

Heat Shock Influences on Parasitization of the Angoumois Grain Moth *Sitotroga cerealella* Oliv. (Lepidoptera, Gelechiidae) Eggs by the Egg Parasitoid *Trichogramma principium* Sug. et Sor. (Hymenoptera, Trichogrammatidae) Females

S. Ya. Reznik and N. P. Vaghina

Zoological Institute, Russian Academy of Sciences, St. Petersburg, 199034 Russia

Received April 24, 2006

Abstract—Experiments have showed that heat shock (45°C during 30 min) caused a twofold increase in mortality of *T. principium* females recorded during two days after the shock. The percentage of females that have started parasitization of the grain moth eggs (among all survived females) sharply decreased (40–50% and 30% in controls and in treated females, respectively). Thus, the switch from egg retention to parasitization of less preferred hosts was not the result of “pathological” changes in a senescent or dying wasp, but a “normal” stage of adult life. Females that have already started parasitization before the heat shock, continued to parasitize after the shock (differences between treatments and controls were not significant). Obviously, the heat shock influence on oviposition was mostly indirect (as a cue factor) and the wasps that have already started parasitization, were insensitive to this cue. These data resemble the loss of sensitivity to the diapause-inducing factor which was often observed after re-activation. We conclude that the results of our study confirm analogies between egg retention and reproductive diapause.

DOI: 10.1134/S001387380608001X

In ectotherms, temperature is one of the most important environmental factors (Chernyshev, 1996). In most of insects, the upper limits of optimum temperature vary from 25 to 35°C. Higher temperatures usually cause a decrease in survival, fecundity, and other biological parameters, while long-term exposure to the temperatures exceeding 40–45° results in the lethal effect. This low resistance to temperature extremes is widely used as a basis for effective and ecologically safe methods of the control of insect pests (Arbogast, 1981; Hansen, 1992), but in biological plant protection it often has a negative role as a limiting factor for the efficiency of insect entomophages.

Species of the genus *Trichogramma* Westw., one of which was the object of our study are widely used for biological control of various agricultural and forestry lepidopteran pests (Wajnberg and Hassan, 1994; Smith, 1996). However, in certain cases their efficiency may be limited by extremely high temperatures. Special investigations (Chihrane et al., 1993; 1997; Chihrane and Laugé, 1994, 1996; Chihrane et al., 1997; Carrière and Boivin, 1997) have showed that even relatively short-term exposure of *Trichogramma* pupae or adults to sublethal high temperatures resulted

in a sharp decrease in their fecundity, life duration, and movement activity, and consequently reduced the efficacy of these beneficial parasitoids.

Our earlier studies (Reznik et al., 1998, 2001a, 2001b) demonstrated that *Trichogramma principium* Sug. et Sor. females offered eggs of the Angoumois grain moth *Sitotroga cerealella* Oliv. often showed a delay in parasitization ranging up to 10 days. The duration of this pre-parasitization period depended on the stage of development and on the number of host eggs (Reznik and Umarova, 1990, 1991). Our experiments (Reznik and Vaghina, 2006) have also demonstrated that within the optimum range, the rate of induction of parasitization linearly increased with temperature.

The aim of the present study was to investigate the influence of heat shock, i.e., short-term exposure to extremely high (sublethal) temperature on induction and stability of parasitization.

MATERIALS AND METHODS

In all the experiments, we used a laboratory strain of *T. principium*, which was cultivated for many

Table 1. Influence of heat shock preceded the first contact with the host on survival and parasitization of the grain moth eggs by *Trichogramma principium* females

Biological parameters	Control	Heat shock	Significance of the difference
Mortality during 30 min after the heat shock (%)*	0 (0–0)	11 (2–13)	$p < 0.001$
Mortality during 48 h after the heat shock (%)*	10 (5–18)	18 (16–31)	$p < 0.001$
The proportion of parasitizing females (%)*	50 (47–60)	27 (24–33)	$p < 0.001$
Mean fecundity of all parasitizing females (eggs / female)**	28.5 ± 7.8 ($n = 168$)	23.3 ± 7.5 ($n = 69$)	$p < 0.001$
Mean fecundity of parasitizing females which survived more that 48 h after the shock (eggs / female)**	29.0 ± 7.7 ($n = 144$)	24.0 ± 7.6 ($n = 56$)	$p < 0.001$

Notes: * Median and quartiles, ** mean ± SD and sample size.

