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Effect of Light Quality and Light Intensity and Various Sugars on the Sexual Expression and Some Observations on the Red Pigment in Equisetum Gametophytes

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EFFECT OF LIGHT QUALITY AND LIGHT INTENSITY AND VARIOUS SUGARS ON THE SEXUAL EXPRESSION AND SOME OBSERVATIONS ON THE RED PIGMENT IN EQUISETUM GAMETOPHYTES

BY

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ABSTRACT

The purposes of this study were to explore what effect light quality and light intensity and various sugars have on the sexual expression of Equisetum gametophytes and to determine some characteristics of the red pigment in Equisetum gametophytes.

Light quality and light intensity were employed to seek the presence of a morphogenetic factor involved in the sexual expression of Equisetum gametophytes. The gametophytes were grown in mass culture in petri dishes and singly in test tubes under red light and white light or under high light intensity and low light intensity. Light quality was employed when the Equisetum species was Equisetum hyemale. When the species was Equisetum arvense, light intensity was employed. A higher percentage of antheridial gametophytes in mass culture in petri dishes than singly in test tubes under either red light or high light intensity would indicate an interaction among the gametophytes due to a diffusible substance and thus would indicate that light quality or light intensity was probably involved in a mechanism which activated a morphogenetic factor determining the sex of Equisetum gametophytes. In mass culture in petri dishes, the morphogenetic factor activated by either red light or high light intensity would influence the sexual expression of other gametophytes in the same petri dish; whereas, singly in test tubes the gametophytes are isolated from each other so that any morphogenetic factor that is produced could not influence the sexual expression of the gametophytes. The factor would probably be some sort of diffusible substance comparable to the antheridogens of ferns. The results were ambiguous.

Mannitol, sucrose and glucose were added separately to Bold's basal

medium to determine what effect these sugars have on the sexual expression of Equisetum gametophytes. The results were ambiguous.

The red pigment often associated with Equisetum antheridial production was isolated by column chromatography and some characteristics were determined using visible light spectrophotometry. It is definitely not rhodoxanthin, as reported, but could not be identified other than to be a carotenoid.

This study showed that Equisetum gametophytes are a difficult system with which to experiment. It seems the sensitivity of Equisetum spores to light quality and light intensity can vary.

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INTRODUCTION

Equisetum, a vascular cryptogam, has a life cycle of two alternating generations, the sporophyte generation and the gametophyte generation. The sporophyte generation bears the strobili that shed the spores. The gametophyte generation, the sexual phase, bears the gametangia (archegonia and antheridia) which form the gametes (egg and sperm).

Uncertainty over the nature of sexuality in Equisetum gametophytes is seen in the morphology textbooks. Some authors (Bold, 1957; Campbell, 1913) have considered them to be unisexual. Other authors (Eames, 1936; Foster and Gifford, 1957; Haupt, 1953) have considered them bisexual. Hauke (1967) believes that Equisetum gametophytes have the potential to be bisexual, but most gametophytes are unisexual and those that are bisexual are postmature and were once unisexual. There is only one species of Equisetum which has bisexual gametophytes from the beginning (Hauke, 1963 and 1969).

Factors other than genetic seem to play an important role in the sexual expression in Equisetum gametophytes. Several authors (Campbell, 1913; Schratz, 1928; Walker, 1931; Eames, 1936; Williams, 1938; Haupt, 1953; Scott and Ingold, 1955; and Doyle, 1970) have reported crowded and unfavorable conditions increase antheridial formation in Equisetum gametophytes. Factors such as nutritional deficiencies, hormonelike substances, and the accumulation of waste products may influence sexual expression in Equisetum gametophytes.

It may be that a hormonelike substance may control sexual expression in Equisetum gametophytes. Already antheridogens that control the forma-

tion of antheridia have been found in fern gametophytes (Döpp, 1950; and 1959; Nøf, 1956, 1959, 1960 and 1961; Voeller, 1964). Because fern gametophytes are much like those of Equisetum in that they are both photosynthetic, dorsiventral and terrestrial, substances comparable to those in ferns may be operating in Equisetum.

