

## RESEARCH

## Transovarial Effect of Novaluron on *Tribolium castaneum* (Coleoptera: Tenebrionidae) After Termination of Direct Contact

A. Trostanetsky,<sup>1</sup> M. Kostyukovsky, and E. Quinn

Department of Food Quality and Safety, ARO, The Volcani Center, Bet Dagan, 50250, Israel

<sup>1</sup>Corresponding author, e-mail: anatoly@agri.gov.il

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**ABSTRACT.** The insect growth regulator novaluron (Rimon 10 EC, Makhteshim-Agan Ltd, Israel) is used against many field pests on corn, vegetables, orchards, forests, and cotton plantations. Previously, we studied various effects of novaluron on stored grain pests. Termination in *Tribolium castaneum* (Herbst) eggs hatching after treating adults with novaluron and following restoration after adult transfer to untreated media was observed. The objective of this study was to investigate the restoration of *T. castaneum* egg hatch following transfer of adults from treated media to untreated favorable and unfavorable media. The time needed for hatching restoration of 50% of eggs laid by adults transferred from novaluron (1 ppm) treated flour to untreated flour (RT<sub>50</sub>) was 2.7 d. RT<sub>50</sub> for those transferred to untreated wheat grain was 4.1 d. RT<sub>90</sub> in flour was 3.6 d, in grain—6.1 d. Varieties of RTs in grain and in flour with non-overlapping confidence intervals indicate that RTs were significantly different. Delay of eggs hatching restoration for adults transferred from treated flour to unfavorable media (Petri dishes with limited amount of flour, lying of eggs not detected) was observed. RT<sub>50</sub> in flour was 2.1 d and RT<sub>90</sub>—3.1 d, while RT<sub>50</sub> in the unfavorable media was 3.4 d and RT<sub>90</sub> 6.5 d. Delayed effect of egg hatching restoration after adult transfer to unfavorable media provides evidence of the significant role of insect physiological state in novaluron excretion and (or) degradation by *T. castaneum* females.

**Key Words:** chitin synthesis inhibitor, egg hatching restoration, novaluron, *Tribolium castaneum*

Red flour beetle, *Tribolium castaneum* (Herbst) is a widespread stored grain insect. It damages flour and bakery, bran, cereals, and dried fruit, while whole dry grain stays almost untouched. The beetle has a stable carbolic smell, which passes from the contaminating grain to the flour. Red flour beetle is originally much more pesticide resistant than other stored product insects, and this resistance can rapidly increase more than 10-fold. Its short development cycle and easiness of laboratory cultures maintaining on a simple medium has made this species a popular choice as a model organism for studying pesticide effects (Zettler and Cuperusi 1990, Assie et al. 2007, Wijayaratne et al. 2012).

One of the main demands for insecticides is their influence only on insect metabolic processes. Development disruption insecticides form the insect growth regulators (IGRs) group. Chitin synthesis inhibitors (CSI) belong to the IGRs group. CSIs are insecticides (usually benzoyl-phenyl urea based substances) that inhibit chitin production. One of them is novaluron. This insecticide (Rimon 10 EC, Makhteshim-Agan Ltd, Israel) is used against many field pests to protect corn, vegetables, orchards, forests, and cotton plantations. Novaluron activity is based on larvicidal effect. Various effects of novaluron on stored grain pests were studied (Kostyukovsky et al. 2003, Arthur and Fontenot 2012). The results showed significant decrease in rice weevil *Sitophilus oryzae* L. population, even though the pest develops inside the seed and larva has no direct contact with the pesticide (Kostyukovsky et al. 2003). The same results were observed for other CSIs (McGregor and Kramer 1976; Desmarchelier and Allen 1992; Elek and Longstaff 1994; Oberlander et al. 1997; Elek 1998a,b).

We found that exposure of *T. castaneum* adult to treated flour may serve as a good model for our understanding of CSIs effect on internal feeders. Termination in eggs hatching after treating adults with novaluron and restoration after adult transfer to untreated media was shown. There is the correlation between duration of adults contact with treated media and novaluron concentration with hatching restoration. This most probably resulted from direct contact penetration of novaluron into the insect, and then into the eggs. The eggs hatching in

*T. castaneum* was significantly reduced even at 0.3-ppm novaluron concentration. (Kostyukovsky and Trostanetsky 2006, Trostanetsky and Kostyukovsky 2008). The same results were observed for other CSIs, such as triflumuron (Yasir et al. 2012) and lufenuron (Mishra et al. 2013). Restoration of hatching eggs after the termination of CSIs contact with adults was noted (Desmarchelier and Allen 1992, Kostyukovsky and Trostanetsky 2006, Alyokhin et al. 2009). However, more detailed studies have not been conducted. The objective of this study was to investigate the restoration of egg hatching of *T. castaneum* following transfer of adults from treated media to untreated favorable and unfavorable media.

