Late-season induction of diapause in *Nezara viridula* and its effect on adult coloration and post-diapause reproductive performance

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Abstract

Nezara viridula (L.) (Heteroptera: Pentatomidae) recently expanded its distribution range in Japan and reached Osaka. In the southern temperate zone, the species overwinters in the adult stage and reproductive diapause is associated with a body colour change from green to russet. In Osaka, the reproductive diapause is only induced in September–October and nymphs from late egg masses are destined to die during winter. However, the fate of adults emerging late in the season remained unknown. Survival, body colour change, and post-diapause reproduction were studied under quasi-natural conditions in Osaka in 1999–2000 in those adults that attained adulthood as late as in November. Two experimental cohorts were used: in the Outdoor cohort, insects were reared outdoors starting in their second instar (28 September); in the Laboratory cohort, nymphs and subsequently adults were reared from the same day under diapause-inducing conditions (L10:D14 at 25 °C), then acclimatized (5 days at 20 °C and 5 days at 15 °C; L10:D14) and transferred outdoors on 1 December. Adults in both cohorts did not reproduce in autumn and survived the winter with a low mortality. More than 20% of adults in the Outdoor cohort failed to change body colour from green to russet during winter, apparently because of the low ambient temperature, suggesting that the environmental conditions required for colour change do not completely coincide with those required for diapause induction, and that the colour of the adults is not always a reliable indicator of diapause in this species. After overwintering, females from the Outdoor cohort produced significantly fewer egg masses and eggs and had a significantly shorter period of oviposition than females that entered diapause under short-day conditions in the Laboratory cohort. Thus, if progeny from the late egg masses attain adulthood late in the autumn, these adults have high chances of successful overwintering, but their reproductive output after the winter diapause is significantly reduced.

Introduction

Many insect species are expanding their distribution ranges northward following the current climate warming (Parmesan & Yohe, 2003; Root et al., 2003). Newly established populations may find themselves under a new and unusual physical environment. Not only temperature and day-length conditions are new, but also the coupling of the seasonal patterns of temperature and day-length is different from those these populations have been adapted to. Photoperiod is a natural cue which is most widely involved in the environmental control of seasonal development, and particularly, diapause induction, in insects (Danks, 1987). However, under new environmental conditions insects may incorrectly interpret the day-length signals in their seasonal positioning, which may result in an imperfect timing of reproduction and dormancy. An induction of winter diapause too early in the season will cause an underexploitation of the warm season's conditions (e.g., Shimizu & Kawasaki, 2001), and may increase the cost of diapause because of the lengthening of the dormant period or high spring mortality, whereas a delay in diapause induction

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may cause the death of physiologically unprepared (non-dormant) individuals when cold conditions arrive in autumn. It is also known that successful overwintering of a species with a reproductive diapause requires a complete period of the pre-diapause feeding, which may be rather long in some species (e.g., about 18 days in the pentatomid *Podisus maculiventris* Say; Saulich, 1999). A shortened and thus incomplete preparatory period may result in reduced winter survival and post-diapause performance.

The southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) is a multivoltine seed-sucking species that occurs throughout tropical, subtropical, and southern temperate regions of the world (Todd, 1989; Panizzi et al., 2000). The adults of the species overwinter under litter, bark, or other shelters in a reproductive diapause (Kiritani et al., 1966a; Jones & Sullivan, 1981). The induction of diapause in temperate populations is controlled by photoperiod and is associated with a seasonal polyphenism: the adults change body colour from green to brown or russet (Ali & Ewiess, 1977; Harris et al., 1984; Musolin & Numata, 2003a). In Japan, *N. viridula* has long been known in the southern part of the archipelago (Kiritani, 1971), but recently expanded its distribution range and reached Osaka (Musolin & Numata, 2003b). Laboratory experiments revealed a highly variable photoperiodic response of diapause induction in this species in Osaka; a proportion of adults were reproductive even under a short-day photoperiod of L12:D12 at 20 °C and 25 °C (Musolin & Numata, 2003a). Such photoperiodic response determines a relatively late diapause induction (if compared with several other seed-sucking heteropterans native to the Osaka region; Numata & Nakamura, 2002) and reproduction in some females that reached adulthood in the wild as late as in September–October. Late-season reproduction results in the death of a significant proportion of progeny in the nymphal stage during winter (Musolin & Numata, 2003b). However, the fate of the bugs that have managed to attained adulthood late in the season (e.g., in November) remains unknown. Even if only a fraction of these late-emerging adults can successfully survive winter and reproduce the next season, this will justify the late diapause induction and risky reproduction in autumn. In the present study, the fate of late-season *N. viridula* was investigated and the effect of the late emergence of the adults on their body coloration and post-diapause reproductive performance was assessed.