Recently Hauke (1971) has shown that light quality affected sexual expression in Equisetum hyemale gametophytes and that light intensity affected the sexual expression in Equisetum arvense gametophytes. One purpose of this study was to further explore the effect of light quality on sexual expression in Equisetum hyemale gametophytes and of light intensity on Equisetum arvense gametophytes.

Hauke (1971) has shown that sucrose, a sugar, also affects the sexual expression in both of these species of Equisetum. Another purpose of this study was to determine what effect sugars have on these species of Equisetum gametophytes. Glucose, mannitol and sucrose were the sugars chosen for study. If the effect of these three sugars were the same, the effect would be assumed to be osmotic. The effect would be considered nutritional if mannitol showed no effect and the other sugars did. If only one of the sugars produced an effect, this would indicate some special morphogenetic effect.

A red pigment has been observed in Equisetum gametophytes (Eames, 1936; Chatterjee and Ram, 1968). The red pigment is seen under the microscope in chromoplasts in cells adjacent to antheridia, so that it seems the red pigment is related to the sexual expression of Equisetum gametophytes. The final purpose of this study was to determine some characteristics of this red pigment.

METHODS AND MATERIALS

Spores and inoculation of cultures. Equisetum gametophytes were grown from spores on Bold's basal medium agar (BBMA) prepared from stock solutions (Bold, 1967). Sucrose, mannitol and glucose were added separately to BBMA in 0.5 per cent (w:v) concentrations. In employing axenic conditions, the nutrient media, the glassware and the tools were sterilized in the autoclave for twenty minutes at 17 lbs. The spores of Equisetum hyemale were obtained from strobili of fertile shoots growing in a marsh in Galilee, Rhode Island. Equisetum arvense spores were obtained from strobili of fertile shoots growing alongside Fortin Road in Kingston, Rhode Island. Fresh spores were used whenever possible, but when they were not available, strobili stored under low temperature were used. Closed strobili were surface sterilized in 50% clorox solution for 5 minutes. The strobili were first rinsed three times in sterile water with 1% Tween 80 before they were soaked in the 50% clorox solution with 1% Tween 80 for five minutes. The strobili were again rinsed three times in sterile distilled water with 1% Tween 80. Surface sterilized strobili were stored in sterile glycerol at -10 C, if preserved. A spore suspension was prepared by dissecting open the strobili and adding about 20 ml of sterile distilled water.

Equisetum gametophytes were grown in sterile disposable plastic petri dishes and in sterile glass test tubes. The petri dishes were inoculated with a few drops of spore suspension, evenly distributed by small pipettes. Single germinating spores were introduced into the test tubes aseptically from the petri dishes. The inoculated petri dishes were

kept in white enamel trays 22x38x6 cm. The test tubes, each with a single germinating spore, were stored in test tube racks.

Growth and analysis of gametophytes. The Equisetum gametophytes were grown in two growth chambers. The first chamber contained a combination of eight 40-watt Cool White (Westinghouse) fluorescent tubes and four 25-watt incandescent bulbs. The second chamber contained six fluorescent tubes (Sylvania F48T12-CW-VHO) and four 25-watt incandescent bulbs. The chambers had an alternating period of 12 hours of light and 12 hours of darkness with temperatures of 25.5 and 15.5 C respectively. The light intensity under which the gametophytes were grown was measured with a YSI-Kettering model 65 radiometer (Yellow Springs Instrument Co., Inc.).

