Biology of *Heterodera avenae* Wollenweber in France. IV. Comparative study of the hatching cycles of two ecotypes after their transfer to different climatic conditions

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SUMMARY

Two geographically distinct populations or ecotypes of *Heterodera avenae* display a shift in their hatching cycle in France. In a mediterranean type climate, hatching of the southern population at Villasavary mainly occurs during winter. The maximum hatch takes place in spring in the northern population at Nuisement-sur-Coole where the climate is more continental in type. When reciprocal transfers are made of populations of different geographical origin, or transfer to an intermediate location (Rennes with a markedly oceanic climate) hatching rhythms are basically unaltered, but dates of initiation of hatch and peak juvenile emergence change. Successive cycles of hatching seem to depend on mechanisms of induction and interruption of physiological resting periods, i.e. diapause or dormancy, due to temperature variations. The results of this investigation also show that in a water saturated atmosphere, summer temperatures recorded in the southern location are detrimental to the viability of the juveniles in cysts, especially of the northern ecotype.

RÉSUMÉ

Biologie d' Heterodera avenae *Wollenweber en France.*
IV. *Comparaison des rythmes d'éclosion des deux écotypes après transfert dans différentes situations climatiques*

Deux populations géographiques distinctes ou écotypes d'*Heterodera avenae* présentent en France un réel décalage dans les rythmes d'activité. En climat à tendance méditerranéenne, la population de Villasavary (méridionale) éclot essentiellement pendant la période hivernale. En situation septentrionale sous influence continentale, la population de Nuisement-sur-Coole présente un maximum d'activité au printemps. Le transfert des populations en situation géographique opposée ou intermédiaire (Rennes, sous forte influence océanique) ne modifie pas fondamentalement les rythmes d'éclosion de ces deux populations. Le décalage se manifeste aussi bien au niveau des dates de déclenchement ou d'arrêt de l'éclosion que des périodes d'éclosion les plus intenses. Ces deux écotypes traduisent une importante spécialisation chez ce parasite dont les cycles successifs d'éclosion relèvent étroitement de mécanismes d'induction et de levée de repos physiologiques (diapause et (ou) dormance) sous l'effet des variations de la température. Les résultats de l'étude montrent également qu'en atmosphère saturée, les températures estivales enregistrées dans la situation méridionale sont préjudiciables à la viabilité des juvéniles contenus dans les kystes, plus particulièrement dans le cas de l'écotype septentrional.

The cereal cyst nematode *Heterodera avenae* Wollenweber is widespread in most cereal-growing regions of the world (Meagher, 1977; Ritter, 1982). This species is extremely polymorphic consisting of a number of physiological forms which differ in virulence (Andersen & Andersen, 1982), in hatching cycles and heat requirements (Clarke & Perry, 1977).

Referring to previous studies, Evans and Perry (1976) described two different hatching behaviours according to the geographical origin of populations : *i)* winter activity in Southern Australia (mediterranean climate); *ii)* spring activity in North America and Northern Europe.

These two hatching patterns were observed in France. In a southern population from Villasavary (Aude), hatching essentially occurred in winter whereas a

