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# Embryonic development in *Neodiprion sertifer*

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## Abstract

Progress of embryonic development in *Neodiprion sertifer* (Geoffroy) was examined in eggs collected in the field during winter and in eggs incubated under controlled conditions in the laboratory. Thirteen embryonic stages can be distinguished with morphological criteria. The development continues throughout winter but requires different temperature thresholds. The first phase proceeds at 5 °C and obviously demands cold because temperatures over 20 °C cause death or malformations. By contrast, in about middle of the development (morphological stage VI) the temperature of 20 °C becomes favourable and supports rapid growth. Hypothesis that the embryos overwinter in a full developmental arrest is rejected on the basis of these results. Term "pseudo-diapause" is suggested for the slow but continuous development.

**Key words:** *Neodiprion sertifer*, embryonic development, diapause, quiescence, pseudo-diapause.

## Introduction

The sawfly *Neodiprion sertifer* (Geoffroy) (Hymenoptera Diprionidae) causes considerable damage to young pine stands in most of Europe and large regions of Asia. In Italy it preferably attacks *Pinus sylvestris* L. and *P. mugo* Turra up to elevations 1,500 m above sea level. In high altitudes and in the North, for example in the Japanese Alps and in central Norway, it has a 2-year life cycle (Cecconi, 1924; Pschorn-Walcher, 1965, 1970, 1982; Morimoto and Nakamura, 1989) but in Italy it is univoltine. The larvae develop rapidly in spring and upon reaching their full size spin cocoons in the soil. They resume development after a summer aestivation yielding egg-depositing adults in the autumn. The eggs are inserted into pine needles where they overwinter. Larvae of a new generation hatch in spring.

The hibernation period lasts in the region of our study five to six months, from October to April. The progress of embryonic development during this time is a subject of dispute. Brygider (1952) asserted that a well developed embryo of *N. sertifer* enters diapause, whereas Breny (1957) claimed that the embryonic development ceases at a non-fixed stage and that winter dormancy of this diprionid is a case quiescence controlled by the osmotic pressure within the pine needle. Niklas (1956) reported that in Germany the embryonic development of *N. sertifer* continues in winter as long as the ambient temperature does not fall below 0 °C.

In our previous work (Baldassari *et al*., 2003) we showed that the egg stage of *N. sertifer* can be shortened to 106 days when the egg cluster is kept at a constant temperature 15 ± 1 °C and a typical winter photoperiod of 10 h light and 14 h darkness. This finding proved that the eggs of *N. sertifer* do not need a period of chilling for completion of their embryonic development. Moreover, since the plants bearing eggs were continuously watered, changes in osmotic pressure were unlikely to provide a stimulus for the activation of embryonic development. The period of about three months, which was required to complete the embryonic development, could not be shortened by an increase of temperature. The upper lethal temperature threshold is apparently rather low because no larvae hatched when the pine seedlings with sawfly eggs were grown at 20 ± 1 °C and 10:14 h photoperiod (unpublished data). In order to understand how *N. sertifer* eggs actually spend the autumn and winter seasons, the present paper compares embryonic development of specimens reared in the laboratory with those collected in the field.

## Materials and methods

### Collection site and meteorological data

Eggs were collected in a 25-year old pine stand of *P. sylvestris* grown 300 m above sea level at 43° 55' 25" N.L. on a slope facing south-west. The stand is close to the village of Mercato Saraceno on the Appennino Tosco-Romagnolo in the Province of Forlì-Cesena. Meteorological data were obtained from the instrument shelter of the forestry service "Corpo Forestale dello Stato" situated about 5 km from the pine stand at 250 m above sea level. The mean temperature was calculated from the maximum and minimum temperature recorded on the particular day.

### Monitoring of adults

Two sticky traps "Lund 1" baited with synthetic pheromone (Anderbrant *et al*., 1989) were placed in the pine stand to monitor the period of adult flight. The bait consisted of a plastic microvial containing 1 ml (2S,3S,7S) 3,7-dimethyl-2-pentadecanyl acetate (> 99% pure) that was kindly provided by Prof. H.-E. Högberg of the Department of Natural and Environmental Sciences, Mid Sweden University Sundsvall, Sweden. The traps were placed about 2 m above ground on September 25 in 1997 and September 17 in 1998 and checked weekly until the end of November. About 50 randomly collected pine twigs were simultaneously examined to establish the start of egg-laying. The number of males caught per week was expressed as a mean of catches in the two traps.

