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The diapause of the birch engraver, Scolytus ratzeburgi Janson (Col., Scolytidae), its termination by chilling, and manipulation with ecdysterone

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The birch engraver, *Scolytus ratzeburgi* JANSON, collected in Switzerland was shown to enter diapause as a mature last instar larva. Neither a natural long day photoperiod (LD) nor a relatively high rearing temperature of 21°C can prevent the induction of the diapause. This obligate diapause seems to be genetically controlled and can not be terminated by LD but only by a chilling period of about 45 days at 4°C. Larvae which are not mature at the time they are chilled do not enter diapause but may do so after they are brought back to 21°C and reach maturity. They then need a second chilling period for diapause termination. Field temperatures during the larval growth therefore seem to be responsible for the 1- and 2-year life cycles found in the birch engraver.

The developmental state of the larvae was determined by injecting 0.6 µg of ecdysterone which induces a precocious moult. Immature last instar larvae or mature larvae that have freshly entered diapause moult stationarily, though 10% of them show tiny wing and leg buds after 60 days of diapause. If diapausing larvae are chilled for 120 days they moult either stationarily (35% with tiny wing and leg buds) when injected with ecdysterone immediately after chilling or to 100% larval-pupal intermediates when injected after having been kept at 21°C for 4 days after chilling. The fact that it is not possible to induce a normal pupal moult in chilled diapausing larvae by an ecdysterone injection indicates that the development of pupal features needs a certain time that cannot be reduced without harmful effects. A possible hormonal regulation mechanism of the larval diapause is discussed.

The birch engraver, *Scolytus ratzeburgi,* attacks mainly weak or newly felled birch. However, little is known about its biology. TREDL (1915) reported one generation per year only, and winter to be spent in the larval stage. This might indicate that hibernation of *S. ratzeburgi* occurs in larval diapause. Monovoltinism in insects, i. e. a single generation per year, is often guaranteed by an obligate or facultative diapause (LEES, 1955; DANILEVSKII, 1965; BECK, 1968). However, RING (1977) found more than one larval *S. ratzeburgi* instar during winter and therefore supposed that these larvae overwinter by their ability to supercool. In order to find out whether *S. ratzeburgi* has a true larval diapause and how it may be maintained and terminated, larvae were chilled for different periods and injected with the moulting hormone ecdysterone. The hormone induces the moulting of the larvae and thus serves the determination of their developmental state.

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MATERIAL AND METHODS

Insects

Birch logs *(Betula pendula* ROTH), infested with *Scolytus ratzeburgi* JANSON, were collected at St. German, Wallis, Switzerland, either in July immediately after the attack by the adults or at the end of October when larvae were expected to be mature. To delay desiccation, the logs were coated with paraffin at both ends and stored in glass containers covered with gauze either at room temperature (21°C) or chilled (4 °C). The photoperiods were natural long day (LD) during July and August (≥ 15 h light per day), continuous darkness during the chilling period, and controlled LD (16 h L: 8 h D) after chilling. The emergence of the adults at 21°C was checked every day.

Ecdysterone injections

Ecdysterone (β-ecdysone) from Rohto Pharmaceutical Company, Osaka, Japan, was dissolved in distilled water and injected just behind the head capsule with a rounded glass capillary mounted on a motor driven syringe. Control larvae were injected with distilled water only or remained untreated. After the injections, treated as well as control larvae were stored on moist filter paper in round glass dishes. Treated insects were controlled every day following the injection.

In order to determine the effective concentration of ecdysterone causing larvae to moult, they were treated with 5, 50, 100, 500, and 1000 ng of ecdysterone, dissolved in 0.5 µl of distilled water. Larvae injected with distilled water, which served for control, or with 5 ng of ecdysterone did not respond at all and injection of 50 or 100 ng induced only 25–30% of the larvae to moult within the period of 5 to 10 days after treatment. However, most larvae injected with 500 ng moulted within 5 days after injection. Although 1000 ng of ecdysterone caused all larvae to moult, some of them were not able to get rid of their old cuticle. Therefore the smaller dose of 600 ng of ecdysterone was used in all further experiments to force larvae to a precocious moult.

