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VARIATIONS IN GLYCEROL CONTENT IN RELATION TO COLD-HARDINESS IN THE LARVAE OF *PETROVA RESINELLA* L. (*LEPIDOPTERA, TORTRICIDAE*)

The majority of insects overwinter in a supercooled state. The cold-hardiness of such insects depends on the ability of the haemolymph to supercool. The lower the supercooling point (S.C.P.), the lower temperatures tolerated by an insect, and hence the higher its cold-hardiness (Ушатинская, 1957). Only few insect species withstand a complete freezing of tissues. The majority of insects, including *Petrova resinella* L., are freezing-susceptible.

The haemolymph supercooling point is depressed by various substances dissolved in the haemolymph, such as sugars, amino acids, polyhydric alcohols. Salt (1959) showed that glycerol played a direct role in cold-hardening the larvae of *Bracon cephi* by depressing the melting point of the haemolymph to as low as — 17.5 °C and by lowering the supercooling point to —47.2°. Various investigators (Salt, 1958, 1959; Sømme, 1964; Baust, Miller, 1970; Хансен, 1971) have established that increased glycerol levels in the haemolymph of various insects raise their cold-hardiness.

The cold-hardiness of insects depends largely on their overwintering ecology, chiefly on their hibernation quarters. The insects hibernating above snow level, which experience low below-zero temperatures, are usually more cold-hardy. Nearly all such insects (80% of the species studied) contained large amounts of glycerol in winter. However, the insects overwintering beneath the snow cover had an extremely low polyhydric alcohol content — 0.1 per cent, or less. At the same time the majority of the species examined lacked glycerol, and had only the sorbitol, mannitol and dulcitol group (Хансен, 1971).

The aim of the present investigation was to establish changes in the glycerol content in relation to cold-hardiness during hibernation as well as in the period preceding and following it.

The pine twig moths (the group of *Evetria*) embrace a large number of species which possess an exceptional ability to supercool. Five species, all of which hibernate above snow level (in the crowns of pines), in mid-winter have a supercooling point ranging within the limits of —46° . . . . . —48° (Куузик, 1971). These species also include *Petrova resinella*, which has a two-year generation. It overwinters in the second instar (in even years) and in the last instar (in odd years) as larvae in resin galls of pine twigs where there virtually obtains the temperature of the ambient air (West, 1936). The resin gall may only smooth short-term variations in the temperature.

Material and methods

The larvae of *Petrova resinella* were collected from young pine cultures established in the Rae raised bog (Harju District, Estonian SSR). The larvae of the last instar were studied from autumn to spring in 1969—1970, and the larvae of the second instar in 1970—1971. The experimental material necessary for the winter months was collected in November. The pine twigs with larvae in resin galls were kept under outdoor conditions. The larvae were separated from the galls immediately before the experiment.

Samples of insects were prepared for analysis by the method described by Salt (1959). Polyhydric alcohols were identified by a chromatographic technique. Ascending chromatograms were run on Leningradskaya-S paper with *n*-butanol-acetic acid-water (4 : 1 : 2) as the solvent. After air-drying, the chromatograms were sprayed with a solution of 1 per cent sodium metaperiodate, and after 3—4 minutes they were oversprayed with a fresh-prepared solution of 1 per cent potassium permanganate. After 5 minutes the residue of the reagents was washed with water, and the chromatograms were dried in the air. By this method glycerol and other polyhydric alcohols appeared as dark-yellow spots on a white background. The chromatograms showed that *P. resinella* larvae contained only glycerol, and therefore no separation seemed to be necessary, and the glycerol content could be identified at once quantitatively by the colorimetric method described by Renkonen (1962). Analyses were performed in 3—10 replications; in most cases one larva specimen was sufficient for the conduction of one analysis.

In the present investigation glycerol concentrations are given as percentages of fresh weight.

The supercooling points were measured with a copper-constantan thermocouple at a cooling rate of 1° per minute. The mean supercooling point was determined in series of 10 specimens.

Results and discussion

Both the haemolymph glycerol content and the supercooling point vary seasonally (Table 1, Fig. 1).

