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Imaginal wing disc morphogenesis, a sign of diapause development in the European corn borer

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Summary. In the European corn borer, subtle changes in imaginal wing discs during diapause constitute observable indications of diapause development, in experimental as well as in field-grown larvae. Wing disc diapause development is dependent mainly on temperature, and its total achievement is a necessary condition for good diapause termination. By applying these observations, we have improved a method that provides homogeneous populations of larvae that can resume their development rapidly in any season.

Key words. European corn borer; wing disc development; diapause development; diapause termination.

Larvae of the European corn borer, *Ostrinia nubilalis* Hbn. cause great losses in corn yields. Mature fifth-instar larvae enter a photoperiodically induced diapause lasting 7 or 8 months during the winter season^{1,2}.

In order to differentiate diapausing from non-diapausing field or laboratory larvae, three physiological symptoms of the diapause syndrome have been studied:

- The stainability of the protocerebral neurosecretory cells (NSC) by Victoria blue (VB) and paraldehyde fuchsin (PF), is a phenomenon which is observable in non-diapausing larvae, and which progressively disappears during the first 3 or 4 months of diapause³.

- The hemolymphatic titer of trehalose, measured by gas-liquid chromatography, is a factor that is 5–10 times higher in diapausing larvae than in non-diapausing larvae³.

- In diapausing larvae, the development of the imaginal wing discs structures seems to be arrested at different early phases. The most advanced, rather poorly differentiated phase is called III3⁴.

Before diapause termination, diapausing larvae must undergo a maturation termed 'diapause development'⁵. Hence diapause appears to be 'a dynamic state during which specific physiological processes occur'⁶. We propose that the subtle changes in the morphogenesis of wing discs during diapause, constitute an observable indication of the progression of diapause development in the larvae of *O. nubilalis*.

Previous examples of morphogenetic changes during diapause have been cited⁷. Particularly, in diapausing embryos of *Cnephasia pumicana*, the mesenteron must develop before diapause termination⁸.

Post-diapause development is characterized by the reversal of the three physiological criteria for diapause. Firstly, the protocerebral NSC regain their stainability, then the titer of hemolymphatic trehalose decreases, and finally the imaginal wing discs resume development. This succession of events is invariable: wing buds do not develop without preliminary changes at the neurosecretory and metabolic levels in the wild as well as in the laboratory³.

Evidence of wing disc development during diapause. Field-grown populations from experimental infestations in Versailles (France) were studied from September to June, in 1984–1985 and in 1985–1986. In both cases, as early as October, all collected larvae entered diapause. Their trehalosemia was high and development of their wing buds was arrested at stages I, II and III1 (fig. 2). From January, III2 stages appeared and became more numerous. The first III3 stages were observed at the beginning of March (fig. 2). At

this time, protocerebral NSC were weakly or not at all stainable. Trehalosemia remained high. Physiologically, the larvae appeared to remain in diapause.

