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Factors affecting sporulation of *Gracilaria cornea* (Gracilariales, Rhodophyta) carposporophytes from Yucatan, Mexico

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Abstract

Carpospore shedding was studied in *Gracilaria cornea* in order to determine maximum spore output potential for mariculture purposes. The combined effects of temperature (23, 26 and 29 °C), daylength (8:16, 12:12 and 16:8 light:dark), photon irradiance (darkness, 20 and 40 μmol m^{-2} s^{-1}) and spore release method (spontaneous release, osmotic shock and drying) were tested. Maximum spore shedding in cystocarpic *G. cornea* occurred within the first three days depending on temperature. A reduction in spore release periodicity was more evident at 29 °C. Carpospore shedding was mainly affected by temperature and daylength. A higher number of carpospores was released per cystocarp at 26 °C than at 23 or 29 °C. Short day conditions (8:16 L:D) produced a higher number of carpospores at 26 °C, even at the lowest irradiances tested (darkness and 20 μmol m^{-2} s^{-1}). A combination of 26 °C and short days (8:16) gave the highest carpospore discharge per cystocarp. There was no significant difference between the spore release methods. These results could be applied to promote the establishment of extensive cultivation of *G. cornea* from spores as an alternative to vegetative propagation in Yucatan coast.

Introduction

Most *Gracilaria* spp. which are harvested or cultivated worldwide are utilized for the commercial production of agar, combining a fast growth rate and ease of vegetative reproduction (Kain & Destombe, 1995). The overexploitation of wild biomass of economically important agarophytes, as well as the urgent need of dependable sources of defined raw material, has led to the expansion of controlled cultivation of *Gracilaria*. Commercial cultivation is done on a very large scale in several countries, such as Chile (Avila & Seguel, 1993), China (Ren et al., 1984) and Taiwan (Chiang, 1981). Pilot scale cultivation is currently being carried out in medium sized farms mainly in Namibia, Venezuela and Malaysia (Armisen, 1995; Dawes, 1995; Alveal et al., 1997). At present, the culture methods for *Gracilaria* rely on vegetative fragments, but cultures are subject to a drop in their productivity with time (2–3 yr) and this may be due to the ageing of the thalli or excessive repeated harvesting (Buschmann et al., 1995; Glenn et al., 1996). The need for alternative methods such as spore culture is evident; on this basis, several studies have demonstrated the feasibility of growing *Gracilaria* from spores, and this coupled with algal selection, offers the possible production of genetically engineered strains of *Gracilaria* in the future (Friedlander & Dawes, 1984; Destombe et al., 1993; Dawes, 1995).

In order to compete in the biomass trade business, the cultivated *Gracilaria* must be more productive and possess special properties (e.g. better processing characteristics, agar yield, gel strength). *Gracilaria cornea* J. Agardh from the Yucatan peninsula has been recently recognized as a high quality agar producer (Freile-Pelegrin & Robledo, 1997a, b). For optimal cultivation of *Gracilaria* efficient seeding, fast growth rate and genetic improvement are of prime importance to the farmer. In this regard, seeding from spores can be achieved only if the methods of reproduction are properly understood. The use of diploid carposporophytes of *G. cornea* was considered because they are present throughout the year in natural populations, the carpospores are released in profusion from cystocarps

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which could be easily recognized and collected by fishermen.

Many experiments which attempt to define the influence of abiotic factors on spore release have been restricted to testing single factor effects; for this reason, we studied the effects of temperature, light, and daylength on carpospore release on *Gracilaria cornea*, as a potential aid for intensive mariculture in the Yucatan peninsula.

Materials and methods

Gracilaria cornea is one of the 8 species of gracilarioids occurring in the Caribbean (Norris, 1985). In the Yucatan peninsula this species grows attached to rocks or dead coral, forming a narrow fringe 20 m wide and several hundred meters long parallel to the coast-line. The diploid phase that develops directly on the female thallus, the carposporophyte, is evident all year in the area, and was selected as seed stock material. Healthy mature cystocarpic thalli of *G. cornea* were collected from a natural shallow (1.5–3 m depth) bed located 3 km west of Dzilam de Bravo, Yucatan (21° 03′ N, 88° 57′ W).