Table 2. Influence of heat shock effected between two host exposures on survival and parasitization of the grain moth eggs by *Trichogramma principium* females

Biological parameters	Control	Heat shock	Significance of the difference
Mortality during 30 min after the heat shock (%) *	0 (0–0)	10 (4–15)	$p < 0.001$
Mortality during 48 h after the heat shock (%) *	8 (5–12)	25 (14–41)	$p < 0.001$
The proportion of females which continued parasitization after the shock (%)*	100 (96–100)	99 (85–100)	$p = 0.12$
The proportion of females which started parasitization after the shock (%)*	40 (30–48)	28 (20–35)	$p < 0.001$
Mean fecundity of all females which started parasitization (eggs / female)**	26.3 ± 8.8 ($n = 144$)	22.3 ± 8.8 ($n = 62$)	$p = 0.002$
Mean fecundity of females which started parasitization and survived more that 48 h after the shock (eggs / female)**	26.8 ± 8.8 ($n = 135$)	23.0 ± 8.3 ($n = 52$)	$p = 0.001$
Mean fecundity of all females which continued parasitization (eggs / female)**	12.7 ± 5.8 ($n = 166$)	11.2 ± 5.6 ($n = 125$)	$p = 0.003$
Mean fecundity of females which continued parasitization and survived more that 48 h after the shock (eggs / female)**	13.1 ± 5.6 ($n = 154$)	11.5 ± 5.5 ($n = 109$)	$p = 0.002$

Note: For designations, see Table 1.

generations on the eggs of the grain moth, *S. cerealella*. *Trichogramma* developed and all experiments (excluding the heat shock periods) were conducted at a constant temperature of 20° C under light regime of L : D = 18 : 6.

At the beginning of each experiment, emerged adults of *T. principium* were allowed to mate during 12–24 h and then were individually placed into small (8 mm in diameter, 45 mm in length) test tubes. Honey (50% solution) was streaked on the glass as a food

source. *S. cerealella* eggs were presented according to the design of the experiment (see below) as an artificial “batch” of 50–60 eggs pasted to a thick paper strip. Each experiment included two 48 h periods (exposures) separated by a short-tem heat shock (45°C during 30 min). In controls, the heat shock was replaced by a 30 min long interval between two exposures under the same constant conditions. If females were presented with a possibility to parasitize (once during the first experiment and twice during the sec-

ond experiment), at the end of the given exposure paper cards with the grain moth eggs were taken out of the test tubes.

In the first experiment, during the first 48 h exposure *T. principium* the females were not presented with a possibility to parasitize, the grain moth eggs were offered only after the heat shock, during the second exposure. Seven replicates of the first experiment were conducted, each including ca 100 females. In the second experiment, the females were presented with a possibility to parasitize during both exposures, but during the heat shock period, host eggs were absent. Eight replicates of the second experiment were conducted, each including 100–150 females.

After completion of *Trichogramma* larvae development, darkened (parasitized) eggs of the grain moth were counted for each card separately. In our experiments, we used the parasitoid : host ratio about 1 : 50. Under these conditions, *T. principium* females usually laid a single egg per each egg of the grain moth. Therefore the number of parasitized hosts was taken for the approximate number of eggs laid by *Trichogramma* female (fecundity). Thus, for each exposure of each replicate of each experiment, the percentage of parasitizing (i.e., those which laid at least one egg) females was calculated and fecundity of each parasitizing female was recorded.

