Journal of Ecological Research, **2**, 1-4 (2000)

EFFECTS OF TEMPERATURE AND THALLUS DENSITY ON SIZE AND SEX RATIO OF THE C-FERN

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ABSTRACT

The male to hermaphrodite ratio was greater at high than low thallus densities in the C-fern, *Ceratopteris richardii*. However, density did not have a significant effect on the thallus size of males or hermaphrodites. Males were significantly smaller than hermaphrodites at both densities tested. Spores germinated at 27° C, but not at 10° C.

Keywords: C-Fern, Ceratopteris richardii, *density-dependence, intraspecific competition, thallus size.*

INTRODUCTION

As population density increases, the rate of growth and average size of individuals can be adversely affected. Knowing and understanding these effects can help crop farmers, fisheries, and animal ranchers maximize their economic yield. In this experiment, C-Fern spores were placed in rooms of different temperatures and were plated in different densities so that the effects of density and temperature upon their thallus size and sex ratio could be studied.

C-Ferns are genetic strains derived from the tropical plant *Ceratopteris richardii,* which grows rapidly and is easy to culture in a limited amount of space (Carolina Biological Supply Company, 1999; Warne and Hickok, 1999). In this experiment, I tested three hypotheses. Hypothesis #1 was that individuals in less dense plates should grow to be larger and have a higher ratio of hermaphrodites to males than those in more dense plates. Hypothesis #2 was that C-Ferns in the colder environment should be smaller and have a lower hermaphrodite to male ratio. Hypothesis #3 was that density should have a greater effect than temperature on fern growth, such that ferns in low-density plates at low temperatures would be larger than ferns in high-density plates at high temperatures. The null hypothesis was that neither density nor temperature would have an effect on size and sex ratio of the C-Ferns.

In C-Ferns, the spores are genetically identical, but differentiate into hermaphrodites and males. Males are formed if the young plant receives a pheromone, antheridiogen, produced by older hermaphrodites (Scott and Hickok, 1987). Therefore, spores unaffected by pheromones will become hermaphrodites, but any plants that are exposed to high enough concentrations of antheridiogen will become male. Therefore, environmental factors such as density or temperature could have a great effect on the sex ratio, if the amount of antheridiogen the plants were exposed to was affected.

METHODS AND MATERIALS

Wild-type C-Fern spores were cultured in C-Fern medium (both from Carolina Biological Supply Company) placed in 20 watchglasses and 60x15 mm Petri dishes using sterile pipettes (following Warne and Hickok, 1999). The medium was melted in a hot water bath to facilitate pouring, which was carried out in a clear area with no drafts. Unfortunately, because of insufficient medium, the dishes were not filled very full, which may have caused some problems later.

Two vials of spores supplied by the Carolina Biological Supply Company were filled with water to the 2 mL mark, and a sterile pipette was used to drop two or four drops of the spore-water mixture onto the plates. Half of the 20 plates were given two drops and half were given four drops, with the plates chosen at random. The pipette was always tipped at about a 45° angle in an attempt to make drop size as consistent as possible. The plates were then spread with plastic spreaders so that the spores were more evenly distributed. The spreaders were sterilized between each spreading so that plates would not get extra spores from a previous plate's spreading. On average, the two-drop plates had 68.4 spores per plate and the four-drop plates had 278 spores per plate. Hence, the four-drop plates actually had, on average, four times as many spores as the two-drop plates. All of the plates were cultured for three weeks under light. Half of the two- and four-drop plates were in a warm room (~27° C) and the other half were in a cold room (~10° C).

All males and hermaphrodites (determined by thallus morphology, following Warne and Hickok, 1999) were counted for each plate. Male and hermaphrodite thallus lengths (± 1 μm) were determined by taking random samples, using a grid overlaying each plate and a random-number generator. Three plants of each sex were chosen per plate and were measured using a stage micrometer. Plants that were too firmly embedded in the medium to pull free and measure were omitted. The results were analyzed using chi-square tests (to compare sex ratios) and two-sample t-tests (to compare thallus sizes) (Minitab Inc, 1991).

RESULTS

None of the spores cultured at 10° C germinated. Therefore, data were available only for the plates cultured at 27° C.

