bundles of intranuclear microfilaments were specifically induced in *Dictyostelium* in response to DMSO, this explanation cannot hold for *Acanthamoeba* since DMSO is never used in this experimental procedure. One could postulate that induction of intranuclear bundles of microfilaments in *Acanthamoeba* is related to cellulose synthesis and cyst wall formation from endogenous carbon sources since there seems to be a close correlation of these events. Indeed, this same mechanism may account for induction in *Dictyostelium* since cellulose synthesis under starvation conditions, though unintentional by the investigator, was a necessary condition for the slime mold in every case where DMSO was used.

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EFFECTS OF VERNALIZATION AND PHOTOPERIOD ON FLOWERING IN ECHINACEA TENNESSEENSIS, AN ENDANGERED SPECIES

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Abstract

Echinacea tennesseensis (Compositae) is a rare plant species endemic to a few cedar glades in the Central Basin of Tennessee. To further understand the life cycle ecology of this species, its requirements for flowering were investigated. Vernalization is not required for flowering, and plants kept in a heated greenhouse in winter flowered during the next growing season. *Echinacea tennesseensis* is a long day plant. None of the plants grown in a growth chamber at a 10-h daily photoperiod flowered, whereas 90% of those grown at an 8-h daily photoperiod plus a 2-h light break in the middle of the dark period flowered.

Introduction

Echinacea tennesseensis (Beadle) Small (Compositae) is endemic to cedar (limestone) glades of middle Tennessee where, at present, only four natural populations are known to occur; all of these are located in the adjoining counties of Wilson, Rutherford and Davidson (Quarterman and Hemmerly, 1971; Hemmerly, 1976; Paul Somers, pers. comm.). The species is the only native Tennessee plant on the official list of endangered species in the United States (Federal Register, 15 December 1980).

Echinacea tennesseensis is considered to be in danger of extinction, and if this were to happen the gene pool unique to this species would be lost forever. Of course, the best way to preserve a species is to preserve it in its native habitat. However, for a species as rare as *E. tennesseensis*, the chances of it becoming extinct in the wild are high. Thus, a knowledge of how to propagate this rare species is highly desirable. With such information, should the existing populations of the species be destroyed it could be propagated in cultivation and possibly reintroduced into its native habitat. To propagate a species, such as *E. tennesseensis*, that does not reproduce vegetatively, the requirements for completion of the life cycle, *i.e.*, seed to seed, must be known. In his study of the autecology of *E. tennesseensis*, Hemmerly (1976) clearly defined the seed germination requirements for the species, but he did not investigate the requirements for flowering. Thus, we have determined the requirements for flowering of this rare plant species.

Methods

Vernalization

Mature seeds (achenes) were collected by Dr. Tom Hemmerly in late summer of 1975 from *E. tennesseensis* plants growing in a cedar glade in Wilson County, Tennessee, and in early November they were planted on

soil in a nonheated greenhouse in Lexington, Kentucky. This greenhouse had no heating or air-conditioning, and the windows were kept open all year. Temperatures in the greenhouse were recorded continuously with an electric thermograph. The soil was watered daily, except when it was frozen during portions of the winter. The seeds germinated in the early spring of 1976, and on 11 April the seedlings were transplanted to individual 15-cm-diameter clay pots filled with soil. The plants were grown in the nonheated greenhouse until the vernalization study was started. They developed vegetatively during the summer of 1976 and flowered during the spring of 1977. The vernalization study was started in the autumn of 1977 using these plants.

On 17 September 1977 (heated control) and on the other dates indicated in Figure 1, 15 plants were transferred to a heated greenhouse where day temperatures ranged from 20-30 and night temperatures from 15-20°C. Fifteen plants were kept in the nonheated greenhouse (nonheated control) throughout the study. Plants were watered daily and examined for flowers at weekly intervals. A plant was considered to be in flower when the ligules of the ray flowers were 3 cm long. From the thermograph records in the nonheated greenhouse, we calculated the number of hours that each set of plants was exposed to temperatures between 0.5 and 10°C. These temperatures generally are optimal for vernalization of most species, but effective temperatures can range from a few degrees below 0 to a few above 10°C. (Leopold, 1964).

