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Shell Disease in the Oyster *OSTREA EDULIS* LINNE, 1758 (BIVALVIA) in the Lake Donuzlav (Black Sea)

Keywords: oyster Ostrea edulis, shell disease, marine parasitic fungus Ostracoblabe implexa, mycelium, conidia, Donuzlav lake, Black Sea.

Shell disease of the Black Sea oyster *Ostrea edulis L.* has known to be widespread since the end of 70-s of the XX century [2]. The disease was revealed in the areas of the main oyster reserves – in the bays Yegorlyksky, Garylgachsky, Karkinitsky, and in the lake Donuzlav. During the 1980 – 1984 period the number of ill molluscs in Garylgachsky bay increased from 38% to 72%, while in Yegorlyksky bay alive animals were not found at all [2]. It was established that the oysters taken from Garylgachsky and Karkinitsky bays could not be used for reproduction as the disease agent was transmitted with larvae [5]. In the Northwestern part of the Black Sea natural oyster reserves decreased 9-11 times from 1973 to 1975 [3]. O edulis was included in the list of state-protected endangered species [7]. Nowadays, only thinned oyster beds are available in some areas. According to the data of 2004 single alive specimens and the shells of dead oysters attached to the rocks were found in Sudaksky bay and near cape Utrish [6].

The agent of the oyster shell disease is the marine fungus *Ostracoblabe implexa* Bornet & Flahault [8]. Shell disease begins with the pathological changes of morphology and microstucture of shell, continues with further atrophy of the tissues, and ends up as mollusks' death [11]. Some oysters can suffer from disease for several years [1].

In natural conditions fungal spores were not found. The attempts to cultivate the fungus on nutritive media were not successful [9, 11, 12].

The article seeks to study the ways of the pathogenic fungus *O. implexa* spreading in natural conditions.

MATERIAL AND METHODS

In 2008 oysters *O. edulis* were obtained in Donuzlav lake (near Novoozerny village) at the depth of 0.5 – 5.0 metres for cultivation in a nursery. Thin fragments isolated from the affected sites of the shells were rinsed with distilled water for 24 hours. The samples were examined under the dissecting microscope MBI-6 and some of them were chosen for a following examination under a JSM-6060 La electron scanning microscope in the Centre of common use of the electronic microscopes of HASU in Kiev.

RESULTS AND DISCUSSION

All the molluscs had soft and exfoliated growth zone. У всех моллюсков растущий край раковин был мягким, расслоенным. Taking into consideration that the molluscs' valves closed slowly in water, one can assume that either adductor muscle attachment or the hinge is affected. Shell disease was revealed when a female animal with a shell of 67.1 mm high was dissected. Раковинную болезнь выявлено при вскрытии самки с высотой раковины 67.1 мм. The disease symptoms were found on left valve, near the hinge and on the growth margin of right valve. This typical shell disease was described in the literature [8, 9]. However, the oyster's gonad was well developed (pre-spawning stage). It was mentioned earlier [2, 11] that oysters infected by shell disease can germinate [2, 11]. On the microphotos of fragments of the affected valves two types mycelium are shown. In the yellow-green fragments obtained from the nacreous layer of the growing shell margin the fungal mycelium is thin, straight, and ramified (Fig. 1a); one can see nodes (Fig. 1b) and septa (Fig. 1c) on some hyphae. Numerous foramens in the nacreous layer are the places of hyphae penetration (Fig. 1d).