Table 1

Seasonal changes in the glycerol content and in supercooling points in the larvae of *Petrova resinella*

Overwintering last-instar larvae			Overwintering second-instar larvae		
Date	Glycerol, %	Supercooling point (°C)	Date	Glycerol, %	Supercooling point (°C)
Sept. 13	Trace				
Sept. 22	Trace	−13.5±0.3			
Oct. 7	0.1±0.0	−15.0±0.4			
Oct. 23	0.2±0.0 A	−16.4±0.8	Oct. 22	0.0±0.0 A	−18.8±0.8
	14.1±2.1 D	−29.1±0.8		9.9±1.1 D	−34.9±2.1
Nov. 5	27.2±0.3	−40.6±1.1			
Nov. 17	26.7±0.7	−41.5±0.8	Nov. 20	28.5±0.5	−47.2±0.5
Dec. 29	27.5±0.8	−43.9±0.7	Dec. 22	27.9±2.3	−47.4±0.3
Jan. 27	30.1±0.4	−45.7±0.8	Jan. 12	26.7±1.7	−48.5±0.6
Feb. 16	28.9±1.3	−46.5±0.3	Feb. 22	27.5±0.5	
March 6	28.5±0.5	−43.5±0.8	March 18	26.1±0.5	−46.1±0.5
March 27	15.9±1.3	−41.9±0.9	March 30	5.3±0.5	
April 10	10.1±1.9 D	−37.1±0.8	April 8	3.7±0.2 D	
	0.5±0.2 A	−25.0±0.7		1.6±0.2 A	
			April 22	0.3±0.2 A	−19.4±0.2

A — active larvae
D — diapausing larvae

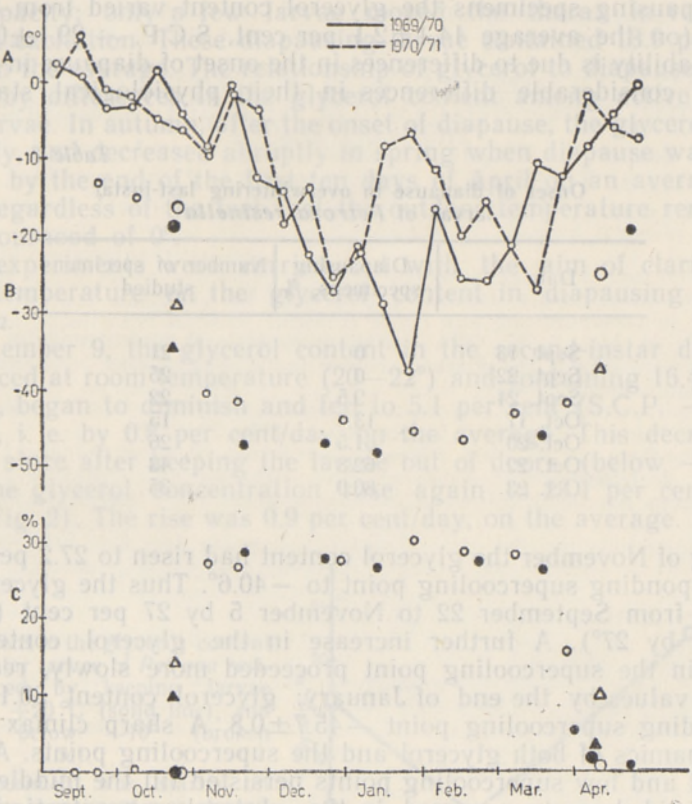

Fig. 1. *A* — temperature of the air, minima of every ten days of a month; *B* — seasonal variations in supercooling points of the larvae of *Petrova resinella*; *C* — seasonal variations in the glycerol content of the larvae of *Petrova resinella*.
o — larvae of the last instar, ● — larvae of the second instar, ○ — active larvae, △ — diapausing larvae.

P. resinella belongs to the species with an obligatory diapause. Its deep diapause lasts till the middle of March. Diapausing larvae of *P. resinella* can easily be distinguished from the non-diapausing ones by body shape. The diapausing larvae are shorter and thicker than the developing larvae. The ratio of body width to body length is 0.42 in the former, and 0.25 in the latter (Куузик, 1971). The diapausing larvae overwintering in the second instar cannot be distinguished from the developing ones by body shape. Distinction is possible only by the degree of their activity. In diapause, the larvae of *P. resinella* lose their moving capacity, which is not restored in deep diapause even at temperatures above the threshold of activity.

When following the onset of diapause in *P. resinella* larvae (Table 2), it becomes clear that by the end of October the majority of the larvae had entered diapause.

At that time great individual variations occurred in the glycerol content as well in the supercooling points. The active larvae of the last instar contained 0.2±0.0 per cent of glycerol (S.C.P. — 16.4±0.8°).