### Sampling of egg, embryo staging and morphometric measurements

Eggs for morphological evaluation were collected from January to the hatching of larvae in 1998. First eggs of the following generation were found and collected from October 28 to December 30 of 1998. Samples of 10-20 needles containing eggs were collected every 6-15 days until hatching. For the morphological studies, the needles with eggs were immediately submersed in Bouin's fixative. The eggs were gently dissected from the needles after 24 h at 23 °C, kept in Bouin's solution for another 24 h, rinsed twice in 70% ethanol, and stained with Grenacher's boracic carmine for 72 h at 32 °C. After two washes in 70% ethanol, the eggs were soaked for 2 days in 70% ethanol containing 1% hydrochloric acid to destain the yolk to milky white, while the embryo and the serosa remained red.

The rate of embryonic development in the field was assessed with a staging scale based on external characteristics, similarly as described by Shafiq (1954) for the sawfly *Pteronidea ribesii* Scopoli. In order to measure the embryos, the eggs were transferred into Faure's fluid and cut frontally along the length, just above the embryo. The body length, the maximum width of thorax, the width of head capsule, and the width of posterior abdomen were measured under a dissecting microscope with the aid of an eyepiece scale. Embryonic growth rate was expressed as a ratio between maximum thorax width and the visible body length multiplied by 10,000. This parameter is similar to the "*quotient de croissance*" adopted by Breny (1957) except that we used maximum width of thorax and not of the first abdominal segment that is not easily distinguishable in early embryos.

### Development time for eggs in field and laboratory

The developmental capacity of eggs collected in the field in November through March was examined by transferring some of the pine-twigs bearing the eggs to a climatic room of 20 ± 1 °C, 12 h photophase, and 70% R.H.. The cut ends of twigs were plunged in water and egg hatch was recorded every day.

Some observations were done on the eggs deposited by sawflies on the pine seedlings grown under laboratory conditions as described by Baldassari *et al*. (2003). The seedlings with eggs were exposed either to 5 °C and an 8:16 h L:D photoperiod or to 16 or 21 °C associated with a 14:10 h L:D photoperiod. In the first experiment, the plants with eggs were exposed to 16 °C for 1, 7 and 15 days, respectively, transferred to 5 °C for 66 days, and eventually kept at 18 °C until the sawfly hatched. In a second experiment, a seedling with eggs was initially maintained at 16 °C for 5 days and another one for 20 days. Both plants were then subjected to 5 °C for 63 days and then kept at 18 °C. In a third experiment, a plant with freshly deposited eggs was exposed to 5 °C for 76 days and then maintained at 21 °C until the hatching of larvae. Two other plants were initially exposed to 16 °C, one for 16 and the other for 12 days, then to 5 °C for 76 days, and eventually were transferred to 21 °C. Yet another seedling with eggs was grown at 16 °C continuously.

Accumulated thermal sum of temperatures above 0 °C experienced by the eggs during their whole development (in days) was calculated for different rearing conditions as well as for the field environment. Mean temperatures recorded from the 31st October to the 7th April, which is the estimated egg incubation period in the nature, were used in the latter case.

## Results

Daily fluctuations of temperature were considerable in the experimental pine stand in September and October and in February through April (figure 1). The temperature fell under 0 °C in 71 days during the 1997/1998 winter, and a minimum of -8 °C was recorded as late as

