Neth. J. Pl. Path. 82 (1976) 173–180

Temperature and mortality in the eggs of Adoxophyes orana (Lepidoptera, Tortricidae)

G. W. ANKERSMIT, B. C. VAN DER POL and J. K. WATER

Laboratory of Entomology, Agricultural University, Wageningen

Accepted 19 May 1976

Abstract

In September, a high mortality among eggs of *Adoxophyes orana* was observed. Temperature effects were believed to be responsible. In controlled conditions, constant temperatures of 13 or 14 °C proved lethal to most eggs, but mortality was about halved when temperature changes were gradual. Low temperature (5 °C or less) for 6 h was lethal when eggs were exposed within a few hours of oviposition. This was also due to a rapid change in temperature. Survival of eggs of *A. orana* is most affected by long cool periods and is slightly affected by cold nights.

Introduction

In attempts to establish a life-table for *Adoxophyes orana* under field conditions, a high egg-mortality was observed in September. As some frosty nights preceded this phenomenon, the low temperatures were believed to offer an explanation. They are known to affect egg hatchability.

Johnson (1940) showed that exposure for 40 days to 4.2, 9.8 or 11.2 °C was lethal to most eggs of the bedbug, *Cimex lectularius*. Kitlaus (1961) found a high egg-mortality in *Leptinotarsa decemlineata* when eggs were exposed to temperatures from 4 to 7 °C. Also prolonged exposure to 10–13 °C was lethal. Temperatures near to the developmental threshold were lethal to eggs of *Oncopeltus fasciatus* (Lin et al; 1954), *Plodia interpunctella* (Cline, 1970) and *Anagasta kuehniella* (Daumal et al; 1974). Similar results were obtained by Watters (1966) with eggs of *Tribolium confusum*. As a rule, susceptibility to low temperatures seems highest at the beginning of embryogenesis. In spite of its widespread occurrence, the ecological significance of mortality induced by low temperature has received little attention.

A. orana has two oviposition periods. The first occurs throughout June and the second in August and September. Usually eggs are laid at temperatures above 13 °C but most at much higher temperatures. Though many eggs can be laid during the night, they can also be produced in the late afternoon. If cool nights do affect hatchability, the duration of periods of cold exposure should be relatively short. In the Netherlands, cold nights can be rather common in early June or in September and night frost may even occur. Moreover on a leaf surface, temperature may even be a few degrees lower than registered at 1.50 m in standard meteorological observations. We tried to imitate these cold periods by exposing eggs of various ages to temperatures below the threshold of development of 10.6 °C (De Jong et al., 1965).

Prolonged exposure to moderate temperatures also seems to affect egg hatchability in several species (Lin et al., 1954). Average temperature in June is between 15 and 16 °C and in September between 14 and 15 °C. Periods of several weeks with lower temperatures do occur and it was decided to study the effect of prolonged exposure to 13 °C, a temperature a little above the developmental threshold but frequently occurring naturally.

Materials and methods

Egg batches of *A. orana*, deposited on polythene sheets, were collected from a culture reared on an artificial medium (Ankersmit, 1968) at 20 °C, 70% relative humidity and 16.5 h light per day. Under these conditions, many eggs are laid in a few hours preceding the 'light-off' moment, and some also after the light was switched on. Eggs were collected in both periods within an hour after deposition. During the dark period, no eggs were collected, except in trials in which knowledge of age was less critical. Only such egg batches were used in the calculations that showed some indications of developing embryos. This was to exclude eggs laid by unfertilized females. Pieces of the sheets with eggs were placed in vials (diameter 15 mm, height 50 mm) in a climate room at 20 °C and 70% relative humidity. The vials were than closed with a cork stopper and placed under experimental conditions. Adaptation from the climate room conditions to, for instance, 5 °C took 10 min. This delay is small relative to most exposure periods. The climate cabinets had a maximum variation in temperature of ± 1 °C.

Results

Trials to imitate prolonged cool periods. Eggs not more than one day old were given a cycle of 16 h at 20 °C and 8 h at 6–7 °C for 16 days. The average temperature was then 15.5 °C. At that temperature, development of the eggs should last 15 to 16 days. After this period, the eggs were placed at 20 °C for 5 days to ensure complete development. The number of eggs was 561. Only 12% hatched against 73% in a control group of 626 eggs that was exposed to 20 °C only. A mosaic of differences in development was observed. In 60% of the eggs, no development was seen at all, in 20% only eye spots were visible; in 7%, black head capsules were apparent. In later trials, a similar variability was found.

