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INDUCTION OF CONJUGATION AND SPORE FORMATION IN SPECIES OF SPIROGYRA (CHLOROPHYCEAE, ZYGNEMATALES)

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SUMMARY

Based on experiences of GROTE (1977) with *Spirogyra majuscula*, methods are described to induce the mating process and the formation of spores in several *Spirogyra* species.

Nitrogen depletion and light appear to be the key factors for the induction of spores in *Spirogyra* species. In the present study experiments in batch cultures were carried out with clonal material growing in defined medium or water from field localities. 31 *Spirogyra* species could be induced to spore formation which is about 40% of the total number of species as yet recorded in The Netherlands. The amount of spores produced showed considerable inter- and intraspecific variation. The influence of environmental factors like temperature and light intensity on the spore production was also studied in some species. For *Spirogyra hassallii* the combined effects of light intensity and temperature were studied with the aid of a temperature/light gradient plate. Ripened spores were formed under a broad range of temperature and light conditions.

The role of N-depletion and its ecological implications are discussed.

1. INTRODUCTION

For the identification of Zygnematalean algae information on the process of conjugation and spore formation is a prerequisite. As yet the filamentous Zygnematalean algae remain rather neglected in field studies as the species are mostly recorded in sterile state making identification impossible.

There are many reports in the literature on attempts to induce sexuality in *Spirogyra*. These have had varying degrees of success. Dilution of the culture medium often led to conjugation and it appeared that nitrogen depletion in particular played an important role. Attention was also given to light intensity, photoperiod, temperature, pH, and other environmental factors (BENECKE 1908; CZURDA 1933; ALLEN 1958; REICHART 1962; PESSONEY 1968; GROTE 1977; YAMASHITA & SASAKI 1979).

Based on experiences of CZURDA (1933) and REICHART (1962), GROTE (1977) developed a successful standardized method for induction of sexual reproduction in *Spirogyra majuscula*, for use in physiological studies of sexual differentiation of plant cells.

The paper at issue describes our experience in applying the method of GROTE (1977) to species other than *Spirogyra majuscula*. Some modifications on that method are presented.

We have also tested the well-known method of STARR (1964), developed for desmids on numerous *Spirogyra* strains.

The results presented provide a tool for achieving more success in field and laboratory studies on this widely and often abundantly occurring group of algae. Moreover, they may be useful for teaching purposes, as *Spirogyra* is often used in courses on plant morphology or phycology. (The strains of our collection are disposable.)

2. MATERIALS AND METHODS

2.1. Algal material and culture media

The clonal isolates used originated from various field localities in The Netherlands. The cultures were stored in tubes with Pringsheim's soil-water medium containing $CaCO_3$ (STARR 1964) at 4 °C and low light intensity at 8:$\overline{16}$.

As culture media were employed: for vigorous vegetative growth a modified Woods Hole medium (STEIN 1973) and for induction of mating a modified "Reichart/Grote" medium (GROTE 1977), which is devoid of a nitrogen source. The composition of these media is presented in *table 1*.

2.2. Induction of mating in the "Reichart/Grote" medium

Material to be induced was precultured in glass boxes with Woods Hole medium for about two weeks at 16 °C, photoperiod 12:$\overline{12}$, and light intensity 3000–4000 Lux. For illumination cool white fluorescent tubes (Philips TL 33) were used.

Table 1. Composition of culture media. The indications between brackets represent the original values.

