreme photoperiodic regime of L10:D14, which corresponds to conditions in a sweet pepper greenhouse at the time \textit{A. cucumeris} usually is released. Selection for non-diapause in a Dutch commercial strain of \textit{A. cucumeris} at L10(23°C):D14(16°C) was unsuccessful; several hundreds of females were raised under this regime but all entered diapause (Y. M. van Houten, unpubl.). In searching for a non-diapause strain of \textit{A. cucumeris}, a population was sampled in northern New Zealand, a region where this mite naturally occurs and where conditions that induce diapause in greenhouses in the Netherlands are rare. A colony of the New Zealand population was found to enter diapause at a moderate level (41%), in a laboratory assay at L10(23°C):D14(16°C).

In this article we report selection experiments aimed at obtaining strains of \textit{A. cucumeris} and \textit{A. barkeri} that do not enter diapause under winter light- and temperature-conditions that are realistic for commercial greenhouses (in the Netherlands). After selection for (complete) non-diapause in the ‘New Zealand’ strain of \textit{A. cucumeris} and the ‘sweet pepper’ strain of \textit{A. barkeri}, we performed a number of small laboratory experiments to estimate the potential of the selected lines as control agents of \textit{F. occidentalis}. Rates of predation and oviposition on a diet of young thrips larvae of the two selected lines were compared to those of the unselected, parental strains and the commercial Dutch \textit{A. cucumeris} strain. Finally, the selected line with the highest rates of predation and oviposition was subjected to a small-scale greenhouse experiment: establishment on a sweet pepper crop by the selected non-diapause line was compared to that of the commercial Dutch \textit{A. cucumeris}-strain during mid-winter.

\section*{Materials and methods}

\textbf{Mites.} The commercial strain of \textit{A. cucumeris} was obtained from a laboratory colony maintained at the Glasshouse Crops Research Station in Naaldwijk, the Netherlands. The second strain of \textit{A. cucumeris} was collected from wild winged-leaved \textit{Thunbergia} (\textit{Thunbergia alata} Bojer) near Auckland, New Zealand, in February 1991. \textit{Amblyseius barkeri} was collected in a sweet pepper greenhouse, in the western part of the Netherlands, in February 1989. Subsequent to their collection, all strains were reared on plastic units as described by Overmeer et al. (1982), in a climate room. Wet tissue paper was wrapped around the edges of the surface to serve both as a water source and as a barrier. An additional Tangletrap® barrier on top of the tissue paper prevented the mites from escaping. Pollen of the broad bean (\textit{Vicia faga} L.) was used as food.

\textbf{Interstrain crosses.} In Australia \textit{A. bellinus} (Womersley) occurs. This species is closely related to \textit{A. cucumeris}; only a few small morphological differences have been described (Schicha, 1976). To ensure that the mites collected in New Zealand are members of the species \textit{A. cucumeris}, we performed a number of crosses with the Dutch commercial ‘Naaldwijk’ strain of \textit{A. cucumeris}. A cohort of eggs of both strains was placed separately on plastic units (Overmeer et al., 1982), under long-day illumination (L16:D8) at 25°C. After the eggs had developed into deutonymphs
Selection for non-diapause. Selection for non-diapause was carried out on units identical to the rearing units, in photoperiod- and thermoperiod-controlled incubators. Cohorts of eggs, ranging from 0 to 24 h old since oviposition, were placed under short-day conditions (L10(23°C):D14(16°C)), supplied with broad bean pollen. For A. cucumeris this diet was supplemented with β-carotene, since A. cucumeris fed on broad bean pollen alone does not respond to photoperiod (Overmeer et al., 1989) or combined photo- and thermoperiod (van Houten, 1991). Two days after the first adult females appeared, the mites were fed with pollen of iceplant, Mesembryanthemum species, which has also been shown to be an adequate food source for A. cucumeris (Overmeer et al., 1989) and A. barkeri (P.C.J. van Rijn & M. Jones, unpubl.). In this way egg production by individual females could easily be assessed, as the intestines of mites feeding on iceplant pollen become purple. In non-diapausing females, the white egg stands out clearly against the surrounding purple intestines; diapausing females, on the other hand, do not feed and remain pale. It was ascertained that sufficient males were present to inseminate all females. Absence of egg production was taken as criterion for diapause incidence. Non-diapausing females were transferred to new arenas from which eggs were collected for the next generation.