Cheesecloth was used to regulate light intensity but did not affect light quality. Two layers of cellophane "K" 210 FC Red from DuPont were used to produce the effect of red light on antheridial gametophytes. Two layers of red cellophane transmitted 1.6% of the light at 380 nm, 1.2% at 400 nm, 0.36% at 420 nm, 0.16% at 440 nm, 0.04% at 460 nm, 0.01% at 480 nm, 0.01% at 500 nm, 0.01% at 520 nm, 0.04% at 540 nm, 0.16% at 560 nm, 1% and 580 nm, 12% at 600 nm, 44% at 620 nm, 69% at 640 nm, 74% at 660 nm, 76% at 680 nm, and 76% at 700 nm. The data supplied by DuPont was for the use of one layer of red cellophane and was readjusted to take care of two layers of red cellophane.

The gametophytes were allowed to grow about six weeks before they were harvested to determine the percentage of antheridial gametophytes that formed. The percentage of archegonial gametophytes formed was not determined because of the difficulty in observing archegonia in Equisetum gametophytes. Antheridial gametophytes were however identified without difficulty by observing antheridia under a dissecting microscope.

Whenever there was any doubt, the gametophyte was observed under a microscope using 100X magnification to eliminate any uncertainty. Those gametophytes that were not identified as antheridial gametophytes were considered either archegonial or neutral.

Pigment studies. In identifying the red pigment, the pigmented gametophytes were ground up in a Waring blender in 100% acetone. After the homogenate was filtered through a sintered glass funnel, the filtrate was diluted with light petroleum ether (bp. 30 to 60 C) and washed free of acetone in a separatory funnel. The filtrate was stored over Na_2SO_4 for one hour and evaporated to dryness in a flash evaporator. The residue was taken up in 3 ml of light petroleum ether and chromatogrammed on a column of pure cellulose. The chromatography was carried out in a dark cool room. A developing solvent of light petroleum ether and acetone (4:1, v:v) was used to elute the red band. The eluate containing the red pigment was treated in exactly the same manner as the original acetone homogenate and the resulting residue was taken up again in 3 ml of light petroleum ether and chromatogrammed as before, but using a column of aluminum oxide (activity of 2). The red band was eluted and the eluate was treated again in exactly the same manner as the original acetone homogenate and the dried purified residue was taken up again in 3 ml of light petroleum ether. An absorption curve was determined between 300 nm and 700 nm using a Cary recording model 15 spectrophotometer. This procedure is an adaptation from that of Jagels (1970) and Foppen (1969). A chemical test was performed to determine if the red pigment could be reduced by $NaBH_4$ as rhodoxanthin can be. The red pigment in light petroleum ether was stored over a few grains of $NaBH_4$

for four hours, after which an absorption spectrum in the visible light range was taken of the red pigment in light petroleum ether to determine if $NaBH_4$ affected the absorption spectrum of the red pigment. A change in the absorption spectrum of the red pigment in light petroleum ether would indicate that $NaBH_4$ had reduced the red pigment. A physical test was performed also to determine if the red pigment had the same partition coefficient value as rhodoxanthin in light petroleum ether and 95% methanol. The test consisted of determining to which layer, light petroleum ether or 95% methanol, the red pigment had a greater affinity.

The red pigment in chromoplasts of overwintering stems of Equisetum hyemale and in young vegetative stems of Equisetum arvense was also extracted and tested in the same way.

Statistics. Clopper and Pearson's 95% confidence belts were employed to determine if significant differences occurred between percentage numbers obtained in each of the experiments. A 95% confidence belt is the range of percentage numbers within which the true percentage number may be found with 95% confidence. Clopper and Pearson's 95% confidence belts are used in determining the significance of percentage figures. A 95% confidence belt is based upon the percentage number and the total number of gametophytes used to calculate the percentage number. If the confidence belts of two percentage figures did not overlap, the difference between the two percentage figures were said to be significant. If on the other hand the confidence belts did overlap, the differences were said not to be significant.