Woods Hole	mg/l	Reichart/Grote	mg/l
$CaCl_2\ 2H_2O$	36.8	$CaSO_4\ 2H_2O$	50.0
$MgSO_4\ 7H_2O$	37.0	$MgSO_4\ 7H_2O$	10.0
$NaHCO_3$	100.0 (12.6)	$NaHCO_3$	1000.0 (1400)
K_2HPO_4	8.7	K_2HPO_4	20.0
$NaNO_3$	85.0		
$Na_2SiO_3\ 9H_2O$	– (28.4)		
Na_2EDTA	4.4	Na_2EDTA	25.0
$FeCl_3\ 6H_2O$	1.5 (3.1)	$FeSO_4\ 7H_2O$	10.0
$CuSO_4\ 5H_2O$	0.01	H_3BO_3	10.0
$ZnSO_4\ 7H_2O$	0.02	$ZnSO_4\ 7H_2O$	1.0
$CoCl_2\ 6H_2O$	0.01	MoO_3	5.0
$MnCl_2\ 4H_2O$	0.2	$MnCl_2\ 4H_2O$	5.0
$Na_2MoO_4\ 2H_20$	0.006		
Thiamine HCl	0.1		
Biotin	0.0005		
Cyanocobalamin	0.0005	Cyanocobalamin	0.04
HEPES	500.0 (TRIS 250)	HEPES	1000.0 (TRIS 9.6)

After preculturing until luxurious vegetative growth was reached, some material was transferred into glass boxes containing the "Reichart/Grote" mating medium at c. 21 °C, photoperiod 16:$\overline{8}$ (in some cases 12:$\overline{12}$, or continuous light), and 5000–8000 Lux. During the first three days after transfer the pH was adjusted to 7–8 by supplying mating medium acidified with HCl. After about 5 days the pH remained between 7 and 8. About two weeks later, spores could be detected if the trial was successful. Quantification of spore production was estimated by random viewing 15 optical fields (objective lens 16×) and counting total number of cells and spore-containing cells. The percentage of spores was determinded as:

$$\frac{2 \times \text{number of cells containing spores}}{(2 \times \text{number of cells containing spores}) + \text{vegetative cells}} \times 100$$

2.3. Induction of mating according to the "Starr" method

Precultured material was transferred into small open glass boxes (diameter 4.5 cm) containing soil-water medium or into autoclaved and 50% diluted water originating from field localities where *Spirogyra* species richly occur. Seven small boxes were placed together in one large glass box (diameter 19 cm) containing a 5% solution of $NaHCO_3$, under light intensities between 5000 and 8000 Lux. Under these conditions the pH remained between 7 and 8, and there was no need for adjustment. It appeared that the supply of the $NaHCO_3$ solution is not a prerequisite for success. Often induction was achieved by transferring precultured material into small glass boxes under high light intensity and with diluted water from a field locality which contained 1–2 mg/l NO_3^-.

2.4. Induction of mating in different temperature/light combinations

In some experiments induction was carried out in a modified version of Edwards and van Baalen's light-temperature gradient plate (Edwards & Van Baalen 1970). A strain of *Spirogyra hassallii* was used orginating from a pasture ditch close to Nijmegen. The material, precultured in Woods Hole medium at 12 °C, was transferred to petri-dishes (∅ 9 cm) containing water from a ditch near Amsterdam in which several *Spirogyra* species are a main component of the algal vegetation. The ditch water contained 0.7 mg N/l. Photoperiod was 14:$\overline{10}$. After 14 days spore production was quantified in 36 combinations of light and temperature.

2.5. Variations in culture conditions and supply of CO_2 or saccharose

In attempts to enhance spore production or to try other induction conditions, experiments were made to investigate the effects of (1) CO_2 (1–5%) supply; (2) supply of 0.2–1% saccharose in the mating medium; (3) drying up on agar plates; (4) supply of red light (Philips TL 40 W/15); (5) starvation in Woods Hole medium; (6) continuous supply of mating medium using a peristaltic pump; (7) keep-

Table 2. List of species which were induced to spore formation. The nomenclature is as in the flora of KADLUBOWSKA (1972). In the species marked with an asterisk spontaneous mating was observed.

