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Phenology of Equisetum

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THE PHENOLOGY OF EQUISETUM

By

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Phenologies of ten *Equisetum* species (*E. arvense*, *E. telmateia*, *E. sylvaticum*, *E. pratense*, *E. fluviatile*, *E. palustre*, *E. laevigatum*, *E. variegatum*, *E. hyemale*, and *E. scirpoides*) were studied by periodic visits to populations in western Montana and coastal Washington at similar latitudes. Observation of growth, spore release, and stem dieback, and examination of rhizomes for sterile and fertile buds, and dissection of buds to determine stage of strobilus development were used to determine life cycle timing.

Five phenological patterns were found. *E. arvense*, *E. telmateia*, *E. sylvaticum*, and *E. pratense* have dimorphic stems, spore release during spring, and form basal buds and strobili during summer. Buds and strobili with spores overwinter, expanding in spring. *E. fluviatile* has monomorphic, annual stems expanding in spring from basal buds. Strobili develop in fall and overwinter, or in spring as buds elongate. Spore release is in July. *E. laevigatum* and *E. palustre* have monomorphic stems expanding in spring from basal buds that form the previous summer and overwinter with conical apices. In spring, strobili develop as the buds elongate, with spore release in early summer. *E. hyemale* and *E. scirpoides* have evergreen stems overwintering with terminal strobili and with basal buds that have conical apices which form the previous summer. Spore release during spring is followed by bud elongation and strobilus development. *E. variegatum* has evergreen stems with abortive strobili and no spore release. Buds and strobili form throughout the growing season. Buds with conical apices, incompletely expanded stems with premeiotic strobili or conical apices, and completely expanded stems with strobili and spores overwinter. For all species, spore release occurs during long days, meiosis occurs in late spring and summer, (only *E. variegatum* had November meiosis) and after the previous year's spores have been released. Strobili overwinter in buds of *E. sylvaticum*, *E. pratense*, *E. arvense*, *E. telmateia*, and *E. fluviatile*, or terminating evergreen stems in *E. hyemale*, *E. scirpoides*, and *E. variegatum*. Buds overwinter with possibly fertile conical apices in *E. fluviatile*, *E. variegatum*, *E. scirpoides*, *E. hyemale*, and *E. palustre*.

I would like to thank Dr. David Bilderback for his insights and advice. I would also like to thank Dr. Charles Miller and Dr. Andrew Sheldon for their suggestions, and my parents for their encouragement. Also, thanks to Janet Sullivan, Brad Willis, Dixie Stark, and Julie Huck for helping me dig rhizomes on cold, windy days. And thanks to Dr. Susan Munch for serving on my committee.

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Introduction

During the Carboniferous period, 345-280 million years ago, the Subdivision Sphenophytina reached worldwide distribution with arborescent and herbaceous forms coexisting. By the late Triassic, approximately 200 million years ago, the Sphenophytina were relatively small and herbaceous and their distribution and numbers had dwindled. Presently, the genus *Equisetum* is all that remains of the Sphenophytina and consists of about 25 herbaceous species. Since the genus may have been present during the Carboniferous and has not undergone any significant change since then, it is one of the oldest extant genera of vascular plants (Foster and Gifford, 1974).

Equisetum grows along rivers, railroad embankments, and in swamps and woods. It is recognized by its cylindrical, jointed, aerial stems that are composed of repeating segments of ridged internodes. Leaves are whorled and sheath the nodes. Stems are rough because of silicaceous deposits in the epidermal cells. In cross-section, aerial stems are seen to have three sets of canals. The largest is the central canal; its size varies between species. In *E. fluviatile* its diameter is large enough to make the internodes practically hollow. Under the ridges and associated with the vascular bundles are the carinal canals. The vallecular canals are located under the grooves between the ridges. All members of the *Equisetum* genus are rhizomatous perennials. Vegetative propagation occurs by rhizome growth and from arrested tuberous branches which occur at rhizome nodes (Foster and Gifford, 1974). Intercalary meristems at the nodes of aerial stems can give rise to new roots and shoots.

Fertile stems are terminated by strobili composed of a central axis with whorls of hexagonal, peltate sporangiophores. Each sporangiophore has six sporangia which contain green spores with hygroscopic extensions known as elaters. The elaters may become entangled with those of other spores, causing many spores to be dispersed in groups, or they may assist in sporangial dehiscence (Foster and Gifford, 1974).

Fertile and sterile stems are dimorphic in some species. The sterile, or vegetative stems are green and branched and persist throughout the growing season. The fertile stems are non-photosynthetic with apical strobili and either die or become branched and green after spore release.