Materials and methods

Origin and routine maintenance of insects

Twenty-four adults *N. viridula* were collected from a soybean field on the campus of the Osaka City University (Japan, 34.7°N; 135.5°E) in August 1999 and reared under quasi-natural conditions. The field-collected adults were reared in round transparent plastic containers (diameter 150 mm, depth 90 mm) with openings in the lids covered with mesh for aeration. The containers were placed in the university campus on metal shelves open on three sides and sheltered from rain, direct sunlight, and any artificial illumination. The air temperature was recorded with a minimum/maximum thermometer. Adults were supplied with dry seeds of soybean, *Glycine max* (L.) Merrill, shelled raw peanuts, *Arachis hypogaea* L., and water containing 0.05% sodium L-ascorbate and 0.025% L-cysteine (Noda, 1991). Food and water were replaced every other day. Six fresh egg masses (ca. 60 eggs in each) produced by the field-collected females on 15–16 September were transferred to a laboratory incubator (a photoperiod of L16:D8 at 25 °C). Nymphs hatched on 22–23 September and synchronously moulted to the second instar on 27–28 September. They were reared in round transparent plastic containers (diameter 90 mm, depth 45 mm) with openings in the lids covered with mesh for aeration. Nymphs in each egg mass were divided into two groups, and each group was assigned to the Outdoor or Laboratory experimental cohort (see below). Nymphs, and subsequently adults, in both cohorts were provided with the same diet as the field-collected adults.

The Outdoor cohort

On 28 September 1999, ca. 150 second-instar nymphs were transferred to the quasi-natural conditions (as above) and reared there. The density of nymphs was kept at 40–60 per container for the second instar and was then gradually reduced to 5–6 per container by the final (fifth) instar. After adult emergence on 16–22 October, the bugs were sexed and each female–male pair was transferred to a new container (diameter 90 mm, depth 45 mm) and reared as described above. In total, there were 50 pairs in this cohort.

Mortality and changes of adult body colour were recorded daily until December 1999, and every other day during the winter (December–February). The following body colour grades were used: green, intermediate (approximately 40–60% of the body surface is light russet), and russet (Musolin & Numata, 2003a).

On 22 December 1999, all the adults in the Outdoor cohort were categorized according to the body colour and transferred into transparent plastic containers (diameter 150 mm, depth 90 mm) at a density of 8–10 pairs per container and provided with food and water as described above. Several leaves of deciduous trees were also added to provide shelter for overwintering. Later, if the adults changed colour, they were transferred to the corresponding colour grade container. During the course of the experiment there were up to five containers for each colour grade.

The Laboratory cohort

On 28 September 1999, ca. 130 second-instar nymphs were transferred to diapause-inducing short-day conditions of L10:D14 at 25 °C (Musolin & Numata, 2003a). Nymphs, and subsequently adults, were treated as in the Outdoor cohort. Adults (in total, 49 female–male pairs) emerged on 16–22 October. Starting on 21 November, adults were acclimatized by exposing them to a series of decreasing temperatures (5 days at 20 °C, then 5 days at 15 °C, both at a photoperiod of L10:D14; these temperatures roughly corresponded to the natural decrease in the outdoor temperature during the last 10 days of November). On 1 December, when the natural day-length in Osaka was 10 h 00 min, adults were transferred to the quasi-natural conditions and categorized according to their body colour into transparent plastic containers (as described above for the Outdoor cohort). During the course of the experiment there were up to five containers for each colour grade.

Record of life history parameters

In spring, when copulation was observed, the pair was transferred into a separate smaller transparent container (diameter 90 mm, depth 45 mm). Mortality, copulation, oviposition, and colour changes were recorded daily from March until the end of September 2000 when only two females remained alive and the experiment was terminated.