Spirogyra cleveana Transeau	*S. laxa Kütz.
S. colligata Hodgetts	S. majuscula Kütz.
S. denticulata Transeau	S. maxima (Hass.) Wittrock
S. distenta Transeau	*S. mirabilis (Hass.) Kütz.
S. fennica Cedercreutz	S. neglecta (Hass.) Kütz.
S. fluviatilis Hilse	S. nitida (Dillwyn) Link
S. frankliniana Tiffany	S. pratensis Transeau
S. gibberosa Jao	*S. scrobiculata (Stockmeyer) Czurda
S. gracilis (Hass.) Kütz.	S. setiformis (Roth) Kütz.
*S. grevilleana (Hass.) Kütz.	*S. singularis Nordstedt
*S. hassallii (Jenner) Petit	S. pseudospreeiana Jao
*S. inflata (Vauch.) Kütz.	S. tenuissima (Hass.) Kütz.
S. insignis (Hass.) Kütz.	*S. teodoresci Transeau
*S. juergensii Kütz.	*S. varians (Hass.) Kütz.
S. kuusamoensis Hirn	*S. weberi Kütz.
S. lagerheimii Wittrock	

ing field material in filtered water from the locality; (8) using mating medium in which another species was induced to sexuality (assuming that inducing chemical substances were liberated).

3. RESULTS

3.1. List of species which could be induced to spore formation

In *table 2* a list is presented of species which could be induced to spore formation, with the "Reichart/Grote" and/or "Starr" method. The amount of spore production is not indicated, as the list presents a synopsis of a number of attempts which had varying degrees of success. At least one trial was made on each strain from a collection of about 300 isolates. When a species reached sexual stages with the Reichart/Grote method, it mostly did so in the modified "Starr" method, but there may have been differences in the amount of spores between the two methods. In one or more strains of the species indicated with an asterisk, spontaneous mating occurred in the full strength Woods Hole medium in the preculture conditions, following transfer from the cool storage condition.

We observed that artificial spore induction was successful in only about 20–30% of all attempts. It must be kept in mind that most strains were isolated in sterile state from various localities, hence it is impossible to know the actual number of species in the collection of 300 strains. The actual number will be far less than 300, presumably no more than 50–100. Those species which could be induced to sexuality were also rather frequently found with reproductive stages in field situations.

The positive results were obtained with clonal material. Hence the species presented in *table 2* are clearly homothallic. The possibility that heterothallic

