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Daylength, temperature and nutrient control of tetrasporogenesis in *Asparagopsis armata* (Rhodophyta)

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Abstract: The formation of tetrasporangia in *Falkenbergia*-phase tetrasporophytes of *Asparagopsis armata* Harvey (Rhodophyta : Bonnemaisoniales) isolated from introduced populations in Ireland and Italy, and from native populations in Victoria, Australia, was examined in temperature-controlled water-baths at 2 °C intervals from 11–23 °C and at 1- or 0.5-h daylength intervals from 8–12 h. At a daylength of 8 h, Irish plants formed tetrasporangia only at (15–)17(–21) °C, Italian plants at 17–21 °C and Australian plants at 13–17 °C. However, after an incubation period of 5 wk, only the Italian strain consistently showed 100% reproduction under these conditions. At 17 °C, the critical daylength for tetrasporogenesis is 8–9 h for the Irish and Australian strains and 9–10 h for the Italian strain. These results indicate that all three isolates differ in their response to environmental variables. It is likely that the Mediterranean and Irish populations thus represent separate introductions into the Northern Hemisphere and that Australia was not the source of these plants. The abundance of gametophytes of this species at Messina, Sicily, in April and May is explained by a reproductive "window" of daylength and temperature for tetrasporogenesis from mid-November to late January. In Victoria, Australia, temperatures are inductive all year but daylength at the water surface is never <9.5 h. However, it is considered that tidal ranges of 2–3 m cause the effective daylength in winter to fall below the inductive threshold. In northern Europe, the restricted geographical distribution of gametophytes is probably determined by tidally and/or topographically induced shorter daylengths in late October and November, and also by higher temperatures in the intertidal on warm days. Previously reported responses to N and P levels in enriched seawater media were not confirmed and may have resulted from incubation at suboptimal temperatures. Arsenic and iodine supplementation appears to be necessary for reproduction only in the Irish strain.

Key words: *Asparagopsis armata*; Control of reproduction; Daylength and temperature effects; *Falkenbergia*-phase; Introduced seaweed; Nutrient effect

Introduction

The marine red alga *Asparagopsis armata* Harvey (Bonnemaisoniaceae, Bonnemaisoniales) was endemic to the Southern Hemisphere until it was introduced into the Atlantic and the Mediterranean in the early part of the present century (Feldmann &

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Feldmann, 1942). The species is now widely distributed in the eastern north Atlantic from the British Isles south to Senegal, including the Canary and Salvage Islands, and possibly the Azores (Price et al., 1986; South & Tittley, 1986), and is widespread in the Mediterranean, although gametophytes are regularly found only in the western basin.

J. Feldmann & G. Feldmann (1939a,b, 1941, 1942) discovered that the separately described species *Falkenbergia rufolanosa* (Harvey) Schmitz was the tetrasporangial phase of *Asparagopsis armata* by germinating the carpospores of the latter entity and obtaining plants that resembled the former. A similar life history for *A. taxiformis* (Delile) Trevisan was established in culture for plants from Japan by Chihara (1961), who grew carpospores and obtained a *Falkenbergia*-phase, and for plants from Venezuela by Rojas et al. (1982), who found that tetrasporogenesis occurred at 24 °C, 8 : $\overline{16}$ h.

Reproduction in the *Falkenbergia*-phase of *A. armata* is reported to be rare and restricted to autumn. The only published records for the British Isles are: McLachlan (1967), who found fertile plants in Cornwall on the south coast of England in mid-October 1966; De Valera et al. (1979) who collected fertile plants at Finavarra, Co. Clare, Ireland, in October 1976; and Guiry et al. (1979) who reported that populations in Devon and Cornwall were forming tetrasporangia in November 1972. In northern France, J. Feldmann (1954, p. 94) remarked that plants of the *Falkenbergia*-phase are not fertile in the area around Roscoff, but further south, in Portugal and Morocco, fertile plants are found from December to March (Gayral, 1958, p. 288; Ardré, 1970, p. 143), and gametophytes are regularly found on the Atlantic coasts of Morocco (Gayral, 1958), Portugal (Ardré, 1970), Spain (Seoane-Camba, 1965) and France (Feldmann, 1954).

The perceived lack of reproductive tetrasporophytes in the British Isles and radically different distributional patterns of gametophytes and tetrasporophytes gave rise to early speculation that both phases reproduced independently by vegetative means (Dixon, 1964, 1965). De Valera & Folan (1964) further suggested that a decrease in the fertility of Irish gametophytes of *A. armata* may have occurred in the period since its introduction, implying vegetative reproduction by the gametophytes, but concluded that their material was not adequate to confirm this; they were also of the opinion that Irish gametophytic plants were becoming smaller and less robust. The *Provisional atlas of the marine algae of Britain and Ireland* (Norton, 1985, Maps 76 and 77), indicates that the *Falkenbergia*-phase of *A. armata* is now widely distributed on western and southern coasts from Shetland to Cornwall, but the *Asparagopsis*-phase gametophytes are confined to restricted areas of the west and south of Ireland, and in Britain are known only from the south-west coast.

In an attempt to establish the environmental parameters for reproduction in the *Falkenbergia*-phase of *A. armata*, Oza (1977, 1989) grew a strain from Roscoff, Brittany (from the culture collection of H.A. von Stosch), under a range of temperature, day-length and nutrient conditions. Tetrasporangia were obtained only at 15 ± 0.5 °C, 6 : $\overline{18}$ or 8 : $\overline{16}$ h, in media with low levels of nitrates or phosphates, and containing small amounts of As_2O_3 and KI. Lüning (1981), using the same strain, obtained reproduction

at 15 °C, 8 : $\overline{16}$ h, in enriched seawater media with low levels of nitrate (< 700 μM NO_3), and found that a night-break was effective in suppressing tetrasporangial formation (confirming the photoperiodic nature of the response), but did not state whether arsenic or iodine were required for reproduction. Using the same strain, Knappe (1985) carried out a more detailed study of the effects of daylength at 15 °C, quantifying the response by counting spores.

Casual monitoring of an intertidal population of the *Falkenbergia*-phase of *A. armata* in Galway Bay at 1- and 2-wk intervals from September through December 1985 (Breeman & Guiry, unpubl. data) indicated that no sporangial production occurred during this period, even though several waves of reproduction took place at this time in the related, introduced species *Bonnemaisonia hamifera* Hariot (Breeman & Guiry, 1989). As the temperature optimum for maximum reproduction in *Bonnemaisonia hamifera* is reported to be 15 °C (Oza, 1977, 1989; Lüning, 1981; Knappe, 1985), this lack of reproduction in *Asparagopsis armata* seems to be in conflict with the published data.