Survivorship, pattern of adult body colour changes, and post-overwintering reproductive performance were compared between the two experimental cohorts to determine the effect of the late-season induction of diapause on basic life-history traits. In order to assess reproductive performance, the following reproductive parameters were calculated: number of egg masses produced by each female, egg mass size (i.e., number of eggs in each egg mass), total number of eggs produced by each female, period of oviposition (i.e., the period between the days when the first and the last egg masses were laid by a female).

Statistical analysis

Proportions were compared statistically by the χ^2-test and the various parameters of post-diapause reproduction by the Mann–Whitney U-test (Zar, 1999).

Results

Winter survival and colour changes

In the Laboratory cohort, 49 females attained adulthood on 16–22 October and 45 of them had changed body colour from green to russet by late November, still under laboratory short-day conditions at 25 °C (Figure 1; data on males are similar and not shown). One female remained green until late March and only then changed colour.

Females did not copulate or oviposit under laboratory or outdoor conditions until mid-May. Winter and spring survival was high: 45 (91.8%) females survived until 1 March and 40 (81.6%) until 1 June. Most of the females that died before mid-May were russet. Starting from mid-April, the females changed body colour back to green and by 2 June all of them were green.

In the Outdoor cohort, 50 females emerged between 30 October and 25 November, and their emergence was not as synchronized as in the Laboratory cohort (Figure 1). Apparently, the delay in emergence and lack of synchronization were conditioned by the autumn decrease in ambient temperature: starting from mid-October, the daily maximum temperature did not exceed 24 °C, and daily minimum temperature was mostly below 14–15 °C, the developmental zero for nymphal growth estimated based on the laboratory data from Musolin & Numata (2003a). These temperatures and the resulting mean daily temperatures were considerably lower than 25 °C, a constant temperature at which nymphs grew and moulted into adults in the Laboratory cohort. Starting from late November, some females changed body colour to the intermediate grade, but only six females did so by late December. The proportion of dark-coloured adults started to increase further only from early January and reached its maximum by late February to late March. During December–March the proportion of green females varied within a range of 20–86%. Some of these females never changed colour to the intermediate grade or russet. Two green females and two green males were dissected in late December. All of them had internal reproductive structures in a diapause (i.e., non-reproductive) state and a well-developed fat body. Their winter and spring survival rates were high and did not differ significantly from those in the Laboratory cohort (χ^2-test, P>0.05). More than 50% of the 14 females that died up to mid-May were russet. In early April, the proportion of green females started to increase steadily, and by 25 May all of them were green.

Females in the two experimental cohorts showed a significant difference in coloration when they started to copulate in spring. In the Outdoor cohort, of 36 females, only one was still intermediately coloured on the day of its first copulation, whereas in the Laboratory cohort, 17 of 40 females were intermediately coloured or russet on the day of their first copulation (χ^2-test, P<0.05). However, by the day when the females produced their first egg masses, all of them were green in the Laboratory cohort, and only one was intermediately coloured in the Outdoor cohort (χ^2-test, P>0.05).