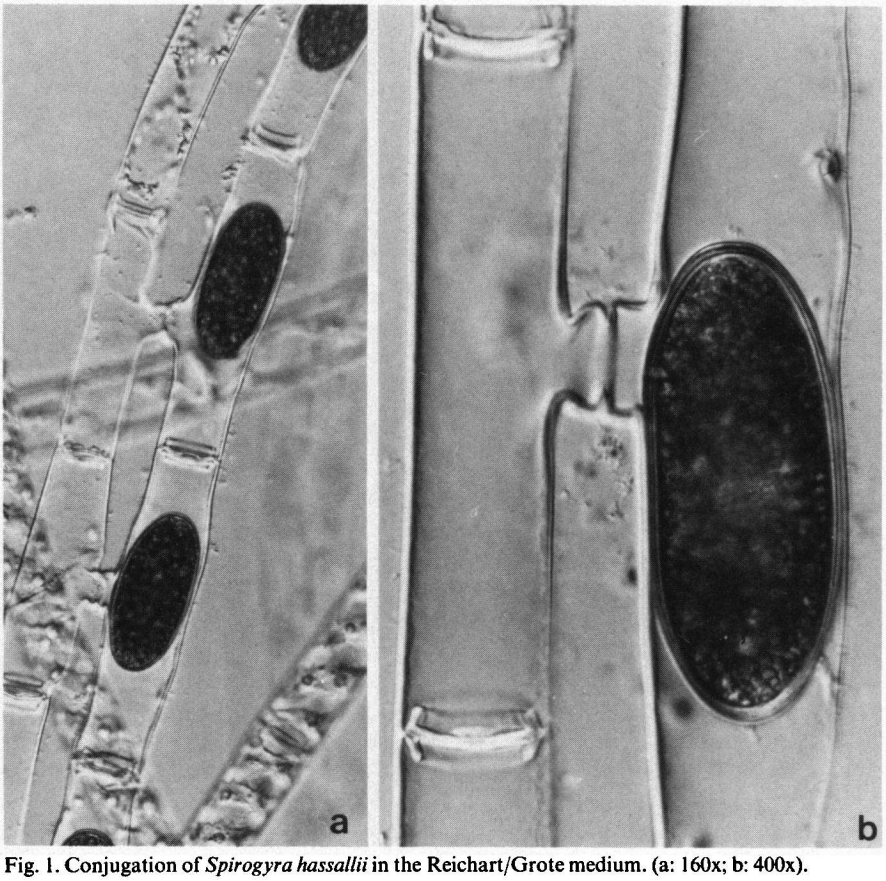

Fig. 1. Conjugation of *Spirogyra hassallii* in the Reichart/Grote medium. (a: 160x; b: 400x).

species occur cannot be excluded, which may partly explain the moderate success.

The morphology of reproductive structures and the form and measures of spores were mostly not aberrant from what is known from field material. Sometimes only conjugation canals were formed but no spores. One strain belonging to *Spirogyra hassallii* appeared to be easy induceable and in nearly all attempts a large number of ripened spores was formed (*fig. 1*).

3.2. Attemps to enhance spore production

A number of experiments was made to test several factors which appeared successful in other algae and according to other authors. From these experiments no clear pattern resulted. All methods used (see 2.5) yielded some results, except supply of saccharose and using mating medium in which another species was induced. At *Spirogyra majuscula* continuous supply of mating medium appeared to enhance spore production with more than 15% as compared with the standard method.

