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Simulation of Diapause Induction in Spider Mites (*Tetranychus urticae* and *T. kanzawai)* by Reproducing Field Environments in the Laboratory

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ABSTRACT

Backgroud: Diapause as the basic mechanism, helps insects and mites to synchronize their life cycles with local seasonal changes. Field tests are desirable to determine the timing of diapause accurately, but the environments are variable, making it challenging to validate the reproducibility of results.

Materials and Methods: We designed an environmental simulator that reproduces the field variation in the laboratory based on the installation of 20 years of climate data in the Hokkaido region. We computed a regression equation to predict the light intensity and temperature between the laboratory simulator and field data and its accuracy was clearly demonstrated.

Findings: Photoperiodic reaction curves of *T. urticae* and *T. kanzawai* indicated a generic short-day reaction type with critical photoperiods at 18.0°C of approximately 13.5 h for *T. urticae* and 12.5 h for *T. kanzawai*. Diapause of *T. urticae* and *T. kanzawai* in the field was induced on 22 and 23 September, respectively, which is close to the dates of diapause induction estimated by using critical photoperiods (19 and 30 September, respectively). Little variation between observed and estimated critical photoperiods of the mentioned species may be due to minor deviations in temperature in the simulator.

Conclusion: The present study demonstrates the importance of factoring in variable temperatures in the field for accurate prediction of the timing of diapause induction in spider mites. Our experimental system may also be useful in pest mite forecasting by predicting its diapause termination and spring emergence in any geographic area where environmental data are available.

Keywords: Tetranychidae, diapause induction, light intensity, simulator, photoperiod

INTRODUCTION

An understanding of the pattern of diapause is fundamental to an understanding of population growth, migration, speciation, species distribution, and species interaction (Butler et al. 1978). Detailed knowledge of insect seasonality is required for accurate forecasts of seasonal activity which is essential for population management. With facultative diapause, day length (photoperiod), temperature, diet, and moisture have all been implicated as being involved in the induction of diapause, but of these several factors, day length and temperature have proved to be of the highest importance (Beck 1980; Tauber et al. 1986; Danks 1987). It is well known that a broadly latitudinally distributed insect species with winter diapause will tend to develop into geographical populations exhibiting adaptive photoperiodic response characteristics. It is not astonishing that diapause may have arisen independently many times during natural selection since the components of diapause have a different genetic basis in various insects (Hoy 1978).

Several spider mite species can enter reproductive diapause in reaction to the physical environment, i.e., short light periods and low air temperature (Veerman 1985; Takafuji et al. 2003). Photoperiodic diapause possibly progressed on numerous instances as insects and mites extended their distributions into higher latitudes with a prominent winter season (Saunders 2009). As all insect photoperiodic mechanisms are probably circadian-based, their development from a prior homodynamic life cycle in the tropics must have employed the pre-existing (and ubiquitous) circadian system.

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most important and economic polyphagous herbivorous arthropods, infesting on more than 1,100 plant species in over 140 plant families (Grbić et al. 2011; Migeon & Dorkeld 2014). In addition, *Tetranychus kanzawai* Kishida is distributed all over Asia, Oceania, North America and Mexico, where it is of considerable economic importance (Bolland et al. 1998) and also a serious pest of a variety of crops such as solanaceous and rosaceous plants in Japan (Ehara & Gotoh 2009). Both species were collected from the northern areas of Japan, Hokkaido, and the extension of the distribution into southern areas suggests that the diapause attributes of the northern populations may have been modified as compared with those in the southern ones. *T. urticae* did not enter diapause in a photophase ranging from 14 to 16 h in Hokkaido and the diapausing females reached 96-100% in a photophase of 10 h (Gotoh 1986). However, diapause in females of *T. urticae* reached 50% in late September in 1981 and 1983, but it reached 100% in early September in 1982. Since a daylength at the same time of year in the same region does not vary from year to year, the different timing of diapause induction may have been influenced by other factors, most likely different temperatures. However, this difference cannot be verified by a conventional experimental system that relies on photoperiod control and is maintained at a constant temperature in the laboratory.

An environmental simulation system (ESS) developed by our group can reproduce the fluctuating temperatures and photoperiods in any region where environmental data are available, making it possible to experimentally verify the effects of the field environments on organisms in the laboratory (Nishide et al. 2017a,b; Ghazy et al. 2018, 2019). ESS offers the possibility of accurately predicting the timing of diapause induction in spider mites. In this study, we installed 20 years' environmental data from the Hokkaido region in ESS to evaluate the timing of diapause induction of *T. urticae* and *T. kanzawai* under simulated field environments.