Approximately 567 carposporophytic fragments (3–4 cm length) of *G. cornea* were used in the experimental design. Clean fragments bearing mature cystocarps (n = 7) were used for each combination of temperature, irradiance and daylength. In order to stimulate spore discharge, three sporulation induction methods were used: **Spontaneous discharge**, carposporophytic fragments were placed on glass Petri dishes (5 cm diameter) with sterile seawater (34 ‰) and left until carpospores spontaneously discharged; **osmotic shock**, carposporophytic fragments were submerged in high salinity seawater (60 ‰) for 30 min and returned to normal seawater (34 ‰) contained in glass Petri dishes; **drying**, carposporophytic fragments were dried for 1 h in the shade and re-immersed in seawater.

All Petri dishes containing the carposporophytic fragments were placed in a temperature-light cross gradient table under 81 different combinations of temperature (23, 26 and 29 °C), photon irradiance (darkness, 20 and 40 μmol m^{-2} s^{-1}), daylength [(short day 8:16, long day 16:8 and 12:12 light:dark (L:D)] and spore induction method (spontaneous release, osmotic shock and drying). Three separate experiments were made, one for each daylength, darkness being the control between experiments. Spore counts were done under a stereo microscope using a counting chamber at 24 h periods during 4 days (96 h). Each day the carposporophytic fragments were rinsed, transferred to a new Petri dish and returned to the experimental conditions described above. For each condition the mean number of carpospores released per cystocarp was recorded.

Statistical analysis were carried out using one-way MANOVA to determine differences between treatments (Johnson & Wichern, 1992). Significant levels ($p < 0.05$) between the different variables were determined in the analysis.

Results

The maximum spore shedding in cystocarpic *G. cornea* occurs within the first three days depending on temperature. Maximum carpospore release with the spontaneous discharge method occurred at 26 °C and 40 μmol m^{-2} s^{-1} under short days 8:16 (Figure 1), but release both in darkness at 29 °C and with 20 μmol m^{-2} s^{-1} under 12:12 daylength at 29 °C did not differ significantly ($p < 0.05$). Spore discharge was lowest at 23 °C in short day (8:16).

Under osmotic shock treatment maximum carpospore shedding was observed at 26 °C, 8:16 at 20 μmol m^{-2} s^{-1}, but not did differ ($p < 0.05$) from that in darkness (Figure 2). At 23 °C, spore discharge was reduced and delayed. Spore release at 29 °C, was reduced in 16:8.

For the drying method maximum spore output was found at 26 °C, short days (8:16) and 40 μmol m^{-2} s^{-1} (Figure 3), although output at the same photoperiod and temperature at 20 μmol m^{-2} s^{-1} or at 23 °C in long days (16:8) at 40 μmol m^{-2} s^{-1} did not differ significantly ($p < 0.05$).

Significant differences resulting from conditions and methods tested are summarized in Table 1. Temperature and daylength were the most effective factors though irradiance also had a significant effect. It seems that the spore induction methods were indistinguishable. The best grouping condition to induce the highest carpospore discharge per cystocarp in *G. cornea* was obtained in 26 °C under short day (8:16).

Discussion

Spore discharge in *G. cornea* was evident until the second and third day depending on the temperature.

