

## Effects of temporary cooling on larvae of *Idaea inquinata* (Scopoli) (Lepidoptera: Geometridae)

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**Abstract:** *Idaea inquinata* develops on dried plants with a preference for medicinal plants. The survival of second and fourth instars larvae of *I. inquinata* exposed to 6 and  $-18 \pm 1^\circ\text{C}$ , for different periods of time, was observed. Groups of 20 larvae were placed at  $6 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  R.H. for periods of 15, 18, 20, and 25 days and then put in thermostatic chamber at  $26 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  R.H. Tests were controlled after 1, 2, 3, 8, 15 and 23 days until adults emergence. The same procedure was used for tests at  $-18^\circ\text{C}$  with exposure times of 4, 8 and 24 hours. For all the tested periods at  $6 \pm 1^\circ\text{C}$ , mortality higher than 95% was observed in second instar larvae after 1 day at  $26^\circ\text{C}$ . A complete (100%) mortality was observed on the second day at  $26 \pm 1^\circ\text{C}$  after treatment at  $6 \pm 1^\circ\text{C}$  for 18, 20, 25, on the third day for 15 days treatment. Few fourth instar larvae, placed for 25 days at  $6 \pm 1^\circ\text{C}$ , were alive after 8 days at  $26 \pm 1^\circ\text{C}$ , but they didn't emerge as adult; when exposed for 15, 18 and 20 days few individuals developed to adult. Larvae of *I. inquinata* were susceptible to the treatment at  $-18 \pm 1^\circ\text{C}$ . A complete (100%) mortality was observed after 24 hours, when second and fourth instar larvae were treated for 4 hours at  $-18 \pm 1^\circ\text{C}$ .

**Key words:** Rusty wave moth, Geometridae, cold treatments

### Introduction

*Idaea inquinata* (Scopoli) shows a great ability for development on dried plants with a preference for medicinal plants. Larvae choose apical leaves and flowers, characterized by a higher nutritional value (Candura, 1931a, b). *I. inquinata* develops also on bran, maize flour, and wheat kernels (Locatelli *et al.*, 2005).

Postembryonic development of *I. inquinata*, at 26 and  $29^\circ\text{C}$ , 50 and 70% R.H., lasted between 20 and 90 days. The increase of temperature, from 26 to  $29^\circ\text{C}$ , the increase of relative humidity from 50 to 70%, and the different photoperiods, 16:8 and 0:24 (light:dark), result in a significant decrease of larval and of pupal development time (Limonta *et al.*, 2010). Since larvae penetrate the substrate and adults are little active, it is difficult to detect infestations and, therefore, this species can be a serious pest in warehouses where dried plants are stored. Medicinal plants can be heavily damaged and made unusable to essence extraction.

A more detailed knowledge of the tolerance to adverse environmental conditions is important for integrated pest management. In this research the survival of second and fourth instars larvae of *I. inquinata* exposed to 6 and  $-18 \pm 1^\circ\text{C}$ , for different period of time, was observed.

## Material and methods

*Idaea inquinata* was collected on medicinal plants in a warehouse in Milano, and reared for 6 years on an artificial diet in a thermostatic chamber at  $26 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  R.H., and a photoperiod of 16:8 (light:dark). The ingredients of the rearing diet were: 60g bran, 30g corn flour, 30g wheat flour, 8g wheat germ, 7g dried yeast, 40g glycerine and 30g honey. Eggs were selected and placed individually in a Petri dish with a little amount of food in order to make visible the head capsule after moulting. Second instar larvae were ready after 12.5 days and fourth instar larvae after 25.5 days (Table 1).

Table 1. Mean ( $\pm$ S.D.) development period of different stages and instars of *Idaea inquinata* (Scopoli) reared at  $26 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  R.H.

Stadium	Development period (days)	Min-max (days)
Egg	$7.3 \pm 0.54$	7-9
First instar larva	$5.2 \pm 0.38$	5-6
Second instar larva	$7.1 \pm 0.41$	6-8
Third instar larva	$5.9 \pm 0.48$	5-7
Fourth instar larva	$8.5 \pm 2.00$	6-13
Pupa	$9.6 \pm 0.74$	8-11

Groups of 20 larvae of second or fourth instar were placed, with a little quantity of the diet, in a base of a Petri dish (diameter 10cm) closed with a textile net. A sufficient amount of diet was periodically provided. Larvae were put at  $6 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  R.H. for 15, 18, 20, and 25 days and then placed in thermostatic chamber at  $26 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  R.H. Tests were controlled after 1, 2, 3, 8, 15, and 23 days. The number and the stage of the surviving insects were recorded. Death was defined as lack of movement when individuals were probed with soft forceps.

The same procedure was used for tests at  $-18 \pm 1^\circ\text{C}$  with exposure times of 4, 8, and 24 hours. Each test was replicated four times. Data were submitted to ANOVA and to Duncan's multiple range test (SPSS 17).

## Results and discussion

The survival of second instar larvae of *Idaea inquinata*, after different exposure periods at  $6 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  R.H., are reported in Table 2. Significant differences were not observed for all the considered exposure periods after one and two days at  $26 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  R.H. (1 day:  $F_{3,12} = 0.6$ ,  $P = 0.627$ ; 2 days:  $F_{3,12} = 1.00$ ,  $P = 0.426$ ). Exposures of 15, 18, 20, and 25 days at  $6 \pm 1^\circ\text{C}$  caused a mortality higher than 95% after 24 hours at  $26^\circ\text{C}$ . Second instar larvae, kept at  $6 \pm 1^\circ\text{C}$  for 15 days, died after three days at  $26 \pm 1^\circ\text{C}$ , while for the other exposure periods 100% mortality was observed on the second day.