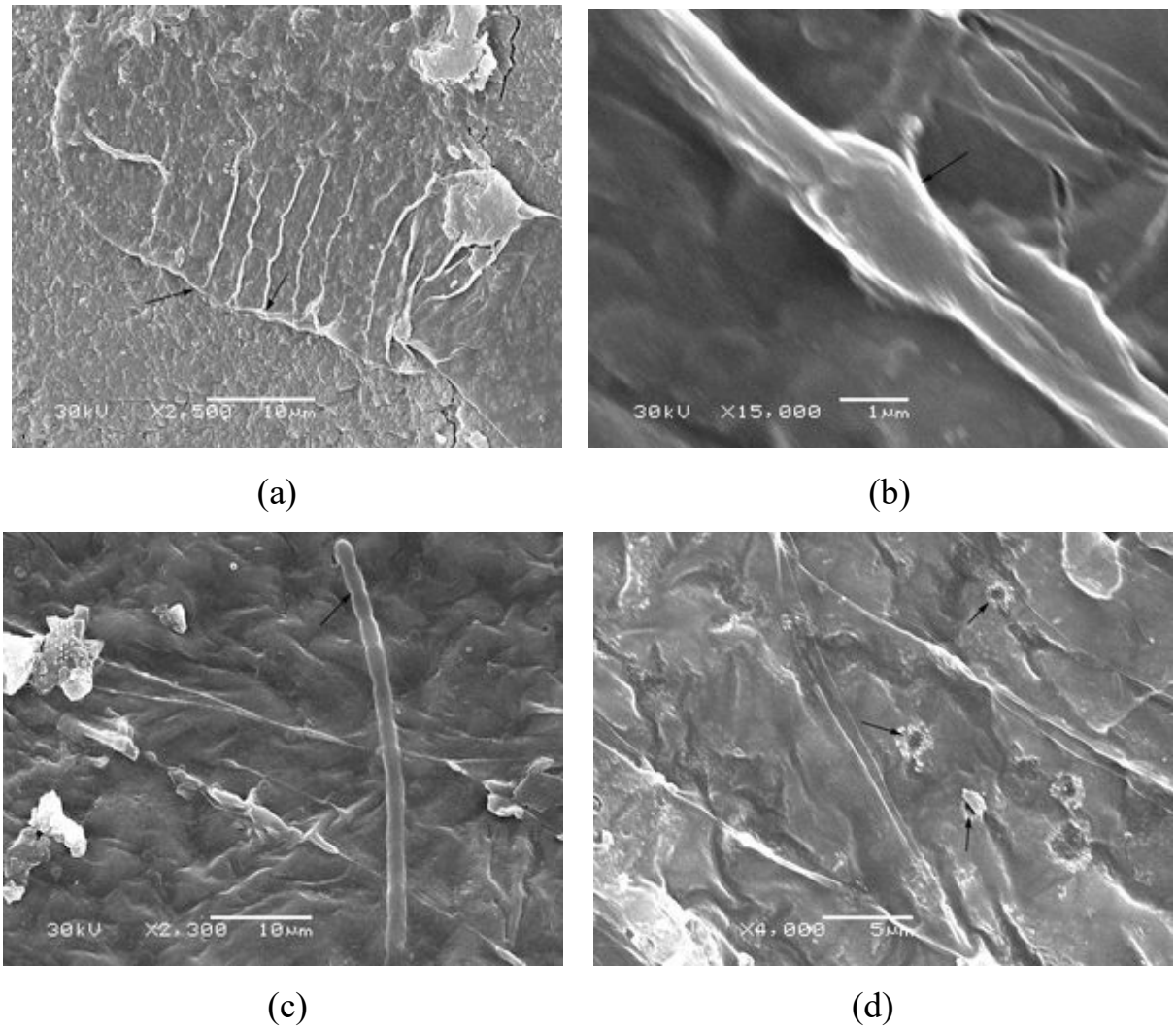

Fig. 1 Mycelium of fungus *Ostracoblabe implexa* in the growing margin of oyster shell *Ostrea edulis*: (a) – mycelium ramification; (b) – hyphae nodes; (c) – growing margin of hypha with septae; (d) – shell nacreous layer damaged by fungal mycelium.

Another type of mycelium was found in the black areas of the affected shells (in a conchiolin node). This type of mycelium is represented with ramified hyphae of various width, partially or completely embedded into the shell matrix (Fig. 2). The hyphae are covered with groups of round conidia 3.1 - 3.2 x 3.2 - 3.5 mkm in diameter containing numerous cells.