RESULTS

Induction and termination of the diapause

Mid July birch logs which had been attacked by adults of the birch engraver a few days before, were brought into the laboratory and reared at a constant temperature of 21°C and natural LD. Two months later all larvae were mature but none had pupated. After another month the larvae had still not pupated, indicating their being in diapause.

In order to break the diapause, the larvae were chilled at 4°C for different lengths of time. The minimal time needed was between 30 and 45 days: whereas a chilling period of 30 days could not break the diapause at all, chilling for 45 days induced pupation and adult emergence beginning at 24 days after a supplemental incubation at 21°C. The period of adult emergence lasted 19 to 25 days (Fig. 1).

The first males appeared one day earlier than the first females. The sex ratio was 46% males to 54% females. No larva could be found in the bark when it was removed after the beetles'emergence ceased.

A prolongation of the chilling period to 90 or 105 days reduced only the period of first adult emergence by 2 and 4 days respectively. The period of adult emergence showed no correlation to the length of the chilling period.

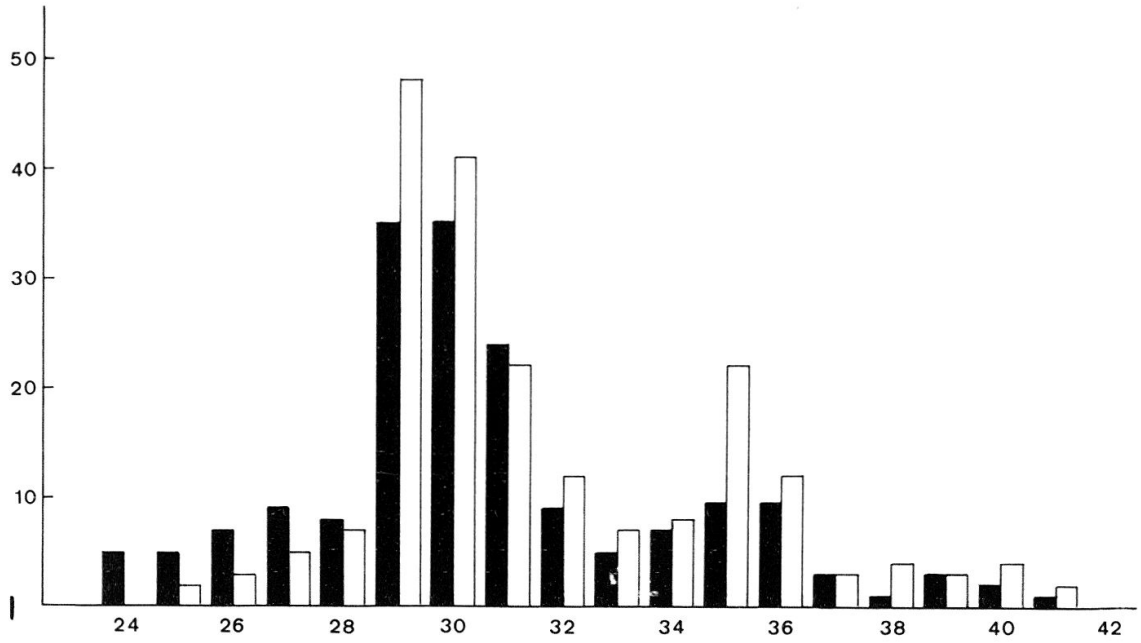

Fig. 1: Emergence of adult males (black columns) and females (light columns) of *S. ratzeburgi* after a chilling period of 45 days at 4°C. Ordinate = number of emerging adults; abscissa = days at 21°C.