### Material and Methods

**Insects.** *T. castaneum* culture had been reared under laboratory conditions for many years without any contact with insecticides. It was reared on wheat flour and ground grain. The insects were maintained in 0.8-liter glass jars at 30 ± 0.5°C and 65 ± 5% r.h. in dark.

**Chemical.** Novaluron, 1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl) urea, supplied by Makhteshim-Agan Ltd as Rimon 10 EC, was used for the experiments.

**Treatment.** Thirty milliliter of 16.7-ppm novaluron (Rimon 10 EC) in acetone solution were fractionally introduced into 500 g of wheat flour and were thoroughly mixed. The container was then washed with 10 ml of acetone, afterwards poured into the flour as well. Final novaluron concentration in the flour was 1 ppm. The wheat flour with acetone alone was used as a control. The treated culture was ventilated to evaporate all the acetone from the samples. Approximately 2,000 adults of *T. castaneum* were transferred into 0.8-liter glass jars with 300-g novaluron-treated or -untreated (control) flour. The jars were covered with airpenetrable paper and stored in dark at 30 ± 0.5°C and 65 ± 5% r.h. Two wks later, adults were transferred to a various media, without novaluron.

**Effect of the Medium on Eggs Hatching Restoration.** The first experiment was conducted to compare the eggs hatching restoration in

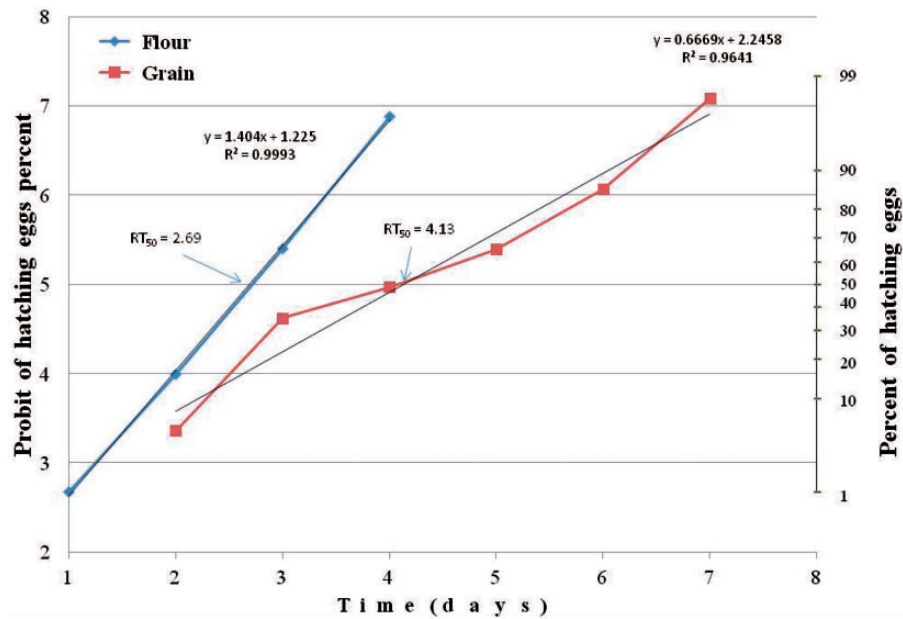


Fig. 1. Restoration of egg hatch of *T. castaneum* following transfer from treated with novaluron flour to untreated flour or untreated grain.

wheat flour and wheat grain medium after transfer of adults from novaluron-treated flour. Insects from jars with treated (experiment) and untreated (control) flour were divided into two groups and transferred into jars with untreated flour (experiment and control) or untreated grain (experiment and control).

Every day 60 unsexed adults *T. castaneum* were taken from each of the four jars and transferred to 70-ml jars with 30 g of flour, 20 adults a jar. The 0- to 24-h-old eggs laid were separated from the flour by 70-mesh sieve. Fifty eggs from each replicate were placed in especially designed plastic slides with individual cell for each egg and clip-on with glass cover and maintained in dark at  $30 \pm 0.5^\circ\text{C}$  and  $65 \pm 5\%$  r.h. The egg hatch was counted under a binocular microscope.