After about one year under short-day conditions (L10(23°C):D14(16°C)), selected A. cucumeris were transferred to a laboratory room with natural daylength and constantly high temperatures ranging from 25 to 30°C. At such high temperatures diapause is not induced in A. cucumeris (Morewood & Gilkeson, 1991). The mites were reared on bran mites as described by Ramakers & Van Lieburg (1982). After 18 months under these conditions, the incidence of diapause in the mites was tested again, in order to get an impression of the stability of non-diapause in the selected A. cucumeris line. Cohorts of eggs ranging from 0 to 24 h old, were placed on plastic units on water-saturated pads of cotton wool, either under short-day conditions (L10(23°C):D14(16°C)) or, as a control, under long-day conditions (L14:D10, 19°C). Mites were fed with broad bean pollen, supplemented with β-carotene. Two days after adult females appeared the mites were fed with iceplant pollen, after which egg production could be assessed.

Rates of predation and oviposition. Predation and oviposition rates were determined on cucumber leaf discs (4.5 cm²), placed upside down on water-saturated pads of cotton wool, in a climate room at 25°C, 70% r.h. and long-day illumination (L16:D8). Each leaf disc was infested with 12 first instar larvae of F. occidentalis (0.5–0.6 mm). Preliminary experiments with A. cucumeris had shown that at this prey density, the functional response to thrips larvae is at its plateau level (P.C.J. van Rijn, unpubl.). This means that a small decrease in prey number does not affect predation rate. A single gravid young female mite was placed on each leaf disc. The predators were transferred daily to fresh leaf discs with 12 newly-emerged thrips larvae, for three days. The old leaf discs were examined to record the number of eggs laid and the number of thrips larvae killed. Data of the first day were omitted from calculations of predation and oviposition rates, to minimize a potential effect of the mites’ food source prior to the experiment.

Establishment in the greenhouse. Establishment of the selected non-diapause A. cucumeris in a sweet pepper crop was studied in a small greenhouse with two rows of eight sweet pepper plants. Mites from the selected strain were introduced on the plants in one row, whereas mites from the commercial strain of A. cucumeris were introduced on the plants in the other row. Small electrical heaters in the greenhouse kept the temperature at 23°C for 10 h during the light period, and at 16°C for 14 h during the dark period. Adult thrips (F. occidentalis) had been released into the greenhouse, so that ample prey (i.e., young thrips larvae) was present on all plants by the start of the experiment. In the fourth week of January 1992 – the plants were c. 1 m high and had just started to flower, which means that pollen is available as alternative food – 25 adults females were released on each plant. To monitor establishment of the predatory mites, eggs were counted on 64 leaves.
that were sampled from the upper parts of all plants (4 leaves/plant) once a week.

Results

Interstrain crosses. Reciprocal crosses between the New Zealand strain of *Amblyseius cucumeris* and the Dutch commercial strain were successful and egg hatch was almost complete (Table 1). This is also true for the two subsequent generations. Based on these results we conclude that the mites collected in New Zealand belong to the species *A. cucumeris*.

Selection for non-diapause. Selection for non-diapause was successful, both for the New Zealand strain of *A. cucumeris* (Fig. 1) and the Dutch strain of *A. barkeri* (Fig. 2). Diapause incidence in the *A. cucumeris* strain decreased from 41% initially to 0% by the tenth generation. Selection against diapause in *A. barkeri* went even faster: in about six generations diapause incidence dropped from 67% to 0%.

Rates of predation and oviposition. The predation rate on first instar thrips larvae of females of the New Zealand strain of *A. cucumeris* was lower than the predation rate of females of the Dutch commercial strain (viz., 4.4 ± 0.3 vs. 6.0 ± 0.2 larvae/female/day, on average; Table 2). Oviposition rates of females of the two strains were comparable: 1.9 ± 0.1 and 2.2 ± 0.1 eggs/female/day, respectively (Table 2).