Equisetum is homosporous. Spores vary little in size and vary little within a species (Duckett, 1970). Light is necessary for their growth and germination (Hauke, 1977). They are viable for only a few days and germinate to form photosynthetic gametophytes which can be male, female, or bisexual. Heterospory was once thought to control sexual differentiation, but research did not support this idea (Duckett, 1970). *Equisetum* gametophytes begin as either males or females. Male plants first develop antheridia and do not usually develop archegonia. Female gametophytes will produce antheridia as they get older. If bisexual, the archegonia develop before the antheridia. A diffusable metabolic product other than an antheridogen apparently has some effect, but the basis of sexual determination is still unclear (Hauke, 1977). The multiflagellated sperm swim through water to reach the eggs. Several eggs of different archegonia on the same gametophytic plant can be fertilized. Following fertilization, embryos and then young sporophytes develop (Foster and Gifford, 1974).

Equisetum is divided into two subgenera: Hippochaete and Equisetum. The Hippochaete have stomata sunken below the epidermal surface and monomorphic stems. Most members of the subgenus Equisetum have dimorphic stems and their stomata are flush with the epidermal surface (Hauke, 1963).

Phenology is the science of the relationship between climate and periodic biological phenomena. There is little previous work on phenologies and life cycle timing in Equisetum. Hauke, (1963, 1978), listed the timing of spore release and stem dieback. How does the environment affect the reproductive biology and life cycle of Equisetum? When are the strobili initiated, how do they develop, and does photoperiod influence strobilus initiation?

The objectives of this study were to answer these questions and to determine the timing of events in the life cycles of different Equisetum species.

Materials and Methods

This study consisted of field observations and laboratory work.

Field observations consisted of periodic visits to *Equisetum* populations from April, 1983 until December, 1984. Populations within five miles of Missoula, Montana were visited at intervals of approximately two weeks throughout the growing seasons of both years. Frozen soil prohibited collections after early December in 1983 and 1984 and before March in 1984. More distant populations were visited with less frequency.

Field observations were recorded in a field notebook and by photography. Growth measurements were taken at intervals from marked stems with a centimeter ruler. Aerial stems were marked with pink or red yarn and/or with red or blue "Pactra" enamel; this was done to determine age of stems and timing of dieback. *Equisetum* was collected by picking aerial stems and digging up rhizomes with their attached buds and aerial stems. Approximately 5-10 stems were collected within a radius of less than two meters, and 5-10 additional stems or buds were collected in a similar manner approximately 3.5 meters from the first collection. Sampling varied with density and extent of the population.

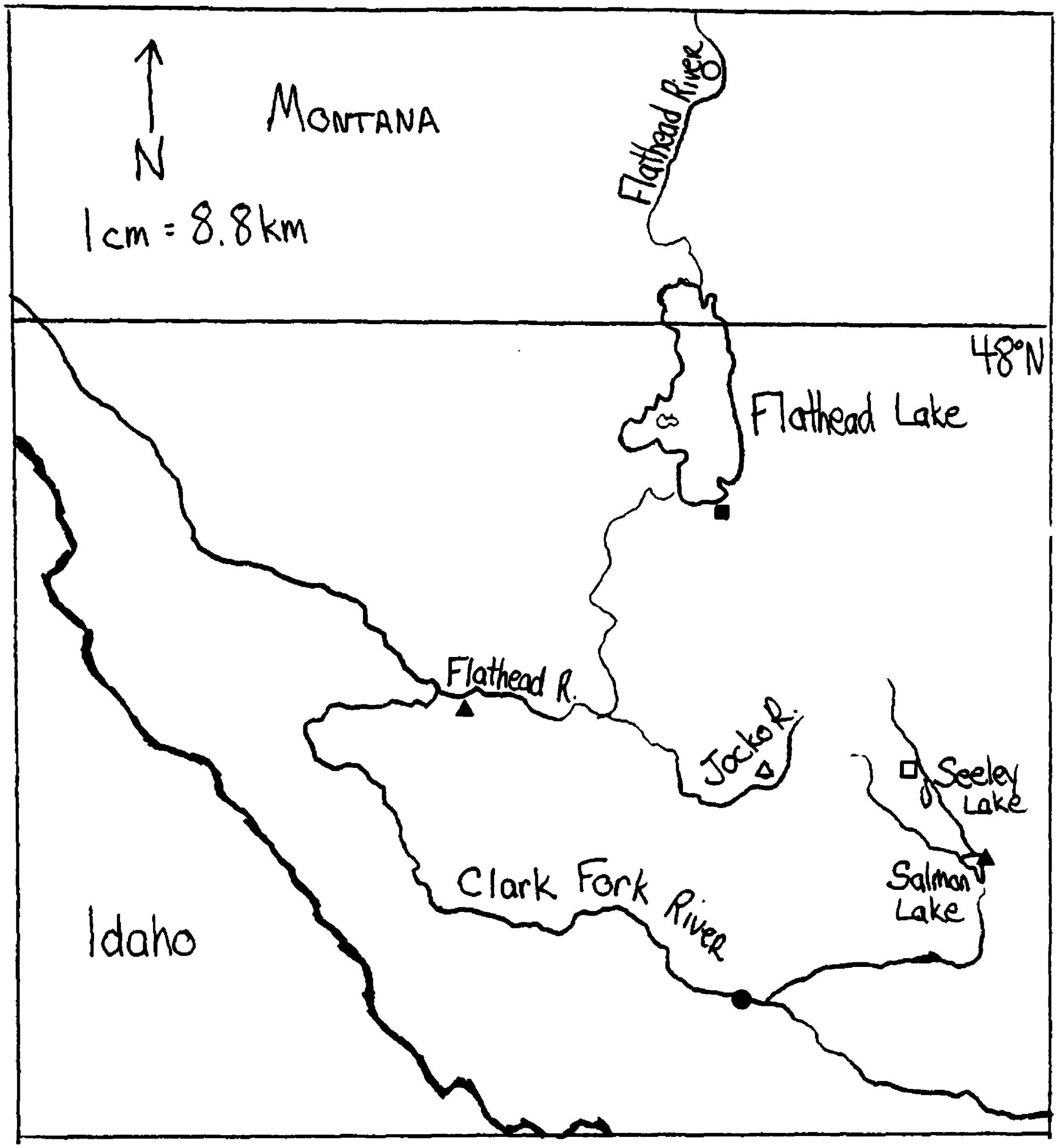
Nine *Equisetum* species inhabit western Montana. *E. telmateia* inhabits coastal Washington. The phenologies of these ten species were investigated. The latitude at Missoula, Montana is 46°52', at Gray's Harbor, Washington it is 46°55', and at the northernmost study site near Glacier National Park it is 48°24'. This causes all populations to have similar photoperiods, facilitating a phenological study. Timing of seasonal changes is similar for the habitats of the western Montana populations.