Several questions are thus apparent with regard to the distribution and reproduction of *Asparagopsis armata* in Europe: (1) If induction of tetrasporangia is possible at 15 °C, then why is reproduction so uncommon in plants of the *Falkenbergia*-phase in the wild near the northern limit of distribution of *A. armata*? (2) Why are gametangial plants very confined in their geographical distribution near the northern limits of the species and to the Mediterranean even though the tetrasporophytes are more widely distributed? (3) Were European populations of the species introduced from Australia and are they the result of a single introduction? (4) What are the effects of nitrate, phosphate, iodine and arsenic levels in enriched seawater media at optimum temperatures?

Materials and Methods

Origin of cultures

Plants of the *Falkenbergia*-phase of *Asparagopsis armata* were isolated into unialgal culture using reproductively sterile field-collected plants from the west coast of Ireland collected on 6 February 1985 (Culture GALW 510, New Quay, Co Clare; 53°N, 09°13′W) and from Sicily, Italy, on 4 June 1987 (Culture GALW 566, near Messina; 38°11′N, 15°33′E). Unialgal cultures were obtained by continuously excising the apices of rapidly growing plants at 16 °C, 16 : $\overline{8}$ h, 2–5 μmol photons $\cdot m^{-2} \cdot s^{-1}$. Carpospores from cystocarpic plants of *A. armata* collected in the intertidal of Victoria, Australia, on 28 October 1987 (Culture GALW 604, Sorrento; 38°20′S, 144°45′W) were inoculated into plastic dishes, grown at 17 °C, 14 : $\overline{10}$ h, 20–25 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$, and a unialgal isolate of the *Falkenbergia*-phase obtained. Cultures were subsequently rendered clonal by selecting a single plant of each as a stock culture, and these were grown at 16 °C, 16 : $\overline{8}$ h, 25 μmol photons $\cdot m^{-2} \cdot s^{-1}$ with the light period digitally set to start at 0800 GMT. Plants were propagated vegetatively by excising

vegetative apical portions of the plants; culture media and dishes were replaced every 3–4 wk.

ENRICHED SEAWATER MEDIA

Filtered sterile seawater was enriched using Guiry & Cunningham's (1984) modification of von Stosch's enriched seawater medium (von Stosch, 1963). Quarter-strength medium (VS_5) was used in which 5 ml of stock solution (see Guiry & Cunningham, 1984) were added to each l of seawater. The final VS_5-enriched seawater medium contained 22.14 mg $NO_3 \cdot l^{-1}$ (0.36 mM) and 17.64 mg $PO_4 \cdot l^{-1}$ (0.19 mM). Background levels of nitrate and nitrite in the medium during the experimental period ranged from 1.21 to 51.04 μg $NO_3 \cdot l^{-1}$ and 0.22 to 66.34 μg $NO_2 \cdot l^{-1}$. The effects of arsenic and iodine on tetrasporangial formation were assessed by supplementing the medium with As_2O_3 (98 $\mu g \cdot l^{-1}$) and KI (16 $\mu g \cdot l^{-1}$), based on the concentrations used by von Stosch (1963) and Oza (1977).

FLUORESCENT STAINING

Determination of whether tetrasporangia were produced from new or old cells was carried out by labelling the cell walls of the filaments with calcofluor, a cellulose-specific stain (Waaland & Waaland, 1975), using 0.01% Fluorescent Brightner (Catalogue No. CI 40622; Sigma Chemical, Poole, Dorset, UK). A concentration of 0.2 mg $\cdot l^{-1}$ was made by dissolving 0.2 mg of the Fluorescent Brightner in a few drops of distilled water and transferring to 200 ml of enriched seawater medium. Minced fragments of the Irish isolate (Culture 510) were grown in this solution for 1 wk. The plants were then washed three times with fresh medium lacking the dye and plants were incubated at 17 °C, 8 : $\overline{16}$ h, to induce tetrasporangial formation. Plants were examined for fluorescence under an epifluorescent microscope (Leitz Labrolux 22D).

EXPERIMENTAL PROCEDURE

For each experiment, tufts of the *Falkenbergia*-phase were taken from stock cultures and minced with an alcohol-sterilized razor-blade on a glass microscope slide until a homogeneous mass of cuttings 1–2 mm in length was obtained. These plants were then suspended in fresh seawater medium and 50–60 cuttings pipetted into sterilized 250-ml, wide-mouth, conical flasks containing 200–250 ml of enriched seawater.

Flasks were incubated in water-baths (Compact Low Temperature, Model RMT6, Lauda, Germany) under cool-white fluorescent bulbs (a single Philips PLC-20 W or two Thorn 15 W) delivering 14–20 μmol photons $\cdot m^{-2} \cdot s^{-1}$ to the surface of the water in the water-baths. Flasks were rotated daily to ensure an even photon dosage and to obviate any minor temperature fluctuations. A minimum of 50 plants $\cdot$ flask^{-1} was assessed for tetrasporangial formation in relation to size after 5 wk $\pm$ 1 d of incubation.

Daylength in the water-baths was controlled by digital timers set to begin the appro-

priate light period at 0800 GMT. Water temperatures were monitored with submerged thermistors and recorded every 15 min with Squirrel Data Loggers (Grant Instruments, Cambridge, UK); at no time did water temperatures in any parts of the water-baths vary by $> \pm 0.2\,^{\circ}C$. Irradiances (photosynthetically active radiation) were measured using Li-Cor light meter Model 185-B and a Li-Cor 190S quantum sensor.

Contingency tables were used to compare the presence or absence of tetrasporangial production using the null hypothesis that no significant difference occurred at the 95% confidence level ($P < 0.05$). χ^2 tables were used to determine significant differences between all paired treatments.

Results

POSITION OF TETRASPORANGIAL PRODUCTION

Tetrasporangial formation (Fig. 1) occurred only on newly formed branches of the Irish isolate of *Asparagopsis armata* (Fig. 2A,B) as cells stained with Fluorescent Brightner did not form tetrasporangia. Some leaching of the Fluorescent Brightner onto new cells was apparent (Fig. 2B), but this was minimal.

