

Sexual reproduction of the invasive green alga *Caulerpa racemosa* var. *occidentalis* in the Mediterranean Sea

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Abstract – Since the beginning of the 1990s, a pan-tropical species *Caulerpa racemosa* var. *occidentalis* has been recorded on the numerous new locations throughout the Mediterranean, specifically on its northwestern part where until then it had never been observed. Such extensive spreading is probably supported by successful sexual reproduction. Field observations of fertile plants and synchronous gamete release of *C. racemosa* var. *occidentalis* were carried out in Saronikos Gulf (Greece, Aegean Sea) at the beginning of July 1999. The fertile plants were detected due to reticulate depigmented protoplasmic masses and cylindrical papillae. The alga is monoecious. The both sex gametes were released synchronously from all the fertile plants approximately 14 min before sunrise and formed a green gamete cloud. Although both sex gametes were present, low gamete compatibility was noted during the laboratory examination. © 2001 Ifremer/CNRS/IRD/Éditions scientifiques et médicales Elsevier SAS

Résumé – **Reproduction sexuée de l'algue envahissante *Caulerpa racemosa* var. *occidentalis* en Méditerranée.** Depuis le début des années 1990, l'algue tropicale *Caulerpa racemosa* var. *occidentalis* a été observée dans de nombreux nouveaux sites de Méditerranée nord-occidentale où elle était jusqu'alors inconnue. Une dispersion d'une telle ampleur est probablement due à une reproduction sexuée particulièrement efficace. Des observations de plants fertiles de *C. racemosa* var. *occidentalis* et des lâchers simultanés de gamètes ont été menés dans le golfe de Saronikos (mer Egée) au début du mois de juillet 1999. Les thalles fertiles se distinguent par leur protoplasme dépigmenté et la présence de papilles cylindriques. L'algue est monoïque. Les gamètes mâles et femelles sont expulsés de façon synchrone environ 14 min avant le lever du soleil et forment un nuage vert. En dépit de la présence des deux sexes, une faible compatibilité entre les deux types de gamètes a été observée au laboratoire. © 2001 Ifremer/CNRS/IRD/Éditions scientifiques et médicales Elsevier SAS

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1. INTRODUCTION

The presence of *Caulerpa racemosa* var. *occidentalis* (J. Agardh) Børgesen in the Mediterranean Sea has been reported for the first time in Tunisia by Hamel (1926). From that time till the beginning of the 1990s, the alga has been sporadically noted only throughout the eastern basin of the Mediterranean (Boudouresque et al., 1990; Djellouli et al., 1998).

Since the beginning of the 1990s, within a few years, the distribution of *C. racemosa* var. *occidentalis* has amazingly extended throughout the eastern and western part of the Mediterranean, usually forming a dense and large population with invasive behaviour: Egypt (Aleem, 1992), Cyprus (Argyrou et al., 1999; Hadjichristophorou, et al., 1997), Malta (Stevens, 1999), Turkey (Cirik and Öztürk, 1991), Greece (Chrysovergis and Panayotidis, 1998; Panayotidis and Montesanto, 1994, 1998), Italy (Alongi et al., 1993; Bussotti et al., 1996; Piazzini et al., 1994, 1997), France (Verlaque et al., 2000) and Balearic Islands (Ballesteros et al., 1999). Such a widespread and rapid spreading is probably supported by successful sexual reproduction. Therefore the basic aim of this study was to prove sexual reproduction by field and laboratory observations.

2. MATERIAL AND METHODS

Field observations were made by snorkel in a shallow bay (figure 1) in Saronikos Gulf (Greece, Aegean Sea), between 1st and 7 July 1999. The rocky substratum between 0.5 and 1.5 m deep was almost completely covered by *C. racemosa* var. *occidentalis*. The algae was surveyed in the afternoon for detecting, collecting and marking fertile plants, and during the next early morning, before the sunrise for the observation of gamete release. The sea water temperature was 26°C.

With the aim to observe gametes, the fertile thalli were collected in the afternoon before gamete liberation, and kept in an aquarium at the ambient temperature and seawater without artificial aeration.