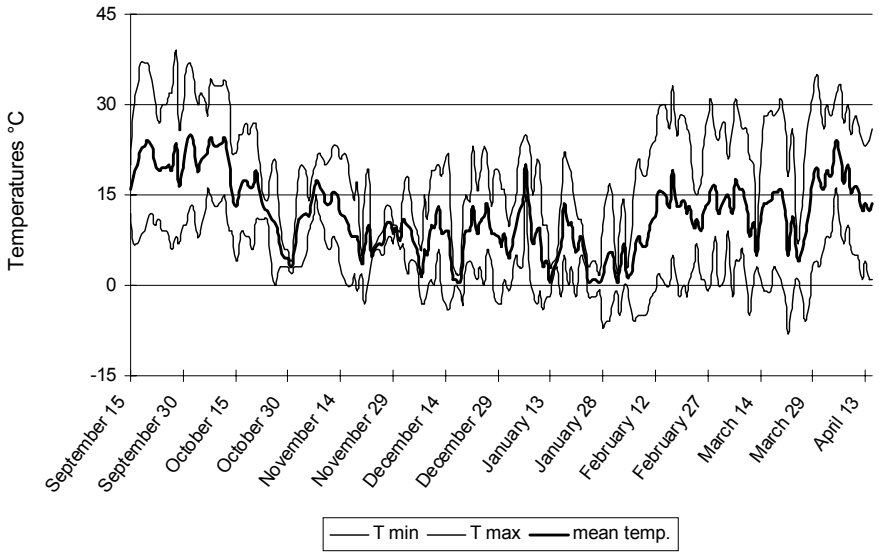


**Figure 1.** Maximum, minimum and mean daily temperatures recorded during the autumn 1997 and winter 1998 close to the pine stand.

**Table 1.** Monthly mean temperatures recorded at the instrument shelter 5 km far from the collection site during three years.

| | 1997 | 1998 | 1999 |
|---|---|---|---|
| September | 24.27 | 22.08 | 22.23 |
| October | 16.61 | 16.02 | 14.71 |
| November | 10.53 | 11.65 | 8.25 |
| December | 7.89 | 5.74 | 6.63 |
| January | 6.44 | 6.53 | 5.50 |
| February | 10.64 | 10.16 | 7.07 |
| March | 15.26 | 12.60 | 12.63 |
| April | 14.82 | 17.92 | 14.97 |

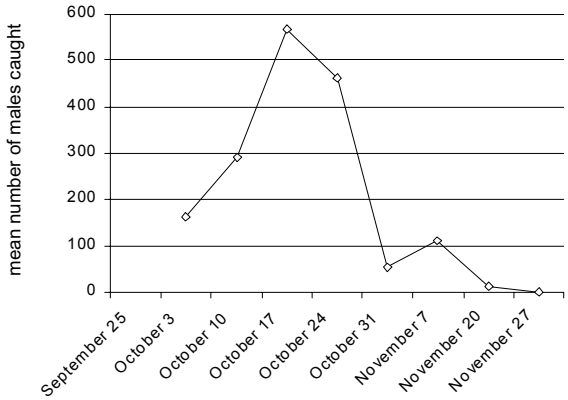


**Figure 2.** The mean number of males caught in two "Lund 1" sticky traps baited with synthetic pheromones placed in the experimental pine stand in autumn 1997.

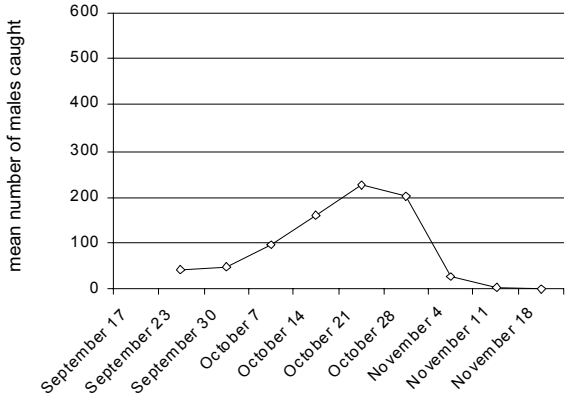


**Figure 3.** The mean number of males caught in two "Lund 1" sticky traps baited with synthetic pheromones placed in the experimental pine stand in autumn 1998.

March 22, but the mean temperature was maintained above the freezing point. Table 1 demonstrates that monthly mean temperatures were similar in three successive years of our study.

In 1997, a large number of *N. sertifer* males were found in the sticky traps already on September 25 but first females walking on the twigs were observed on October 24. On the same day a female was also found in a trap. Apparently newly laid eggs were detected on October 31 and newly hatched larvae on April 7, 1998. In the autumn of this year, the first males appeared in the traps on September 17 and the first egg cluster was found on October 28. The period of flight of males reached a peak around October 27 and 21 in 1997 and 1998, respectively (figures 2 and 3). No more males were caught after November 17 and 18 in the respective years. The total number of males collected in the traps was 3316 in 1997 and 1611 in 1998.