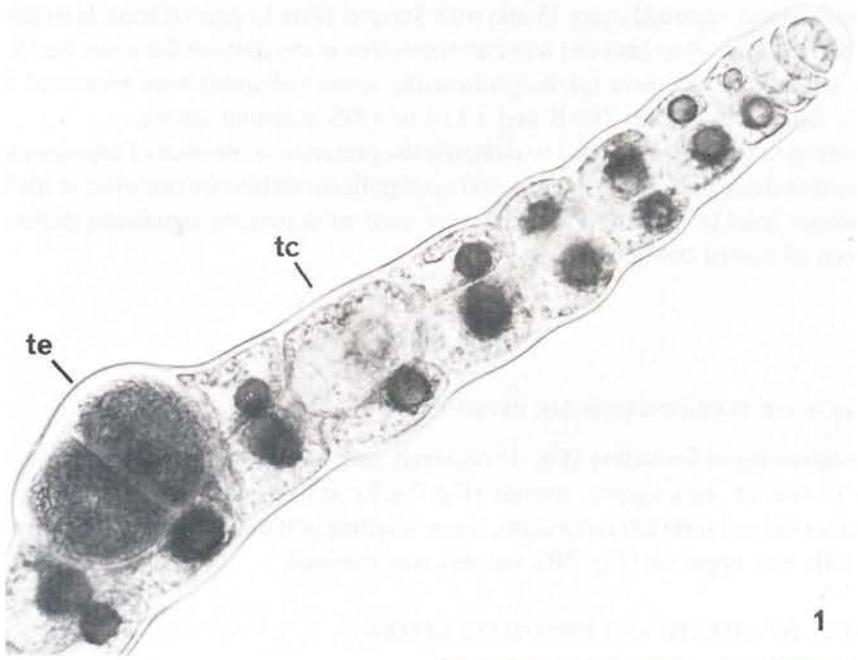
EFFECTS OF NITRATE AND PHOSPHATE LEVELS

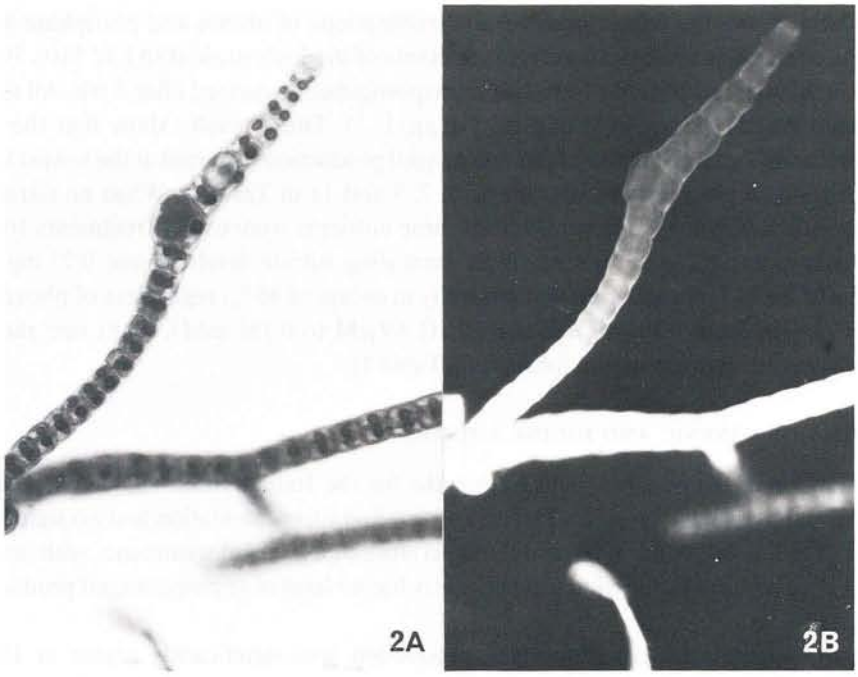
Table I shows the effects of different combinations of nitrate and phosphate levels on reproduction in various size classes of plants of the Irish strain (GALW 510). Plants were incubated at 17 °C, 8 : $\overline{16}$ h, and tetrasporogenesis assessed after 5 wk. All media included As_2O_3 (98 $\mu g \cdot l^{-1}$) and KI (16 $\mu g \cdot l^{-1}$). These results show that the only significant effect on reduction of tetrasporangial production occurred at the lowest levels of nitrates and phosphates (Treatments 3, 7, 9 and 11 in Table I). When no nitrate or phosphate or very low concentrations of these nutrients were used (Treatments 10 and 11), tetrasporangia were absent. With increasing nitrate levels above 0.21 $mg \cdot l^{-1}$ (3.85 μM NO_3^-) reproduction was generally in excess of 80%, regardless of phosphate levels ranging from 0.18 to 17.64 $mg \cdot l^{-1}$ (1.89 μM to 0.186 mM). Plant size showed no relation to tetrasporangial formation (Table I).

EFFECTS OF ARSENIC AND IODINE ADDITIONS

At optimum temperatures and daylengths for the Italian strain (17–19 °C, <9 h), nitrate and phosphate levels and arsenic and iodine supplementation had no significant effect (Figs. 3, 4). At the suboptimal temperature of 21 °C, all treatments with arsenic and iodine addition resulted in a significantly higher level of tetrasporangial production ($P < 0.05$).

In the Irish strain, tetrasporangial production was significantly higher at 17 °C, 8 : $\overline{16}$ h when arsenic and iodine were added (Fig. 3), but no significant difference in tetrasporangial production was evident at 17 °C, 9 : $\overline{15}$ h (Fig. 3) and 19 °C, 8 : $\overline{16}$ h





Figs. 1–2. Tetrasporangia (t) and a tetrasporocyte (tc) in *Asparagopsis armata* from Ireland in culture. Dark areas in vegetative cells are secretory cells.

TABLE I

Effects of nitrate and phosphate levels on tetrasporangial production in various size classes of plants of Irish strain of *Falkenbergia*-phase of *Asparagopsis armata*. Plants were grown at 17 °C, 8:$\overline{16}$ h, 25–30 μmol photons · $m^{-2} \cdot s^{-1}$, and assessed after 5 wk. Percentage reproduction of total plants is given in final column. Y, reproduction; N, no reproduction.

Treatment			Reproduction	Branch size (mm)				%
No.	NO_3	PO_4		0 – 1	1 – 2	2 – 3	3+	
1	22.14 mg · l^{-1}	17.64 mg · l^{-1}	Y	–	1	6	50	88
	(0.36 mM)	(0.19 mM)	N	–	1	1	6	12
2	22.14 mg · l^{-1}	1.76 mg · l^{-1}	Y	4	8	6	31	91
	(0.36 mM)	(18.5 μM)	N	–	1	3	1	9
3	2.29 mg · l^{-1}	1.76 mg · l^{-1}	Y	–	1	3	33	57
	(38.5 μM)	(18.5 μM)	N	–	2	2	22	43
4	0.23 mg · l^{-1}	1.76 mg · l^{-1}	Y	–	5	6	44	83
	(3.85 μM)	(18.5 μM)	N	–	1	3	7	17
5	22.14 mg · l^{-1}	0.18 mg · l^{-1}	Y	–	2	2	50	89
	(0.36 mM)	(1.89 μM)	N	–	–	–	7	11
6	2.29 mg · l^{-1}	0.18 mg · l^{-1}	Y	2	–	10	36	79
	(38.5 mM)	(1.89 μM)	N	–	–	2	11	21
7	0.23 mg · l^{-1}	0.18 mg · l^{-1}	Y	–	1	1	7	14
	(3.85 μM)	(1.89 μM)	N	–	3	8	42	86
8	2.29 mg · l^{-1}	17.64 mg · l^{-1}	Y	–	1	8	44	87
	(38.5 μM)	(0.19 mM)	N	–	–	1	7	13
9	0.23 mg · l^{-1}	17.64 mg · l^{-1}	Y	–	–	3	7	15
	(3.85 μM)	(0.19 mM)	N	2	3	24	29	85
10	0.02 mg · l^{-1}	0.18 mg · l^{-1}	Y	–	–	–	–	0
	(0.32 μM)	(1.89 μM)	N	–	3	8	46	100
11	0.00 mg · l^{-1}	0.02 mg · l^{-1}	Y	–	–	–	–	0
	(0.00 μM)	(0.2 μM)	N	2	7	10	36	100

(Fig. 4). The addition of nitrate and phosphate significantly reduced tetrasporangial production only at 19 °C but not at the optimum temperature of 17 °C at 8 : $\overline{16}$ h (Fig. 3).

