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EPIDEMIOLOGICAL SURVEY OF THE BROWN RING DISEASE IN CLAMS OF ATLANTIC COAST : ROLE OF TEMPERATURE IN VARIATIONS OF PREVALENCE

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La Maladie de l'Anneau Brun (MAB) chez la palourde japonaise, Ruditapes philippinarum, est une maladie bactérienne, provoquée par un vibrion, Vibrio tapetis, lequel perturbe le processus d'élaboration coquillière. Cette maladie, décrite à partir de 1987 dans les populations naturelles et cultivées de palourdes de la façade atlantique Française, a été détectée depuis en Grande Bretagne, en Espagne et occasionnellement en Italie. Cette maladie affecte aussi l'espèce autochtone, Ruditapes decussatus. Une échelle de classification des symptômes macroscopiques de la MAB (stades de maladie et de guérison) a été établie en vue de son utilisation dans les études épidémiologiques et les expérimentations au laboratoire. Dans le cadre d'un programme européen (FAR, AQ-3-763, 1992-1995), un suivi épidémiologique de la MAB a été mené dans différents sites de la façade atlantique Française. Un des facteurs clés, la température, a été continuellement mesuré, par l'intermédiaire de sondes automatiques déposées dans chaque site. Le long de la façade atlantique Française, il existe une nette frontière au niveau de la rivière, la Loire; les prévalences de la MAB sont comprises entre 0 et 3% pour les sites situés au sud de la Loire alors qu'elles atteignent 45% au nord de la Loire. Ces variations de prévalences inter-sites peuvent être expliquées en partie par les valeurs des températures extrêmes différentes entre les sites du Nord et du Sud de la Loire. Au laboratoire, des expériences de contamination, réalisées à différentes températures (8, 14, 21°C), ont permis de montrer l'effet de la température sur le développement de la MAB; ainsi, après 4 semaines d'inoculation, toutes les palourdes sont malades à 14°C alors que seulement 20% présentent la maladie à 21°C. Les expérimentations en milieu naturel et au laboratoire confirment l'influence de la température sur le développement de la MAB.

INTRODUCTION

The objective of this study was to evaluate the role of temperature in the variations of Brown Ring Disease (BRD) prevalence in clams.

Our BRD results (etiology, symptomatology) are first and briefly presented; then the epidemiological survey and laboratory experiments to test the potential role of temperature on the prevalence of this disease.

THE BRD : ETIOLOGY AND SYMPTOMATOLOGY

The Brown Ring Disease (BRD) in the manila clam, *Ruditapes philippinarum* is a bacterial disease which perturbs the calcification process. Following classical Koch's postulate for pathogen identification, a bacterial strain was detected as the agent responsible for this disease. This bacterium was characterized as belonging to the genus *Vibrio* and has been termed *Vibrio* P1 and recently named *Vibrio tapetis*.

The macroscopic sign which characterises this disease is a conchiolin deposit adhering to the inner surface of the shell, forming a characteristic brown ring deposit. Four weeks after *V. tapetis* inoculation in healthy clams, 90% of clams exhibited the typical brown ring deposit. Microscopic syndrome can be also observed mainly in the external peripheral compartment (i.e. compartment located between the external epithelium of the mantle and the inner face of the shell). In all diseased clams, the periostracal lamina shows alterations: it is invaded by cell debris, bacteria and areas of darker melanin-like pigmentation are observed. This disorganized periostracal lamina is not a good substrate for the biomineralization process, and therefore it is accumulated forming a deposit on the inner face of the shell. In order to recover from the disease, the clams regenerate their shells by covering the deposit with calcified secretions like the nacrezation process. A classification system based on the macroscopic description of the syndrome has been established for use in epidemiological and experimental studies. Seven conchiolin deposit stage (CDS) are proposed, based on the assessment of an index which take simultaneously into account the extent and the thickness of the deposit. Three shell repair stages (SRS) were established to evaluate the progression of recovery.

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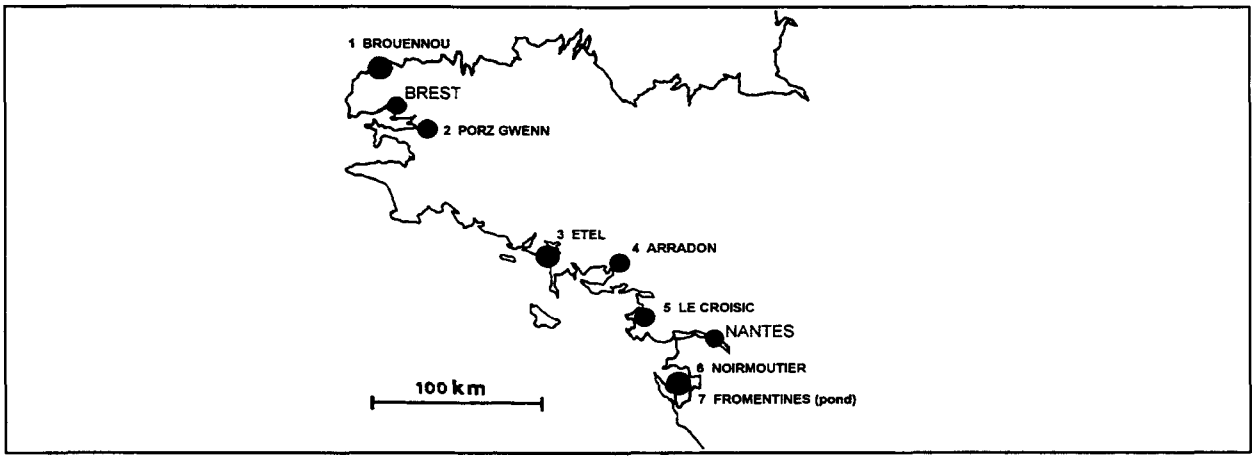
EPIDEMIOLOGICAL SURVEY OF THE BRD IN CLAMS

This disease occurs in wild and reared clam populations in France, England, Spain and occasionally in Italy, but has never been reported in Japan where this species originates from. The BRD has also been detected in another clam species (*Ruditapes decussatus)* from France and Spain. To test the effect of temperature on the BRD development, experimental seeds of clams were performed. BRD prevalence and temperature were monitored.