Table 2. Mean number ( $\pm$  S.D.) of second instar larvae of *Idaea inquinata* (Scopoli) surviving exposure periods of 15, 18, 20, and 25 days at  $6 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  R.H., after 1, 2 and 3 days at  $26 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  R.H (ANOVA: 1 day:  $F_{3,12} = 0.6$ , n.s.; 2 days:  $F_{3,12} = 1.00$ , n.s.).

Exposure period (Days)	Mean number of surviving larvae ( $\pm$ S. D.)		
	Days		
	1	2	3
15	$0.3 \pm 0.5$	$0.3 \pm 0.5$	$0 \pm 0$
18	$0.8 \pm 0.96$	$0 \pm 0$	-
20	$0.3 \pm 0.5$	$0 \pm 0$	-
25	$0.2 \pm 0.5$	$0 \pm 0$	-

Stratil & Reichmut, 1984, observed mortality of first instar larvae of *Cadra cautella* (Walker), after 35 days at  $10^\circ\text{C}$ ; at the same condition, larvae of *Plodia interpunctella* Hübner survived, and 100% mortality was observed after 21 days at  $8^\circ\text{C}$ , while larvae of *E. elutella* Hübner died after 35 days at  $6^\circ\text{C}$ .

Few fourth instar larvae, exposed at  $6 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  R.H., for 25 days, survived until the eighth day at  $26 \pm 1^\circ\text{C}$  and  $70\%$  R.H. (Tab. 3). With exposure periods of 15, 18, and 20 days few individuals completed the development to adult (Tab. 4). After 15 days at  $6 \pm 1^\circ\text{C}$  three individuals developed to adults; while after 18 and 20 days only one individual emerged as adult. Similar results were observed on different moths by other authors. Mature larvae of *C. cautella* were not able to complete development when exposed for 5 days to mean temperature of  $0^\circ\text{C}$ , or 40 days to mean temperature of  $6^\circ\text{C}$  (Burges, 1956). Mature larvae of *Ephestia kuehniella* (Zeller), collected in fields by Jacob & Cox (1977), developed to pupae at  $10^\circ\text{C}$  but failed at  $7.5^\circ\text{C}$ . Etman (1990) observed 100% mortality of first instar larvae of *Corcyra cephalonica* (Stainton) exposed at  $5^\circ\text{C}$  for five days.

Larvae of *I. inquinata* showed a high susceptibility to exposure at  $-18 \pm 1^\circ\text{C}$ , a hundred per cent mortality of first and fourth instar larvae was observed after 4 hours exposure.

Results agree with observation of Naeemullah *et al.* (1999) on non diapausing larvae of *P. interpunctella* exposed to  $-10$  and  $-20^\circ\text{C}$ ; a hundred percent mortality was observed at  $-20^\circ\text{C}$  after 1 hour, at  $-10^\circ\text{C}$  after 8 hours. All the stages of *E. kuehniella* were dead after 24 hours at temperatures between  $-18$  and  $-19^\circ\text{C}$  (Mathlein, 1961).

Table 3. Mean number ( $\pm$  S.D.) of fourth instar larvae of *Idaea inquinata* (Scopoli) alive after 15, 18, 20, and 25 days at  $6 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  R.H., in the controls after 1, 2, 3, 8, 15, 23 days. In the first three days only larvae were observed, in the following ones also pupae and adults were found (details in Table 4).

Exposure period (Days)	Mean number of alive larvae ( $\pm$ S.D.)					
	Days					
	1	2	3	8	15	23
15	$3.8 \pm 0.96a$	$3.8 \pm 0.96a$	$2.5 \pm 1.29$	$1.5 \pm 0.58$	$1.3 \pm 0.5$	$0.8 \pm 0.5$
18	$2.3 \pm 1.89ab$	$2.3 \pm 1.89ab$	$2.3 \pm 1.89$	$1.3 \pm 1.89$	$1.3 \pm 1.89$	$0.3 \pm 0.5$
20	$1.0 \pm 1.41b$	$0.8 \pm 0.96bc$	$0.8 \pm 0.96$	$0.8 \pm 0.96$	$0.5 \pm 0.58$	$0.3 \pm 0.5$
25	$0.3 \pm 0.5b$	$0.3 \pm 0.5c$	$0.3 \pm 0.5$	$0.3 \pm 0.5$	$0 \pm 0$	$0 \pm 0$

1day:  $F_{3,12} = 5.568$ ,  $P < 0.01$

2 days:  $F_{3,12} = 7.059$ ,  $P < 0.01$ ; n.s. for the other controls.

Table 4. Number of alive individuals, after exposure of fourth instar larvae of *Idaea inquinata* (Scopoli) at  $6 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  R.H., after 8, 15 and 23 days at  $26 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  R.H. (\*pupa, \*\* adult).

Exposure period (Days)	Replicates	Days		
		8	15	23
15	A	1	1*	1**
	B	2	2	1**
	C	1	1	0
	D	1	1*	1**
18	A	1	1*	0
	B	0	-	-
	C	4*	4*	1**
	D	0	-	-
20	A	0	-	-
	B	1*	1*	0
	C	2	1*	1**
	D	0	-	-

Second instar larvae of *I. inquinata* are more affected by low temperatures than fourth instar larvae. Also in *Grapholita prunivora* (Walsh) fourth instar larvae are more tolerant to cold than the other instars (Neven, 2004).

In this research larvae were exposed directly to low temperature, they couldn't bore in the substrate to escape cold. When larvae are inside dried plants, the time necessary to cool the product should be considered. The treatment period will be influenced by the plant species and by the part of the plant considered (leaves, roots, seeds). To obtain 100% mortality of fourth instar larvae, it must be provided that the temperature throughout the product is below  $6 \pm 1^\circ\text{C}$  for 25 days.

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