In the Australian strain, tetrasporangial production was so low after the experimental period of 5 wk that differences may reflect the shorter incubation period. The addition of arsenic and iodine did result in a significant increase in tetrasporangial production at 13, 15 and 17 °C using an 8 : $\overline{16}$-h photoperiod (Fig. 4). The addition of nitrate and phosphate resulted in a significant decrease and thus contradicted the above results for the 15 and 17 °C treatments while stimulating tetrasporangial production at 13 °C, 8 : 16 h if arsenic and iodine were also added. At 17 °C, 9 : $\overline{15}$ h, only the addition of nitrate and phosphate resulted in tetrasporangial production of any magnitude (Fig. 3).

Fig. 1. Light micrograph of a tetrasporangium on a branch tip (× 45).
Fig. 2. Bright field (A) and Fluorescent Brightner-induced fluorescence (B) of same plant. Only newly formed branch is forming tetrasporangia as evidence by absence of fluorescence (× 35).

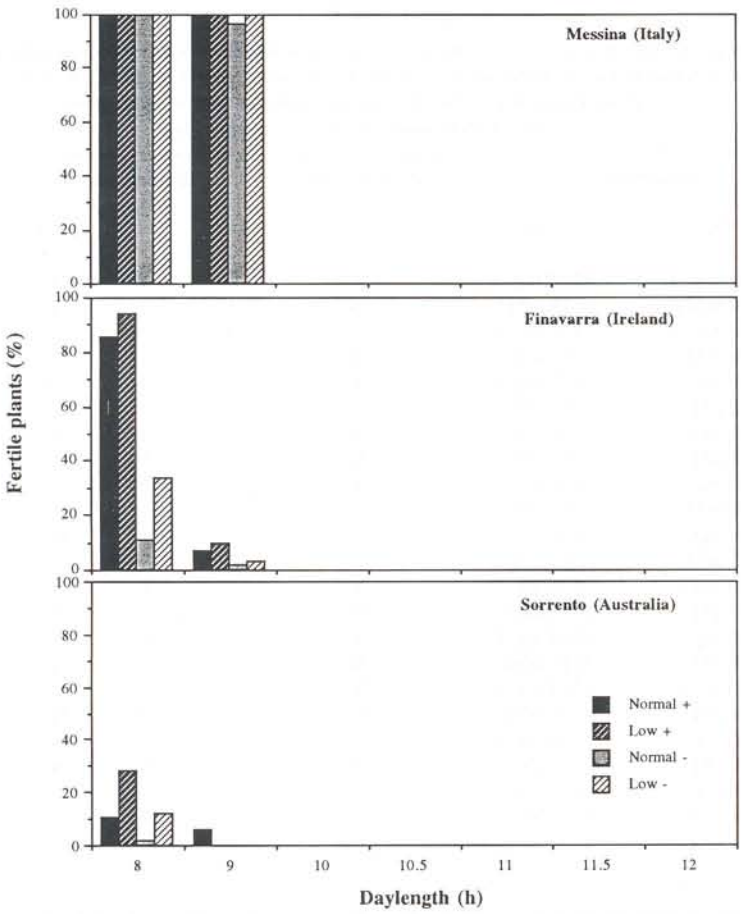


Fig. 3. Effects of daylength on three strains of *Falkenbergia*-phase of *Asparagopsis armata*. Plants were incubated at 17 °C at daylengths shown. Normal, a normal-strength medium was used; low, N and P were reduced as given in Materials and Methods. + and −, presence/absence of arsenic and iodine.

DAYLENGTH AND TEMPERATURE EFFECTS

The three geographical strains tested showed marked differences in their response to daylength at 17 °C (Fig. 3). The Italian strain showed 100% reproduction at 8 and 9 h, whereas the Irish strain showed >80% at 8 h and <20% reproduction at 9 h. The Australian strain, on the other hand, showed <30% reproduction at 8 h and reproduction took place at 9 h only in the high-nutrient medium with As and I supplementation.

Temperature differences between the strains were also apparent at 8-h daylengths (Fig. 4). The Italian strain showed 100% reproduction at 17 and 19 °C, with no reproduction taking place at <17 °C, reproduction being significantly reduced at 21 °C

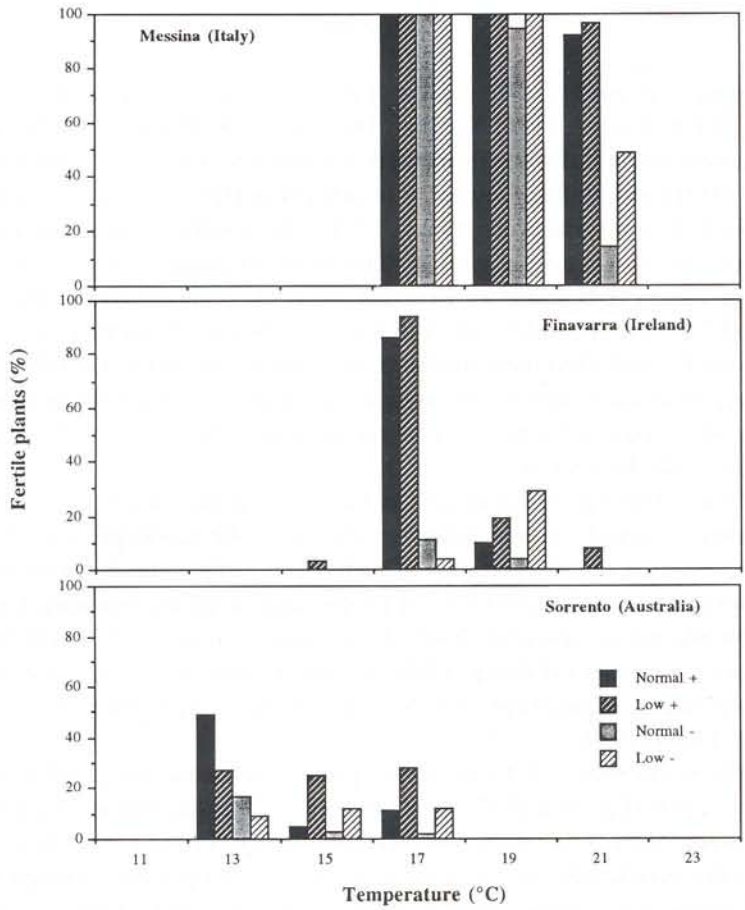


Fig. 4. Effects of temperature on three strains of *Falkenbergia*-phase of *Asparagopsis armata* grown at a daylength of 8 h. Normal, a normal-strength medium was used; low, N and P were reduced as given in Materials and Methods. + and −, presence/absence of arsenic and iodine.