In diapausing specimens the glycerol content varied from 5.9 to 20.9 per cent (on the average 14.1±2.1 per cent, S.C.P. — 29.1±0.8°). This great variability is due to differences in the onset of diapause in the larvae, reflecting considerable differences in their physiological state. By the

Table 2

Onset of diapause in overwintering last-instar larvae of *Petrova resinella*

Date	Diapausing specimens, %	Number of specimens studied
Sept. 13	0	21
Sept. 22	0	25
Sept. 24	9.5	22
Oct. 17	13.3	15
Oct. 20	61.5	26
Oct. 22	62.8	43
Oct. 23	80.0	35

beginning of November the glycerol content had risen to 27.2 per cent, and the corresponding supercooling point to −40.6°. Thus the glycerol content increased from September 22 to November 5 by 27 per cent (the S.C.P. decreased by 27°). A further increase in the glycerol content and a decrease in the supercooling point proceeded more slowly, reaching the following values by the end of January: glycerol content 30.1 per cent, corresponding supercooling point −45.7±0.8. A sharp climax is lacking in the dynamics of both glycerol and the supercooling points. A high glycerol level and low supercooling points persisted till the middle of March, when a rapid decrease occurred in the glycerol concentration, together with a rise in the supercooling point. At the end of the first ten days in April, when part of the larvae had become active, great individual variations occurred in the glycerol content as well as in the supercooling point, which were due to different reactivation levels of the larvae.

Seasonal variations in the glycerol content and in the supercooling points in the *P. resinella* larvae overwintering in the second and the last instar were similar (Fig. 1). Likewise, noticeable differences in specific indicators were lacking. Seasonal changes in supercooling points coincided with the data published by Kuusik (1970).

The apparently normal pattern in diapausing insects is to accumulate or retain glycerol regardless of the temperature. This has been shown by Sømme (1964, 1965) for various insects, placed at a temperature of 20° during diapause. In post-diapause, glycerol is lost even at temperatures down to 0° and −5°. While in most species glycerol metabolism seems to be related to diapause, it has also been suggested that glycerol and diapause are not always correlated (Salt, 1961; Sømme, 1965). It has been found that glycerol was lost in specimens placed at 20° at a time when all of them were in diapause (Takehara, 1963; Sømme, 1965), but the decrease of glycerol in a diapausing insect was slower than in a post-diapausing one.

Experiments with *P. resinella* showed that the presence of glycerol in the larvae is connected with diapause. The pine twigs which had been brought indoors on October 7 together with last-instar larvae whose glycerol content was 0.1 per cent, were kept at +20° for 41 days. The twigs were placed in the water, and sufficient humidity was maintained in the breeding chamber. During that time the majority of the larvae had lost the moving capacity; only a few larvae moved the thorax in response to mechanical excitation. These diapausing larvae contained 13.9 per cent of glycerol, on the average. The relationship of glycerol to diapause was also confirmed by differences in the glycerol content among active and diapausing larvae. In autumn, after the onset of diapause, the glycerol content rose sharply, and decreased abruptly in spring when diapause was broken, decreasing by the end of the first ten days of April on an average to 0.5 per cent, regardless of the fact that the outdoor temperature remained in the neighbourhood of 0°.

A few experiments were carried out with the aim of clarifying the effect of temperature on the glycerol content in diapausing larvae of *P. resinella*.

On December 9, the glycerol content in the second-instar diapausing larvae, placed at room temperature (20—22°) and containing 16.4 per cent of glycerol, began to diminish and fell to 5.1 per cent (S.C.P. −22.8°) in two weeks, i. e. by 0.8 per cent/day, on the average. This decrease was reversible, since after keeping the larvae out of doors (below —10°) for 3 weeks, the glycerol concentration rose again to 23.1 per cent (S.C.P. −42.2°) (Fig. 2). The rise was 0.9 per cent/day, on the average.

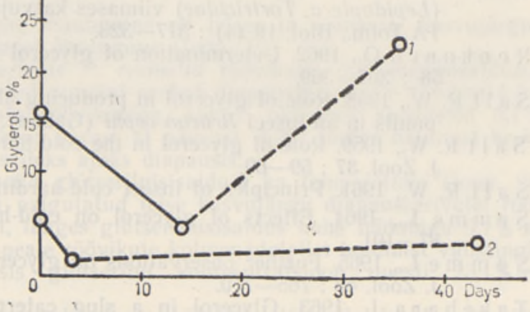

Fig 2. Variations in the glycerol content of second-instar larvae of *Petrova resinella* produced by keeping larvae previously at +20 °C (solid line), and subsequently below —10° (broken line).