Study sites (Figures 1-3) were:

Species	Site	Description
E. telmateia	1.a.	R10W,T17N, Gray's Harbor County, Washington. Shore of Gray's Harbor, 2 miles west of Hoquaim. In coal along Highway 109.
		Other collection- July, 1983, near Eugene, Oregon.
		Observation - March, 1983, Olympia, WA.
E. arvense	2.a.	R19W, T13N, Sec. 22, SE¼. Missoula County, Montana. Northern shore of Jacob's Island, along south bank of Clark Fork River, in sand.
	2.b.	R19W,T13N,Sec.21, SE¼. Missoula County, MT. Gravelly soil at former site of railroad tracks. Along irrigation ditch south of Clark Fork River.
	2.c.	R19W,T13N,Sec.22,NE¼, Missoula County, MT. Seep at base of Waterworks Hill. Swamp along Interstate 90, south facing.
E. variegatum	3.a.	R19W, T13N, Sec.22,SE¼, Missoula County, MT. North shore of Jacob's Island along south bank of Clark Fork River, in sand.
E. laevigatum	4.a.	R19W,T13N,Sec.25,NW¼, Missoula County, MT. Seep at base of Waterworks Hill. South facing swamp along Interstate 90.
	4.b.	R19W,T13N,Sec.25,NW¼, Missoula County, MT. Gravelly soil 25-50 feet inland from Clark Fork River, in Hellgate Canyon.
	4.c.	R19W,T13N,Sec.21,Se¼, Missoula County, MT. Gravelly soil at former site of railroad tracks. Same as 2.b.

Species	Site	Description
E. hyemale	5.a.	R19W,T13N,Sec.22,SE¼, Missoula County, MT. Rocky soil along north bank of irrigation ditch south of Clark Fork River, near Jacob's Island, south facing.
	5.b.	R19W,T13N,Sec.21,NE¼, Missoula County, MT. Gravelly soil at base of Waterworks Hill, west of 2.c.
	5.c.	R19W,T13N,Sec.22,SE¼,Missoula County, MT. Rocky soil along south bank of Clark Fork River.
E. fluviatile	6.a.	R14W,T16N,Sec.30, Missoula County, MT. Northeast shore of Salmon Lake in water of small bay, next to Highway 209.
	6.b.	R25W,T18N,Sec.1, Sanders County, MT. Southern shore of Flathead River in outside curve of river.
	6.c.	R19W,T23N,Sec.8,NE¼, Lake County, MT. In wet ditch on south side of East Shore Road, Hwy 35, 50 yards before road turns north.
E. palustre	7.a.	R19W,T23N,Sec.8,NE¼,Lake County, MT. Same location as 6.c.
E. sylvaticum	8.a.	R16W,T18N,Sec.35, Missoula County, MT. In brush and in clearings on banks of the junction of Marshall Creek and West Fork, Clearwater River, south of bridge
E. scirpoides	9.a.	R18W,T17N,Sec.31,SW¼, Lake County, MT. Middle Fork of Jocko River, floodplain NE of bridge. On hummocky soil caused by cattle.
E. pratense	10.a.	R19W,T13N,Sec.7,NE¼, Flathead County, MT. 15-30 meters south of Blankenship Bridge, in sand on small bluff 20 feet inland from west bank of Flathead River.