3. RESULTS

Fertile plants of *Caulerpa racemosa* var. *occidentalis* were easily detected due to reticulate depigmented protoplasmic masses contrary to homogeneously coloured non-fertile plants (figure 2). Also, the greatest part of the rhizome became white as a result of the migration of cytoplasm into fronds. Approximately 12 h before ga-

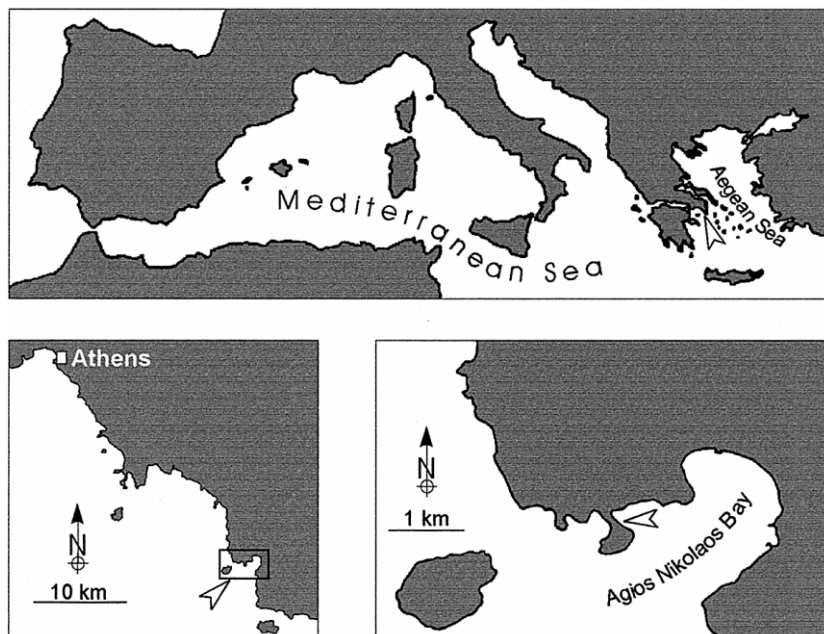


Figure 1. Maps showing the position of the Aegean Sea and the study site (37°43'30"N, 23°54'50"E)

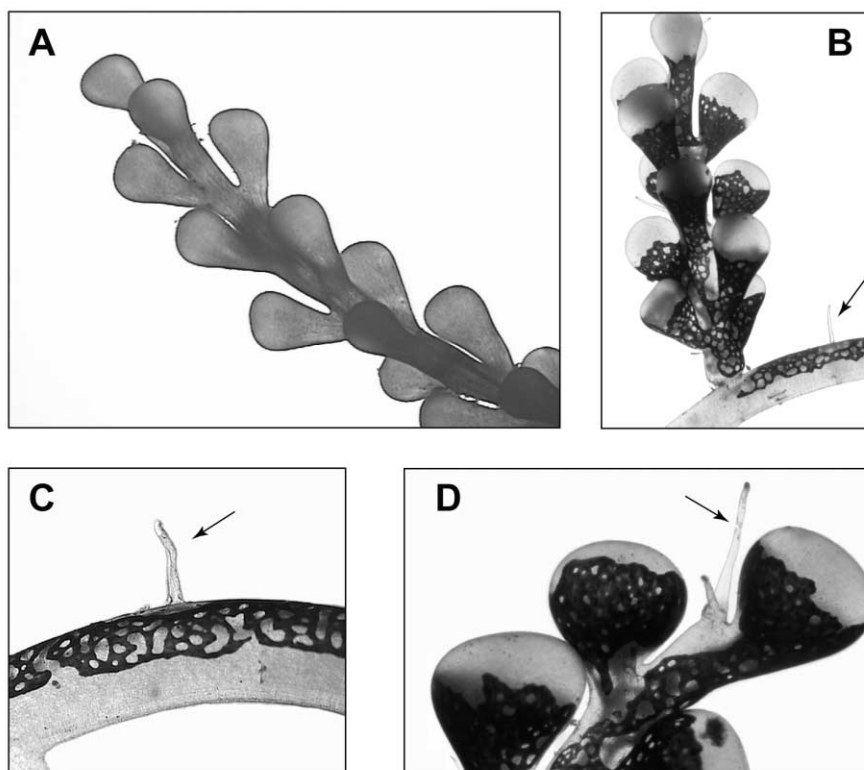


Figure 2. Visible changes of the *C. racemosa* var. *occidentalis* thalli during gametogenesis. Uniformly coloured nonfertile thalli (A); reticulate depigmented thalli of fertile algae (B) one day before spawning; detail of a fertile rhizome (C) and frond (D) with papillae (arrows).