The second experiment was designed to prove the hypothesis that unfavorable conditions may delay the secretion of novaluron from the insect organism. For this experiment insects from jars with treated (experiment) and untreated (control) flour were divided into two groups, as it was done in the first experiment, and transferred into two jars with untreated flour (experiment and control) and Petri dishes (20 *T. castaneum* per dish) containing 0.1 mg of flour each (50 dishes for the experiment and 50 for control). In the preliminary experiment, it was found that *T. castaneum* do not lay eggs under the conditions created in the dishes. Every day 60 *T. castaneum* were collected from the jars and transferred to flour medium as described earlier. Insects from six Petri dishes (three for experiment and three for control) were also transferred into jars with flour. The quantity of daily eggs laid after transferring the insects from the dishes to the flour was decreased during the experiment. Thus, *T. castaneum* adults from two dishes (40 adults) were transferred into each jar from the seventh day. The 0- to 24-h-old eggs separation and hatching percentage calculation was done as described earlier. Both experiments were conducted in three replicates.

**Statistical Analysis.** All results were corrected with Abbott's (1925) formula, which takes into account the egg hatching in parallel control assays. The regression analyses were done using Excel. POLO-PC probit analysis program (Le Ora Software 1987) was used for  $RT_{50}$  and  $RT_{90}$  (the time needed for eggs hatching restoration up to 50 and 90%, respectively, after the insects exposure to novaluron) with corresponding 95% confidence interval (CI) and slope with the standard error calculation.

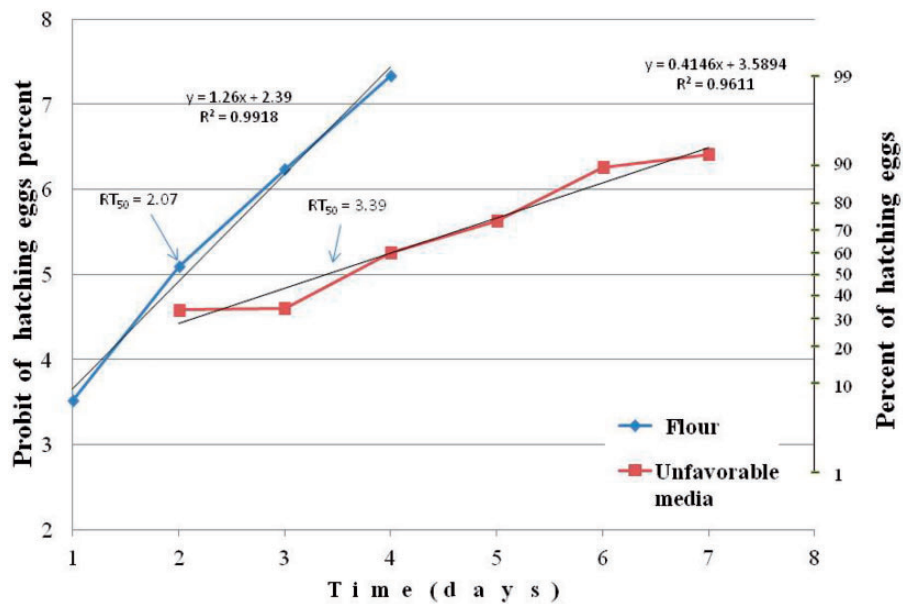
## Results

Eggs hatching percentage in the control was  $92.6 \pm 0.7\%$ . We should note that for the control insects, transferred from flour to flour this percent was higher than for those transferred from grain or Petri dishes to the flour. In the first experiment, we compared the restoration time of normal eggs hatching after transferred *T. castaneum* adults to different novaluron-free medium (flour and grain). The time needed to restore eggs hatchability was significantly different on these media (ANOVA:  $F = 4.67$ ;  $df = 1, 16$ ;  $P = 0.046$ ). The time needed for hatching restoration of 50% eggs laid by adults transferred from novaluron (1 ppm) treated flour to untreated flour ( $RT_{50}$ ) was 2.7 d (95% CI: 2.6–2.8; slope  $1.40 \pm 0.02$ ). The time of 50% restoration for those transferred to untreated grain was 4.1 d (CI: 3.5–4.6; slope  $0.67 \pm 0.06$ ).  $RT_{90}$  in flour—3.6 d (CI: 3.4–3.8; slope  $1.40 \pm 0.02$ ), in grain—6.1 d (CI: 5.5–7.3; slope  $0.67 \pm 0.06$ ) (Fig. 1). Similar results were obtained in the second experiment. The same restoration rate for *T. castaneum* transferred to flour and the delay of eggs hatching restoration for adults transferred to unfavorable media (Petri dishes with limited amount of flour, lying of eggs not detected) were observed. The time needed to restore eggs hatchability was significantly different on these media. (ANOVA:  $F = 17.63$ ;  $df = 1, 16$ ;  $P = 0.00068$ ). For the latter, the restoration up to the control level was the same as for those transferred to grain in the first experiment:  $RT_{50}$ —3.4 d (CI: 2.8–3.8),  $RT_{90}$  6.5 d (CI: 5.8–7.6); slope  $0.41 \pm 0.04$ .  $RT_{50}$  on flour was 2.1 d (CI: 2.0–2.2) and  $RT_{90}$ —3.1 d (CI: 2.9–3.3); slope  $1.26 \pm 0.08$  (Fig. 2).