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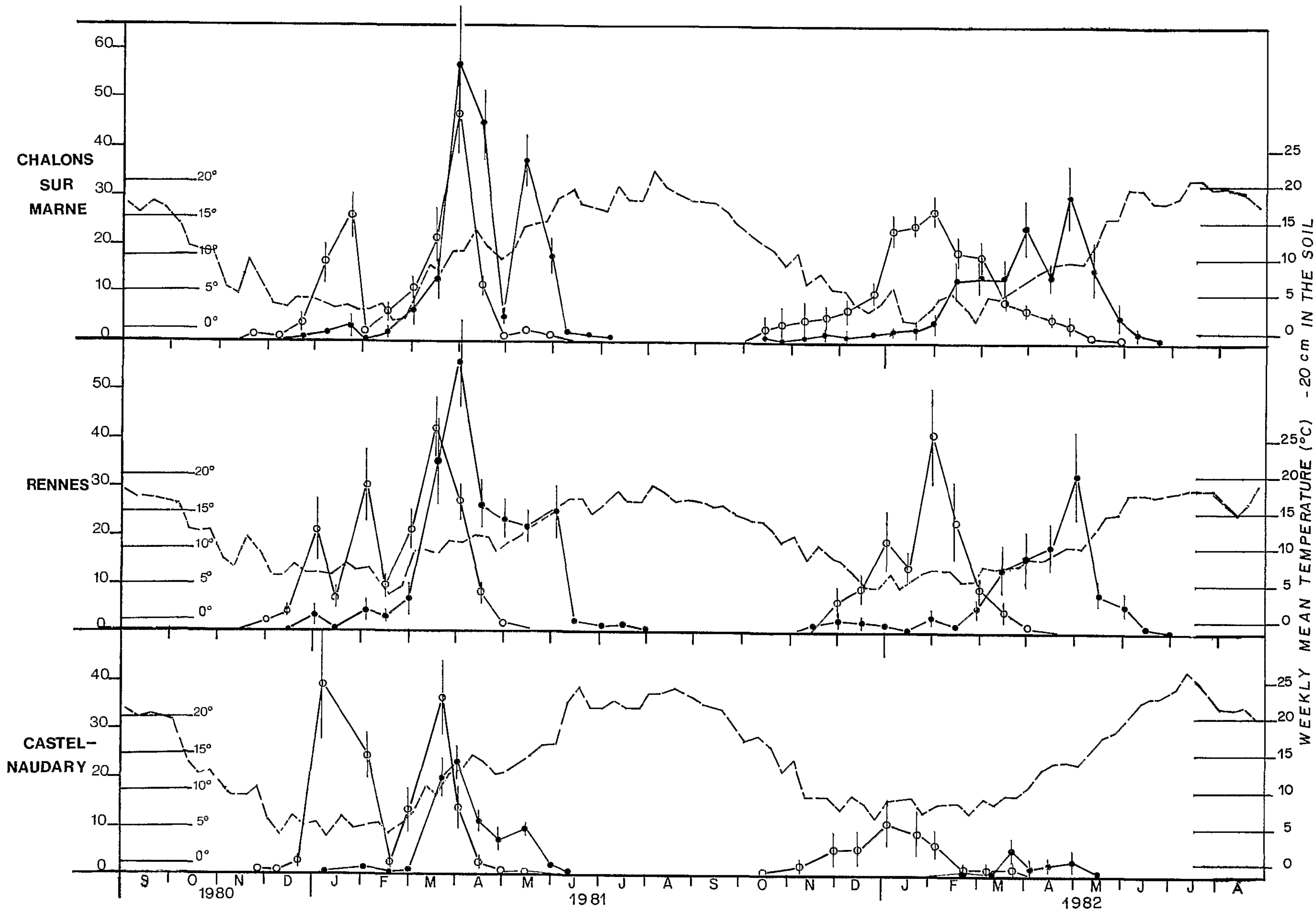

Fig. 1. Hatching cycles of two ecotypes of *Heterodera avenae* in three locations : average number of hatched juveniles per cyst and standard deviation of the mean > 1; northern (●——●) and southern (○——○) ecotypes. Weekly mean temperature (-----).

northern population from Nuisement-sur-Coole (Marne) released its juveniles in spring (Rivoal, 1978). These two population also differ in their virulence, corresponding to pathotypes Ha 41 for Villasavary and Ha 12 for Nuisement-sur-Coole (Rivoal, 1977; Andersen & Andersen, 1982).

Experiments carried out in the laboratory and under natural conditions uncovered the factors in both populations determining this activity which appeared to be due to induction or interruption of two kinds of diapause as a function of seasonal temperature variations. The first was obligate and occurred in summer on newly-formed juveniles of the southern population; it ceased by autumn when temperatures fell below 10°. The second one was facultative and was induced by summer temperatures in the northern population, stopping naturally between 5° and 15°. After exposure to low temperature, juveniles tended to lasting mass hatching when moved to 10° or 15° (Rivoal, 1979). The combined action of these diapauses and of the temperature variations determined several successive annual cycles for juvenile emergence (Rivoal, 1983).

The hatching cycles of these two populations were maintained even after removal to a climatically intermediate location (Rennes), which suggests they express a true adaptation to particular environmental conditions (Rivoal, 1983). For that reason the term ecotypes was used (Rivoal, 1982) : Villasavary, the southern ecotype, would favour survival of populations located in mediterranean climate, while behaviour of the Nuisement-sur-Coole population corresponds to the hatching pattern observed in *H. avenae* in a more of less temperate oceanic climate.