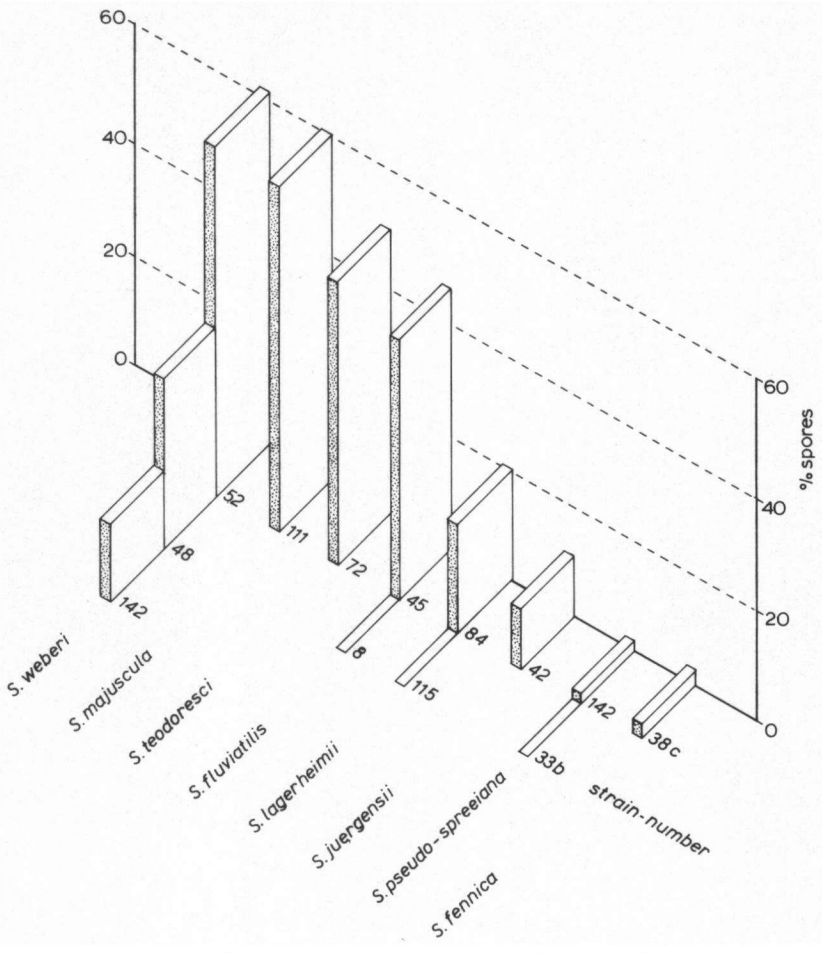

Fig. 2. Spore production in different *Spirogyra* species and in different strains of the same species. Culture conditions: Reichart/Grote medium, photoperiod 16:$\bar{8}$, 20 ± 2 °C, 5000 Lux.

3.3. Intra- and interspecific differences in amount of produced spores

In one series of experiments spore production was quantified in eight species. In four of the eight species two or three strains originating from different localities were used. The results are shown in *fig. 2*. From this figure it appears that the amount of spores shows considerable variation between species, and also within species. In this case the method used gave the best results with the species *Spirogyra weberi*, *S. majuscula*, *S. teodoresci*, and *S. fluviatilis*. Within *S. fluviatilis*, *S. lagerheimii* and *S. pseudospreeiana* one strain was successful, but the others not at all.

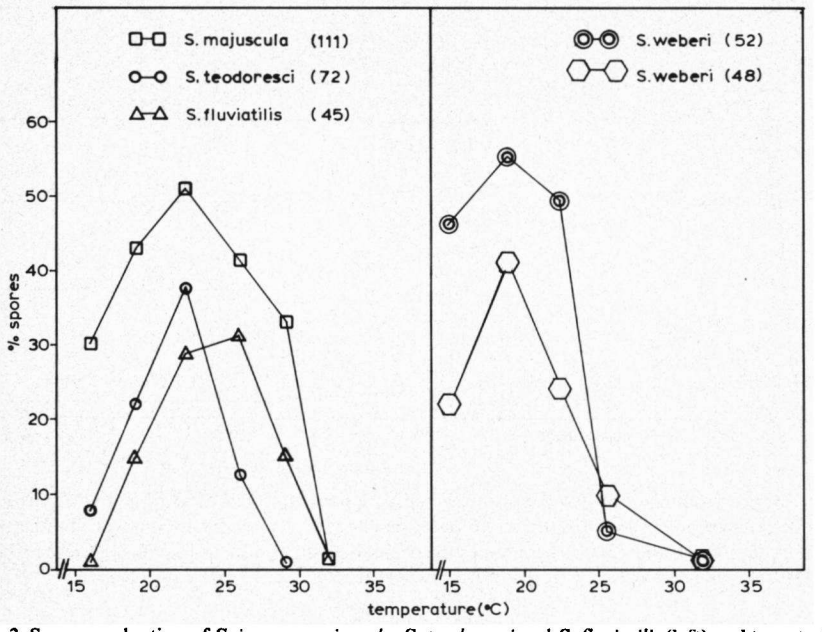

Fig. 3. Spore production of *Spirogyra majuscula*, *S. teodoresci* and *S. fluviatilis* (left) and two strains of *S. weberi* (right) at different temperatures. Culture conditions: Reichart/Grote medium, constant illumination of 8000 Lux.

3.4. Influence of temperature on the amount of spores

In nine strains belonging to seven species spore production was recorded at different temperatures.

It appeared that ripened spores were formed within a range from 15 to 32 °C. However, at 29 and 32 °C only in two cases (*S. majuscula* and *S. fluviatilis*) ripened spores were produced, while in most cases at these temperatures only conjugation tubes were formed.