It is well known (Reznik et al., 1996) that the percentage of parasitizing females and their fecundity significantly vary even in sequential generations of laboratory lines. Hence, nonparametric characteristics (mortality, percentage of females which started and continued oviposition) were separately estimated for each replicate and then averaged with medians and quartiles, the number of replicates being taken for a sample size. For these characteristics, the significance of the difference between the control and treatments was estimated by the Mantel-Haenszel test (Afifi and Azen, 1982). The significance of differences in fecundity was estimated by the two-way ANOVA test, replicate being also considered as a factor, but in the tables total sample sizes and averaged results of all replicates of each experiment are given. All statistical procedures were calculated with SYSTAT 10.2.

RESULTS

First, it should be noted that the heat shock (45°C during 30 min) caused about 10% mortality of

treated individuals. In addition, mortality during the next 48 h increased about twofold. The difference in mortality between treated and control individuals was significant in both experiments (Tables 1 and 2), while the difference between two experiments was not significant. Thus, the sublethal effect of the heat shock on the most of individuals of the studied *T. principium* strain was clearly demonstrated.

In the first experiment, the heat shock preceded the first contact with the host which resulted in almost twofold decrease in the percentage of females that started parasitization (Table 1). A decrease in fecundity of ovipositing females was also significant, although not as pronounced. As noted above, some of the treated and control females died during 48 h long exposure (period of contact with the host). Thus, the decrease in fecundity could be partly caused by mortality of females. However, when the females that died before the end of exposure were not taken into consideration, the difference between the experiment and the control being still significant, although relatively small (Table 1).

In the second experiment, the wasps were subjected to the heat shock after the first period of contact with the host, when certain females had already started parasitization. Naturally, proportions of females that had started parasitization before the shock in control and in experiment were practically equal constituting, respectively, 35.9 and 35.0%. Practically all females that parasitized during the 1st exposure, parasitized also during the 2nd exposure, the difference between the experiment and the control was insignificant (Table 2). As for the females which did not parasitize before the shock, the percentage of individuals which started parasitization during the 2nd exposure in the experiment was much lower than that in the control. Fecundity of females which had started parasitization was almost equal to that in the first experiment, the difference between treated individuals and controls being also approximately the same (Tables 1 and 2). Females that continued parasitization started during the 1st exposure, manifested much lower fecundity, the difference between treated individuals and controls being less pronounced but still significant.

DISCUSSION

Thus, the heat shock caused a sharp decrease in the percentage of *T. principium* females which started parasitization. Fecundity of parasitizing females also, although not as markedly, decreased. However, fe-

males that had already started parasitization, usually continued it after the heat shock. These data in combination with the results of the previous studies allow us to draw several conclusions.

It is known (Tyshchenko, 1980) that various (particularly adverse) environmental factors could exert two kinds of influences on insects: direct (vital) and indirect (cue). One could suppose that the heat shock directly influenced oviposition by *Trichogramma* causing some pathological effects, such as partial male sterility and female oogenesis suppression in *Trichogramma brassicae* Bezdenko (Chihrane et al., 1993; Chihrane and Laugé, 1994, 1996). However, the hypothesis for the direct traumatic effect does not match with the fact that females which started parasitization, continued it after the shock. Moreover, the shock caused only a slight decrease in fecundity of females that started or continued parasitization, suggesting good preservation of mature ovarian eggs ready to be laid and continuation of more or less intensive oogenesis. Hence, in this case the heat shock exerted mostly an indirect (cue) effect. The ability to lay eggs, as such, was constant or just slightly reduced, while the main effect was manifested in a sharp decrease of the probability to switch from “delay state” to “parasitization state.”