mete release, the protoplasmic network became mosaically bicolored in light green and brownish orange.

The changes in protoplasmic consistence were followed by the formation of numerous, slightly white cylindrical papillae, which appeared as outgrowths on the frond axis, branchlets and rhizomes (figure 2).

Liberated gametes formed a large green cloud 1–1.5 m around the mother plant. It usually dispersed within 5–10 min. During liberation, all protoplasmic material streamed out and only white, ghost thalli persisted (holocarp). All of the fertile plants released their gametes synchronously approximately 14 min before sunrise: the first gametes cloud appeared 17 min and the last 12 min before sunrise.

Maintaining the fertile plants in dark in the laboratory, we could postpone the liberation for about 2 h in comparison to the gamete release in natural conditions. Liberation started a few minutes after illumination.

Microscope observation of the gamete samples showed two types of biflagellate gametes in the same plant: one (female) slightly larger contained an orange stigma, while the other was smaller and without stigma (male). The presence of both sex gametes in the same plant was expected on the basis of bicolored protoplasmic network caused by the sexual segregation of the gametes throughout the thallus and reddish stigma in female gametes (Enomoto and Ohba, 1987; Goldstein and Morall, 1970).

In spite of both sex gametes presence, we observed just a low percentage (< 5 %) of apparent gamete conjugations to form a planozygotes. Because of such a low percentage, the apparent conjugation could be an artefact due to incomplete cleavage of gametes during gametogenesis, which at the moment of observation looked like planozygotes. Further cultivation of the gametes and planozygotes were not done.

4. DISCUSSION

Our results confirm previous reports of *Caulerpa racemosa* sexual reproduction, which showed that the species was monoecious, producing both sex gametes on the same plants: Caribbean specimens of *C. racemosa* (Forsskål) J. Agardh (Clifton, 1997; Clifton and Clifton, 1999; Goldstein and Morall, 1970), *C. racemosa* var. *laetevirens* (Montagne) Weber van Bosse and *C. racemosa* var. *peltata* (Lamouroux) Eubank from Japan (Enomoto and Ohba, 1987; Ohba and Enomoto, 1987; Ohba et al., 1992), *C. racemosa* var. *uvifera* (C. Agardh) J. Agardh from South India (Iyengar, 1933, 1940).

The apparent low gamete compatibility observed in the studied Mediterranean strain of *C. racemosa* var. *occidentalis* could be caused by the inadequacy of the laboratory conditions (ambient temperature, lack of aeration) or by stress due to manipulation of the fertile plants.

Our results also confirm the observations on sexual reproduction of Caribbean *C. racemosa* (Clifton, 1997; Clifton and Clifton, 1999) regarding the time of the gametes release. Nevertheless, for the Caribbean plants this occurred 32 min after sunrise in distinction from 14 min before sunrise for the Mediterranean species. This difference is probably due to the shorter period of dawn in the tropical regions.

At the beginning of the 21st century, it seems that the introduced *Caulerpa* species constitutes a serious threat for the Mediterranean marine biological, ecological and landscape diversity (UNEP, 1999). In less than two decades *C. taxifolia* (Vahl) C. Agardh has covered a large area of the Western Mediterranean Sea, although reproducing only by fragmentation (Meinesz and Hesse, 1991; Meinesz et al., 1998). *Caulerpa racemosa* var. *occidentalis* supported by sexual reproduction, together with its remarkable capability of adjustment to the ecological factors such as temperature, substratum and depth (light) (Verlaque et al., 2000), will probably perform a much faster invasion dynamic throughout the Mediterranean Sea.

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