RESULTS

Effect of red light on sexual expression in Equisetum hyemale gametophytes. The results of the experiments on the effect of red light on the sexual expression in Equisetum hyemale gametophytes are recorded in Table 1. In the experiments with isolated gametophytes, the results did not show any significant differences in the percentage of antheridial gametophytes formed singly in test tubes under red light and under white light using Clopper and Pearson's 95% confidence belts (Clopper and Pearson, 1934). In the fourth, fifth and sixth sets of experiments in which Equisetum gametophytes were grown in both petri dishes and test tubes, the results did not show any significant differences in the percentage of antheridial gametophytes formed in mass culture in petri dishes and singly in test tubes under red light. It was expected that there would be a higher percentage of antheridial gametophytes in mass culture than singly in test tubes. One reason the results did not show any significant differences might be that too few gametophytes were counted singly in test tubes for each set of experiments to determine differences. Finally in the third and fifth sets of experiments the results showed a higher percentage of antheridial gametophytes in mass culture under red light than under white light, but in the fourth and sixth sets of experiments, the results did not show such significant differences. However, contamination might have affected the fourth experiment and using spores obtained in the field in the middle of the winter after the strobili had become frozen might have affected the results of the sixth experiment.

Effect of light intensity on sexual expression in Equisetum arvense gametophytes. The results (Table 2) of the experiments with the effect of light intensity on the sexual expression in Equisetum arvense gametophytes did not show any significant differences in the percentage of antheridial gametophytes formed in mass culture in petri dishes and singly in test tubes under either high or low light intensities. Conversely there were no significant differences under high light intensity and low light intensity in mass culture in petri dishes or singly in test tubes.

Effect of various sugars on the sexual expression in Equisetum hyemale gametophytes. The data from six sets of experiments to determine the effect of various sugars on sexual expression of Equisetum hyemale gametophytes are presented in Table 3. The first set of experiments showed that sucrose and glucose had no effect on the formation of antheridial gametophytes but that mannitol stimulated antheridial production. In the second set of experiments the results showed mannitol inhibited antheridial gametophyte development, but glucose had no effect. The results from the third set of experiments showed glucose and sucrose increased the formation of antheridial gametophytes, but mannitol had no effect. The fourth set of experiments showed that glucose had no significant effect on the formation of antheridial gametophytes. In the fifth set of experiments the results showed that glucose, sucrose and mannitol all increased antheridial gametophyte formation. Finally the results from the last set of experiments showed that mannitol inhibited the formation of antheridial gametophytes, but glucose had no effect. It must be noted that contamination might have been a factor in these results,

since it is difficult to maintain axenic cultures in the growth chambers over long periods of time (5 to 6 weeks) and many cultures were lost completely to fungi.

Effect of various sugars on sexual expression in Equisetum arvense gametophytes. In the experiments determining the effect of various sugars on the sexual expression in Equisetum arvense gametophytes, the data (Table 4) showed that glucose definitely increased the formation of antheridial gametophytes under both high light intensity and low light intensity. Mannitol increased the formation of antheridial gametophytes under high light intensity but not under low light intensity. Finally sucrose appeared to have no effect on antheridial formation under either high light intensity or low light intensity.

Characteristics of the red pigment isolated from Equisetum. It was determined that the red pigments isolated from Equisetum hyemale gametophytes and from both Equisetum hyemale and Equisetum arvense sporophytes had the same visible light absorption spectrum in light petroleum ether. Red pigment had an absorption spectrum with peaks at 452, 475 and 505 nm. The partition coefficient measured in light petroleum ether and 95% methanol gave a value of 0:100 for this red pigment. Also the red pigment was shown not to be reduced by $NaBH_4$.

Table 1. Effect of red light on the sexual expression in Equisetum hyemale gametophytes.

Experiment Number	Glassware	Color of Light[a]	Intensity of light, ergs/ cm^2/sec	Number of gametophytes[b]	Number of antheridium gametophytes	Percent antheridium gametophytes	95% confidence belt[c]
1	Test tubes	White	5,000	19	9	47	24-70
1	Test tubes	Red	5,000	23	8	35	15-60
2	Test tubes	White	5,000	7	3	43	12-77
2	Test tubes	Red	5,000	11	4	36	9-68
3	Petri dishes	White	5,000	152	11	7	4-13
3	Petri dishes	Red	5,000	198	43	22	14-28
4	Petri dishes	White	20,000	50	5	10	3-23
4	Petri dishes	Red	20,000	40	10	25	13-43
4	Test tubes	White	20,000	10	4	40	12-75
4	Test tubes	Red	20,000	5	1	20	2-60

Table 1. (cont.) Effect of red light on the sexual expression in Equisetum hyemale gametophytes.