MATERIALS AND METHODS

To determine the timing of diapause induced by the field environments, we used ESS that reproduces the outdoor photoperiods formed by changes in light intensity and temperatures over time in the laboratory (Nishide et al. 2017a,b; Ghazy et al. 2018, 2019). ESS consisted of a computer (PC, XC cube EZ 65, Aopen Inc., Tokyo, Japan), a refrigerator (JF-NU40B, Haier Japan Inc., Osaka, Japan), an hair dryer (Tescom Denki Inc., Tokyo, Japan), white LEDs (GW5BTF50k00, Sharp Inc., Osaka, Japan), a temperature-humidity sensor (THA-3151, T & D Co., Nagano, Japan), an illumination sensor (ISA-3151, T & D, Co., Nagano, Japan), a data logger (TR-74Ui, T & D Co., Nagano, Japan), and a control unit (Figure 1). Temperatures were regulated by on/off controls of the refrigerator and the hair dryer. Light intensity was regulated by pulse-width modulation of LEDs (Suzuki et al. 2011). Temperature and light intensity were recorded every 10 seconds. Setting data of temperatures and photoperiods in ESS was based on the mean hourly values of 20 years from 1981 to 2000 in Sapporo, Hokkaido (Meteorological Data System Co., Kagoshima, Japan). We set ESS to reproduce the temperatures and photoperiods precisely and continuously from the beginning till the end of experiment. As the sunlight is strong and the LEDs used in this study cannot reproduce the same output of light, the light intensity during day time was set at a constant value. To get the exact period for diapause induction of *T. urticae* and *T. kanzawai* in field, the environment was reproduced at five dates (21 August, 1 September, 6 September, 11 September, and 16 September).

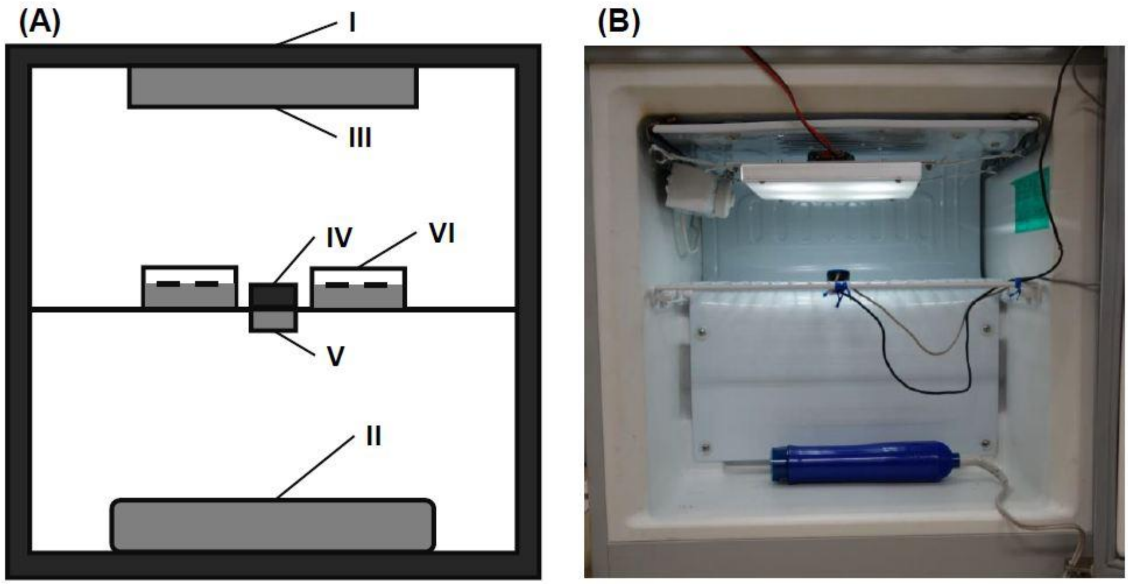

Figure 1. Schematic diagram (A) and photograph of inner structure (B) of the simulator. (I) mini refrigerator, (II) hairdryer, (III) white LED, (IV) illumination sensor, (V) temperature-humidity sensor and (VI) leaf disk on wetted cotton in Petri dish (9 cm diameter).

Statistical Analysis

The critical photoperiod was generated based on logistic analysis consisting of a generalized linear model. To better understand the significance of correlations between the field and laboratory data, relative light intensity and temperature on each date were subjected to correlation analysis. All analyses were carried out using R version 3.1.2 (R Core Team 2014).

RESULTS

Validation of ESS data

Expectations concerning the ability to match the data collected from field and laboratory can be developed by attempting to fit regression equations to validate the data. We computed a regression equation to predict temperature between ESS-in-laboratory and field data (Fig. 3). The regression analysis showed the positive relationship in temperatures between field and ESS with the R^2 value of 0.795.