in media without arsenic and iodine additions. The Irish strain reproduced over much the same temperature range, but the levels of tetrasporangial production were much lower: at 17 °C, >80% of the plants in media containing added arsenic and iodine formed tetrasporangia. At 19 °C, <30% of plants reproduced with significantly highest tetrasporangial production in media containing no additional nitrate and phosphate and arsenic and iodine. At 21 °C, a small number of plants reproduced in the low-nutrient medium with added As and I, but no reproduction was observed in the other media. Similarly, at 15 °C, <5% of plants in the low-nutrient medium with As and I formed tetrasporangia, and none of the other media showed any response. The Australian strain reproduced at a different temperature range (13–17 °C) than the Italian and Irish strains (17–21 °C), but in no instance did >50% of Australian plants form tetrasporangia after 5 wk of incubation.

Discussion

Our experimental results confirm those of previous studies of *Asparagopsis armata* (Oza, 1977, 1989; Lüning, 1981; Knappe, 1985; Bonin & Hawkes, 1987), viz., that tetrasporogenesis in the *Falkenbergia*-phase is controlled by daylength and temperature. Oza (1977, 1989) concluded that low levels of either N or P (10% normal strength) were necessary for tetrasporangial production at 15 °C. Our results, using a combination of N and P concentrations, do not entirely support his findings; however, our investigations of the effects of N and P were carried out at the optimum temperature for the Irish strain (17 °C; Fig. 4), and it is likely that under suboptimal conditions (15 °C), low levels of N and P could affect the response in the manner reported by Oza (1977, 1989). This comparison assumes that the strain he used, which was isolated from Roscoff on the north-western coast of France, is similar in its temperature, daylength and nutrient requirements to the Irish strain.

Dring (1974, 1984) has summarized the literature on the effects of high nutrient concentrations on reproduction in algae. It is clear from the results presented here that caution is necessary in the interpretation of such effects as they may be partly or wholly due to suboptimal incubation conditions or to other chemical factors in enriched media. Low-nutrient effects have also been reported in tetrasporogenesis in the *Trailliella*-phase of *Bonnemaisonia hamifera* (Lüning, 1980), but this requires reinvestigation given the narrow temperature requirements for tetrasporogenesis also found in this entity (Breeman & Guiry, 1989).

It is clear from our results that nitrate, phosphate, arsenic and iodine additions at 17 and 19 °C, 8 : $\overline{16}$ h (Fig. 4) and 17 °C, 9 : $\overline{15}$ h (Fig. 3) in the Italian strain have no significant effect. At 21 °C, 8 : $\overline{16}$ h, however, additions of As and I to the low and high N and P media significantly increased the numbers of tetrasporangia formed (Fig. 4). In the Irish strain at the optimum temperature (17 °C), As and I additions to low and high N and P media significantly increased tetrasporangial formation (Fig. 4), but this was not apparent at 19 °C, 8 : $\overline{16}$ h, probably due to the overall low levels of reproduction. A medium with low levels of N and P to which As and I had been added was the only combination that produced even a marginal response at 15 and 21 °C in the Irish strain (Fig. 4). This supports the conclusions of Oza (1977) and Lüning (1981) in that the former used a very dilute medium (10% normal-strength von Stosch medium with As and I added) and the latter a dilute Provasoli's enriched seawater (with $\approx 700\ \mu$M N; it is not stated whether As and I were added) at 15 °C, 6 : $\overline{18}$ or 8 : $\overline{16}$ h. Strict comparison of our and Oza's and Lüning's results is not possible as these authors chose to assess reproduction in terms of sporelings formed and small numbers of sporangia tend to translate into relatively large numbers of sporelings. In the Australian strain, an apparently suboptimal response was obtained by us at 13, 15 and 17 °C, 8 : $\overline{16}$ h and at 17 °C, 9 : $\overline{15}$ h, and, although As and I additions significantly affected the responses, reproductive levels were generally so low that one cannot determine if these differences are real.

The effects of arsenic and iodine additions on induction of tetrasporogenesis in the Irish strain are particularly interesting. Von Stosch (1963) showed that in culture arsenic and iodine were necessary elements for the development of gametophytes of *Asparagopsis* with a normal morphology and Codomier et al. (1979) found that concentrations of 5 μM KI or KIO_3 were optimal for growth of gametophytes, but that initial concentrations in excess of 15 μM inhibited growth. Knappe & Werner (1975) later indicated that the gametophytes were up to 10 times more efficient than the tetrasporophytes in scavenging iodine from culture media. Arsenic is present in various forms in aquatic environments (Sanders, 1979; Anderson & Bruland, 1991; Lee et al., 1991) and, in the natural environment, there should not be any problems with availability in the small quantities required. It is not clear, however, how arsenic and iodine levels could affect the photoperiodic mechanism except perhaps by means of enzyme inhibition or promotion.

In previous studies of tetrasporogenesis in the *Falkenbergia*-phase of *Asparagopsis armata* various combinations of temperature and daylength were used. Oza (1977, 1989) experimented with daylengths of 4, 6, 8, 10, 12, 14 and 16 h at 9, 12, 15, 18 and 21 °C, but only obtained tetrasporangia in appreciable numbers at 15 °C, 6 : $\overline{18}$ h and 15 °C, 8 : $\overline{16}$ h. Lüning (1981) used temperatures of 5, 10, 15 and 20 °C in combination with daylengths of 8, 12 and 16 h, and his plants only reproduced at 15 °C, 8 : $\overline{16}$ h. Knappe (1985) examined the effects of daylength at 3, 4, 5, 6, 8, 9 and 10 h at 15 °C, quantifying the response by counting spores; after 28 d, spores had formed at daylengths of 4–9 h, but not at 3 and 10 h; maximal numbers of spores were formed at 6 h. After 42 d, sporangia were also formed at 3-h daylengths. Our results are consistent with those of Oza (1977, 1989), Lüning (1981) and Knappe (1985) with regard to the critical daylength for tetrasporogenesis and lead to the conclusion that the Irish and French strains have the same requirements and that they are of similar genetic origin.

In our studies, tetrasporogenesis occurred over relatively narrow temperature bands: 13–17 °C in the Australian strain, (15–)17(–21) °C in the Irish strain and 17–21 °C in the Italian strain, and then only in daylengths of ≤ 9 h. It is clear that the temperature requirements in the Irish and Italian strain, although different, are more similar to each other than they are to the Australian strain.