In the first trial the temperature amplitude of nearly 14 °C was rather high for Dutch conditions, so in the next trial we imitated more closely a cool September day by changing temperatures gradually from a minimum of 8 °C and 95% relative humidity during the night (03:00 h) to 19 °C and 55% r.h. during the day (15:00 h). The photoperiod was 13 h per day (24 h) with the lights on at 07:00 h. Average daily temperature was then 13.5 °C. The results (Table 1) show a distinctly lower egg-hatch under the variable-temperature regime than at constant temperature of 20 °C. When, however, we placed the eggs within an hour of oviposition under constant 13 °C, hatch was extremely low. Even oviposition under the variable temperature regime seemed detrimental to viability of eggs.

Eggs not more than one day old were subject for periods as mentioned in Table 2 to

Table 1. Effect on egg-hatch of temperatures varying around an average of 13.5 °C and constant at 13 or 20 °C.

Oviposition temperature (°C)	Development temperature (°C)	Number tested	% hatch
8–19	8–19	3608	44a
8–19	20	3781	69b
8–19	13	1699	4c
20	8–19	2418	47a
20	13	3539	6c
20	20	3267	87d

Values with the same letter are not significantly different at P = 0.05.

Tabel 1. Invloed van de temperatuur variërend om een gemiddelde van 13,5 °C en constant op 13 of 20 °C op het uitkomen van de eieren.

Table 2. Effect of long periods at 13 °C and then 20 °C on egg hatchability.

Number of days at 13 °C	0	4	8	12	16	26
Number of eggs tested	556	690	511	548	529	544
Number of days:						
till hatching-actual	9–10	12–14	13–15	16–17	19–20	21–26
according to thermal summation	10	12	14	16	19	24
Hatched eggs (%)	87	44	66	57	6	1

Tabel 2. Invloed van lange perioden bij 13 °C en daarna bij 20 °C op het uitkomen van de eieren.

Table 3. Effect of long periods at 20 °C and then at 13 °C on egg hatchability.

Number of days at 20 °C	0	2	4	6	8	10
Number of eggs tested	544	470	446	464	518	556
Number of days:						
till hatching-actual	21–26	17–25	14–21	11–14	10–11	9–10
according to thermal summation	24	20	16	13	11	10
Hatched eggs (%)	1	17	54	45	75	87

Tabel 3. Invloed van lange perioden bij 20 °C en daarna bij 13 °C op het uitkomen van de eieren.

13 °C and than transferred to 20°C or the reverse (Table 3). The figures show again that keeping the eggs for a long period at 13 °C was fatal. According to De Jong et al. (1965), the developmental threshold is 10.6 °C, so even temperatures above that threshold may be lethal. This was studied further in trials with eggs not older than 16 h subject to the temperatures mentioned in Table 4. The results confirm the previous finding of a deleterious effect of 13 °C and indicate a remarkable improvement in hatchability of the eggs between 13 and 15 °C. Differences in egg hatch between 13, 14 and 15 °C were significant at P = 0.05.

Trials to imitate cool nights. Eggs of three different ages were subject to temperatures ranging from −2 to + 10 °C. Age of the eggs, temperatures, number of temperature treatments, their duration and the percentage of eggs hatched are presented in Table

Table 4. Influence of constant temperatures on egg hatch. Controls at 20°C were begun simultaneously for each treatment.

Temperature (°C)	Test eggs		Control eggs (20°C)	
	number tested	% hatched	number tested	% hatched
13	1520	3	1220	86
14	1140	19	1500	90
15	2650	72	2070	87
25	1360	93	1460	87

Tabel 4. Invloed van constante temperaturen op het uitkomen der eieren.

Table 5. Percentage egg hatch after short cold treatments at intervals of 24 h between periods at 20°C.