**Table 2.** The stages of embryonic development of *N. sertifer*.

| Stage | Description |
|---|---|
| I | Formation of the germ band |
| II | The germ band contracts and differentiates a broad protocephalic region |
| III | Differentiation of two protocephalic lobes |
| IV | The edges of protocephalic lobes bend upwards |
| V | Lateral edges of the anal region begin to rise up and backwards; head and thorax exhibit rudimental segmentation |
| VI | Dorsal closure of the anal segment; segmentation of the abdomen |
| VII | Thickening of the cephalic and thoracic limb buds; differentiation of the labrum and stomodaeum |
| VIII | Elongation of the head appendages (mandibles, maxillae, and labium) and legs; invagination of the proctodaeum |
| IX | The head is clearly demarcated from the thorax and the head appendages have moved anteriorly; dorsal closure completed |
| X | The posterior end of the embryo turns ventrally |
| XI | Differentiation of the eyes |
| XII | Sclerotisation of the mandibles |
| XIII | Sclerotisation of the head capsule and tanning of the general body cuticle |

The embryonic development of *N. sertifer* appears to start immediately after the egg deposition and proceed throughout the winter. Thirteen stages of embryogenesis could be distinguished based on the size and shape of the germ band, the progress of dorsal closure, and development of eyes and appendages (table 2 and figure 4).

Embryos of the first stage were found very exceptionally, indicating that the start of embryogenesis is very fast (table 3). Most eggs examined in October and November contained embryos at the stage of well-formed germ band with a broad protocephalic region that becomes bilobic with the lateral lobes bent upwards (stages II-IV). Embryos with rudimental segmentation in the thoracic region (stage V) first appeared in mid-November. Stage VI, characterised by the initiation of dorsal closure at the end of abdomen, is reached by a few embryos at the beginning of December but by others only in middle January. Most embryos examined at the end of January possessed distinct appendages and