1 — the experiment was started on Dec. 9, *2* — the experiment was started on March 30.

The below-zero temperatures checked the termination of diapause even in March and April. In the larvae which were brought from outside into room temperature on March 30, and contained 5.3 per cent of glycerol at that time, the glycerol content diminished in 3 days to 1.3 per cent. Thus the rate of decrease was on an average 1.3 per cent/day. By that time all the larvae had terminated diapause. Thereafter the larvae were held at a temperature of −12° for 40 days. By the end of this period the glycerol content had risen only to 3.8 per cent. The re-synthesis of glycerol was very slow, 0.06 per cent/day, on the average.

At the end of the first ten days of April the disappearance of glycerol at room temperature was still faster. In the larvae containing an average of 14.7±1.17 per cent of glycerol, this was completely lost within 2 days (Hansen, Kuusik, 1970).

Thus in midwinter, when larvae were in deep diapause, the decrease in the glycerol content at 20—22° was considerably slower and the re-synthesis of glycerol after the return of larvae to a low temperature was markedly faster than in spring.

It is evident from the data presented above that the temperature regulates the glycerol content in *P. resinella*. This dependence on the temperature, however, is not so direct as in non-diapausing species of insects in which the rise of glycerol is completely controlled by the temperature (Dubach et al., 1959; Sømme, 1964). In such insects glycerol is formed only at low temperatures and is completely lost at room temperature. The neurosecretory mechanism of the metabolism of diapause described in the reports of Harvey (1962) and Wilde (1965) enabled one to explain the rise of glycerol in the haemolymph also at temperatures above 0°. It can be assumed that during diapause the dependence of the glycerol content on the temperature is conditioned by the depth of diapause: the deeper the diapause, the less the glycerol content depends on the temperature. To obtain a clearer picture of this problem, it will be necessary to conduct further studies with different insect species.

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MUUTUSED MÄNNI-VAIGUMÄHKURI *PETROVA RESINELLA* L. (*LEPIDOPTERA, TORTRICIDAE*) RÖÖVIKUTE GLÜTSERIINISISALDUSES SEOSES KÜLMAKINDLUSEGA

Resümee

Petrova resinella röövikud talvituvad mändide võras, ülalpool lumepiiri ning taluvad hästi talviseid madalaid temperatuure.

Oktoobri lõpuks oli enamik röövikuist juba diapausis. Aktiivsed, veel toituvad viimase kasvujärgu röövikud sisaldasid sel ajal 0,2% glütseriini, allajahtumispunkt (AJP) oli —16,4 °C. Diapauseerivatel isenditel varieerus samal ajal glütseriinisisaldus 5,9—20,9%-ni (keskmiselt 14,1%, AJP —29,1°). Glütseriinisisalduse suur individuaalne varieeruvus kajastab suuri erinevusi röövikute füsioloogilises seisundis ning on tingitud sellest, et diapaus saabub erinevatel aegadel. Novembri alguseks tõusis glütseriinisisaldus juba 27,2%-ni, AJP aga langes —40,6°-ni. Seega tõusis glütseriinisisaldus 22. septembrist kuni 5. novembrini 27% (AJP langes 27° võrra). Glütseriinisisalduse edasine tõus ja AJP langus toimus aeglasemalt. Jaanuari lõpuks jõudis glütseriinisisaldus 30,1%-ni, s.o. maksimumi, AJP oli —45,7°. Nii glütseriini kui ka AJP dünaamikas puudub terav kulminatsioon. Kõrge glütseriinisisaldus ja madalad AJP-d püsisid kuni märtsi keskpaigani, mil esines glütseriini kontsentratsiooni kiire langus koos AJP tõusuga. Aprilli esimese dekaadi lõpul, kui osa röövikuid oli juba muutunud aktiivseks, täheldati jällegi nii glütseriinisisalduses kui ka AJP-s suurt individuaalset varieeruvust, mis oli tingitud erinevustest röövikute reaktiveerumises.

Glütseriinisisalduse ja AJP sesoonne muutumine oli teises ja viimases kasvujärgus talvituvatel männi-vaigumähkuri röövikutel ühesugune (joon. 1).

Erinevused aktiivsete ja diapauseerivate *P. resinella* röövikute glütseriinisisalduses näitavad, et glütseriini tekkimine on sellel putukal seotud diapausiga. Seda kinnitasid ka katsed viimases kasvujärgus talvituvate röövikutega, kes 7. oktoobril sisaldasid 0,1% glütseriini ja kelle glütseriinisisaldus tõusis pärast 41 päevast +20° juures hoidmist keskmiselt 13,9%-ni. Enamik röövikuist oli selleks ajaks diapausis.