Temperature (°C)	Age of eggs	Duration of cold period/Number of periods					
		2h/1	2h/2	2h/3	6h/1	6h/2	6h/3
−2	6–7 days	66	85	92	84	61	82
	1–2 days	53	25	42	28	0	0
−1	6–7 days	96	75	84	58	83	67
	1–2 days	85	83	7	25	29	0
0	6–7 days	86	64	94	84	96	92
	1–2 days	31	25	44	54	69	93
	0–16 h	13	3	3	20	4	3
+2	6–7 days	36	80	85	88	68	70
	1–2 days	77	80	82	71	63	32
	0–16 h	54	56	38	43	23	21
+5	0–16 h	60	58	41	24	33	33
+10	0–16 h	62	51	67	75	63	60

Tabel 5. Percentage uitgekomen eieren na kortdurende koudebehandelingen met tussenpozen van 24 uur bij 20°C.

5. In each trial, at least three egg masses were used consisting of at least 150 eggs in total. The results were often variable.

After angular transformation of the percentages, the analysis of variance showed that, egg hatchability of eggs younger than 16 h, was lower at lower temperatures ($P = 0.005$). When duration of the treatment increased, egg hatch decreased ($P = 0.05$). Also a higher number of treatments resulted in a lower hatchability ($P = 0.025$). An effect of age of the eggs was found ($P = 0.05$) as eggs 6–7 days old always had little mortality, and mortality was highest in the eggs whose cold treatment started within 16 h of deposition. With eggs not more than 1–2 days old, hatchability decreased as temperature decreased ($P = 0.05$). Eggs 6–7 days old were remarkably resistant to freezing and could thus survive night frosts.

In a later trial an attempt was made to find the age at which temperatures of −2°C might still affect survival. The ages tested and the duration of cold treatment are given with the percentage egg hatch in Table 6. In each trial, at least three egg batches were used with in total of at least 158 eggs. Analysis of variance showed an effect of

Table 6. Percentage egg hatch after cold treatments ($-2\,^{\circ}$C) of different duration at different ages.

Treatment (h)	Age (days)							
	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8
0.5	2	73	88	94	52	92	83	85
2.0	0	53	87	72	86	75	76	78
6.0	0	28	55	60	83	73	84	83

Tabel 6. Percentage uitgekomen eieren na koudebehandeling ($-2\,^{\circ}$C) van verschillende duur op verschillende leeftijden.

duration of treatment (P = 0.025) and of egg age between 1–2 and 2–3 days (P = 0.05). So eggs older than 1–2 days would not suffer much from such night frosts.

As susceptibility decreased with age, an attempt was made to determine more accurately the influence of low temperatures during the first 24 h after oviposition. Eggs with an age known to within an hour were subject exposure to 5 °C for 6 h. Per trial, at least 663 eggs were used. The results (Fig. 1, lower line) show a gradual decrease in mortality with age. So in the first few hours after oviposition, eggs are extremely susceptible to cold. This trial was repeated a year later under similar conditions (Fig. 1, upper line). Susceptibility was then somewhat less but the slope of the line was nearly the same. If the high susceptibility were connected with a certain phase in embryonic development, this period of high susceptibility could be prolonged by exposing the eggs to a temperature close to the developmental threshold of 10.5 °C. At 13, 15 or 20 °C, one would expect 2.1 %, 2.9 % or 5.6 % development, respectively, per 12 h according to De Jong et al. (1965). So we would expect a

Fig. 1. Change in susceptibility to 5 °C for 24 h after oviposition.

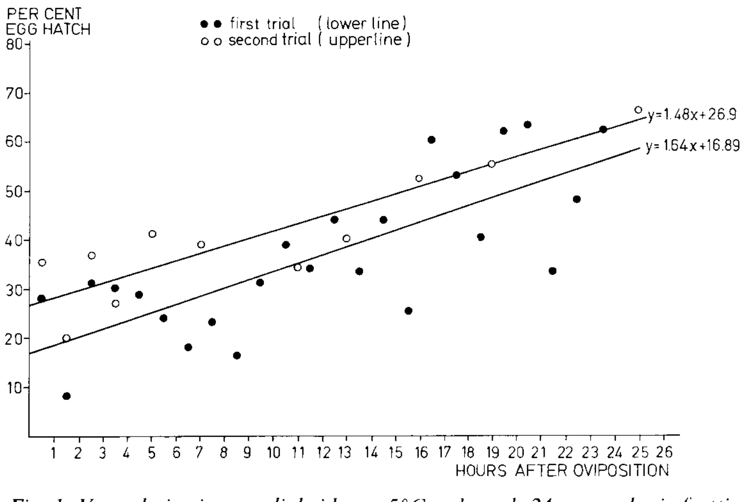

Fig. 1. Verandering in gevoeligheid voor 5 °C gedurende 24 uur na de ei afzetting.