Asparagopsis armata was first described from Garden Island, Western Australia (W. H. Harvey, 1855), and is apparently native to New South Wales, Victoria, Tasmania, South Australia and Western Australia (W. H. Harvey, 1862; Lucas & Perrin, 1947; Levring, 1953; Huisman & Walker, 1990), New Zealand and the Chatham Islands (Levring, 1955; Chapman, 1969; Bonin & Hawkes, 1987), and Chile (Santelices & Abbott, 1978; Santelices, 1988). In Europe, both the *Falkenbergia* and *Asparagopsis* phases of *Asparagopsis armata* were discovered at four centres, i.e., Algeria, Côte des Albères (Mediterranean France), Biarritz (Bay of Biscay, Atlantic) and on the Cherbourg Peninsula in the English Channel, at about the same time in the early 1920s (see Feldmann & Feldmann, 1942, for a literature review of the sequence of events up to 1941). These almost simultaneous occurrences gave rise to early speculations that

the populations might be the result of separate introductions (e.g., Westbrook, 1930; Svedelius, 1933). Of course, there is always the possibility that the time of their detection was a function of the observers; in other words, field-workers were searching for the species after being made aware of the possibility of its presence (see Feldmann & Feldmann, 1942, p. 105). It must be admitted, nevertheless, that a large and distinctive entity such as the gametophytes of *Asparagopsis* could not easily be overlooked.

At present, the *Falkenbergia*-phase of *A. armata* is widely distributed in the Mediterranean (e.g., Feldmann & Feldmann, 1942, Fig. 8; Giaccone, 1978; Furnari & Scammacca, 1970; Güven & Öztig, 1971; Meñez & Mathieson, 1981; Gallardo et al., 1985; Giaccone et al., 1985; Athanasiadis, 1987), but gametophytes are known only from the western Mediterranean [1] (e.g., Feldmann & Feldmann, 1942, Fig. 8; Gayral, 1958; Meñez & Mathieson, 1981; Aranda et al., 1984; Giaccone et al., 1985). Dixon (1964, 1965) and Dixon & Irvine (1977) concluded (largely, it seems, on the basis of the work of Schiffner, 1931) that distinctions between *A. armata* and *A. taxiformis* were not entirely clear, despite the opinion of J. Feldmann (1939, pp. 277–278) that some of the herbarium material cited by Schiffner was badly prepared and thus misleading.

Asparagopsis taxiformis is widespread in the warmer waters of the Northern and Southern Hemispheres, and in the eastern Atlantic is known from Madeira, the Salvage and Canary Islands southwards (Levring, 1974; Price et al., 1986). This species is restricted to the south-eastern Mediterranean (Feldmann, 1942), and there appear to be very few literature records of gametophytes other than the original description from Alexandria in Egypt (Delile, 1813, p. 115; 1824, p. 401). Aleem (1945) reported that at Alexandria "The species flourishes during July and August particularly in 2–3 m depth." Aleem (1951, pers. comm.) later reported both the *Asparagopsis* and *Falkenbergia* phases at 10–30 m in May and June. Gametophytes are also reported from Libya (Nizamuddin et al., 1979, citing an early record by De Toni).

Various opinions on the conspecificity of the two species of *Asparagopsis* have led to *Falkenbergia*-phase plants being referred to *A. armata* as if this were the only species occurring in the Mediterranean (e.g., Diapoulis & Verlaque, 1981; cf. Athanasiadis, 1987, p. 29). There are, however, some records of *Falkenbergia* from the western Mediterranean prior to 1920 and the type locality of *Falkenbergia hillebrandii* (Bornet in Ardissone) Falkenberg, widely regarded as the tetrasporophyte of *A. taxiformis*, is the island of Elba, off the north-west coast of Italy (Ardissone, 1883)[2].

With regard to the possible conspecificity of *A. armata* and *A. taxiformis*, Bonin & Hawkes (1987, p. 587) have concluded that the two entities are morphologically and

[1] Due to the practice of giving the name *Asparagopsis armata* to the tetrasporophyte phase, it is sometimes not clear from various floristic accounts whether authors are referring to the gametophyte or tetrasporophyte phase.

[2] As the *Falkenbergia* phases of *Asparagopsis armata* and *A. taxiformis* are said to be indistinguishable (e.g., Dixon, 1964), it is conceivable that, as to type, *Falkenbergia hillebrandii* represents the former rather than the latter species and the original collection from Elba represents an early record of the introduction of *A. armata* into the Mediterranean.

ecologically distinct: *A. armata* is primarily an epiphyte, attaching to other plants by means of modified branches referred to as barbs; *A. taxiformis* is usually associated with sand-covered habitats, having a well-developed rhizomatous system for anchorage, and lacks barbs. Other authors (Santelices & Abbott, 1978; Santelices, 1988; Huisman & Walker, 1990) have maintained the two species on the basis of similar distinctions.

In the British Isles, the *Falkenbergia*-phase of *Asparagopsis armata* was first found in the vicinity of Galway Harbour in 1939 (De Valera, 1942; Drew, 1950; De Valera & Folan, 1964), and *Asparagopsis*-phase gametophytes were discovered two years later at Muigh Inis (Mweenish), at the head of Galway Bay (De Valera, 1942; Drew, 1950). The *Falkenbergia*-phase was subsequently discovered in Britain by C.C. Harvey & K.M. Drew (1949) on Lundy Island off the coast of north Devon, and the gametophytes were located at a number of sites on the Lizard Peninsula in Cornwall in 1950 (Drew, 1950). The tetrasporophyte has spread independently, probably by vegetative fragmentation, in the British Isles since these initial introductions (Dixon, 1964, 1965; Dixon & Irvine, 1977; Tittley in Irvine et al., 1975; Farnham, 1980; Norton, 1985). However, it is very unlikely that the gametophytes regularly perennate, as implied by various authors (e.g., Dixon, 1964, 1965; De Valera & Folan, 1964), because *Asparagopsis*-phase plants have not at any time been found in Ireland from October–May. If such perennation were possible, it seems reasonable to suppose that gametophytes would be as widespread as the tetrasporophytes.

With regard to the incubation period for tetrasporogenesis in our strains of the *Falkenbergia*-phase of *Asparagopsis armata*, it should be noted that 5 rather than 6–8 wk is a more appropriate incubation period for the assessment of a response in an autumn-reproducing species because of the major changes in daylength and temperature that rapidly take place at this time of year. Temperature and daylength graphs for Messina, Sicily, Italy, Galway Bay, Ireland, and Sorrento, Victoria, Australia, are shown in Figs. 5–7, respectively. The temperatures available for most localities are the maxima recorded for surface, open water, and take no account of smaller bodies of inshore water or tidal pools, which, as we shall see later, may be more relevant. Similarly, the daylength curves are those at the surface of the water and assume that the effective daylength starts at a solar elevation of 0° ("daylight", but not including "civil twilight" in Dring, 1984). Figs. 5–7 also show the maximum and minimum temperatures at which reproduction may take place in the tetrasporophytes and the maximum inductive daylength.