Selection for non-diapause did not seem to influence the predators’ performance on a diet of thrips larvae. Predation rates were equal for the unselected and selected line of the New Zealand strain of *A. cucumeris* (4.4 ± 0.3 vs. 4.5 ± 0.4 thrips larvae/female/day, on average; Table 2). Oviposition rate of females of the selected line of this strain was somewhat higher: 2.4 ± 0.1 eggs/female/day, as opposed to 1.9 ± 0.1 for females of the unselected line. There were no striking differences in predation or oviposition rates between unselected and selected lines of *A. barkeri*. Females of the unselected line killed on average 2.6 ± 0.2 thrips larvae/female/day, females of the selected line 2.8 ± 0.3. Oviposition rate of females of the unselected line was on average 1.5 ± 0.1 eggs/female/day, and females of the selected line laid on average 1.4 ± 0.2 eggs/female/day. Females of *A. cucumeris* showed higher predation and oviposition rates than females of *A. barkeri* (Table 2; see also van Houten *et al.*, 1995).

Establishment in greenhouse. The selected ‘non-diapause’ strain of *A. cucumeris* appeared to establish successfully in our small sweet pepper greenhouse under short-day conditions. Two to three weeks after introduction of the predators, numbers of eggs of the ‘non-diapause’ strain started to increase sharply (Fig. 3). This is to be expected for predatory mites that do not enter diapause. It would take about two weeks for eggs, laid by the introduced adults females, to develop into ovipositing females. If predatory mites enter diapause, gradually decreasing egg numbers are to be expected. After all,
Table 1. Crosses between the New Zealand strain of Amblyseius cucumeris ("NZ") and the Dutch commercial strain of A. cucumeris ("C")

<table>
<thead>
<tr>
<th></th>
<th>$\varphi_C \times \delta_{NZ}$</th>
<th>$\varphi_{NZ} \times \delta_C$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>F$_1$</td>
</tr>
<tr>
<td>number of pairs</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>number of unsuccessful crosses</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>number of eggs</td>
<td>70</td>
<td>172</td>
</tr>
<tr>
<td>% eggs hatched</td>
<td>98</td>
<td>98</td>
</tr>
</tbody>
</table>

Table 2. Rates of predation and oviposition of three Amblyseius cucumeris strains and two Amblyseius barkeri strains on a diet of first instar Frankliniella occidentalis-larvae, on cucumber leaf discs (4.5 cm$^2$) at 25°C. Predation rate: mean number of larvae killed per female per day; oviposition rate: mean number of eggs laid per female per day. N = number of females; s.e. = standard error

<table>
<thead>
<tr>
<th>Predator strain</th>
<th>N</th>
<th>Predation rate mean ± s.e.</th>
<th>Oviposition rate mean ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblyseius cucumeris Naaldwijk$^1$</td>
<td>23</td>
<td>6.0 ± 0.2 $^a$</td>
<td>2.2 ± 0.1 $^ab^2$</td>
</tr>
<tr>
<td>Amblyseius cucumeris New Zealand</td>
<td>23</td>
<td>4.4 ± 0.3 $^b$</td>
<td>1.9 ± 0.1 $^a$</td>
</tr>
<tr>
<td>Amblyseius cucumeris New Zealand ‘non-diapause’</td>
<td>16</td>
<td>4.5 ± 0.4 $^b$</td>
<td>2.4 ± 0.1 $^b$</td>
</tr>
<tr>
<td>Amblyseius barkeri$^1$</td>
<td>21</td>
<td>2.6 ± 0.2 $^c$</td>
<td>1.5 ± 0.1 $^c$</td>
</tr>
<tr>
<td>Amblyseius barkeri ‘non-diapause’</td>
<td>20</td>
<td>2.8 ± 0.3 $^c$</td>
<td>1.4 ± 0.2 $^c$</td>
</tr>
</tbody>
</table>

$^1$ Data from van Houten et al. (1995)
$^2$ Means within a column followed by the same letter do not differ significantly (t-test, P < 0.05).

oviposition rate of the introduced females would gradually diminish, as a consequence of increasing age, and the hatched eggs would develop into diapausing (and non-egglaying) adults. This is precisely what was seen in the unselected commercial strain of A. cucumeris: egg numbers gradually decreased; these mites did not establish in the sweet pepper crop (Fig. 3).

**Stability of non-diapause.** After rearing for 18 months under non-diapause laboratory conditions, females of the selected A. cucumeris line still showed a high percentage (92%) of ovipositing females when tested under short-day conditions, comparable to the percentage shown under long-day conditions (98%; Table 3). This result suggests that the trait ‘non-diapause’ is stable in these mites for at least 18 months after selection has stopped.