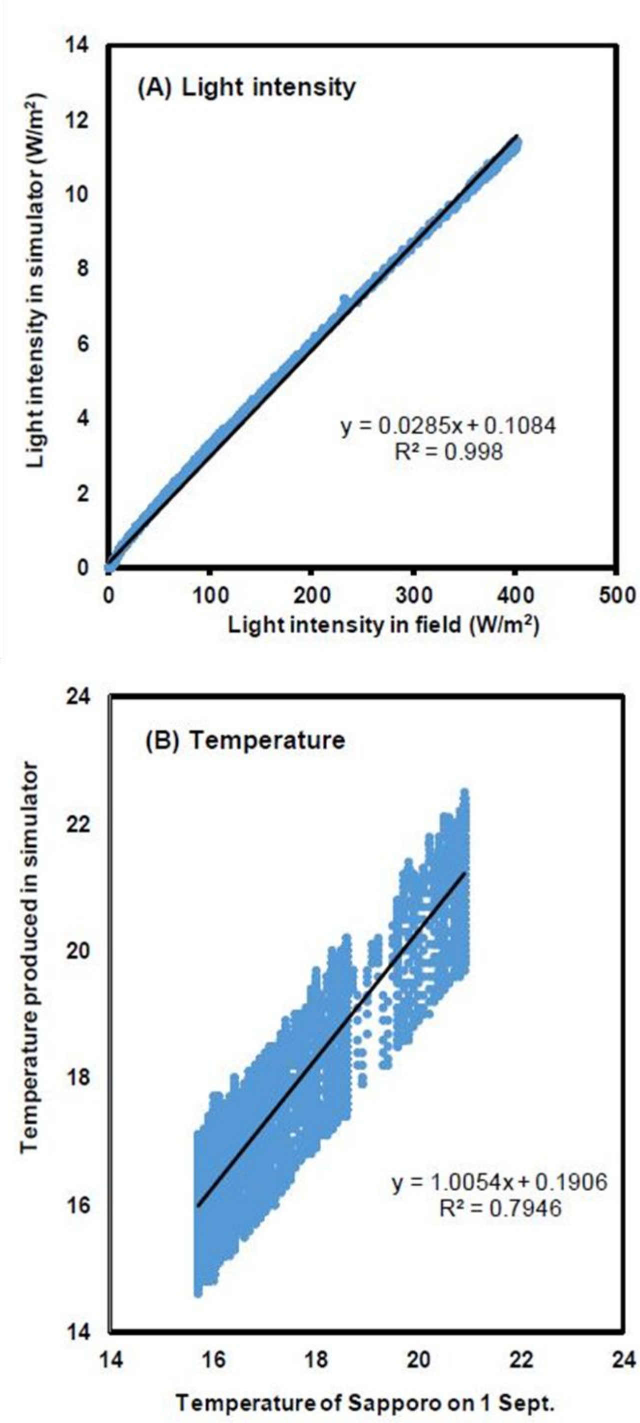

Figure 2. Correlation of light intensity (A) and temperature (B) produced inside the simulator to validate the data with field in Sapporo on 1st September.

Diapause induction

Diapause induction of *T. urticae* and *T. kanzawai* collected in Hokkaido was observed in recreated environmental condition by simulator in laboratory (Fig. 4). No diapause induction was observed in either species in the beginning of September. In the middle of September diapause was 21.7 and 12.8% in *T. urticae* and *T. kanzawai*, respectively. On September 23, the diapause induction of *T. urticae* was slightly lower than that of *T. kanzawai*, but at the end of September both species showed a similar response. Finally, in the early week of October 100% females had entered diapause in both species. The photoperiodic response curve of *T.*

urticae and *T. kanzawai* indicated that a typical short-day response type with a critical photoperiod (at 18.0°C) of approximately 13.5 h for *T. urticae* and 12.5 h for *T. kanzawai*. Diapause induction of *T. urticae* and *T. kanzawai* was observed on 22 and 23 September, whereas the estimated dates of diapause induction were on 19 and 30 September, respectively, based on the photoperiodic response curves. So, the observed date of diapause induction was 4 days later in *T. urticae* and 7 days earlier in *T. kanzawai*.