A delay in parasitization of the less preferred factitious laboratory host (e.g. eggs of the grain moth) is typical of many *Trichogramma* species. The rate of induction of parasitization linearly increases with temperature (Reznik and Vaghina, 2006). However, the average insect life duration decreased with temperature. It is known that in certain insects typical changes in behavior were revealed during the last days of their life (Giebultowicz et al., 1990). In certain cases, a wider host specificity was recorded. For example, *Horogenes chrysostictos* Gmelin (Hymenoptera, Ichneumonidae) females usually avoid superparasitization, but they lose this ability several days before death (Fisher, 1959). Such changes in senescent insects are explained by loss of perception or discrimination ability (Tallamy, 2000). Thus, it is conceivable that the increase in the rate of induction of parasitization with increasing temperature results from the decrease in the mean life duration. Moreover, the increase in the rate of induction of parasitization in *T. principium* females deprived of carbohydrate food, as compared to fed females, and a slightly higher mean life duration in females which delayed parasitization, as compared to parasitizing females (Reznik et al.,

1997, 2003) could be explained in the same way. However, the results of the present study (Tables 1 and 2) spoke against this explanation: the heat shock, reducing the mean life duration, did not stimulate, but inhibited the induction of parasitization. Obviously, the switch from delay to parasitization of a less suitable host is not the result of some “pathological” terminal changes, but a “normal” stage of adult life, similar to reactivation after the reproductive diapause.

A tendency to diapause could be increased by very different adverse environmental factors, but after reactivation, insects often became insensitive to those cues (Danilevskiy, 1961; Tyshchenko, 1983; Zaslavskiy, 1996; Chernyshev, 1996). Just this result was obtained in our study: the heat shock prevents induction of parasitization, but it practically does not influence the continuation of parasitization. Thus, the outcome of the present study could be considered as a further argument in support of the earlier published (Reznik et al., 1998, 2001, 2003) hypothesis of analogies between reproductive diapause and delay in parasitization of a suitable but less preferred host.

ACKNOWLEDGMENTS

This work was partly supported by the Programme “Biological Resources of Russia: Fundamental Bases of Rational Use” of the Department of Biological Sciences, Russian Academy of Sciences.

REFERENCES

1. Afifi, A.A. and Azen, S.P., *Statistical Analysis. A Computer Oriented Approach* (Academic Press, New York, 1979).
2. Arbogast, R.T., “Mortality and Reproduction of *Ephesia cautella* and *Plodia interpunctella* Exposed as Pupae to High Temperatures,” *Environ. Entomol.* **10** (5), 708–711 (1981).
3. Carrière, Y. and Boivin, G., “Evolution of Thermal Sensitivity of Parasitization Capacity in Egg Parasitoids,” *Evolution* **51** (6), 2028–2032 (1997).
4. Chernyshev, V.B., *Insect Ecology* (Moscow, 1996) [in Russian].
5. Chihrane, J. and Laugé, G., “Effects of High Temperature Shocks on Male Germinal Cells of *Trichogramma brassicae* (Hymenoptera, Trichogrammatidae),” *Entomophaga* **39** (1), 11–20 (1994).
6. Chihrane, J. and Laugé, G., “Loss of Parasitization Efficiency of *Trichogramma brassicae* (Hym.: Trichogrammatidae) under High-Temperature Conditions,” *Biological Control* **7** (1), 95–99 (1996).
7. Chihrane, J., Laugé, G. and Hawlitzky, N., “Effects of High Temperature Shocks on the Development and Bi-