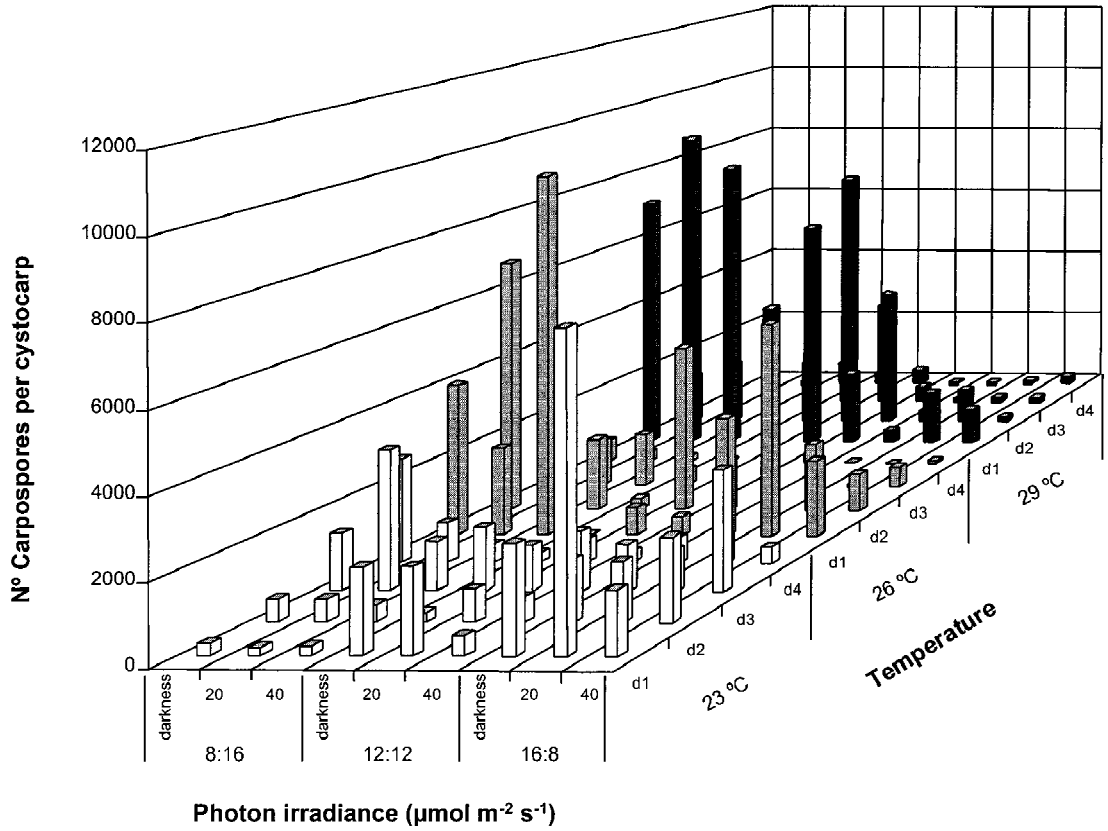

Figure 1. Number of spores released per cystocarp in *G. cornea* with spontaneous release method as a function of temperature, 23 °C (white bars), 26 °C (grey bars), 29 °C (black bars), daylength (8:16, 12:12, 16:8 light:dark) and photon irradiance (darkness, 20, 40 μmol m^{-2} s^{-1}) during 4 day period (d1, d2, d3, d4). Mean number of carpospores discharged per cystocarp for each treatment.

The decline in daily output has been also observed in *Gracilaria* spp. from India (Oza & Krishnamurthy, 1968; Rama Rao & Thomas, 1974). On the other hand, Lefebvre et al. (1987) has found that *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham shed carpospores rhythmically for about a month. This effect can be related to temperature in relation to algal origin (temperate or tropical).

Higher numbers of carpospores shed per cystocarp were obtained in *G. cornea* when compared with those in *Gracilaria corticata* (J. Agardh) J. Agardh (4911 carpospores per cystocarp per day) (Umamaheswara Rao, 1976) and *Gracilaria verrucosa* (Hudson) Papenfuss (19 700 carpospores shed per cystocarpic plant) (Oza & Krishnamurthy, 1968). Maximal spore shedding was obtained at 26 °C, which is the average water temperature in the area. A reduction in the number of spores released was observed at temperatures below or above 26 °C. The same pattern was present in other Gracilariales (Rama Rao & Thomas, 1974). According to Umamaheswara Rao & Subbarangaiah (1981) the peak of spore output is affected by temperature but not by irradiance, desiccation or salinity.

Table 1. Significance levels of carpospore release for *Gracilaria cornea* (one-way ANOVA fixed factors) using pooled means (first two days) for spore induction method, temperature, daylength and photon irradiance

Variable	*F*	*p*
Spore induction method	1.015478	0.362559
Temperature	13.68927	0.000001
Daylength	12.77647	0.000003
Photon irradiance	5.002675	0.006869

A combined effect between daylength and irradiance on carpospore release was observed in *G. cornea* with spontaneous release method. A short day period allowed a higher number of spores to be released, while in general an increase in daylength produced