For five strains belonging to *S. majuscula*, *S. teodoresci*, *S. fluviatilis* and *S. weberi* (two strains) the spore production was quantified within the above mentioned range of temperatures under the same conditions. The results are shown in *fig. 3*. From this experiment clearly defined optimum temperature conditions became apparent, which are situated at 22.5 °C for *S. majuscula* and *S. teodoresci* and at 25 °C for *S. fluviatilis*. For the two strains of *S. weberi* optimum temperature was situated at 20 °C. In all cases spore production stopped at about 30 °C.

3.5 Influence of light intensity on the amount of spores

In five strains belonging to four species (*S. weberi*, *S. teodoresci*, *S. fluviatilis*, and *S. majuscula*) spore production was recorded at different light intensities. Light intensities tested varied between 0 and 27700 Lux, under constant illumination and the optimal or nearly optimal temperatures (see 3.4.) of 20 °C for two strains of *S. weberi*, and 22.5 °C for *S. majuscula*, *S. teodoresci* and *S. fluviati-*

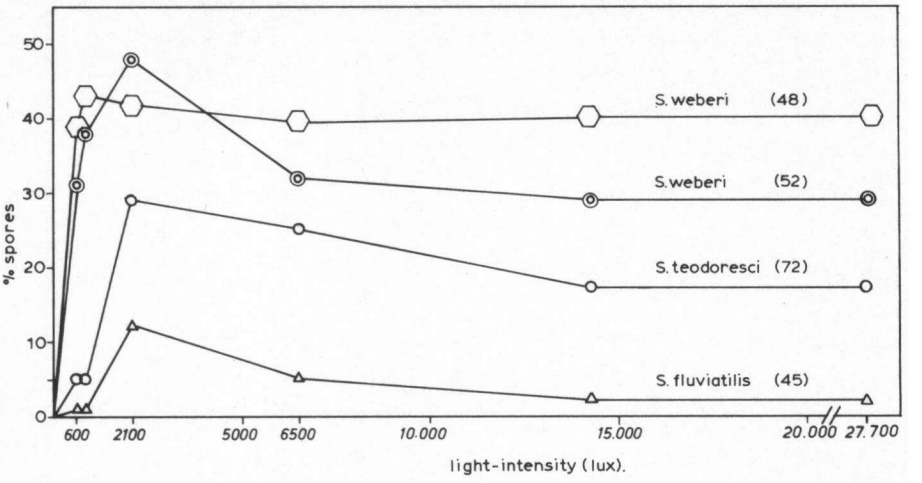

Fig. 4. Spore production of two strains of *Spirogyra weberi* and of *S. teodoresci* and *S. fluviatilis* at different light intensities. Culture conditions: Reichart/Grote medium, constant illumination. *S. weberi* (strains 48 and 52) was kept under the optimum temperature of 19.5 °C for spore production, *S. teodoresci* (72) at 22.5 °C and *S. fluviatilis* (45) at 25 °C.

lis. Conjugation and formation of ripened spores took place under all tested light intensities.

In order to estimate optimal light intensities, in four strains belonging to *S. weberi* (two strains), *S. teodoresci* and *S. fluviatilis* spore production was quantified at the above mentioned light intensities and other conditions. The results are shown in *fig. 4*. For three strains optimal light intensities were situated at 2100 Lux. For one strain of *S. weberi* optimal intensity was situated at 850 Lux. At light intensities above 2100 Lux a slight decrease of spore production was observed. These results clearly show that a certain amount of light is needed for spore production. The decrease of spore production at light intensities above 2100 Lux may be explained by the increase of the pH to values above 8 at the higher light intensities in the batch cultures.

3.6. Investigation into the combined effects of temperature and light intensity in Spirogyra hassallii

A strain of *S. hassallii* was tested on conjugation and spore production in the light-temperature gradient apparatus. (For culture conditions see 2.4.). The result is shown in *fig. 5*.