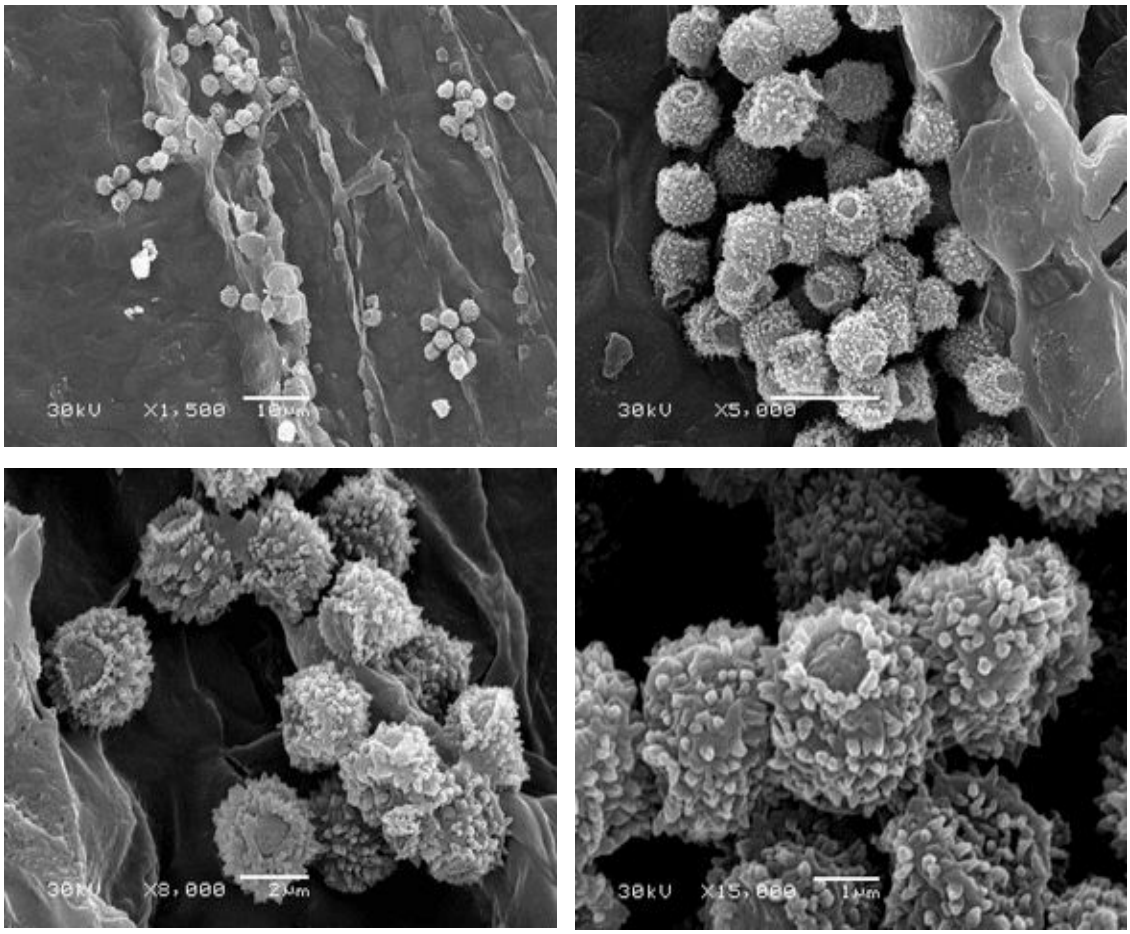

Fig. 2. Conidia and mycelium of the fungus *Ostracoblabe implexa* in the affected black areas of the shell.

Fungal cells are petal-shaped frontview S-shaped sideview, and sharpened at the top. One of the cells' end is plunged inside the conidia, whereas the others are located at different angles, thus forming a round "crown" 1.0 - 1.1 x 1.2 - 1.3 mkm in diameter. The length of the cells is about 0.2 - 0.5 mkm, the maximum width is up to 0.3 mkm.

Shell disease was revealed in other female oyster (with height of the shell 68.3 mm), which was opened just after larva leaving the suprabranchial cavity (Fig. 3).

Fig. 3. Shell disease of oyster *Ostrea edulis*.

The examination of the affected shell areas with the help of electronic scanning microscope did not reveal any conidia in them.

Both availability of fruiting conidia, which were found in the affected female oyster shell in the pre-spawning stage of the gonad development, and unavailability of those in the affected oyster after larva leaving the suprabranchial cavity, might corroborate the parallel maturation of spores of the fungus *O. implexa* and development of the shell larva *O. edulis.* Both larvae and conidia leaving are likely to be simultaneous process. It was established that the level of oyster infection is greater in the areas where the water temperature exceeds 19^{o}C for two weeks [10]. Under such a temperature oysters' larvae leave the suprabranchial cavity of a female oyster.

The plankton stage of the oyster *O. edulis* lasts for about three weeks [4]. During this period larvae are gone with a current for long distances settling on the oyster grounds. Subsequently, healthy oysters can be infected with the fungus from spat. Laboratorial experiments confirmed that mycelium grows into the neighbouring shell providing two oysters contact directly [12]. Furthermore, conidia which were separated and joined to plankton, can attach to the shells of healthy oysters and germinate in the mycelium. Conidia can also appear in the silt of the sea bed and rise with the silt into the water column during storms. This is a way the disease spreads throughout cultivated and natural oyster reserves.

CONCLUSION.

The credible ways of spreading the disease agent (marine fungus-parasite *O. implexa*), which infects the Black Sea shell oyster *O. edulis* are: 1 – through conidia available in the plankton or raised from the sea bed during strong gales; 2 – through larvae spawned by infected female oysters; 3 – a direct contact of a healthy mollusc with the oyster shells affected by fungus.

I dedicate this article to the memory of D.P. Demenko – a colleague of the Centre of common use of electronic microscopes of HASU in Kiev.

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The oyster *Ostrea edulis* L. is an endangered species in the Black Sea. One of the factors causing its extinction is shell disease induced by the fungus *Ostracoblabe implexa.* Micro-photos of two types of fungus mycelium and conidia are presented in the article; their form and size are described; possible ways of spore spreading including that with the help of oyster larvae are discussed.