Experiment Number	Glassware	Color of light[a]	Intensity of light, ergs/ cm^2/sec	Number of gameto-phytes[b]	Number of antheridium gametophytes	Percent antheridium gametophytes	95% confidence belt[c]
5	Petri dishes	White	5,000	272	19	7	4-13
5	Petri dishes	Red	5,000	102	41	40	30-51
5	Test tubes	White	5,000	26	1	4	0-19
5	Test tubes	Red	5,000	12	1	8	0-44
6	Petri dishes	White	15,000	156	32	21	14-28
6	Petri dishes	Red	15,000	101	23	23	14-33
6	Test tubes	White	15,000	14	3	21	4-50
6	Test tubes	Red	15,000	5	2	40	15-85

[a]Red light was not monochromatic but filtered through red cellophane.

[b]Each datum for petri dishes represent the sum of three petri dishes.

[c]From a table by Clopper and Pearson, 1934.

Table 2. Effect of light intensity on the sexual expression in Equisetum arvense gametophytes.

Glassware	Intensity of light, ergs/cm^2/sec	Number of gametophytes[a]	Number antheridial gametophytes	Percent antheridial gametophytes	95% confidence belt[b]
Petri dishes	5,000	300	20	7	4-10
Petri dishes	20,000	400	34	9	6-12
Test tubes	5,000	51	4	8	3-20
Test tubes	20,000	44	3	7	2-18

[a]Each datum for petri dishes represents the sum of 3 petri dishes

[b]From a table by Clopper and Pearson, 1934.

Table 3. Effect of various sugars on the sexual expression in Equisetum hyemale gametophytes.

Experiment Number	Sugar[a]	Intensity of light, ergs/ cm^2/sec	Petri dishes sampled	Number of gameto-phytes	Number of antheridium gametophytes	Percent antheridium gametophytes	95% confidence belt[b]
1	No sugar	1,500	3	415	129	31	25-35
1	Glucose	1,500	2	78	30	38	27-50
1	Mannitol	1,500	1	67	19	67	54-78
1	Sucrose	1,500	1	66	21	32	21-46
2	No sugar	1,500	3	230	84	37	31-44
2	Glucose	1,500	2	173	49	28	21-36
2	Mannitol	1,500	1	56	6	11	4-24
2	Sucrose	1,500	c	---	---	---	---
3	No sugar	5,000	3	171	11	6	3-11
3	Glucose	5,000	1	27	9	33	17-53
3	Mannitol	5,000	2	209	20	10	6-17
3	Sucrose	5,000	1	72	22	31	20-43

Table 3. (cont.) Effect of various sugars on the sexual expression in Equisetum hyemale gametophytes.

Experiment Number	Sugar[a]	Intensity of light, ergs/ cm^2/sec	Petri dishes sampled	Number of gameto-phytes	Number of antheridium gametophytes	Percent antheridium gametophytes	95% confidence belt[b]
4	No sugar	20,000	1	18	2	11	2-34
4	Glucose	20,000	2	219	86	39	31-48
4	Mannitol	20,000	c	---	---	---	---
4	Sucrose	20,000	c	---	---	---	---
5	No sugar	5,000	3	255	23	9	6-14
5	Glucose	5,000	4	797	35	41	37-44
5	Mannitol	5,000	4	668	290	43	36-49
5	Sucrose	5,000	2	133	75	56	44-65
6	No sugar	15,000	3	156	32	21	15-29
6	Glucose	15,000	1	24	2	8	0-30
6	Mannitol	15,000	2	141	7	5	3-14
6	Sucrose	15,000	c	---	---	---	---

[a]0.5% (w:v) concentration

[b]From a table by Clopper and Pearson, 1934.