In order to compare the activity of each ecotype in its natural area and after transfer to an area of contrasting climate, the hatching cycles of both ecotypes were observed at Châlons-sur-Marne (Marne) and Castelnaudary (Aude), both locations close to where the two populations originated, as well as at Rennes. Our purpose was also to determine the influnece of applied experimental conditions such as variations in temperature and saturated atmosphere, which are known to affect *H. avenae* juvenile survival in cysts (Meagher, 1974).

Material and methods

The two populations tested (Villasavary and Nuisement-sur-Coole) have been maintained at Rennes every year since the start of the virulence study (Rivoal, 1977). In 1979-1980 they were reared on the same host, *Triticum aestivum* cv. Hardi as previously described (Rivoal *et al.*, 1978). The new cysts were extracted on September 10th 1980 using centrifugal-flotation in a sugar solution with a density of 1.23, then carefully washed.

For each ecotype and each location, fourteen egg-bearing cysts were immersed separately in small plastic tubes filled with 0.8 cm^3 tap water (Rivoal, 1979). These tubes were placed in a sealed box, then in the soil at a depth of 20 cm in a well with its upper part closed by a removable asbestos cement plate covered with a layer of turf about 5 cm thick.

The experiment was begun on the same day (September 15th 1980) at the three locations : Châlons-sur-Marne, Rennes and Castelnaudary, and ended on July 30th 1982. Cysts were transferred into similar tubes filled with water from the same source (Rennes), approximately every fortnight except in August 1981. Samples were sent to Rennes for counting hatched juveniles.

At the end of the experiment, all cysts were squashed to count the numbers of unhatched juveniles. These were pressed out of their eggshells using the rounded tip of a glass rod and classified into two types : viable when straight in form and with contrasty contents or dead when they showed a zigzag form with strongly vacuolated and clearly disintegrating body contents (Banyer & Fisher, 1976).

Soil temperatures were taken daily at 20 cm depth for the three experimental locations throughout the investigation.

Results

Temperature differences between the three experimental sites

The weekly mean temperatures were always higher at Castelnaudary than at Châlons-sur-Marne and showed differences which varied with the season and could exceed 8° (Fig. 1). In both locations, lowest temperatures were in December, January and February. In February 1981 temperatures fell below 1° at Châlons-sur-Marne, an effect of the continental climate. At Castelnaudary, the lowest mean temperature (3.5°) was recorded in February 1981 but this level was not reached during the following winter when the mean minimum temperature recorded was 6.9°. June, July and August 1981 were warmer at Castelnaudary than at Châlons-sur-Marne, with the difference exceeding 7° during the third week of June. Temperatures over 26° in July 1982 accorded with a mediterranean type of climate at this location. The drop in temperature during autumn and more especially in November occurred earlier at Châlons-sur-Marne than at Castelnaudary. Conversely the rise of soil temperatures in spring was later in the northern location.

The data recorded at Rennes were representative of a location subject to an oceanic climate with temperatures (from December 1980 to February 1981) sometimes higher than at Castelnaudary but with summer temperatures always lower than the other locations.