Another series of logs was collected toward the end of October. They were infested with mature and immature larvae. After a chilling period of 45 days and a supplemental incubation period at 21°C adults of *S. ratzeburgi* emerged as described above. Adult emergence ceased after about 40 days at 21°C. However, when the bark was removed from the logs after 90 days at 21°C the logs still contained mature larvae, but neither immature larvae nor pupae. When these larvae were chilled once more for 45 days 100% of them emerged as adults during the subsequent incubation at 21°C.

Ecdysterone injections

If immature last instar larvae or mature larvae that had entered diapause were injected with 600 ng ecdysterone, they underwent a stationary larval moult. This was shown by the head capsule being of the same size before and after the moult and by the lack of any pupal features (Fig. 2A). On the other hand, if larvae remaining in diapause for at least 60 days were forced to moult, 10% of them showed tiny wing and leg buds (Fig. 2B). Ecdysterone injection in diapausing larvae immediately after chilling for 10, 30 or 120 days resulted in 22, 66, and 35% larvae with tiny wing and leg buds respectively.

In these experiments, the first larvae which moulted underwent a stationary larval moult, whereas many of those moulting later on had wing and leg buds. The rate of this trait was directly correlated to the length of time that elapsed between ecdysterone injection and moulting, indicating that the growth of the buds depends on the time after the ecdysterone injection and not the length of the chilling period.

If larvae were chilled for 120 days and remained for 4 days at 21°C before ecdysterone was injected, they moulted 3 to 4 days later and all individuals showed medium to well sized wing and leg buds after the moult (Fig. 2C). But none of the thus

treated larvae moulted to a complete pupa. Control larvae, which were chilled for 120 days but not treated with ecdysterone, pupated 8 to 10 days after incubation at 21°C (Fig. 2D).