[c]Petri dishes were lost by contamination.

Table 4. Effect of various sugars on the sexual expression in Equisetum arvense gametophytes.

Sugar[a]	Intensity of light, ergs/ cm^2/sec	Number of gametophytes[b]	Number of antheridium gametophytes	Percent antheridium gametophytes	95% confidence belt[c]
No sugar	5,000	405	93	23	20-27
No sugar	20,000	554	180	32	27-37
Glucose	5,000	431	357	83	78-86
Glucose	20,000	503	440	87	84-91
Mannitol	5,000	422	94	22	18-26
Mannitol	20,000	830	380	46	40-50
Sucrose	5,000	633	128	20	16-25
Sucrose	20,000	701	179	26	23-29

[a]0.5% (w:v) concentration.

[b]Each datum represent the sum of 3 to 4 petri dishes.

[c]From a table by Clopper and Pearson, 1934.

DISCUSSION

Effect of red light on sexual expression in Equisetum hyemale gametophytes. After Hauke (1971) had shown that red light increased the formation of antheridial gametophytes in Equisetum hyemale, the author had hoped to show from further experiments that red light was involved in a mechanism that activated a morphogenetic factor which controlled the formation of antheridial gametophytes in Equisetum and that the morphogenetic factor was a diffusible substance comparable to antheridogens in ferns. If red light activated such a morphogenetic factor comparable to the diffusible substances of antheridogens in ferns, the results would have to show (1) a higher percentage of antheridial gametophytes in mass culture in petri dishes under red light than under white light, (2) a higher percentage of antheridial gametophytes in mass culture in petri dishes than singly in test tubes under red light, and (3) no differences in the percentage of antheridial gametophytes formed singly in test tubes under red and white lights.

Red light would have increased the formation of antheridial gametophytes in mass culture and not singly in test tubes because in mass culture the morphogenetic factor activated by red light would influence the sexual expression of other gametophytes in the same petri dish, assuming it is diffusible like antheridogens. In test tubes the gametophytes are isolated from each other so that any morphogenetic factor that is activated by red light could not possibly influence the sexual expression of other Equisetum gametophytes.

The result did show the percentage of antheridial gametophytes

formed singly in test tubes under red light were the same as those formed singly in test tubes under white light. In experiments three and five (Table 1), the mass cultures under red light did have a higher percentage of antheridial gametophytes than those under white light. However experiments four and six did not show this difference between red and white light in mass culture. The lack of statistically significant differences between sexuality of gametophytes in mass culture under red light and single gametophytes under red light is probably only a reflection of the small sample size for test tube cultures. Although light intensity varied between sets of experiments, light intensity was not expected to affect the results because Hauke (1971) has shown that light intensity was not a factor in the sexual expression in Equisetum hyemale gametophytes. Contamination might have affected the results in the fourth set of experiments and using spores obtained in freezing temperatures during the winter might have affected the results in the sixth set of experiments. Duckett (1970) reported that Equisetum telmatia spores stored at 4 C for sixty days produced a higher percentage of antheridial gametophytes. Freezing Equisetum hyemale spores might have affected the sensitivity of these spores to red light.

The results were too ambiguous to either confirm or deny the expectation that red light activated a morphogenetic factor which controlled the sexual expression of Equisetum hyemale gametophytes. These experiments should have been repeated but were not because there were no longer any more viable strobili of Equisetum hyemale. In future experiments light intensity should be kept constant and only fresh viable

strobili should be used.