Post-overwintering reproduction

All the females that survived the winter reproduced in May–June (Figure 1). Adults started to copulate slightly
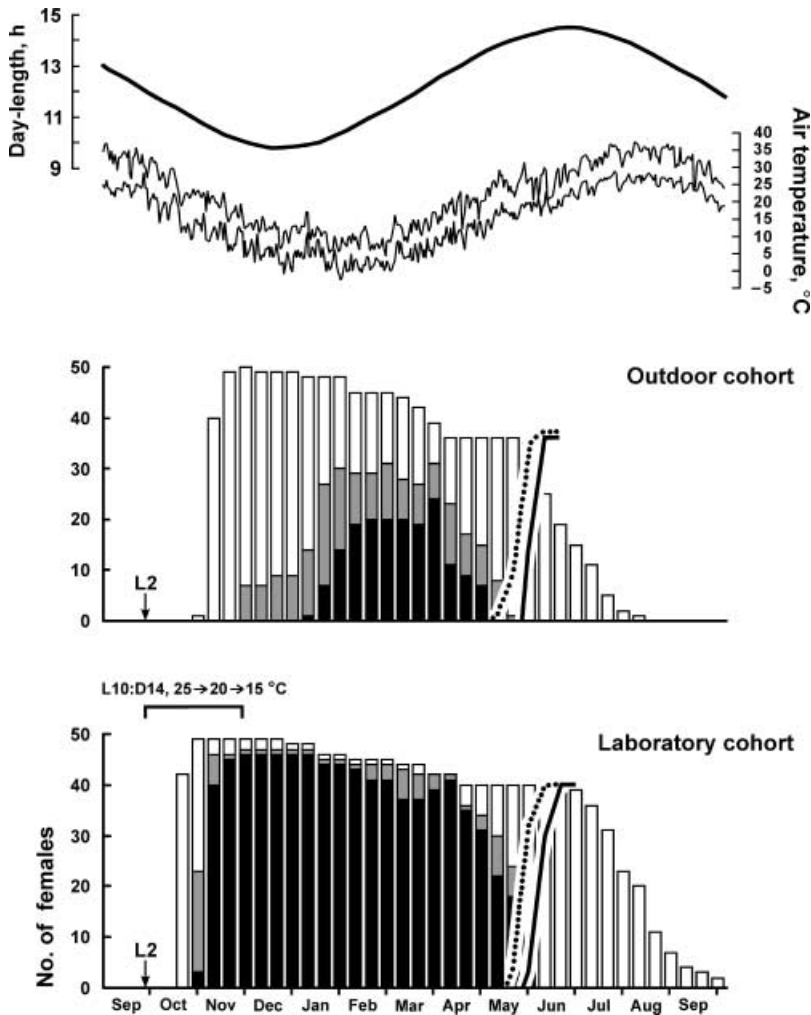
Figure 1 Seasonal development of *Nezara viridula* in the Outdoor and Laboratory cohorts. On 27–28 September (indicated by the L2 arrows) second-instar nymphs were transferred to outdoors (the Outdoor cohort), or reared under diapause-inducing (L10:D14 at 25 °C) and acclimatization (5 days at 20 °C, then 5 days at 15 °C; both at L10:D14) conditions, and on 1 December transferred to outdoors (the Laboratory cohort). Histograms show the number of female adults that emerged and survived, and the relative abundance of colour grades: green (white bars), intermediate (grey bars), and russet (black bars). The dotted lines over the histograms show cumulative numbers of copulating females and solid lines cumulative numbers of ovipositing females after overwintering. Environmental conditions (at the top of the figure): thick line, natural day-length; thin lines, daily maximum and minimum air temperature at the experimental shelves.

earlier in the Outdoor cohort, and the median date of the first copulation was 25 May in that cohort, whereas it was 27 May in the Laboratory cohort (Mann–Whitney U-test; U = 509.5, P = 0.014, days counted from 1 May). Females started oviposition significantly earlier in the Outdoor cohort than in the Laboratory cohort (the median dates were 1 June and 6 June, respectively; Mann–Whitney U-test; U = 264, P<0.01).

There was a pronounced difference in the post-diapause reproductive performance between the two experimental cohorts. Females from the Outdoor cohort produced significantly fewer egg masses and eggs, the size of the egg masses was significantly smaller, and the period of oviposition was significantly shorter than in the Laboratory cohort (Figure 2; Mann–Whitney U-test, P<0.01 for all indexes, U = 216 for number of egg masses, U = 173 for number of eggs, U = 21 684 for egg mass size, U = 240 for the period of oviposition).