- ology of *Trichogramma brassicae* (Hymenoptera, Trichogrammatidae)," *Entomophaga* **38** (2), 185–192 (1993).
8. Chihrane, J., Derrien, A. and Laugé, G., "Locomotor Activity of *Trichogramma brassicae* (Hymenoptera) under the Influence of High-Temperature Shocks," *J. Insect Behav.* **10** (2), 203–211 (1997).
 9. Danilevskiy, A.S., *Photoperiodism and Seasonal Development of Insects* (Leningrad, 1961) [in Russian].
 10. Fisher, R.C., "Life History and Ecology of *Horogenes chrysostictos* Gmelin (Hymenoptera, Ichneumonidae), a Parasite of *Ephestia sericarium* Scott (Lepidoptera, Phycitidae)," *Canad. J. Zool.* **37** (4), 429–446 (1959).
 11. Giebultowicz, J.M., Raina, A.K. and Uebel, E.C. "Mated-like Behaviour in Senescent Virgin Females of Gypsy Moth, *Lymantria dispar*," *J. Insect Physiol.* **36** (7), 495–498 (1990).
 12. Hansen, J.D., "Heating Curve Models of Quarantine Treatments against Insect Pests," *J. Econom. Entomol.* **85** (5), 1846–1854 (1992).
 13. Reznik, S.Ya. and Umarova, T.Ya., "The Influence of the Host's Age on the Selectivity of Parasitism and Fecundity of *Trichogramma*," *Entomophaga* **35** (1), 31–37 (1990).
 14. Reznik, S.Ya. and Umarova, T.Ya., "Host Population Density Influence on Host Acceptance in *Trichogramma*," *Entomol. Exp. Appl.* **58** (1), 49–54 (1991).
 15. Reznik, S.Ya. and Vaghina, N.P., "Temperature Effects on Induction of Parasitization by Females of *Trichogramma principium* (Hymenoptera, Trichogrammatidae)," *Zool. Zh.* **85** (1), 48–53 (2006) [*Entomol. Rev.* **86** (2), 133–138 (2006)].
 16. Reznik, S.Ya., Voinovich, N.D., and Umarova, T.Ya., "Experimental Studies of Dynamics of the Percentage of Ovipositing Females and their Fecundity in Successive Generations of *Trichogramma* (Hymenoptera, Trichogrammatidae)," *Zool. Zh.* **75** (3), 375–382 (1996) [*Entomol. Rev.* **76** (1), 138–143 (1996)].
 17. Reznik, S.Ya., Voinovich, N.D., and Umarova, T.Ya., "Carbohydrate Suppresses Parasitization and Induces Egg Retention in *Trichogramma*," *Biocontrol Sci. Techn.* **7** (2), 271–274 (1997).
 18. Reznik, S.Ya., Umarova, T.Ya., and Voinovich, N.D., "Egg Retention in the Presence of a Host in *Trichogramma* Females," *J. Appl. Entomol.* **122** (9–10), 555–559 (1998).
 19. Reznik, S.Ya., Voinovich, N.D. and Umarova, T.Ya., "Comparative Behavioral Analysis of Ovipositing Females and Females with Egg Retention in *Trichogramma principium* (Hymenoptera, Trichogrammatidae)," *Entomol. Obozr.* **80** (3), 545–555 (2001a) [*Entomol. Rev.* **81** (8), 895–903 (2001a)].
 20. Reznik S.Ya, Voinovich N.D., and Umarova, T.Ya., "Long-term Egg Retention and Parasitization in *Trichogramma principium* (Hymenoptera, Trichogrammatidae)," *J. Appl. Entomol.* **125** (4), 169–175 (2001b).
 21. Reznik, S.Ya., Umarova, T.Ya., and Voinovich, N.D., "Egg Retention in *Trichogramma* (Hymenoptera: Chalcidoidea: Trichogrammatidae): Learning or Diapause?" *Acta Soc. Zool. Bohem.* **67** (1), 25–33 (2003).
 22. Smith, S.M., "Biological Control with *Trichogramma*: Advances, Successes, and Potential of their Use," *Ann. Rev. Entomol.* **41**, 375–406 (1996).
 23. Tallamy, D.W., "Physiological Issues in Host Range Expansion," in *Proceedings of Session: Host Specificity Testing of Exotic Arthropod Biological Control Agents. X Intern. Symposium Biol. Control of Weeds* (Bozeman, Montana, USA, 2000), pp 11–26.
 24. Tyschenko, V.P., "Signal Action of Ecological Factors," *Zh. Obsch. Biol.* **41** (5), 655–677 (1980).
 25. Tyschenko, V.P., "Evolution of Seasonal Adaptations in Insects," *Zh. Obsch. Biol.* **44** (1), 10–22 (1983).
 26. Wajnberg, E. and Hassan, S.A., *Biological Control with Egg Parasitoids* (Wallingford, UK, 1994).
 27. Zaslavskiy, V.A., "Diversity of Environmental Factors Controlling Seasonal Development of Insects, and Probable Unity of Underlying Physiological Mechanism," *Entomol. Obozr.* **75** (2), 233–243 (1996).