Photoperiod

Plants from seeds that germinated in the spring of 1976 were potted individually in 15-cm-diameter pots and grown in the nonheated greenhouse until 18 February 1979. All plants used in this study had flowered in the springs of 1977 and 1978. On 18 February 1979, 30 plants each were placed in a "short-day" and a "long-day" photoperiod regime in light- and temperature-controlled growth chambers. Light intensity at plant (rosette stage) level was approximately 9.0 Klx of cool, white fluorescent light. In the short-day regime, plants received a 10-h light period each day, and in the long-day regime plants received an 8-h light period plus 2h of light ("light break") in the middle of the dark period. It is a well known fact that a light-break given at, or near, the middle of a long dark period inhibits the flowering of short-day plants and promotes the flowering of long-day plants (Salisbury and Ross, 1978). The two chambers were set on a 12/12 h daily thermoperiod of 30/20°C. In the short-day chamber the high temperature period extended from 1 h before the beginning of the photoperiod to 1 h after it ended, while in the long-day chamber the high temperature period extended from 2 h before the beginning of the photoperiod to 2-h after it ended. Thus, the 2-h night interruption period in the long-day chamber was given during the low temperature phase of the daily thermoperiod. All plants were watered daily and checked weekly for flowering. Since none of the 30 plants in the short-day regime had flowered by 5 May 1979, on this date 15 of the plants were transferred to the long-day chamber to determine if they actually were still capable of flowering under appropriate conditions. The other 15 plants were kept in the short-day chamber to serve as controls. The photoperiod study was terminated on 15 September 1979.

RESULTS

Vernalization

Plants of *E. tennesseensis* do not require a vernalization period for flowering (Fig. 1). Ninety two percent or more of the plants in all treatments flowered, including those that received no vernalization (Fig. 2). In the heated greenhouse the first plant flowered during the week ending on 17 April 1978, and the last one flowered during the week ending on 19 August 1978. In the nonheated greenhouse the period during which individual plants began to flower extended from the week ending on 5 June 1978 to the week ending on 2 August 1978.

Photoperiod

Whereas 27 (90%) of the 30 *E. tennesseensis* plants in the long-day chamber flowered, none of those in the short-day chamber did so (Fig. 3). However, nine of 15 plants transferred from the short- to the long-day chamber had flowered when the study was terminated. None of the 15 plants in the short-day chamber flowered.

DISCUSSION

Growing *E. tennesseensis* from seeds to the flowering stage is relatively easy. Seeds exhibit a non-deep dormancy (cf. Nikolaeva, 1969) and will germinate to a relatively high percentage after moist, low temperature pretreatment (stratification), and some seeds will germinate even without stratification. Seeds stratified for 10 and 16 weeks and then incubated at a daily thermoperiod of 25/10°C at a 12-h photoperiod or in continuous darkness germinated to about 45-50%, while nonstratified controls germinated to about 10-30% (Hemmerly, 1976). Hemmerly (1976) concluded that the optimum conditions necessary for maximum seed germination (67%) was a 16-week stratification pre-treatment followed by incubation in light at 25 or 15°C.