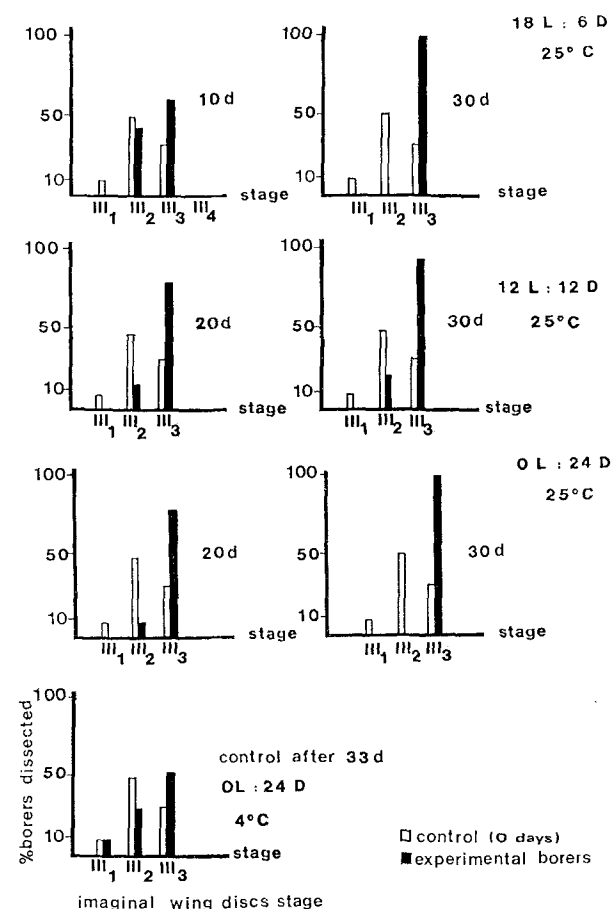


Figure 1. Experimental influence of temperature and photoperiod on the differentiation of the imaginal wing discs during diapause. The development of imaginal wing discs of diapausing control larvae maintained at 4°C and 0L:24D, was compared with those of diapausing experimental larvae placed at 25°C, and at different photoperiods (18L:6D, 12L:12D, 0L:24D). Each batch of experimental borers represents 20 larvae, collected in South-West France (Bordeaux).

Influence of temperature and photoperiod on the diapause development of the European corn borer.

| | | Non-diapausing control | 0L:24D, 4°C | | 18L:6D, 25°C | | | 12L:12D, 25°C | | 0L:24D, 25°C | |
|---|-------------------------------------|------------------------|-----------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|-----------------|------------------|
| | | | 0-d | 33-d | 10-d | 20-d | 30-d | 20-d | 30-d | 20-d | 30-d |
| A | Wing discs (% in III ₃) | — | 30 ^a | 50 ^{ab} | 60 ^b | 85 ^c | 100 ^c | 80 ^c | 95 ^c | 80 ^c | 100 ^c |
| | Time (days) for 50% pupation | 6–7 | > 25 | 25 | 24 | — | 9–10 | 15 | 11 | 15 | 11 |
| B | Stainability of protocerebral NSC | +++ | — | — | — or ± | ± | ± | — or ± | — or ± | ± | ± |
| | Hemolymphatic trehalose (g/l) | 0.8 ± 0.2 (N = 10) | 7.9 ± 2.5 (10) | 7.5 ± 2.5 (10) | 5.8 ± 2.2 (9) | 7.1 ± 2.7 (10) | 11.7 ± 3 (10) | 9.6 ± 2 (10) | 11.3 ± 2.9 (10) | 8.1 ± 2.2 (10) | 12.9 ± 3.1 (10) |

All the experimental borers remained in a diapausing state because they were deprived of water. After 10, 20 or 30 days spent in such conditions, a part of each batch was placed under diapause breakdown conditions (18L:6D, 25°C and water) and time necessary for 50% pupation was noted; the remaining borers were sacrificed. Their hemolymph was sampled, their brain and imaginal wing discs dissected. Protocerebral neurosecretory cells (NSC) are either not (—), or little (±) or strongly stainable (+++). Expression of hemolymphatic trehalose: mean value ± SEM (N = number of replicates). The difference is significant only if the letters following the compared data are different (G-test, $p < 0.05$).

At the end of April and the beginning of May, protocerebral NSC stainability was recovered and the titer of hemolymphatic trehalose dropped. Thus, the first two signs of resumed development were detectable. During this period 75–80% of the population also showed III₃ stage wing buds. Stage III₄ and later stages were not observed before the middle of May (fig. 2). Wing discs then resumed development. The number of III₃ stages declined to 0 during the following 3 weeks: all individuals in the population had resumed development.

Our observations in natural overwintering populations show that 2 phases usually occur in the diapause development of wing discs. A first phase corresponding to the coldest period of the winter, generally until the end of January, is characterized by a very slow development. The second phase takes place during the last two months (March–April) of diapause when the mean ambient temperature and the photoperiod increase. Then the borer wing discs develop more rapidly and are arrested in phase III₃.