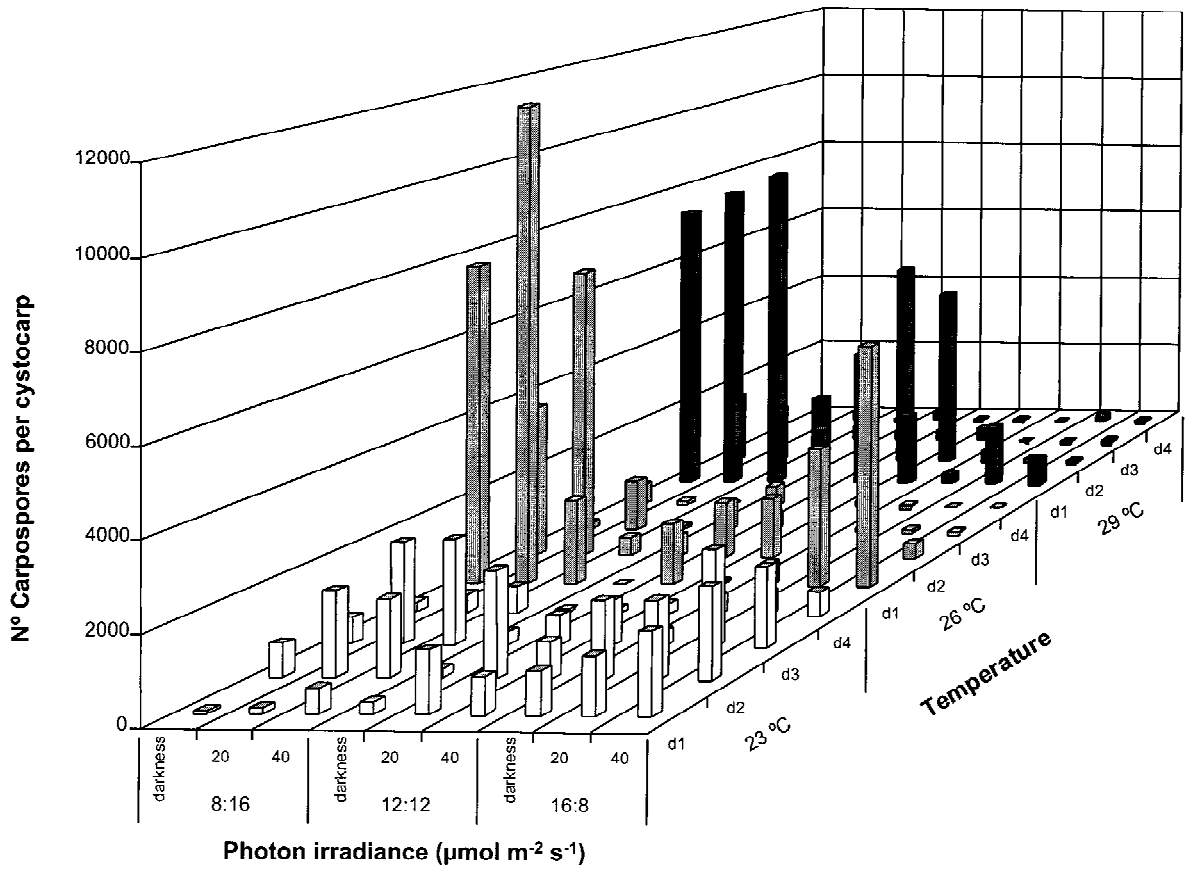

Figure 2. Number of spores released per cystocarp in *G. cornea* with osmotic shock method as a function of temperature, 23 °C (white bars), 26 °C (grey bars), 29 °C (black bars), daylength (8:16, 12:12, 16:8 light:dark) and photon irradiance (darkness, 20, 40 μmol m^{-2} s^{-1}) during 4 day period (d1, d2, d3, d4). Mean number of carpospores discharged per cystocarp for each treatment.

a decrease in the number of spores shed, except for drying at 23 °C and 40 μmol m^{-2} s^{-1}. Increasing irradiance had the same effect on spore released for *Gracilaria foliifera* (Førsskäl) Børgesen (Friedlander & Dawes, 1984) as found for *G. cornea*. These results are different from those obtained by Umamaheswara Rao & Kaliaperumal (1983) for two species of Gelidiales and other red algae, in which low irradiance and longer daylength determined the higher number of spores shed. On the other hand, spore output decreased as irradiance increased in *G. corticata* and *Gracilaria textorii* (Suringar) J. Agardh (Umamaheswara Rao & Subbarangaiah, 1981).

In relation to the spore induction method, spore shedding in red algae is stimulated by salinity changes (Reed, 1995) and desiccation followed by a re-immersion in sea water (see review article by Santelices, 1990). In this study, spore induction method had no significant effect on spore shedding (Table 1). Umamaheswara Rao (1976) reported that sometimes these induction methods do not produce any effect or even inhibit spore release. Mild desiccation followed by re-immersion in seawater was used for spore release in *Gracilaria chilensis* Bird, McLahlan et Oliveira carposporophytes (Infante & Candia, 1988). Spore output in *G. cornea* using drying treatment (1 h) at 26 °C under short day and 40 μmol m^{-2} s^{-1} was comparable to the best conditions obtained with the spontaneous release method. Nevertheless in *G. corticata*, *G. textorii* and *Gracilariopsis sjoestedtii* (Kylin) Dawson 15 min exposure to air inhibited tetraspore release (Umamaheswara Rao & Subbarangaiah, 1981). It is important to note that viability of *G. cornea* spores was not affected by the sporulation method used in this study.