Discussion

Nezara viridula recently expanded its distribution range in Japan and reached Osaka, but the timing of its induction of diapause is still imperfect on the edge of the distribution range: reproductive diapause is induced late in the season (only in late September), and the progeny from 35–100% of the egg masses produced by females in autumn fail to attain adulthood before the arrival of the cold winter weather and are destined to die in the nymphal stage during the winter (Musolin & Numata, 2003b). However, the fate of the bugs that have managed to attain adulthood before the arrival of the cold weather (e.g., in November) remains unknown. The present study demonstrates that if egg masses are produced in mid-September and nymphs attain adulthood in November, the adults do not start reproduction in autumn and are likely to successfully overwinter and survive until the next reproductive season (Figure 1).
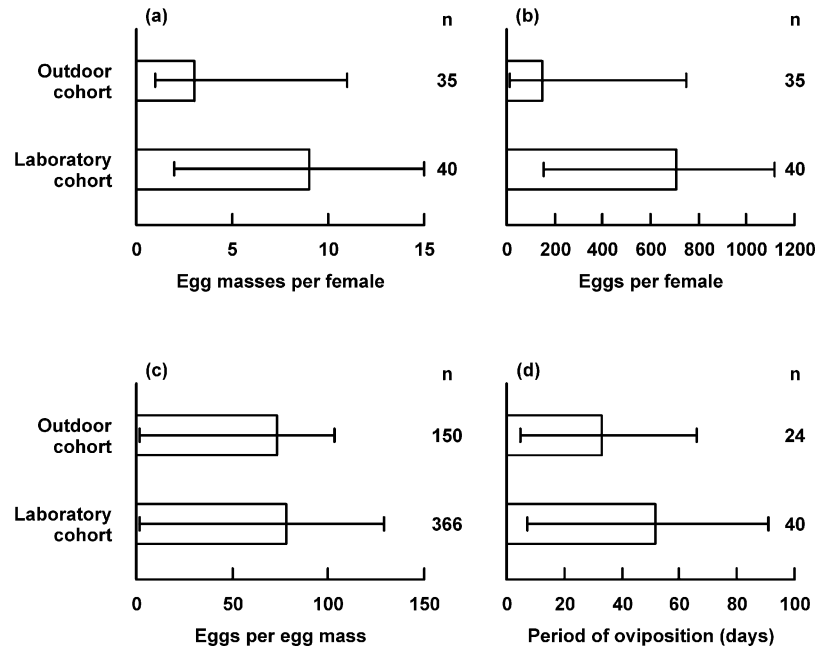
Figure 2 Post-diapause reproduction of *Nezara viridula* in the Outdoor and Laboratory cohorts. (a) Number of egg masses per female, (b) number of eggs per female, (c) egg mass size, and (d) period of oviposition. The medians (with range) are significantly different between the two cohorts in all indexes (Mann–Whitney U-test, P<0.01 for all indexes, see Results for U-values). 'n' refers to the number of females in (a), (b), and (d), and to the number of egg masses in (c). In (d), only females that produced two or more egg masses are included. See Figure 1 and the text for further explanation.

However, a considerable proportion of overwintering adults of the Outdoor cohort failed to change body colour from green to russet as *N. viridula* normally does when it enters diapause (Harris et al., 1984; Jones & Westcot, 2002; Musolin & Numata, 2003a). The incidence of dark-coloured individuals slowly increased until late February/late March despite the lengthening of the natural photophase after the winter solstice and rise in temperature from mid-February. However, even then more than 20% of the adults remained green (Figure 1). The present study suggests that the environmental conditions required for colour change probably do not completely coincide with those required for diapause induction. In this experiment, perhaps, the outdoor temperature was high enough for pre-diapause feeding and the gain of metabolic reserves necessary for overwintering, but too low for the physiological processes associated with seasonal colour change. In spring, most of the adults changed colour from russet to green prior to the start of copulation, whereas some adults were still dark-coloured when they started reproduction. This observation further suggests that the colour of the adults is not always a reliable indicator of diapause in this species.

Post-diapause reproductive performance differed between the two experimental cohorts. Females from the Laboratory cohort entered diapause under favourable laboratory conditions and after overwintering outdoors produced 153–1119 eggs in 2–15 egg masses, which is not significantly different from the highest post-diapause reproductive indexes of *N. viridula* reported earlier (Series 4; Musolin & Numata, 2003b; Mann–Whitney U-test; U = 449.5,

P>0.05 for number of eggs per female; U = 520, P>0.05 for number of egg masses per female). Females from the Outdoor cohort entered diapause under late-season and apparently sub-optimal outdoor temperature conditions. After overwintering, these females produced significantly fewer egg masses and eggs; they were also inferior in other reproductive indexes (Figure 2).

These results indicate that if the progeny of *N. viridula* from egg masses produced as late as in mid-September attain adulthood late in the season (e.g., in November), the adults have high chances of a successful overwintering, but their reproductive output after diapause is significantly reduced compared to that of individuals emerging as adults and entering diapause under more favourable environmental conditions (e.g., in October). This reduced reproductive output may, in turn, affect the fitness of the progeny, as it is known that, for instance, egg mass size has an influence upon survival rates of nymphs in *N. viridula* (Kiritani et al., 1966b).

Acknowledgements

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