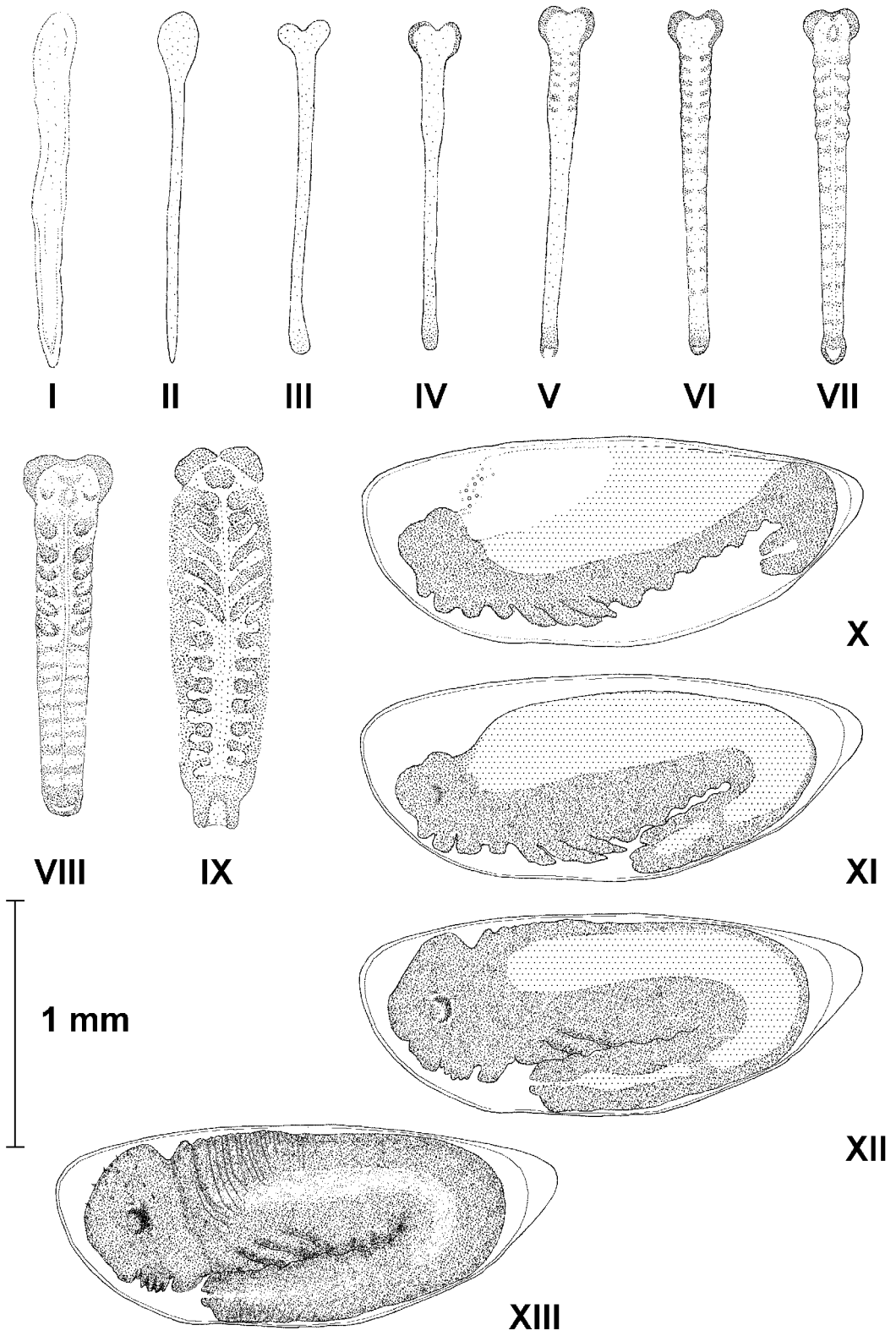


**Figure 4.** The stages of embryonic development of *N. sertifer*.

invaginated proctodeum (stage VIII). Embryos with a clearly demarcated head and complete dorsal closure (stage IX) were found two weeks later. Rapid embryonic growth ensues in the second half of February and the first half of March when the embryos pass through stages X to XIII. In the last stage they seem to rest for 2-3 weeks before hatching at the beginning of March. The data summary in table 3 demonstrates that embryogenesis proceeds continuously throughout winter. The period of egg laying lasts probably more than one month and this initial difference in the egg age is manifested as stage variability in embryos examined at any time point

**Table 3.** The stage of embryonic development achieved by *N. sertifer* in autumn and winter; n means the number of embryos observed.

| Date of sampling | no. | Stages | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | XIII |
| October 28, 1998 | 7 | 1 | 3 | 3 | | | | | | | | | | |
| November 4, 1998 | 6 | | 2 | 3 | 1 | | | | | | | | | |
| November 11, 1998 | 4 | | | | 4 | | | | | | | | | |
| November 18, 1998 | 11 | | | | 3 | 8 | | | | | | | | |
| November 25, 1998 | 6 | | 1 | 4 | 1 | | | | | | | | | |
| December 2, 1998 | 6 | | | 1 | 1 | 3 | 1 | | | | | | | |
| December 9, 1998 | 6 | | | | | | 6 | | | | | | | |
| December 15, 1998 | 6 | | | | | 2 | 4 | | | | | | | |
| December 22, 1998 | 7 | | | | | 1 | 2 | 4 | | | | | | |
| December 30, 1998 | 5 | | | | | 1 | 4 | | | | | | | |
| January 6, 1998 | 6 | | | | | | 1 | 3 | 2 | | | | | |
| January 16, 1998 | 7 | | | | | | 2 | 5 | | | | | | |
| January 21, 1998 | 5 | | | | | | | 1 | 4 | | | | | |
| January 30, 1998 | 5 | | | | | | | 1 | 3 | 1 | | | | |
| February 14, 1998 | 6 | | | | | | | | 1 | 5 | | | | |
| February 22, 1998 | 9 | | | | | | | | 1 | 1 | 7 | | | |
| February 28, 1998 | 3 | | | | | | | | | 3 | | | | |
| March 4, 1998 | 4 | | | | | | | | | 4 | | | | |
| March 10, 1998 | *not measurable* | | | | | | | | | | 9 | 19 | 3 | |
| March 17, 1998 | *not measurable* | | | | | | | | | | | | | 6 |
| March 23, 1998 | *not measurable* | | | | | | | | | | | | | 8 |
| April, 7 1998 | hatching | | | | | | | | | | | | | |

**Table 4.** Morphometric values (mean ± S.E.) of *N. sertifer* embryos in nature. Growth rate (GR) was assessed as a ratio of the maximum thorax width to the visible body length multiplied by 10,000; n means the number of embryos observed.