Teatud määral reguleerib *P. resinella* glütseriinisisaldust ka temperatuur (joon. 2). 9. detsembril toatemperatuuri (20—22°) paigutatud teise kasvujärgu diapauseerivatel röövikutel, kes sisaldasid 16,4% glütseriini, langes glütseriinisisaldus kahe nädalaga 5,1%-ni (AJP —22,8°). See langus oli pöörduv, peale röövikute kolmenädalalist hoidmist välistingimustes (temperatuur alla —10°) tõusis glütseriini kontsentratsioon uuesti 23,1%-ni (AJP —42,2°).

Südatalvel, kui röövikud olid sügavas diapausis, vähenes glütseriinisisaldus 20—22° juures tunduvalt aeglasemalt kui kevadel (vastavalt 0,8 ja 1,3% päevas) ning tõusis peale madalasse temperatuuri tagasiviimist palju kiiremini (keskmiselt 0,9% päevas) kui kevadel, kus glütseriini resüntees oli väga aeglane, keskmiselt vaid 0,06% päevas.

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ТИЙУ ХАНСЕН

ИЗМЕНЕНИЯ В СОДЕРЖАНИИ ГЛИЦЕРИНА В СВЯЗИ С ХОЛОДОСТОЙКОСТЬЮ У ГУСЕНИЦ СОСНОВОГО СМОЛЯНОГО ПОБЕГОВЬЮНА *PETROVA RESINELLA* L. (*LEPIDOPTERA, TORTRICIDAE*)

Резюме

Гусеницы соснового смоляного побеговьюна *Petrova resinella* зимуют в кронах сосен выше снегового покрова и хорошо приспособлены к перенесению низких зимних температур.

Была изучена динамика содержания глицерина и точек переохлаждения (ТП).

К концу октября большинство гусениц уже диапаузировало. Активные, еще питающиеся гусеницы, зимующие в последнем возрасте содержали в это время 0,2% глицерина (ТП —16,4°С). У диапаузирующих особей содержание глицерина варьировало от 5,9 до 20,9% (в среднем 14,1%, ТП —29,1°). Большие индивидуальные колебания объясняются разными сроками наступления диапаузы и отражают большие различия в их физиологическом состоянии. К началу ноября содержание глицерина достигло уже

27,2%, а ТП —40,6°. Таким образом, с 22 сентября по 5 ноября содержание глицерина повысилось на 27% (ТП понизилась на 27°).

Дальнейшее повышение концентрации глицерина и понижение ТП протекало медленнее, достигая к концу января максимума —30,1% глицерина (ТП —45,7°). Высокое содержание глицерина и низкие ТП держались до середины марта, когда началось быстрое уменьшение концентрации глицерина и повышение ТП. В конце первой декады апреля как в содержании глицерина, так и по ТП опять наблюдались большие индивидуальные различия, обусловленные различной степенью реактивации гусениц.

Сезонные изменения глицерина и ТП у гусениц *P. resinella*, зимующих во втором и в последнем возрасте, похожи (рис. 1).

Различия в содержании глицерина между активными и диапаузирующими гусеницами *P. resinella* показывают, что накопление глицерина у этого насекомого связано с диапаузой. Опыты с гусеницами последнего возраста подтвердили это положение. После 41-дневного содержания гусениц при +20° (большинство гусениц в это время диапаузировало) концентрация глицерина повышалась от 0,1% в среднем до 13,9%.

В какой-то степени количество глицерина у *P. resinella* находится под контролем температуры (рис. 2). У диапаузирующих гусениц второго возраста, которые содержали 9 декабря 16,4% глицерина, после двухнедельного пребывания при комнатной температуре (20—22°) ее концентрация падала до 5,1% (ТП —22,8°). После трехнедельного пребывания в природных условиях содержание глицерина снова повышалось до 23,1% (ТП —42,2°).

В середине зимы, когда гусеницы были в глубокой диапаузе, концентрация глицерина при комнатной температуре (20—22°) понижалась гораздо медленнее, чем весной (понижение соответственно на 0,8% и 1,3% в сутки), и повышалась после пребывания в низкой температуре гораздо быстрее (в среднем на 0,9% в сутки), чем весной, когда накопление глицерина было очень медленное (в среднем на 0,06% в сутки).

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