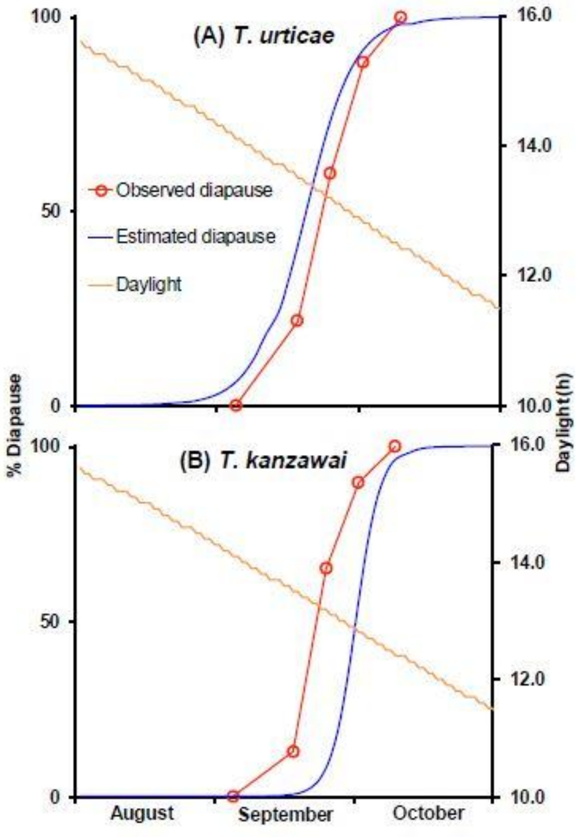

Figure 3. Photoperiodic response curves of diapause induction of *Tetranychus urticae* and *T. kanzawai* inside the simulator experienced with the environment set as field from August to October.

DISCUSSION

The results with the laboratory condition which could be recreated using the ESS are valid and similar with field data indicated that twilight, light intensity and temperatures. Both the temperatures and photoperiods are important in predicting the timing of diapause induction of *T. urticae* and *T. kanzawai*. The estimated critical daylengths for the photoperiodic induction of diapause was slightly different from the observed diapause induction.

The scientific significance of ESS can only be substantiated by rigorous validation with respect to field data. It can be seen from the comparison of temperatures in ESS and field data that the ESS is well suited. This result is possibly presented by the static diapause parameters in ESS that could fully capture the climatic-driven mechanism of diapause. When the diapause effect is included, the ESS demonstrates a substantial improvement in cases of Hokkaido, where diapause shows an established seasonal pattern. Adjusting the ESS with light intensity and temperature-related parameters yields moderate improvement in this study areas and the best performance is achieved with both facets of adjustments applied.

A few cautionary notes should be highlighted. First, the ESS did not account for the accurate light intensity occurring in the field. Generally, the high light intensity observed in the field cannot be produced by the ESS. ESS can create a proportionate light intensity that is fitted with the field light intensity. A change in critical photoperiods for diapause induction with ongoing climate change was already observed in nature (Stoeckli et al. 2012). Several studies on photoperiod-induced diapause induction have concentrated on the operation of the 'circadian clock' that identifies the length of day and night, and the 'photoperiodic calendar' for seasonal changes in photoperiod (Saunders 1981). Outcomes of these and photoperiod change experiments have been related to the operation of the circadian clock and calendar (Koštál 2011; Saunders 2009, 2010a,b, 2013). Although several previous studies have documented seasonal monitoring data, suggesting that *T. urticae* populations from red currant and red clover gradually underwent a change from 10 to 16 h (Gotoh 1986). The diapause induction of field *T. urticae* population from these two food plants corresponded to the timing predicted by the critical photoperiod, when considered the twilight light hours (Beck & Hance 1960). It was also reported that the proportion of diapausing females of *T. urticae* was high in early September and it was low in October, as compared with 1981 and 1983 for red currant populations and with the 1982 for the red clover population (Gotoh 1986). The critical photoperiods of two populations of *T. urticae* appeared to be 13 h, which appeared in mid-September in Sapporo (Gotoh 1986). Our study, using the ESS, also showed that the critical photoperiod was on 22-23 September, which is very close to the critical photoperiod observed in field. The little variation between these two studies was likely due to the slight deviation in temperature in the ESS. The present study accurately simulated the relationship between temperature and relative light intensity in field and in laboratory, and may be used to predict its post-diapause development or spring emergence in future.

Tetranychus urticae and *T. kanzawai* is a significant and frequently infesting agricultural pest in Japan (Gotoh 1986; Shah et al. 2011a,b). Understanding photoperiodic as well as other factors involved in diapause induction in these species is important to improve simulation models of developmental timing and therefore for monitoring and management program. In an attempt to approximate natural photoperiod and temperature change experienced during the life of spider mites in ESS could help us to predict diapause characteristics. Therefore, this study concentrated on temperature, photoperiod and relative light intensity could be recreated in laboratory using ESS similar to field conditions, which are the major factors regulating diapause induction and provided the fundamental information necessary to predict spring emergence.

CONCLUSION

The prediction of diapause induction in the laboratory using the simulator indicated effectiveness and was consistent with the field observations. Temperature, photoperiod, and relative light intensity could be recreated in the laboratory using a simulator similar to field conditions, which are the major factors regulating diapause induction and provided the fundamental information necessary to predict spring emergence. The photoperiodic reaction of diapause induction in both species using the simulator in the laboratory was accurate. It may be a useful instrument for monitoring and management procedures.

CONFLICT OF INTEREST

All authors express no conflict of interest in any part of research, manuscript and submission to the journal.

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