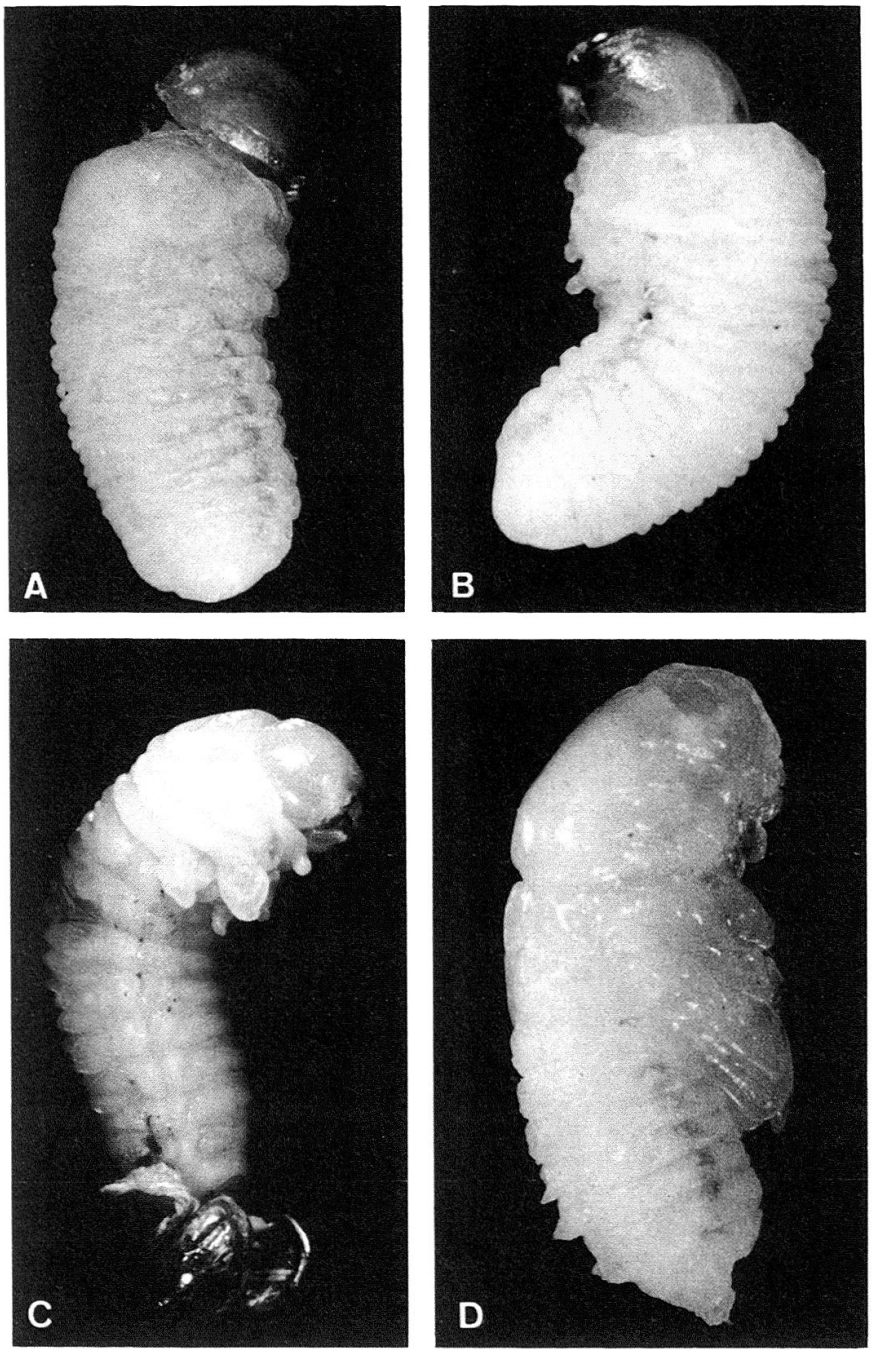

Fig. 2: Different types of moulting after injecting with 600 ng ecdysterone. A = stationary larval moult, B = larval moult with tiny wing and leg buds, C = larval-pupal intermediate form, D = untreated control pupa.

Only mature last instar larvae of the birch engraver, *Scolytus ratzeburgi,* enter diapause. This diapause cannot be prevented by rearing the larvae under long day conditions and thus seems to be genetically controlled. Since it can only be terminated by a chilling period of a at least 45 days at 4°C, it is an eudiapause according to the classification of MÜLLER (1970).

In two other Scolytidae, *Dendroctonus obesus* (MANN.) and *D. rufipennis* (KIRBY), DYER (1970) and DYER & HALL (1977) found diapause to be determined by the rearing temperature of the larvae: no diapause resulted when the larvae were reared at a constant high temperature of 21°C, whereas mature larvae entered diapause at a rearing temperature below a certain threshold. This is not the case in our experiments where 100% of the larvae entered diapause after rearing at 21°C. Diapause in *S. ratzeburgi* is therefore unlikely to be determined by temperature.

When infested logs were collected in autumn, not all of the larvae were mature and thus in diapause. Therefore not all larvae pupated after a chilling period of 45 days. Such larvae obviously overwinter in a state of supercooling as described by RING (1977). In spring, when the temperature increases, these larvae start feeding again, become mature, and then enter diapause. Since these larvae terminate diapause only after a minimal chilling period, they cannot pupate before they have been subjected to low temperatures and therefore may pupate in the next spring only. Our investigations thus show that larvae of *S. ratzeburgi* overwinter either in a state of supercooling or in true diapause, depending on their developmental state in autumn and, furthermore, that this bark beetle develops in a one- or a two-year life cycle, depending on environmental temperature.

The developmental state of the larvae can be determined by forcing them to moult. Following an injection with 600 ng ecdysterone, unchilled immature and mature last instar larvae moult stationarily whereas many of the larvae kept in diapause for 60 days moult to larvae with tiny wing and leg buds, i. e. to a kind of larval-pupal intermediates. This result shows that ecdysterone cannot stimulate the imaginal discs before the larvae have been in diapause for a certain time. Even then it can only stimulate the eversion of the discs but not their growth. The findings also confirm that diapause, which prevents the epidermal and imaginal disc cells from changing the larval to the pupal commitment, cannot be terminated without a minimal chilling period.