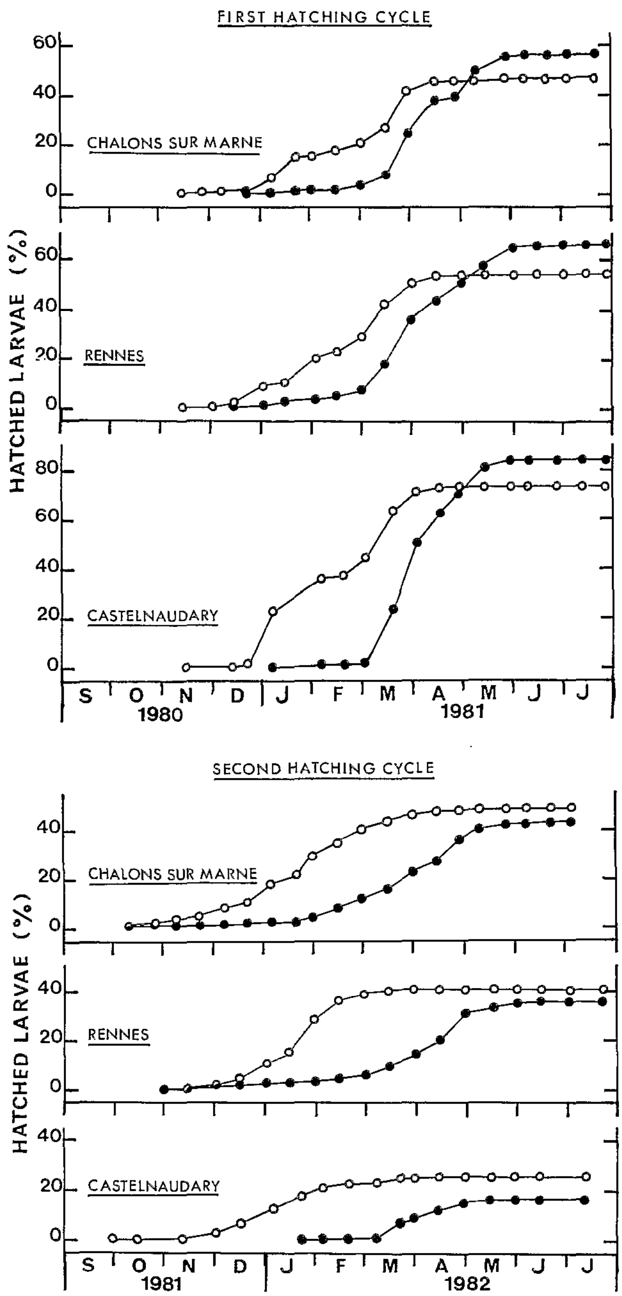

Fig. 2. Hatching cycles of two ecotypes of *Heterodera avenae* in three locations: cumulative percentages of hatched juveniles per cyst for northern (●——●) and southern (○——○) ecotypes.

HATCHING CYCLES OF BOTH ECOTYPES AT THE THREE SITES

Hatching cycles are expressed by counting the successive average hatched juveniles per cyst (Fig. 1) and summing to give the cumulative hatching percentages (Fig. 2).

At Castelnaudary, hatching of the southern ecotype starded during November 1980 when mean soil temperatures dropped below 10°. Juvenile emergence was already substantial in January 1981 while just beginning in the northern ecotype. The activity of the southern ecotype declined markedly by mid February, increased to a high level in March and stopped in late April. The northern ecotype hatched really from March 1981 onwards, paralleling the rise in temperature, reaching a maximum between March and April and continuing until June 1981.

A second hatch occurred in the southern ecotype between October 1981 and March 1982 with maximum emergence in December and January, but the hatching rate was much slower than during the first activity cycle. A similar reduction in hatching rate was noted in the northern ecotype whose activity did not develop before March 1982, i.e. six months after that of the southern ecotype and ceased in May 1982.

At Châlons-sur-Marne, simultaneous hatch of both *H. avenae* ecotypes occurred between November and December 1980. However a rapid increase in rate of hatch in the southern ecotype was observed in January 1981; the northern ecotype failed to hatch massively again until early March. Hatch of the southern ecotype was inhibited during February 1981 as at Castelnaudary, but increased markedly in March. However a big temperature drop in late April resulted in marked decrease of hatching activity in this ecotype whose first cycle ended in June 1981. Temperature rise following winter was associated with hatching activity of the northern ecotype which peaked in March and April 1981. The severe but transient temperature drop in late April 1981 slowed the activity of the northern ecotype which continued up to early July and only ceased when temperatures exceeded 15°.

Both ecotypes began a second hatch in October 1981 which ended between May and June 1982, with however a period of maximum activity in January and February for the southern ecotype and between March and May for the northern one.

At Rennes, two successive hatching cycles were also recorded for each ecotype. Their first hatch started simultaneously in November/December 1980 just as at Châlons-sur-Marne. The southern ecotype stopped hatching in May 1981 while that of the northern one continued until late July. The second cycle began in November 1981 and stopped in April or July 1982 according to the ecotype with maximum hatch earlier in the southern ecotype than in the northern one. For both cycles, hatching of the southern ecotype occurred at the lowest winter temperatures, whereas juvenile release in the northern ecotype occurred with the onset of higher temperatures in spring.