Spore production in *S. hassallii* appeared to occur between 10 and 30 °C, and under light intensities from 105–3700 Lux. Optimum spore production (values from 12–38%) occurred between 10 and 25 °C, and under light intensities from 235–3700 Lux. Under temperature and light conditions outside the optimum range (suboptimum in *fig. 5*), spore production varied between 0.4 and 12%.

The formation of conjugation canals, without spore production, occurred at 5 ° and at 30 °C under nearly all light intensities applied.

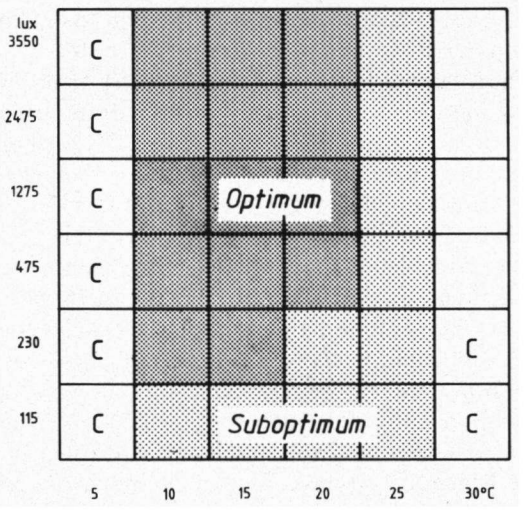

Fig. 5. Spore production in *Spirogyra hassallii* under 36 combinations of light intensity and temperature; in the optimum region spore production varied between 12 and 38%, in the suboptimum region between 0.4 and 12%. C means that only conjugation canals were formed and no spores. The data are a combination of two identical experiments.

4. DISCUSSION

4.1 The meaning of N-depletion and light as primary factors

The results confirm the essential role of N-depletion in induction of conjugation. The effect of N-depletion on sexual reproduction is known for some other freshwater green algae, such as *Chlamydomonas* (SAGER & GRANICK 1954), *Chlorococcum* (O'KELLY 1983), *Pandorina* (RAYBURN, 1974), *Oedogonium* (HILL & MACHLIS 1970, and Desmids (BIEBEL 1964; BIEBEL & CHAMBERLAIN 1970).

The physiological basis of the phenomenon has not yet been understood. UENO & SASAKI (1978) and HOGETSU & YOKOYAMA (1979), stated that light and N-depletion effect the differentiation of vegetative into gametangial cells in *Closterium* species. The combined effect of light and N-depletion would result in the accumulation of starch in gametangial cells. SAGER & GRANICK (1954), also stress the combined effect of light and N-depletion for induction of sexuality in *Chlamydomonas reinhardi*. They postulate that the role of light in gametic differentiation is indirect, providing through photosynthesis energy for the mating process and carbohydrates to tie up excess nitrogenous reserves, and that the concentration of some particular nitrogen fraction or compound determines whether or not gametic differentiation is initiated. Recently O'KELLY (1983), stated that the nitrogen fraction postulated by Sager and Granick is glutamine. A low nitrogen level in the medium would enhance the level of the enzyme glutamine synthetase which may play a role in sexual differentiation of vegetative cells. This enzyme would only be activated when the nitrogen source is not gluta-

mine, as was found by O'Kelly who tested different N-sources in their effect on sexual induction.

The role of light in sexual induction would consist of providing energy for the mating process and the accumulation of carbohydrates in gametangial cells. The accumulation of carbohydrates will be promoted by the high concentration of HCO_3^- in the mating medium. Under conditions of N-depletion the accumulation of carbohydrates would result in a high C/N ratio in conjugating cells, as was shown in *Spirogyra* by YAMASHITA & SASAKI (1979).

Another aspect of N-depletion may be its positive relation with the formation of secundary carotenoids, as was stated by KESSLER (1976) in *Chlorella* species. We assume a link between accumulation of secundary carotenoids and the ability to synthesize sporopollenin, as sporopollenin is built up by carotenoids and/or carotenoid esters (SHAW 1971). Sporopollenin was shown to be a constituent in the cell wall of *Chorella* by ATKINSON et al. (1972), and we (DE VRIES et al. 1983) have shown that sporopollenin is a main constituent in the spore wall of *Spirogyra*. The brown or yellow colour of *Spirogyra* spores also points to the presence of carotenoids.