Effect of light intensity on sexual expression of Equisetum arvense gametophytes. In the experiments testing the effect of light intensity on the sexual expression in Equisetum arvense gametophytes, the author had intended to show that a morphogenetic factor comparable to the diffusible substances of antheridogens in fern controlled the sexual expression in Equisetum gametophytes. Earlier the author had expected to show such a morphogenetic factor was operating in Equisetum hyemale gametophytes but did not reach any conclusions when the results proved to be inconsistant and unreliable. It was decided to use Equisetum arvense when there were no longer available viable spores from Equisetum hyemale. Light intensity and not light quality was employed to seek the morphogenetic factor because Hauke (1971) had shown light intensity and not light quality influenced the sexual expression in Equisetum arvense gametophytes. In order to seek such a morphogenetic factor, the results had to show (1) a higher percentage of antheridial gametophytes in mass culture in petri dishes under high light intensity than under low light intensity, (2) a higher percentage of antheridial gametophytes in mass culture in petri dishes than singly in test tubes under high light intensity, and (3) no differences in the percentage of antheridial gametophytes formed singly in test tubes under high light and low light intensities. In mass culture in petri dishes the morphogenetic factor activated by high light intensity in a gametophyte would influence the sexual expression of other gametophytes in the same petri dishes; whereas, singly in test tubes the gametophytes are isolated from each

other so that any morphogenetic factor that is activated in a gametophyte could not influence the sexual expression of other gametophytes.

The results showed very little difference in the percentage of antheridial gametophytes between those in mass culture in petri dishes and those grown singly in test tubes under either high or low light intensities. Unfortunately, there was also no difference between mass culture under high light intensity either. Therefore, no conclusions could be drawn about the presence or absence of a diffusible morphogenetic factor. It may be that since Hauke got his strobili as soon as they appeared above ground and the author got his later in the season, an aging factor may be affecting the sensitivity of Equisetum arvense spores. The author's spores may have been less sensitive than Hauke's spores to light intensity because they were postmature. It seems working with Equisetum arvense gametophytes it very difficult, because various unknown factors may be affecting the sensitivity of Equisetum arvense spores. These experiments were not repeated because viable spores of Equisetum arvense were no longer available. Usually the strobili of Equisetum arvense are available for only a month each year.

Effect of various sugars on the sexual expression in Equisetum hyemale gametophytes. In setting up the experiments, light intensity was not controlled between sets of experiments because Hauke (1971) had shown that light intensity was not a factor in the sexual expression of Equisetum hyemale gametophytes. The author thought light intensity would not affect the results determining the effect of various sugars on sexual expression of Equisetum hyemale gametophytes.

The results from each set of experiments proved to be completely different from each other if light intensity is neglected. Using spores stored at -10 C in glycerol and spores obtained in the freezing temperatures during the winter might have affected the physiology of Equisetum spores used in the fourth and sixth sets of experiments. Duckett (1970) reported that Equisetum telmatia stored at 4 C for sixty days produced a higher percentage of antheridial gametophytes. Contamination might have been a factor also.

If light intensity is considered in interpreting the results, one can see some sort of trend indicating an interaction of light intensity and the effect of various sugars on sexual expression in Equisetum hyemale gametophytes. Hauke (1971) did not interact various light intensities and various sugars. The possibility of such interaction exists. Glucose and sucrose showed no effect on the sexual expression of Equisetum hyemale gametophytes under 1,500 ergs/cm^2/sec but did under 5,000 ergs/cm^2/sec. The results from experiments using light intensities of 15,000 and 20,000 ergs/cm^2/sec were not interpreted because the spores used in these experiments were either stored at -10 C in glycerol or obtained outside in freezing temperatures during the winter which might have affected the physiology of the spores.

The results under 1,500 ergs/cm^2/sec suggested the effect of glucose and sucrose is not nutritional because neither sugar showed an effect. The effect of these two sugars under 5,000 ergs/cm^2/sec could not be determined to be either nutritional or osmotic because the effect of mannitol was inconsistant. In summary light intensity may have interacted with the various sugars or may have no effect, but at any rate the

author interpreted the results both ways.

These experiments would have been repeated until a conclusion had been obtained, but there were no longer available viable spores. In repeating these experiments, light intensity should be kept constant and the sugars should be added to the agar medium in equal molarity concentrations, rather than equal weight to volume concentrations.