**Discussion**

Our experiments show that laboratory selection for non-diapause was successful, both in the New Zealand strain of A. cucumeris and in the Dutch strain of A. barkeri. After ten and six generations, respectively, diapause incidence had dropped to 0%. It should however be taken into account that this percentage was based on live mites. Some mites disappeared from the rearing units, as they crawled under the surrounding tissue paper and drowned, or climbed on the tissue and got stuck in the glue barrier (see Figs. 1 and 2). This tendency to disperse might somehow be associ-
Table 3. Diapause incidence in the 'non-diapause' line of *Amblyseius cucumeris*, after rearing for 18 months under non-diapause conditions. Female mites were tested under short-day conditions (Light: Dark = 10 (23°C) : 14 (16°C) h) and under long-day conditions (Light: Dark = 14 (19°C) : 10 (19°C) h). N = number of females

<table>
<thead>
<tr>
<th>Regime</th>
<th>Diapause (%)</th>
<th>Non-diapause (%)</th>
<th>Missing or drowned (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>L 10 (23°C) : D 14 (16°C)</td>
<td>2</td>
<td>92</td>
<td>6</td>
<td>174</td>
</tr>
<tr>
<td>L 14 (19°C) : D 10 (19°C)</td>
<td>0</td>
<td>98</td>
<td>2</td>
<td>174</td>
</tr>
</tbody>
</table>

ated with the onset of diapause. For example, Field & Hoy (1985) have shown that diapausing females of *Typhlodromus (= Metaseiulus) occidentalis* (Nesbitt) tend to leave the plants and seek shelter places. If in our experiments diapausing females are eager to find a suitable shelter for 'overwintering' they are more likely to run into the barriers. In this case diapausing females would be disproportionately present among the dead-or-missing mites, and thus the percentages for diapause presence would be underestimations. (As an aside: this reasoning would also provide an explanation for the gradual decrease in percentage of missing-or-dead mites, in the course of continuing selection for non-diapause.) However, it leaves the main result unaltered: within ten generations (almost) no mites entered diapause under short-day light and temperature conditions.

Successful selection for non-diapause in a predatory mite was reported by Hoy (1984). She found that four generations of selection at L8:D16 and 19°C in a strain of *T. occidentalis* resulted in a decrease of diapause incidence from c. 99% to 11%, when tested at L10:D14 and 19°C. In an isolated laboratory rearing of these mites the incidence of non-diapause appeared rather persistent; after 18 months at high temperature and under long-day conditions, diapause incidence had only slightly increased (Field & Hoy, 1986). This result is comparable to our result with 'non-diapause' selected *A. cucumeris*: after 18 months under non-diapause laboratory conditions, diapause incidence had increased only to c. 5%. After two winters in a greenhouse, however, mites of the selected strain of *T. occidentalis* had not maintained their ability to reproduce under diapause-inducing conditions (Field & Hoy, 1986). Field & Hoy hypothesized that this might be due to interbreeding of mites of the selected strain with mites of a wild strain that had been released previously into the greenhouse. Stability of non-diapause under greenhouse circumstances remains to be investigated in our selected *A. cucumeris* strain.

Selection for non-diapause in both *A. cucumeris* and *A. barkeri* did not have much effect on rates of predation and oviposition on a diet of young thrips larvae. For both species it was found that differences between the selected lines and the original lines were insignificant. Therefore, as far as predation rate and oviposition rate are relevant measures for suitability as biological control agents, the selected non-diapause lines seem equally good thrips predators as the original strains. Predation rate and oviposition rate were higher in *A. cucumeris* females than in *A. barkeri* females. This result is in accordance with comparative studies by Ramakers (1988, 1990). He has found that in a sweet pepper crop *A. barkeri* is less successful in controlling thrips than *A. cucumeris*. Based on his and our results, it seems likely that the most promising candidate for greenhouse thrips control early in the season is the non-diapause line of *A. cucumeris*.

In our small-scale greenhouse experiment we took a first step in evaluating the success of the non-diapausing *A. cucumeris* as thrips control agent. It was found that under short-day (= diapause-inducing) conditions, mites of the selected line of the 'New Zealand' strain performed much better than mites of the unselected 'Naaldwijk' strain. This result was confirmed by trials in several commercial cucumber and sweet pepper greenhouses near Naaldwijk, The Netherlands. Non-diapausing *A. cucumeris* were released in February and rapidly established, especially in sweet pepper crops (Y. M. van Houten, unpubl.). Its success as a biological control agent of thrips can only be proven in future practice.
Acknowledgements

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