At Messina (Fig. 5), seawater temperatures are within the inductive range from late September until early July, and daylengths are inductive from mid-November until the beginning of February. Although water temperatures are only marginally inductive from January until May, a reproductive "window" (Lüning, 1981; Guiry & Cunningham, 1984) for this species occurs from early November until late January. At Messina, gametophytic plants of *A. armata* are common in spring and become dominant in the upper subtidal in April and May (Tripodi, pers. comm.) and this correlates well with an autumnal inductive "window" as predicted by our results. Aranda et al. (1984, Fig. 9) reported four waves of tetrasporangial reproduction in plants of the *Falkenbergia*-phase

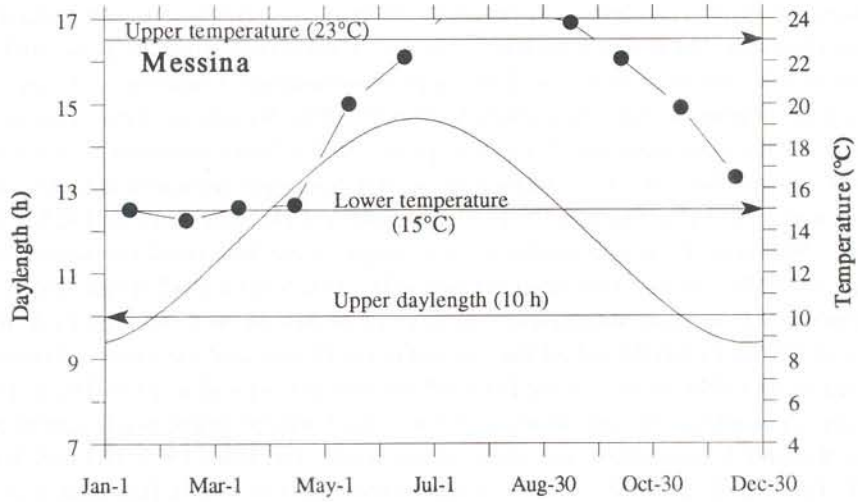


Fig. 5. Temperature (–●–) and daylength curves for Messina Harbour, Sicily. Temperature data are monthly maxima for March 1976–March 1977 (De Domenico et al., 1979). Daylength curve (for a solar elevation of 0°) was calculated using a spreadsheet incorporating formulae given by Dring (1984).

of *A. armata* in the Straits of Gibraltar, but this seems to be contradicted by our results on the strain from Messina as induction would not be possible in spring or summer.

In Galway Bay (Fig. 6), seawater temperatures appear to be inhibitory (< 15 °C) from mid-September to mid-June, with open-water temperatures in the Bay only rising above 15 °C for a short period in July to September. Inductive daylengths are only present

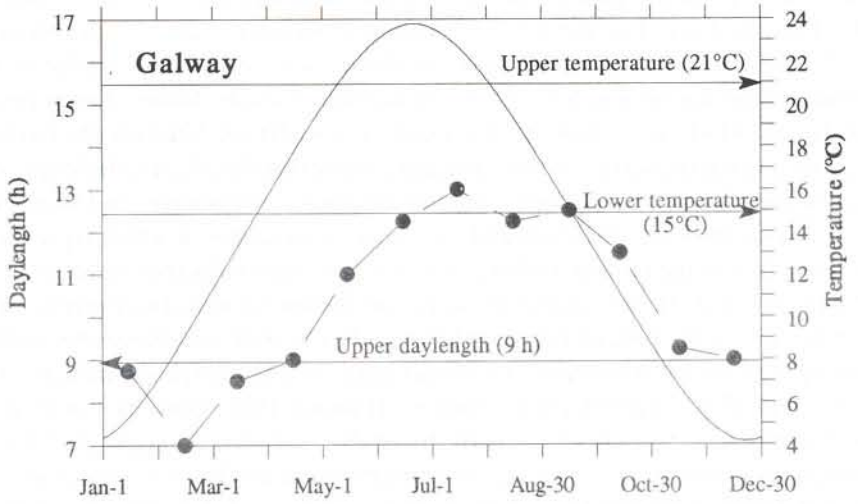


Fig. 6. Temperature (–●–) and daylength curves for Galway Bay, Ireland. Temperature data are maxima for 1987 at Shellfish Research Laboratory, Carna, Co Galway (unpubl. data). Daylength curve (for a solar elevation of 0°) was calculated using a spreadsheet incorporating formulae given by Dring (1984).

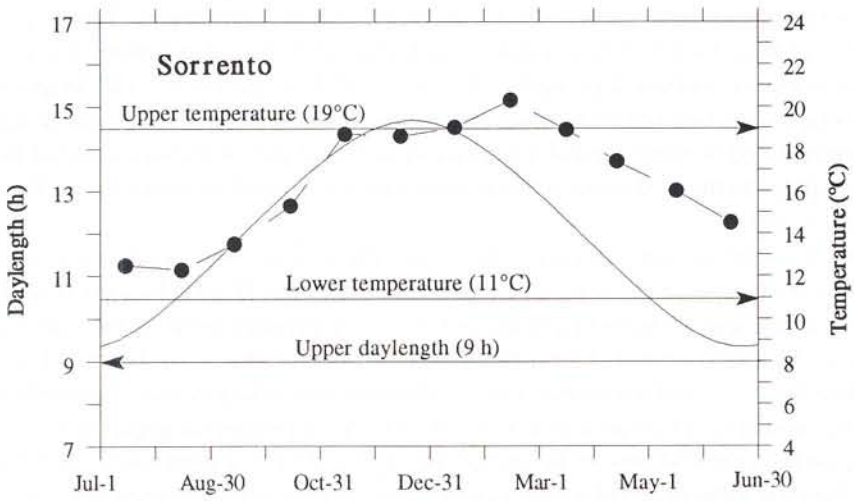


Fig. 7. Temperature (–●–) and daylength curves for Sorrento, Victoria, Australia. Temperature data are maximum values recorded by King (1970). Daylength curve (for a solar elevation of 0°) was calculated using a spreadsheet incorporating formulae given by Dring (1984).