Effect of various sugars on sexual expression in Equisetum arvense gametophytes. The author has more confidence in the results of this one set of experiments determining the effect of various sugars on the sexual expression in Equisetum arvense gametophytes than in the results from the six sets of experiments on the effect of various sugars on the sexual expression of Equisetum hyemale gametophytes because of the larger numbers of gametophytes counted in the Equisetum arvense experiments.

The results of various sugars on sexual expression in Equisetum arvense gametophytes showed that the effect of mannitol, which produced an effect under high light intensity, is osmotic and not nutritional because mannitol is not metabolizable by many plants, but sucrose, which produced no effect, is. It appears light intensity interacts with this osmotic effect of mannitol because mannitol showed the effect under high light intensity but not under low light intensity. The effect of glucose is at least partly osmotic because glucose is also, like mannitol, a monosaccharide and not like sucrose, a disaccharide, but the effect is only partly osmotic because glucose had a greater effect than mannitol and some other additional factor must be involved in the effect of glucose. Because glucose showed a much greater effect than mannitol,

this additional factor may be a direct morphogenetic one.

It is interesting to note that Wollersheim (1957) observed a higher percentage of archegonial gametophytes using Equisetum fluviatile with a medium supplemented with glucose, but the author observed instead a higher percentage of antheridial gametophytes using Equisetum arvense. It appears a different mechanism is involved in the effect of glucose on these two species of Equisetum.

These experiments were done only once because there were no longer available viable spores from Equisetum arvense. In further experiments sugars should be added to the nutrient medium in equal molarity concentrtions rather than equal weight to volume concentrations.

Red pigment isolated from Equisetum. The absorption spectrum of the red pigment in light petroleum ether from both Equisetum gametophytes and Equisetum sporophytes indicated that the pigment was a carotenoid but not rhodoxanthin. Rhodoxanthin in light petroleum ether has a different absorption spectrum than the red pigment in the same solvent. The red pigment has absorption peaks at 452, 475 and 505 nm in light petroleum ether and rhodoxanthin has peaks at 456, 487 and 521 nm in the same solvent (Karrer and Jucker, 1950). Lippmaa (1926a, b and c) had reported the red pigment to be rhodoxanthin in a few Equisetum sporophytes. He had apparently identified and named the pigment rhodoxanthin just by looking at a few drawings by Schimper (1895) of chromoplasts containing this red pigment in Equisetum telmatia sporophytes, for he gave no indication in his article of having extracted the pigment from Equisetum.

The red pigment cannot be considered another cis-trans form of rhodoxanthin because unlike rhodoxanthin the red pigment was not reduced

by $NaBH_4$. This red pigment also has a different partition coefficient value than rhodoxanthin in light petroleum and 95% methanol. The partition coefficient value for the red pigment was determined to be 0:100. Quackenbush (1965) has determined the partition value in hexane and 95% methanol to be 55:45 for rhodoxanthin.

The red pigment could not be identified for the author could not find a red pigment in the literature that had an absorption in light petroleum ether similar to the absorption spectrum of this red pigment in the same solvent. It is possible the red pigment had not yet been identified.

Nuclear magnetic resonance spectrum and infrared absorption spectrum would be the next steps to take in identifying the pigment, but because of the scope of the problem, amount of pure pigment required and equipment required, these steps were not taken.

In Equisetum, antheridial gametophytes are often pigmented red. Under the microscope the red pigment is found in chromoplasts in cells adjacent to the antheridia. It is possible the red pigment is related to the sexual development of Equisetum antheridial gametophytes.

CONCLUSIONS

This study showed that working with Equisetum gametophytes is difficult. However, this study did show that glucose promoted an increase in number of antheridial gametophytes in Equisetum arvense and that the red pigment observed in Equisetum is not rhodoxanthin as reported in the literature but some other red carotenoid not yet identified.

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