A total of 115 males and 205 hermaphrodites were found on the two-drop plates. This sex ratio was significantly different from a 1:1 ratio ($\chi^2 = 12.912$, $P < 0.001$). A total of 492 males and 610 hermaphrodites were found on the four-drop plates. This sex ratio was also significantly different from a 1:1 ratio ($\chi^2 = 6.336$, $P = 0.012$), as well as from the sex ratio observed on the two-drop plates ($\chi^2 = 7.687$, $P = 0.006$).

For each sex, mean thallus size did not differ significantly with density treatment (males: $t = 0.83$, $P = 0.41$; hermaphrodites: $t = 0.66$, $P = 0.52$). However, hermaphrodites were significantly larger than males at both densities (two-drop plates: $t = 3.72$, $P = 0.0016$; four-drop plates: $t = 3.45$, $P = 0.0030$; Fig. 1).

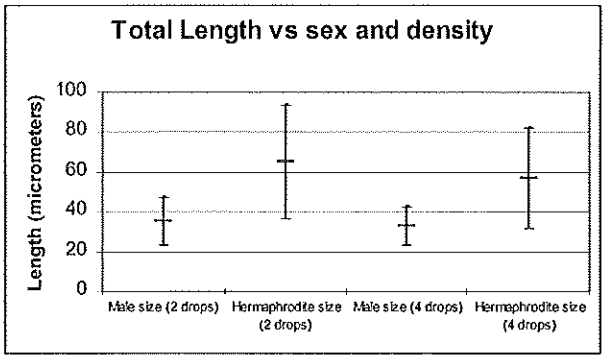

Figure 1. Mean thallus lengths (± 1 S.D.) of male and hermaphroditic C-Ferns grown under low (2-drop) versus high (4-drop) density conditions.

DISCUSSION

Both temperature and thallus density appear to affect C-Fern development. No spores germinated at the lower test temperature (10° C), probably because these tropical plants cannot tolerate low temperatures. Density also significantly affected the sex ratio of C-Ferns, but not their thallus size. At both experimental densities, hermaphrodites were significantly larger than males.

Because no spores in the cold environment grew, the null hypothesis that neither temperature nor density would affect the size and sex ratio of C-Ferns was rejected. Because of this problem, hypotheses #2 and #3 could not be evaluated. Hypothesis #1 was partly rejected and partly verified. Although the sex ratio was more biased toward males at high vs. low density, as expected, no significant difference in thallus size was detected between density treatments. Perhaps males were more frequent in the high density treatment because they are smaller than hermaphrodites, and thus require fewer resources. It is also possible that the pheromone antheridiogen was produced at higher levels in the high density treatment, thus causing relatively more males to develop.

Temperature appears to affect C-Fern development more than density, because while there was no significant size difference between the two densities in the warm environment, there was a significant difference between the growing plants in the warm environment and the dead spores in the cold environment. No minimum temperature for growth was reported on the C-Fern website or on any of the promotional material studied, but this experiment seems to indicate that it is higher than 10° C.

Originally, the "cold" spores were placed in a refrigerator at 15° C, but a malfunction heated them to over 50° C and either that or the transition to the 10° C cooler killed all of them. Therefore, the plates containing these dead spores were later replated with fresh spores from a new vial. It is possible that all of the spores in the new vial were dead and that is why they did not germinate.

The "warm" environment's temperature fluctuated between about 26 and 30° C, because the door had to be left open so that the refrigerators inside the room would not overheat, which was the cause of the above accident. Also, the plants in the cooler received less light than the warmer plants. To see if the cold or lack of light had merely slowed down the germination of the spores in the cooler, they were later placed at room temperature in the sunlight, but no growth was seen after three days and they were judged to be dead.

In addition, there were uneven numbers of spores in the two source vials, but this was inconsequential because all of the spores in the second vial apparently died because of the cold. The number of spores per drop varied for all plates, but 4-drop plates always had more spores on them than 2-drop plates. One of the reasons for poor growth of some spores was that the medium on some of the plates was too thin; all plates had less than the minimum amount of medium that is recommended (Warne and Hickok, 1999).

It is recommended that anyone attempting an experiment of this nature again have stable environments in which to grow the plants, enough medium to plate all dishes equally, and enough petri dishes to go around without having to use watchglasses, as well. To verify my findings, a study should be done with a much larger sample size in each group.

ACKNOWLEDGMENTS

Thanks to Dr. Doug Glazier for the original idea, most of the materials and many hours of figuring out what was going on, Julie Harrison for assisting me with getting the results, and the C-Fern website (Warne and Hickok, 1999) for its pictures.

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