| Date of sampling | no. | length | width of head | max. width of thorax | length of telson | GR |
|---|---|---|---|---|---|---|
| 28/10/98 | 7 | 1379.36 ± 24.75 | 239.99 ± 7.44 | 95.32 ± 6.04 | 96.44 ± 2.82 | 690.48 ± 39.72 |
| 04/11/98 | 6 | 1352.82 ± 34.08 | 217.18 ± 7.49 | 81.12 ± 5.97 | 90.28 ± 4.86 | 600.89 ± 45.33 |
| 11/11/98 | 4 | 1203.01 ± 20.11 | 192.33 ± 13.78 | 74.58 ± 6.79 | 82.43 ± 3.93 | 618.64 ± 50.56 |
| 18/11/98 | 11 | 1188.92 ± 15.24 | 229.08 ± 6.25 | 103.48 ± 4.59 | 83.73 ± 2.27 | 868.68 ± 33. 82 |
| 25/11/98 | 6 | 1303.10 ± 48.98 | 198.87 ± 12.60 | 88.97 ± 3.31 | 73.27 ± 5.23 | 684.69 ± 23.44 |
| 02/12/98 | 6 | 1334.50 ± 32.68 | 287.83 ± 13.85 | 138.42 ± 7.65 | 103.36 ± 5.13 | 1039.43 ± 60.81 |
| 09/12/98 | 6 | 1279.55 ± 13.29 | 304.84 ± 9.16 | 163.54 ± 14.95 | 113.83 ± 1.76 | 1273.91± 105.42 |
| 15/12/98 | 6 | 1392.07 ± 37.30 | 307.46 ± 8.21 | 139.99 ± 7.42 | 112.52 ± 5.23 | 1006.41 ± 50.58 |
| 22/12/98 | 7 | 1379.36 ± 28.13 | 326.34 ± 8.01 | 172.70 ± 9.84 | 123.36 ± 5.06 | 1254.50 ± 73.52 |
| 30/12/98 | 5 | 1400.44 ± 18.17 | 307.72 ± 14.56 | 125.60 ± 4.30 | 102.05 ± 3.51 | 898.87 ± 41.77 |
| 06/01/98 | 6 | 1106.85 ± 46.71 | 211.95 ± 10.53 | 119.06 ± 17.48 | 74.58 ± 11.78 | 1069.28 ± 140.07 |
| 16/01/98 | 7 | 1287.40 ± 12.82 | 236.62 ± 1.12 | 145.79 ± 8.19 | 92.24 ± 3.76 | 1131.87 ± 60.13 |
| 21/01/98 | 5 | 1422.42 ± 13.69 | 273.18 ± 16.16 | 204.10 ± 9.93 | 111.47 ± 6.28 | 1436.09 ± 74.55 |
| 30/01/98 | 5 | 1447.54 ± 36.28 | 271.61 ± 15.62 | 241.78 ± 23.60 | 120.89 ± 4.71 | 1662.73 ± 135.10 |
| 14/02/98 | 6 | 1394.68 ± 22.72 | 345.40 ± 5.73 | 277.37 ± 7.76 | 133.45 ± 10.13 | 1991.43 ± 64.69 |
| 22/02/98 | 9 | 1349.33 ± 20.29 | 302.66 ± 11.19 | 345.40 ± 24.16 | 136. 94 ± 4.74 | 2554.95 ± 167.20 |
| 28/02/98 | 3 | 1454.87 ± 34.32 | 366.33 ± 40.87 | 507.63 ± 64.31 | 183.17 ± 18.87 | 3473.06 ± 358.01 |
| 04/03/98 | 4 | 1844.75 ± 37.10 | 522.03 ± 25.94 | 745.75 ± 18.69 | 329.70 ± 21.26 | 4042.84 ± 67.89 |

throughout the winter. In March, however, all embryos reach the final stage XIII and their hatching is to some extent synchronised, probably by the rise of maximal temperatures to nearly 20 °C.

The morphometric values and the growth rate calculated on their basis also demonstrate a continuum of embryogenesis (table 4). The data depict effective embryonic growth only partially and are less suitable for embryo staging than the morphological features. The measurements of embryo dimensions nevertheless show clearly a rapid embryonic growth in January and February (figure 5). The growth in February is actually somewhat underestimated because the abdomen is bent ventrally at this time and its extension cannot properly