4.2. The influence of other factors on the mating process

Factors such as pH, light intensity, day length, temperature, CO_2 supply, and others may affect the mating process. However, from data from literature and also from our own data, no clear statement on the how and why of observed influences can be made. Each algal group, species and even individual organism may react in its own way. As we have shown, like SCALIONE & HOSHAW (1980) in *Sirogonium* (*Zygnemataceae*), there may be large and constant differences in sexual response between species and between different strains of the same species, presumably reflecting genetic differences in sexual behaviour.

In *table 3* a summary is given of the mating conditions in *Spirogyra*. These

Table 3. Mating conditions of *Spirogyra* in batch cultures.

pH	7–9
temperature	10–25 °C
light intensity	105–27000 Lux
day length	neutral or long day
nutritional substances	N-depletion (0–1 mg/l)

results are in agreement with the sparse earlier records of successful induction experiments in *Spirogyra* (CZURDA 1933; GROTE 1977; ALLEN 1958; PESSONEY 1968).

The reason for the observed increase in spore production under continuous supply of mating medium, as we found in *Spirogyra majuscula*, may be that in this way the pH in the mating medium is kept more of less constant. Otherwise pH shows a tendency to increase to 9, which is beyond the optimum.

4.3. Ecological implications

During field work on the occurrence of *Spirogyra* species in The Netherlands, it appeared that the chance of finding reproductive structures is greatest in spring, especially in the second half of May and the first half of June. The mean air temperature in May is 12.4 °C, and in June 15.5 °C. In small water bodies such as pools and ditches in which many *Spirogyra* species occur, often in dense masses, temperatures in May/June may reach values of 20–25 °C while at the surface of algal mats temperatures of 30 °C or more are no exceptions. Moreover, in such situations large daily fluctuations occur, not only in temperature, but also in oxygen content and pH (HILLEBRAND 1983). These fluctuations are largest in the upper layer of the algal masses (in Dutch called "flab"), causing a vertical gradient in which differences of 10 °C and pH values between 7.5 and 9.9 were observed.

Combining these field observations with the data presented from batch cultures, we suppose that bad mating conditions prevail at the surface layer of algal mats and that mating induction takes place under relatively moderate conditions of temperature and irradiance. Such relatively moderate conditions will prevail in early stages of the formation of an algal mass and at some distance from the water surface. The recorded broad range of temperature and light conditions at which spores are formed, may be linked with the observed fluctuations within *Spirogyra* containing algal masses in the field.

Presumably mating will start after a period of strong vegetative growth, and especially in small stagnant water bodies the available nitrogen may rapidly be consumed by the quickly growing algal filaments causing a local condition of N-depletion within the algal mass which could induce the mating process.

PESSONEY (1968) observed relatively abundant conjugation of *Spirogyra* in temporary water bodies as compared with larger and less temporary situations. This is in accordance with observations in The Netherlands (to be published) where the chance to find *Spirogyra* species with sexual stages is greater in small and/or temporary water bodies like ditches and dune pools as compared to larger water bodies as peat lakes. Whether this phenomenon is connected with a predominance of the volume of the algal mass over the local volume of free water which will be reached more frequently in small than in large water bodies or with other conditions, needs further research.

Like Pessoney, we found that in other Zygnematacean algae such as *Zygnema* and *Mougeotia* species sexual stages are far less frequently found in nature than in *Spirogyra* species. Pessoney also states that artificial N-depletion appeared clearly less successful in *Zygnema* and *Mougeotia* than in *Spirogyra*. The few attempts with our methods on *Zygnema* and *Mougeotia* were also without success.

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