In the European corn borer, wing disc morphogenesis occurs slowly during autumnal-hibernal diapause. However, this phenomenon does not occur at the same rate for all larvae in a given population. During this period, the protocerebral neurosecretory perikarya, the assay of hemolymphatic trehalose, as well as the results of experimental diapause termination show that these larvae are still in the diapause state. During diapause, morphogenesis of the wing discs proceeds, but never goes beyond the III₃ stage (the clamping stage), which usually appears at the end of winter. As described by Beck and Alexander⁹, 'diapause in the European corn borer is not a state of arrested development'.

We have attempted to determine whether the slow differentiation of wing discs in *O. nubilalis* during diapause could be used to measure the progress of diapause development as defined by Andrewartha⁵. In an initial experiment, we observed that larvae from Versailles that were entering diapause failed to pupate, and died after transfer to diapause terminating conditions (18L:6D, 25°C, available water). Conversely late diapausing larvae (6 months in diapause) pupated in 15 days when subjected to the same conditions. These findings are similar to those of McLeod and Beck¹⁰. These authors showed that under diapause terminating conditions, the time to pupation decreased and survival increased as autumn and winter progressed. Our observations on the timing of resumed development of wing discs in field populations of the European corn borer correlate well with these findings.

In order to obtain more conclusive results, we examined the rate of diapause termination in several batches of borers after determination of their mean wing disc developmental state. The results, shown in table A, indicate that the time for 50%

pupation is particularly short when larvae had already reached the stage III₃ of wing disc development. Thus in the European corn borer, the development of the imaginal wing discs provides a good, but not complete, indication of the progress of diapause development.

Physical factors affecting wing disc diapause development. We compared the physiological characteristics of diapausing control larvae maintained at 4°C and 0L:24D on moistened paper, with those of diapausing experimental larvae placed in dry conditions (maintaining the borers in diapause)¹¹, at 25°C and under different photoperiods. The populations used for this experiment originated from the region of Bordeaux (France). They were collected in November, stored in the laboratory at ambient temperature, then at 4°C in a cold room during 2–3 months. At this period, the imaginal wing discs of 30% of the population had reached stage III₃, and their development remained arrested during diapause at this same stage. The larvae showed all the characteristics of diapause. Larvae collected from Versailles and kept in similar conditions never showed such advanced morphogenesis during the winter. Thus it appears that part of the south-western populations of France possess more precocious wing bud development than more northern populations.

Imaginal wing discs from diapausing larvae exposed to 4°C and 0L:24D showed a slight but not statistically significant amount of development during the 33 days of the experiment (fig. 1). In contrast, the imaginal wing discs of some borers, maintained for 10 days at 25°C, advanced to phase III₃. The number in stage III₃ increased significantly when the larvae remained for 20 or 30 days in the same conditions (fig. 1 and table A). It must be noted that the differences in diapause development between the borers maintained under 18L:6D photoperiod and those kept under shorter daylengths were not significant. Throughout the experiment, the protocerebral NSC are weakly (±) or not (—) stained, and trehalosemia was 5–10 times higher than in nondiapausing larvae (table B). These two observations confirm that development of the imaginal wing discs occurs in larvae that undergo diapause, under laboratory conditions as well as under natural conditions. In our experiments, temperature appears to be a factor hastening the rate of wing disc diapause development. At 25°C, photoperiod has no significant influence. Our results differ in certain respects from those obtained by Beck and Alexander⁹. These authors found that an exposure to short-day photoperiods after 10 days of long-day treatment sharply reduced the rate of diapause development, as estimated by the number of days to 50% pupation. This difference underlines the fact that wing disc development constitutes only a part of the diapause development of the larva.