The effect of environmental factors on spore output is of considerable interest to cultivators. In *G. cornea*

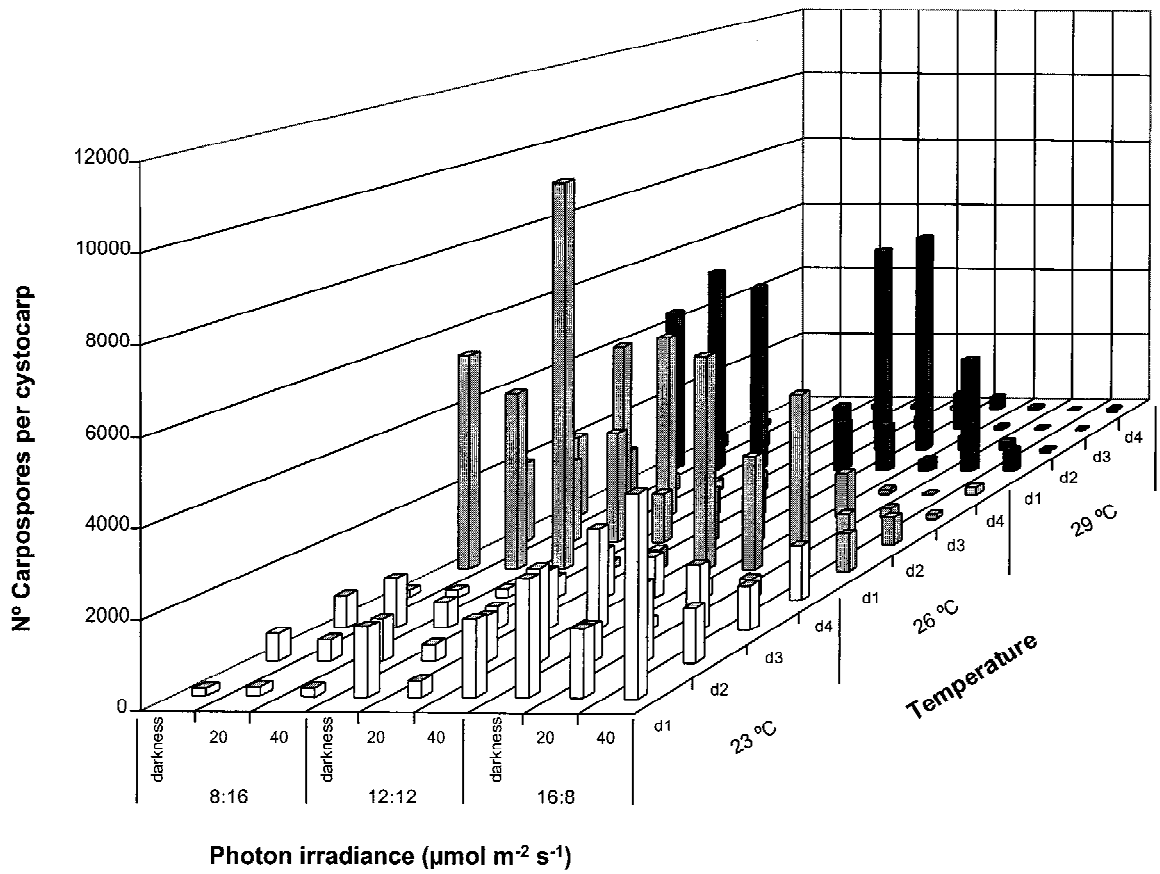

Figure 3. Number of spores released per cystocarp in *G. cornea* with drying method as a function of temperature, 23 °C (white bars), 26 °C (grey bars), 29 °C (black bars), daylength (8:16, 12:12, 16:8 light:dark) and photon irradiance (darkness, 20, 40 μmol m^{-2} s^{-1}) during 4 day period (d1, d2, d3, d4). Mean number of carpospores discharged per cystocarp for each treatment.

the best conditions to maximize carpospore shedding were 26 °C and daylength 8:16 (short day period).

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