EFFECTS OF EXPERIMENTAL CONDITIONS ON LARVAL VIABILITY

Percentages of dead juveniles are compared to all juveniles in cysts (Tab. 1). Most dead juveniles were found in the northern ecotype wherever located, but the difference between both ecotypes was statistically significant only at Castelnaudary. No significant difference was found between both ecotypes at Châlons-sur-Marne and Rennes though mortality was consistently lower at Rennes, 6 % to 13 % depending on ecotype. By contrast numbers of dead juveniles in both ecotypes were significantly higher at Castelnaudary than other locations (P = 0.01). The high juvenile mortality in this southern location was probably related to temperature variations as constant moisture conditions were maintained in the three experimental locations.

Discussion

The transfer to locations with clearly different temperatures did not alter the hatching cycles of the two ecotypes of *H. avenae* but a change did occur in the dates of hatch and the most active periods of juvenile emergence. The southern ecotype hatched at mean temperatures below 10° wherever the experiments were carried out and hatch cycle proceeded from autumn to early spring. The northern ecotype hatched mainly as temperatures rose, from mid-winter to late spring. The results of this investigation suggest differences between the ecotypes of *H. avenae* in hatching behaviour.

With the southern ecotype, it was confirmed that the first hatch cycle depended on breaking an obligatory diapause undergone by newly-formed second stage juveniles over about four months (Rivoal, 1979, 1983). No emergence was observed until mid-November 1980 at Châlons-sur-Marne though temperatures below 10° were recorded there. The later interruption in the first hatch cycle at Châlons-sur-Marne also gave evidence of the influence of the temperature rise above 10° on juvenile emergence. The inhibition of activity of the southern ecotype during part of spring and in summer has been considered in an earlier experiment as a diapause because high temperature (20°) exposure is needed for subsequent hatch of juveniles at low temperatures (Rivoal, 1983). The results of the present investigation show that conditions for diapause breaking (releasing time and temperature) are less strict than those observed with neonate juveniles.

Early hatching of the northern ecotype at Châlons-sur-Marne compared with Castelnaudary confirm that its activity is related to low temperature breaking of a diapause which we had termed facultative (Rivoal, 1979). The first cycle of hatching activity is interrupted earlier at Castelnaudary than at Châlons-sur-Marne suggesting that temperature increase above 15° inhibits this ecotype's hatch which stops during summer and part of autumn. The late second hatch at Castelnaudary compared with Châlons-sur-Marne and Rennes suggests a more complex biological activity than that observed where this

Table 1

Differences in percentages of dead larvae on total numbers of juveniles per cyst of two ecotypes of *Heterodera avenae*, tested during two consecutive years in three locations.

Location	Ecotypes				
	Southern		*Northern*		*L.S.D. P* = 0.05 0.01
	X	SD_X	X	SD_X	
Châlons-sur-Marne (north)	18.3 (11.8) (1) (2)	9.2 (3)	22.4 (15.6)	7.7	NS
Rennes (intermediate)	10.9 (5.8)	9.5	18.0 (13.2)	14.0	NS
Castelnaudary (south)	43.7 (47.7)	11.7	64.9 (80.7)	9.1	8.1 11.0
L.S.D. 0.05 0.01	7.7 10.6		8.0 11.0		

Angle transformed (1) and untransformed (2) mean proportions; standard deviation of the mean (3), on 14 replicates.

ecotype originated. At Châlons-sur-Marne and also at Rennes hatching might be initiated by a break in dormancy occurring as soon as temperature, i.e. below 15°, favoured juvenile emergence (Rivoal, 1979). At Castelnaudary however higher temperatures in summer seem to have induced another diapause, breaking of which requires a longer period with low temperatures.

Meagher (1974) then Banyer and Fisher (1976) reported that in Australia storage of *H. avenae* juveniles in a satured atmosphere was detrimental to viability especially at temperatures over 20°. This was also observed in France, more particularly in the experiment carried out at Castelnaudary where higher summer temperatures recorded than at Rennes or Châlons-sur-Marne seemed responsible for a substantial increase in dead juveniles in cysts. Also, juveniles of the northern ecotype were notably more susceptible to these conditions than those of the southern ecotype. Person and Doussinault (1978) also observed a difference in the susceptibility of these two ecotypes to temperatures exceeding 20°, particularly in development and growth of females.

The least mortality of juveniles of both ecotypes was observed at Rennes where summer temperatures are the lowest. This permitted the study of *H. avenae* hatching rhythms from the same cysts during four consecutive years (Rivoal, 1983). Further work is required to determine whether juvenile mortality resulted from a physical effect of temperature or from the action of micro-organisms which might have developed within the cysts (Banyer & Fisher, 1980).

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