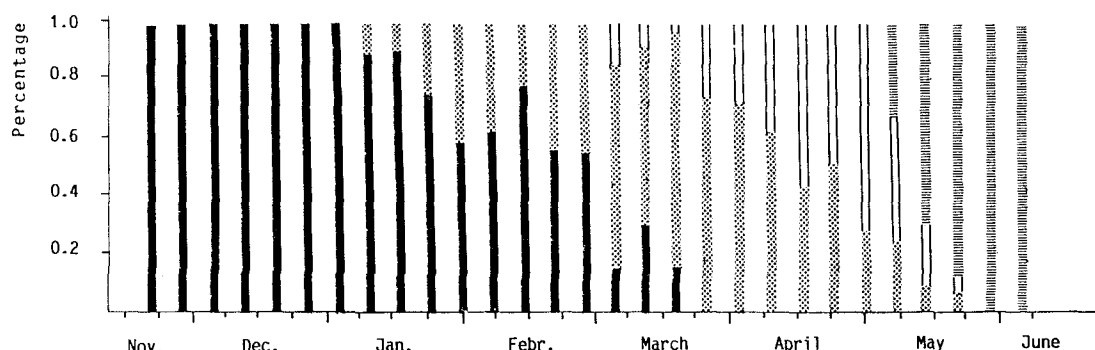


Figure 2. Development of imaginal wing discs in a field-grown population of European corn borer in Versailles (1984-1985). Each data point repre-

sents the dissection of 10-15 borers (■: I, II, III1 stages; ▨: III2 stage; ▤: III3 stage; ▩: III4 and more differentiated stages).

Application. By applying our results, homogeneous batches of larvae that are able to resume development simultaneously with a minimum of 15 days until pupation can be obtained throughout the year. This procedure leads to the completion of diapause development (100% of the wing discs are in the III3 stage). After 3 weeks at 25°C and 12L:12D, diapausing larvae are kept on moistened paper strips, at 4°C, under the same photoperiod, for 6 weeks. Then they are transferred again to 25°C and 12L:12D conditions and deprived of water, for a period of 2 weeks. This procedure can be performed at any time of the year.

Conclusion. In the European corn borer, we show that morphogenesis of the imaginal wing discs occurs during diapause. The progress of wing disc diapause development is an irreversible and cumulative phenomenon; features corresponding to some of the characteristics pointed out by Beck and Alexander⁹ in their definition of diapause development. Diapause termination occurs in favorable conditions when the wing discs of the whole population have terminated their own diapause development. In the wild strains of European corn borer, the examination of wing discs in winter gives a good idea of the potential ability for resumption of development of the larvae. Such indications are used to improve the modelling of population dynamics¹².

In our experimental conditions, the proposed 3 physiological criteria failed to reveal the 2 successive periods of diapause, maintenance period and transitional period, as proposed in

Tauber and al.¹³. Additional studies thus need to be carried out to define, generalize or limit the physiological interest of our proposed criteria and particularly the wing disc development during diapause.

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α_1 -Adrenergic stimulation of ketogenesis and fatty acid oxidation is associated with inhibition of lipogenesis in rat hepatocytes

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Summary. The effect of norepinephrine on fatty acid synthesis ($^3\text{H}_2\text{O}$ incorporation into fatty acids), on fatty acid oxidation to CO_2 and on ketogenesis was studied in isolated hepatocytes of fed rats. After incubation with norepinephrine (50 μM), lipogenesis was lower (5.7 ± 1.1 nmoles $^3\text{H}_2\text{O}$ incorporated into fatty acids/mg dry weight/30 min) than in controls (7.5 ± 1.7 ; $n = 6$, $p < 0.02$). In contrast, ($1\text{-}^{14}\text{C}$) palmitate conversion into total ketone bodies was increased to 10.9 ± 1.8 nmoles/mg/30 min with norepinephrine, vs 8.5 ± 1.6 in controls ($p < 0.05$), and more ($1\text{-}^{14}\text{C}$) palmitate was converted to $^{14}\text{CO}_2$ with norepinephrine than in controls (1.48 ± 0.10 nmoles/mg/30 min vs 1.06 ± 0.11 , $p < 0.05$). The inhibitory effect of norepinephrine on lipogenesis was abolished by addition of the α_1 -receptor blocker prazosin, but not by α_2 or β -blockers. The results demonstrate that the ketogenic effect of norepinephrine is coupled with an inhibitory effect on lipogenesis which may be explained by diminished activity of acetyl-CoA carboxylase, diminished formation of malonyl-CoA and decreased activity of carnitine palmitoyl transferase I.

Key words. Catecholamines; norepinephrine; ketogenesis; fatty acid oxidation; fatty acid synthesis; prazosin; propranolol; yohimbine.