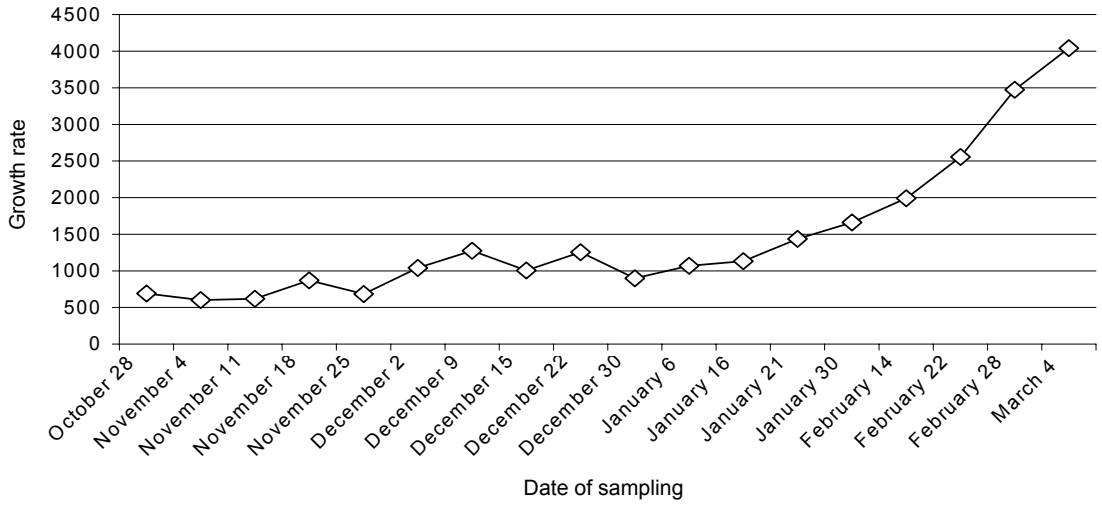


**Figure 5.** The development of *N. sertifer* embryos described by the growth rate.

be measured. At no time can be the rate of embryogenesis be assessed from the morphometric values alone.

The development of eggs in pine twigs collected in the experimental pine stand and transferred to 20 ± 1 °C depended on the time of collection. No larvae hatched from the eggs collected on November 18 but it was impossible to decide whether the death was caused by a sudden egg exposure to a relatively high temperature or by desiccation of the pine needles. Very few larvae hatched after 60 days from the eggs collected on December 2. By contrast, the incubation time was only 10 days and the hatchability rate was high in the eggs collected in the field on January 13 or February 22. Finally, the larvae hatched in four days when the pine twigs with eggs were transferred from the field temperature to 20 ± 1 °C on March 8.

The sums of accumulated degree-days needed for the completion of embryogenesis varied under the tested conditions from 674 to 1577 (table 5). Values established with initial egg incubation at 16 °C, followed by chilling at 5 °C for 63 or 66 day, and final exposure to 18 °C, indicate that the length of incubation at 16 °C has a negative effect on the total length of development and the sum of degree-days. The development to hatching lasted only 87 days when the exposure to 16 °C was for a single day but 98-100 days when it was 20-15 day, respectively. The corresponding degree-day values were 706 and 905-912, respectively. Similar correlations were found when the final incubation occurred not at 18 but at 21 °C (table 5). There is no doubt that embryogenesis proceeds at 5 °C. Possible interpretation of these data is provided in the discussion.

**Table 5.** The effect of different length of cold treatment on the duration of embryonic development, and accumulated thermal sum at the hatching.

| Number of days to complete embryonic development in the nature | | | Accumulated thermal sum |
|---|---|---|---|
| 159 | | | 1577.5 |
| Number of days at 16 °C | Number of days at 5 °C | Number of days to complete embryonic development at 18 °C | Accumulated thermal sum |
| 1 | 66 | 20 | 706 |
| 5 | 63 | 26 | 863 |
| 7 | 66 | 17 | 748 |
| 15 | 66 | 19 | 912 |
| 20 | 63 | 15 | 905 |
| Number of days at 16 °C | Number of days at 5 °C | Number of days to complete embryonic development at 21 °C | Accumulated thermal sum |
| 0 | 76 | 14 | 674 |
| 12 | 76 | 13 | 845 |
| 16 | 76 | 13 | 909 |
| Number of days to complete embryonic development at 16 °C (mean ± S.E.) | | | Accumulated thermal sum |
| 101.5 ± 3.27 | | | 1504 |

## Discussion

Brygider (1952), Breny (1957), and to some extent several other authors attempted to elucidate the process of egg hibernation in *N. sertifer* but the dependence of the length of embryogenesis on temperature has not been addressed in detail. We show that the thermal sum accumulated by embryos to the moment of hatching in nature (1577.5 degrees-day) is comparable to that accumulated in the laboratory at constant temperature of 16 °C (1504 degrees-day). This result suggests a direct relationship between temperature and the rate of development but according to our experience this is true only under certain thermal regimes. Eggs in pine twigs kept continuously at 20 °C typically yielded no larvae or, in rare cases, produced malformed embryos (unpublished). A similar phenomenon was observed in the grasshopper *Austroicetes cruciata* (Saussure) upon egg exposure to certain temperatures at specific times (Andrewartha, 1952).