## Discussion

According to the accepted hypothesis, novaluron, as all the benzoyl-phenyl urea based CSIs, affects the eggs hatching by transmission into the egg during its formation in the insect organism. This is the reason for low laid eggs hatching directly after transferring *T. castaneum* on insecticide free medium (Desmarchelier and Allen 1992; Elek 1998a,b; Kostyukovsky and Trostanetsky 2006; Alyokhin et al. 2008; Trostanetsky and Kostyukovsky 2008, Kim et al. 2011).

In this study, the restoration of *T. castaneum* egg hatch after transfer of adults from treated media to untreated media was investigated. Flour and grain were used in this experiment as a media. It should be noted that the use of insecticides in flour has no practical importance. However, flour may serve as a good model for understanding of CSI's transovarial effect on *T. castaneum* and other stored product insects.



**Fig. 2.** Restoration of egg hatch of *T. castaneum* following transfer from treated with novaluron flour to untreated flour or unfavorable media. Limited amount of flour in Petri dishes was used as unfavorable media (lying of eggs not detected).

The received data is presented on probit-time correlation scale. Probit analysis was used before to describe the correlation between mortality and time of contact with the insecticide. It was described and recognized as appropriate (Preisler and Robertson 1989, Throne et al. 1995). On the other hand, we did not find any mentioning in relevant literature of the use of this analysis to describe the correlation between the time and eggs hatching restoration percentage after the end of insecticide effect. However, our data perfectly correspond with this correlation. The excretion and (or) degradation rate of an insecticide in a living organism is described as an exponential correlation (Gazit et al. 1989, Stamm et al. 2013); therefore, we used a linear axis scale for a while.

According to our current data, eggs hatching restoration time in *T. castaneum* after 2 wk of exposure to 1-ppm novaluron medium was 4 d after transferring to untreated flour. Earlier we showed the correlation between the restoration time and novaluron concentration in the treated medium. After 2 wk of exposure to medium with 100-ppm novaluron the restoration time for *T. castaneum* was more than 3 wk. (Kostyukovsky and Trostanetsky 2006). In this article, we demonstrated that the egg hatching restoration in adults transferred to grain medium was slower than in flour medium. This effect may be caused by unfavorable conditions for insects in the grain medium. We conducted an additional experiment to verify our hypothesis. After 2-wk exposure to novaluron-treated flour *T. castaneum* adults were transferred to Petri dishes with minimal amount of flour, which allows preventing mortality of *T. castaneum* adult, but no eggs were laid. There was a delay in egg hatching restoration in comparison to the control but after 7 d the egg hatching returned to normal. Delayed effect of egg hatching restoration after adult transfer to unfavorable media may be explaining significant role of eggs in novaluron excretion. The literature data do not support this conclusion. CSIs can be excreted into eggs but feces play a more significant role (Ivie and Wright 1978, Medina et al. 2002). After topical application of diflubenzuron on lacewing *Chrysoperla carnea* (Stephens), 20% of the CSI were excreted into the feces while only 1% was excreted into eggs. Diflubenzuron treated *Musca domestica* L. and *Stomoxys calcitrans* L. flies also showed low level of the CSI in eggs (<1%).

It is known that various environmental factors, including commodity characteristics of grain affect the sensitivity of insects to insecticides. It may be explained by physiological state of insects (Athanassiou et al. 2011; Kavallieratos et al. 2012, Subramanyam et al. 2012).

Alyokhin et al. (2009) investigated dependency of novaluron effects on physiological state of the beetles. It was shown, that time of hatching restoration of eggs Colorado potato beetle *Leptinotarsa decemlineata* (Say) after transfer from novaluron-treated foliages to -untreated foliages was 4 d in young beetles and 3 d in old. We suggest that in our experiments physiological state of insect plays the main role in delayed effect of egg hatching restoration after adult transfer to unfavorable media.

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