Hemmerly (1976) reported that some plants in experimental plots in the middle Tennessee cedar glades flowered during their second growing season. In the nonheated greenhouse in our study, 100% of the plants grown from seeds that germinated in the spring of 1976 flowered in the summer of 1977. These plants formed a rosette of leaves during their first growing season, the leaves senesced in autumn, plants remained leafless during winter, and then formed a new rosette and flowered during their second season of growth. The same plants that flowered in their second year of growth, after overwintering in the nonheated greenhouse, flowered again during their third growing season, although they were kept in a heated greenhouse during the second autumn and winter (Figs. 1, 2). Thus, low temperature vernalization is not required for flowering in *E. tennesseensis*. However, plants do exhibit the typical phytochrome-controlled response to photoperiod, and *E. tennesseensis* is, by definition (Salisbury and Ross, 1978), a long-day species. Plants did not flower

FIG. 1. *Flowering of vernalized and nonvernalized plants of Echinacea tennesseensis. From left to right, plants were moved from the nonheated to the heated greenhouse on 17 September 1977, 1 December 1977, 1 January 1978 and 15 February 1978. Photograph was taken 7 June 1978.*

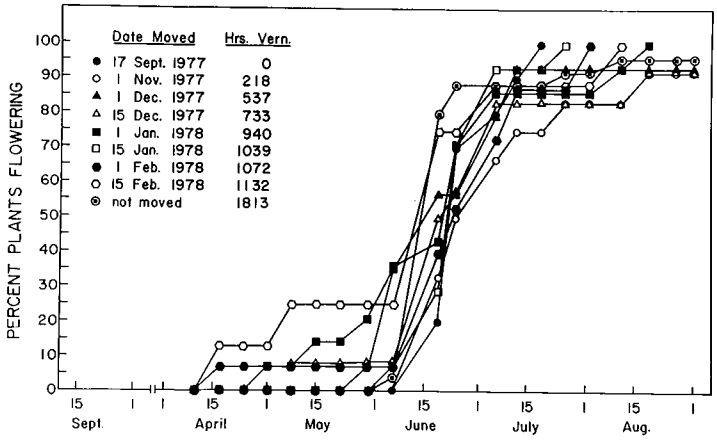

FIG. 2. *Effect of vernalization on flowering of* Echinacea tennesseensis. *The dates on which plants were moved from the nonheated to the heated greenhouse and the cumulative number of hours of vernalization that the plants received in the nonheated greenhouse are given in the table (inset).*

on a 10-h daily photoperiod, but they did flower when subjected to an 8-h daily photoperiod plus a 2-h light break given in the middle of the dark phase of the daily light-dark cycle (Fig. 3). Although the critical photoperiod for flowering has not been determined, data on flowering of plants in the heated greenhouse (Fig. 2) indicate that at least for some plants in the population it is relatively short. A few plants in the heated greenhouse were flowering on 15 April when the photoperiod in Lexington, Kentucky (sunrise to sunset) is 13 h and 11 min. (U.S.G.P.O., 1965). In *E. tennesseensis* flower buds are initiated before the flower stalk elongates; therefore, several days elapsed between floral initiation and the beginning of anthesis in the heated greenhouse. Thus, the critical photoperiod for flowering is less than 13 h and 11 min. Allard and Garner (1940) reported that another member of the genus, *Echinacea purpurea* (L.) Moench, is a long-day species with a low critical day length for flowering.

In the middle Tennessee cedar glades, *E. tennesseensis* begins to flower as early as mid May (Hemmerly, 1976), while in the nonheated greenhouse in our study anthesis began in early June (Fig. 2). Since anthesis in the heated greenhouse began in mid April, it appears that the onset of flowering in spring is delayed by low temperatures, rather than by the daily photoperiod.

Hemmerly (1976) reported that insect visitation was necessary for seed set in *E. tennesseensis*. Plants that flowered in our study did not set seeds; no insects were observed visiting the plants in the growth chambers or greenhouses. McGregor (1968) reported that all other taxa of *Echinacea* are self-sterile and that it was relatively easy to do crossing experiments by rubbing flowering heads of the different taxa together. Thus, one should be able to cause seed set in *E. tennesseensis* by rubbing flowering heads of different plants together.

FIG. 3. *Effect of photoperiod on flowering of* Echinacea tennesseensis. *Nonflowering plant on left was grown under a short-day photoperiod and flowering plant on right under a long-day photoperiod. Scale is in centimeters. Photograph was taken on 18 May 1979.*

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