Table 7. Percentage egg hatch after a treatment with 5 °C for 6 h after previous incubation at 20, 15 or 13 °C.

Age at beginning of cold treatment (h)	Temperature before treatment (°C)		
	20	15	13
0–2	36		
1–2	20		
2–3	37		
3–4	27		
4–6	41		55
6–8	39	46	55
10–12	34	36	59
12–14	40	55	63
15–17	52	38	68
18–20	55	55	61
24–26	66	65	65
control	73		

Tabel 7. Percentage uitgekomen eieren na een behandeling met 5 °C gedurende 6 uur wanneer zij tevoren werden bewaard bij 20, 15 of 13 °C.

physiological age after 6 h at 13 or 15 °C almost to equal that of about 3 h at 20 °C.

Trials in which the eggs were stored for several hours at such near-threshold temperatures did not reveal any prolongation of the susceptible period (Table 7). On the contrary, mortality was always lower when the eggs were first kept at an intermediate temperature. This suggests that at least part of the effect of cold treatment must be due to the rapid change in temperature. But even when we made the transition from 20 to 5 °C very gradually (Table 8) percentage egg hatch was still significantly lower than in the control kept at 20 °C.

A few trials were done with a newly collected strain. The results of these tests did not differ from those with the older laboratory strain, for instance from 648 eggs 0–2 h old only about 20 % hatched after exposure for 6 h to 5 °C.

Table 8. Percentage egg hatch after gradual cooling from 20 to 5 °C for 6 h cold treatment.

Temperature and period	Number of eggs	Egg hatch (%)
20 °C, 6 h 15 °C, 6 h 10 °C, 10 h	1391	62a
20 °C, 1 h 15 °C, 2.5 h 10 °C, 2.5 h	2840	58a
Gradually in 6 h from 20 to 10 °C	1342	50b
Control at 20 °C	2310	73c

Different letters under percentage egg hatch indicate significant difference at $P = 0.05$.

Tabel 8. Percentage uitgekomen eieren na geleidelijke afkoeling van 20 naar 5 °C voor een 6 uur durende koude behandeling.

Discussion

Lethal effect of constant temperatures above threshold. Prolonged exposure to 13 or 14°C was lethal to most embryos. The rapid decrease in mortality between 14 and 15°C was remarkable (Table 4). It suggests the occurrence of a threshold. Eggs that hatch, however, closely follow the thermal summation rule established by De Jong et al. (1965). It seems immaterial whether the 13°C occur at the beginning or end of development. It seems that roughly half the development taking place at too low temperature is detrimental (Table 2 and 3). These results resemble those of Lin et al. (1954) with eggs of *Oncopeltus fasciatus*. None of those eggs hatched at 14°C but 64.8% hatched at 16°C; 2 days immediately after oviposition at 20°C sufficed to raise hatchability to 64% from zero at constant 14°C. During the oviposition period of *A. orana*, prolonged periods with temperatures below 15°C are rare. They may, however, occur at the end of the second flight in September or October.

The results also indicate the vagueness of the concept of developmental threshold, although its phenological usefulness is beyond doubt. Above the threshold, a range of temperatures obviously occurs in which there is some development but hatching does not always ensue.

Effect of a temperature varying around threshold. The programme with the varying temperature was closer to natural conditions. In several years, an average temperature between 13 and 14°C with a maximum of 19°C and a minimum of 8°C has occurred during oviposition periods. Thes conditions allow oviposition. In our trial most eggs were laid in the period with a high temperature before 'nightfall.' We can therefore assume that survival under natural conditions would be affected and hatchability would be halved (Table 1).

In the first trial, we changed temperature from 20 to 6°C. This decreased hatchability to 12% but there we have to consider the effect of rapidly changing temperature seen later in Table 7 and 8. Though we cannot quantify this effect, much of the lower egg hatch must be due to the rapid change.

Effect of short periods of cold exposure. The effect of short periods of cold exposure too is difficult to evaluate for its ecological significance as much is due to rapid change. The results in Table 7 and 8, however, suggest some residual effect of cold. The effect of low temperatures was clearest in eggs younger than 2 days. Older eggs even withstood repeated night frosts of -2°C. Temperatures above zero were most detrimental to newlaid eggs. As it would usually take almost half a day for the temperature to drop from that suitable for oviposition (e.g. 15°C) to 5°C, we can assume that mortality would then be at most 30 or 40% (Fig. 1).