Even after chilling no fully developed pupa is obtained if the larvae are forced to moult immediately after the chilling period. The fact that the percentage of larvae with wing and leg buds increases, if more time elapses between ecdysterone injection and moulting, indicates that the eversion of the buds and their growth starts only after the injection. This is supported by the result that more pupa-like intermediates can be induced in larvae kept at 21°C for 4 days after the chilling period. Obviously even after the termination of diapause the growth and differentiation of the imaginal discs needs time and can only advance if the temperature is rised. Speeding up the moulting process by the ecdysterone injection prevents the full growth and differentiation of the pupal structures.

The different types of moulting of immature and diapausing last instar larvae before and after chilling suggest a hormonal regulation of the diapause of *S. ratzeburgi.* The occurrence of an additional larval instar after forced moulting during the last larval instar and at the beginning of the diapause suggests the presence of a high titer of juvenile hormone (JH) during these periods, as found in diapause induced last instar

larvae of *Cydia pomonella* (L.) by SIEBER & BENZ (1977, 1980). Also similar to the situation in *C. pomonella* (SIEBER & BENZ, 1980), the JH titre seems to decrease during the diapause of *S. ratzeburgi,* as suggested by the partial switch over from the larval to the pupal commitment in the insects that showed tiny wing and leg buds when forced to moult after a diapause period of 60 days. However, true pupal differentiation was induced only after a chilling period of 45 days, i. e. after the diapause was broken. Further investigations would therefore be needed to find out to what extent hormonal changes are responsible for the pupal differentiation in *S. ratzeburgi.*

ZUSAMMENFASSUNG

Der Birkensplintkäfer *Scolytus ratzeburgi* JANSON fällt als ausgewachsene Larve in eine Diapause, die nur durch eine Kühlungsperiode von etwa 45 Tagen gebrochen werden kann. Sie scheint eine genetisch determinierte obligate Diapause zu sein, denn weder Langtag noch eine hohe Zuchttemperatur können sie verhindern. Larven, die im Herbst noch nicht reif sind, gehen nicht in Diapause, sondern überwintern offenbar in einem Zustand von Unterkühlung («supercooling»). Erst wenn im Frühling die Temperaturen wieder höher sind, werden diese Larven reif und gehen in Diapause. Folgt danach keine Abkühlungsperiode, müssen die Tiere fast ein Jahr lang warten, bis ihre Diapause gebrochen ist und sie sich verpuppen und weiter entwickeln können. Je nach Umweltstemperatur kann deshalb der Entwicklungszyklus von *S. ratzeburgi* im gleichen Baum ein- oder zweijährig sein.

Durch Injektion von 600 ng Ecdysteron (= β-Ecdyson oder 20-Hydroxyecdyson) können die Larven zur Häutung gezwungen werden. Unreife Larven und solche, die das Diapausestadium erreicht haben, häuten dabei stationär wieder zu einer Larve, während 10% der Tiere kleine Flügel- und Beinknospen aufweisen, wenn sie vorher schon 60 Tage lang in Diapause waren. Offenbar erhalten die Imaginalscheiben erst in der Diapause die Kompetenz, auf Ecdysteron mit Evagination zu reagieren. Auch wenn die Diapause durch eine Kühlperiode gebrochen wird, häuten sich die Larven stationär bzw. zu 35% mit kleinen Flügel- und Beinknospen, wenn die Ecdysteroninjektion unmittelbar nach der Kühlperiode erfolgt. Werden die Larven nach der Kühlperiode jedoch 4 Tage bei 21°C gehalten und dann injiziert, treten Larven-Puppen-Zwischenformen auf. Die Tatsache, dass nach Ecdysteroninjektion nie vollständige Puppen entstanden, zeigt, dass die pupale Entwicklung eine bestimmte Zeit benötigt und nicht willkürlich gekürzt werden darf. Aufgrund der Resultate wird vermutet, dass der Juvenilhormon-Spiegel im letzten Larvenstadium hoch sei und erst während der Diapause erniedrigt werde.

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