from early November to mid-February, during which time open-water temperatures would be inhibitory. However, Breeman & Guiry (1989, Fig. 8) have shown that temperatures in the intertidal of Galway Bay are occasionally higher than those found in open water, due largely to emersion, particularly during mild autumns and periods of spring tides. At two sites on the southern shores of Galway Bay, these authors showed that the maximum in situ seawater temperatures during 1985 were $>15\,^{\circ}C$ in late September and early October. Daylengths at the water surface (Fig. 6) are still too long at this time (≈ 12 h); however, this daylength curve does not take account of the effect of light reduction in the water column at times of high water, particularly during spring tides. It has been shown that the effects of large tidal ranges, particularly in inshore waters, and of high waters of spring tides occurring at dawn and dusk, are sufficient to reduce the effective daylength perceived by the plants by at least an hour (Dring, 1987; Breeman & Guiry, 1989). Additionally, there may be further effects of position: plants in tidal pools with steep sides or in positions with elevated ground to the west or to the east would experience further reductions in effective daylengths. Certain habitats, due to aspect and topology, may also experience higher temperatures at low water. Thus, a combination of local daylength and temperature effects may explain the restricted distribution of *Asparagopsis armata* gametophytes on the west and south coasts of Ireland and on the south coast of England. Temperature may be the primary range-limiting factor with local tidal and topographical effects determining local distribution and the completion of the sexual life-history cycle. The more restricted distribution of *A. armata* gametophytes as compared to the gametophytes of *Bonnemaisonia hamifera* (Norton, 1985, Maps 78 and 79) is likely to result from a

narrower reproductive window for tetrasporogenesis in *Asparagopsis*. This is a consequence of three factors: lower temperature limits of 15 °C in *Asparagopsis* vs. 12 °C in *Bonnemaisonia*; critical daylengths of ≈9 vs. 11 h, respectively; and longer tetrasporangial induction requirements in *Asparagopsis*. These differences probably account for the reported northern limit of *Asparagopsis* gametophytes on the west coast of Ireland as compared to the west coast of Norway for gametophytes of *B. hamifera* (see Breeman & Guiry, 1989).

On the south-western coast of Australia, where *Asparagopsis armata* is a native species, the temperature curves in the area of Port Philip Heads, Victoria (Fig. 7) are such that the species would have the potential to reproduce most of the year if there were no daylength control of tetrasporogenesis. However, the curve shown in Fig. 7 for daylengths at the surface of the water at this latitude indicates that daylength never reaches inductive levels as it is never <9.25 h. As *Asparagopsis* gametophytes occur commonly on these shores in the austral spring, it is clear that conditions must at some time become inductive. Tidal and, perhaps, topographical effects probably come into operation in May and June (austral autumn). Decreasing the effective daylength by an hour at these times would result in inductive conditions (13–14 °C, 8.25 : $\overline{15.75}$ h).

All three strains examined in this study differ in their daylength and temperature requirements for tetrasporogenesis. Of the three strains, the Australian isolate is the most different, and it seems very unlikely that Australia was the origin of either the Irish or Sicilian strains, particularly as the south coast of Australia in one of the few coasts in the world where the coastline follows latitude rather than longitude, and it is probable that the daylength and temperature responses of *Asparagopsis* are very similar all along the south coast of Australia. Latitudinal ecotypes may not exist in Australia, except perhaps on the Indian Ocean coast. In reality, the temperature and daylength responses of the Australian strain are more suited to European coasts than the strains that presently occur there and *Asparagopsis* gametophytes would be more abundant and more widespread if it were an Australian strain similar to that from Victoria which had been introduced.

According to Bonin & Hawkes (1987), *A. armata* is common throughout New Zealand, except for the Kermadec Islands (30° S, 178° W) and, taking into account the latitudinal spread of the islands and the consequent potential occurrence of temperature and daylength ecotypes, this seems to be a more plausible origin for the European strains. However, Bonin & Hawkes (1987) reported that tetrasporangia were formed in a New Zealand strain incubated at 13 °C, $\overline{12}$: 12 h ("spring conditions"; see Hawkes, 1986). In northern New Zealand, tetrasporogenesis occurred in wild plants from March to September (Bonin & Hawkes, 1987), which is consistent with their experimental results. Bonin & Hawkes's results differ radically from those we obtained with our Australian strain in regard to the critical daylength for reproduction. A temperature of 13 °C is within the reproductive range of the Australian strain, but is clearly outside the ranges of the European strains. However, both the Australian and European strains do not reproduce at daylengths of >9 h. Nevertheless, given the

longitudinal spread of New Zealand (34–47° S), it is possible that latitudinal ecotypes exist there, but further investigations are necessary to establish this as a potential source of the European strains.

Santelices & Abbott (1978) and Santelices (1988) described *A. armata* as occurring in Chile from Iquique (20° S) to Coquimbo (30° S). Although these authors described the species as "tropical to subtropical", open-water temperatures on the Chilean coast at 20 °S vary from 16 °C in winter to 22 °C in summer. However, Chilean populations would have a longer critical daylength than European or Australian populations as the minimum daylength at 20° S in winter would be ≈ 11 h.

Apparently, therefore, none of the known distributional areas of native *Asparagopsis armata* in the Southern Hemisphere are readily identifiable as the origin of the European strains of this adventive species, except perhaps for the southern part of New Zealand. However, it is clear that the widely cited assumption of Australia as the origin of the European strains is not consistent with results presented here.

Although the Irish and Italian strains are more similar to each other in their temperature and daylength responses (Figs. 3, 4), there are sufficient differences to suggest that these strains resulted from separate introductions. If they were derived from a single introduction, one would expect that their physiological responses would not have had time to adapt to local conditions in the intervening 65 yr. That such physiological changes do not occur rapidly is supported by the fact that strains of the *Trailliella*-phase of *Bonnemaisonia hamifera* (also an introduced species) from Ireland and the Canary Isles had the same daylength and temperature responses in culture (Breeman & Guiry, unpubl. data). The possibility that the Mediterranean and Atlantic populations represent separate introductions lends some support to early speculations on the subject (e.g., Westbrook, 1930; Svedelius, 1933), but further studies of other strains from, for example, Biarritz (France), Spain, Algeria and the Mediterranean coast of France, are necessary.

In conclusion, tetrasporogenesis in Irish, Italian and Australian strains of the *Falkenbergia*-phase of *Asparagopsis* show clear responses to daylength and temperature. The Italian strain responds more rapidly and, perhaps, more completely than the Irish strain within roughly similar temperature limits (17–21 °C). The Australian strain shows a slower and less complete response, similar to the response time of the Irish strain but over quite a different temperature range (13–17 °C). Previously reported responses to nitrate and phosphate levels in enriched seawater media were confirmed, but only for the Irish strain, and there is evidence that arsenic and iodine supplements are necessary for reproduction in this strain. The effects of arsenic and iodine need to be separated and identified, and further studies of the responses to the various forms of arsenic found in aquatic environments (see Anderson & Bruland, 1991; Lee et al., 1991) would be of interest. The possible day-time temperature effects extrapolated here from previous work on *Bonnemaisonia hamifera* need to be verified experimentally, particularly as previous studies of temperature effects on photoperiodic responses in higher plants suggest that it is the night-time temperatures that are critical (Breeman et al., 1988).

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