Methodology

Juveniles of the two clam species *R. philippinarum* and *R. decussatus* (mean length 8 mm) originating from the same hatchery were seeded in 7 selected sites (fig 1) of the Atlantic French coasts. The clams were seeded at the end of March 93, approximately at the same tide level (low tide about coeficient of 70), in experimental parcel of 20 m², at a density of 250 individual/m². A periodic sampling (generally 50 individuals) was carried out every 3 months. The 7 sites were selected for their differences in BRD prevalence, and also for the extension of their seasonal temperature range.

Figure 1
Localization of the 7 sites on the French Atlantic coast.



Results and discussion

The experimental survey carried out from July 93 to November 94 shows that:

- Whatever the site and until the end of the survey, the native species, *R decussatus*, is less affected by BRD than the Indo-Pacific manila clam, *R. philippinarum*. The prevalence do not exceed 18% in *R decussatus* whereas levels over 40% are commonly observed in *R philippinarum*.
- High variations of prevalence, depending of the site, are observed; the more affected sites are Brouennou, Etel bas, Arradon, and Le Croisic.
- In the strongly affected sites of Arradon & Etel, maximum BRD prevalences were recorded in May 94, namely 14 months after the experimental seeding. During this period, mean temperature of the sediment upper layer usually varied between 10 and 16 °C, a temperature range which, very interestingly, is optimal for the growth of *V. tapetis*.
- In the site of Brouennou, higher prevalences were measured in February 94, (33%), and were associated with a mass mortality. In this site, the temperature ranged from 8 to 12°C in the upper sediment layer.
- There is a clear boundary at the level of the Loire river: the observed BRD prevalences ranged between 0 and 3% for the sites situated to the South of the Loire.

Those inter-site prevalence variations could be explained by the pattern of minimal temperatures. Minima of winter temperature (< 3°C) were recorded in Noirmoutier. In the other sites, minimal temperatures were between 3 and 9 °C. It is known that a temperature below 3°C totally inhibits the *V. tapetis* growth, and consequently its pathogenic capacity. The very low temperatures recorded in winter 93-94 in Noirmoutier could explain the absence of BRD development in that site.

LABORATORY EXPERIMENTS

T°C and V. tapetis growth

The V. tapetis growth was studied, at different temperature between 4°C and 30°C. The growth (optical density of the culture) was measured by mean of spectrophotometer at 492 nm. This bacteria presents a maximal growth between 8 and 21°C; no growth is noted, even after 5 days for extreme temperature of 4°C and 30°C.

T°C and V. tapetis-clams interactions

Experimental *V. tapetis* contaminations were carried out at different temperatures (8, 14, 21°C). The effect of temperature on the development of the disease is clearly shown by the experiment (figs. 2 and 3). Four weeks after inoculation, the BRD prevalence was maximum (almost 100%) at 8 and 14°C, and reached about 20% at temperature of 21°C. The level of BRD development is the highest (Group 3) at 14°C. At 21°C the situation, is very different in terms of development and prevalence of the disease. It has been shown that at 21°C the pathogenic agent, VP1, is active (it grows well, metabolizes substrates and is motile), but nothing can be concluded concerning its virulence at that temperature. The low BRD development level obtained at 21°C could results either from a weakening of defence system of the host (high percentage of complete recovery, 20% of SRS 3, fig. 3) or from a lowering of the virulence of the pathogen or both.

Figure 2

Evolution of disease prevalence as function of time (days) and temperature in clams inoculated by *V. tapetis*. Group 1 to 3 correspond to a progressive development of the disease

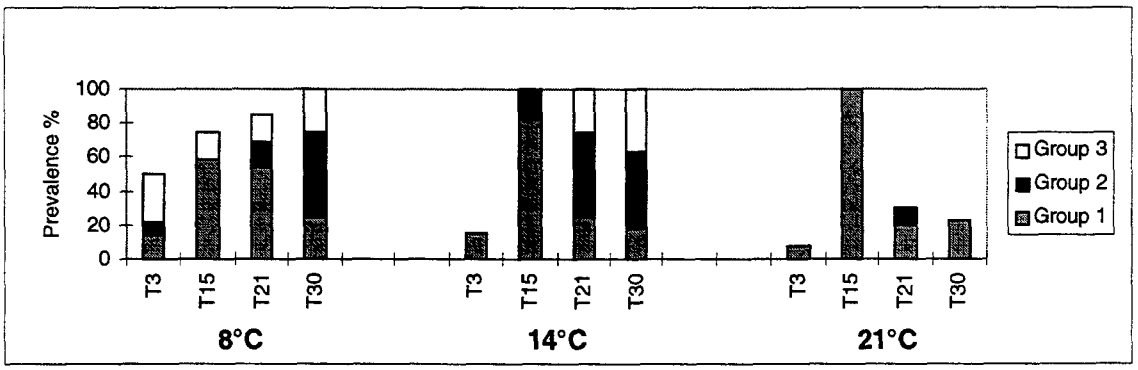


Figure 3

Evolution of BRD recovery (SRS) as function of time (days) and temperature in clams inoculated by *V. tapetis*. SRS 1 to 3 correspond to a progressive recovery of the disease