Our experiments show that the duration of embryonic development is shortened, within certain limits, when the embryo develops for some time at a low temperature. The embryos exposed to 5 °C for 76 days immediately after the egg deposition and only afterwards to 21 °C accomplished their development in 90 days at a degree-day value as low as 674. The thermal sums necessary to complete development, as well as the length of development, increased when the period of relative chilling was preceded by incubation at 16 °C. An initial exposure to 16 °C for 12 and 16 days, followed by incubations at 5 °C for 76 days and at 21 °C until hatching, enhanced the degree-day value to 845 and 909, respectively. Unlike typical diapausing embryos, however, the embryos of *N. sertifer* develop more or less continuously in a wide range of temperatures and the chilling of eggs is not obligatory.

The continuous embryogenesis of *N. sertifer* seems to be characterised by two developmental patterns. The initial development occurs at relatively low temperatures, for example 5 °C, while temperatures around 20 °C are lethal. This mode of temperature dependence changes in embryos around stage VI for whom 20 °C is no longer lethal but become favourable because it accelerates last phases of embryonic development. The exact timing of the switch of temperature dependence and the values of temperature lethal and development thresholds remain to be established.

The temperature of 5 °C may be close to the optimum and 16 °C above the optimum for early embryogenesis. On the other hand, development towards the end of embryogenesis is accelerated upon transfer to 18 and even more to 21 °C, indicating that the optimal temperature value is elevated. The high value of degree-days which was established in the controlled culture at 16 °C in our opinion reflects the fact that this temperature is above optimum for the early and below optimum for the final part of embryogenesis. Nevertheless, the embryos reared at 16 °C complete their development, albeit at a high thermal sum. The degree-day value established for 16 °C is very similar to the value calculated from the field data and possibly applicable in the predictions of timing of *N. sertifer* egg hatch in nature.

The pattern of embryogenesis in *N. sertifer* does not fit into the consolidated classification of insect dormancy (Müller, 1970 reviewed by Thiele, 1973). From a typical diapause it differs by the continuation of development at a wide range of temperatures and it cannot be classified as quiescence because the over-all development is not accelerated by temperature increase. We propose that the eggs of *N. sertifer* overwinter in a diapause-like state that is characterised by two changes in the development dependence on temperature. The situation somewhat resembles diapause-like states of certain insect larvae that are characterised by reduced growth rates (Lees and Tilley, 1992).

Our conclusion differs from the proposal of Breny (1957) who regarded the hibernating embryo as a quiescent stage controlled by the osmotic pressure of the pine needle. In support of his hypothesis he described development of eggs removed from the needles and placed on wet filter paper incubated at 22 °C. Eggs were collected in November and December, i.e. after an undefined period of time spent under natural conditions. The embryos completed development to hatching in 60 days. We obtained similar results with egg-clusters collected in December and placed to 20 °C. According to Brygider (1952), *N. sertifer* enters diapause as well developed embryo. This conclusion was based on analysis of a single egg cluster collected at the end of December, i.e. 60 days after the start of oviposition. The specimen described by Brygider (1952) as a diapausing embryo is comparable to stage VI described in this paper. As a matter of fact the embryo at this stage, far from being in diapause is ready for a rapid growth, at least in our region, if it is exposed to temperature around 20 °C.

The inhibiting effect of elevated temperature on early embryogenesis is a fitting adjustment to temperature fluctuations in different regions occupied by *N. sertifer*. The sawfly first develops only at low temperatures that are typical for the winter months, and only at an advanced stage of embryogenesis the development is promoted by higher temperatures. It cannot be excluded that the switch in temperature sensitivity depends not only on the developmental stage but to some extent also on the length of exposure to low temperature. This phenomenon could be defined as "pseudo-diapause". The use of this term is justified: it is not a true diapause because no morphogenic arrest is evident but low temperatures shorten development as is typical for embryos undergoing winter diapause.

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