Survival of eggs of *A. orana* is most affected by long cool periods and is slightly affected by cold nights.

Samenvatting

Invloed van de temperatuur op eisterfte bij Adoxophyes orana

In september werd in het veld hoge sterfte bij eieren van *Adoxophyes orana* waargenomen. De verklaring werd gezocht in temperatuurseffecten. In proeven kwam bij een cyclus van 16 uur 20°C en 8 uur 6–7°C slechts 12% der eieren uit tegen 73% in de controle (20°C) Constant 13 of 14°C bleek ook hoge sterfte te geven, maar bij 15°C kwam al 72% der eieren uit (Tabel 2, 3 en 4). Een te lage overdagtemperatuur zal dus slechts bij uitzondering de sterfte kunnen veroorzaken daar in september de maximumtemperaturen gewoonlijk meer dan 15°C bedragen.

Een geleidelijk wisselende temperatuur van 8–19°C bleek eveneens een belangrijke eisterfte te veroorzaken (Tabel 1). Koude nachten bleken ook een mogelijke oorzaak te zijn. Eén tot twee dagen oude eieren waren zeer gevoelig voor koude behandelingen met temperaturen tot 5°C (Tabel 5). Ook de duur van de koude had effect. Oudere eieren zijn minder gevoelig en 4 tot 5 dagen na het leggen kunnen ze 6 uur −2°C doorstaan (Tabel 5 en 6). Vooral gedurende de eerste dag neemt de koudegevoeligheid snel af (Fig. 1). Een deel van het effect van de koude moet aan een snelle temperatuursverandering worden geweten daar de sterfte bij de geleidelijke overgang kleiner is (Tabel 7 en 8). Door een lage gemiddelde temperatuur en ook enigszins door koude nachten kan dus sterfte onder de eieren van *A. orana* optreden.

Acknowledgments

The authors are indebted to P. Gruys, J. de Wilde and H. Schooneveld for criticism and comment on the manuscript and to J. C. Rigg for correcting the English text.

References

Ankersmit, G. W., 1968. The photoperiod as a control agent against *Adoxophyes reticulana* (Lepidoptera, Tortricidae). Entomologia exp. appl. 11: 231–240.

Cline, L. D., 1970. Indian – meal moth egg hatch and subsequent larval survival after short exposures to low temperature. J. econ. Ent. 63: 1081–1083.

Daumal, J., Jourdheuil, P. & Tomassone, R., 1974. Variabilité des effets létaux des basses températures en fonction du stade de développement embryonnaire auquel elles sont appliquées chez la pyrale de la farine (*Anagasta kühniella*). Annls. Zool. Ecol. Anim. 6: 229–243.

Johnson, C. G., 1940. Development, hatching and mortality of the eggs of *Cimex lectularius* in relation to climate, with observations on the effects of pre-conditioning to temperature. Parasitology 32: 127–173.

Jong, D. J. de, Beeke, H. & Wondergem, H. J., 1965. Bestrijding van de vruchtbladroller *Adoxophyes reticulana* Hb. Meded. Dir. Tuinb. 28: 539–542.

Kitlaus, E., 1961. Die embryonalen Entwicklung von *Leptinotarsa decemlineata* Say, *Epilachna sparsen* Herbst und *Epilachna vigintioctomaculata* Motsch. var. *niponica* Lewis in Abhängigkeit von der Temperatur. Dt. ent. Z., N.F. 8: 41–62.

Lin, S., Hodson, A. C. & Richards, A. G., 1954. An analysis of threshold temperatures for the development of *Oncopeltus* and *Tribolium* eggs. Physiol. Zoöl. 27: 287–311.

Watters, F. L., 1966. The effects of short exposures to subthreshold temperatures on subsequent hatching and development of eggs of *Tribolium confusum* Duval (Coleoptera, Tenebrionidae) J. stored Prod. Res. 2: 81–90.

Addresses

G. W. Ankersmit: Laboratorium voor entomologie, Binnenhaven 7, Wageningen, the Netherlands.
B. C. van der Pol: Slunterweg 42, Ede, the Netherlands.
J. K. Water: Van Woerdenstraat 15, Vleuten, the Netherlands.