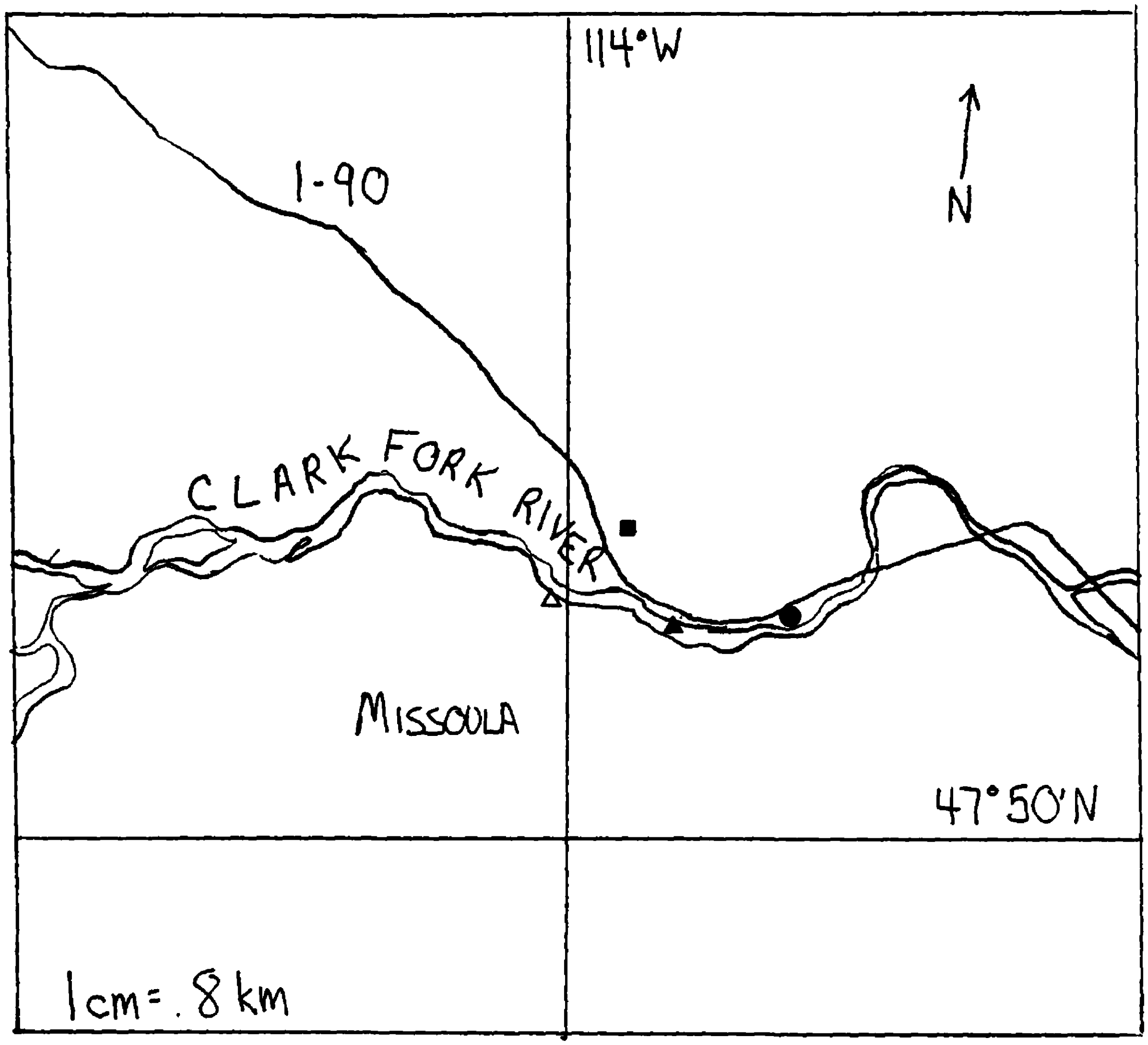
Further investigation of collected plants occurred in the laboratory. The apex was examined to determine if it was vegetative or fertile, and if fertile, the developmental



EQUISETUM STUDY SITES, Western MONTANA

● MISSOULA - See FIGURE 2
▲ *E. fluviatile*
△ *E. SCIRPOIDES*
□ *E. SYLVATICUM*
■ *E. PALUSTRE*
○ *E. PRATENSE*

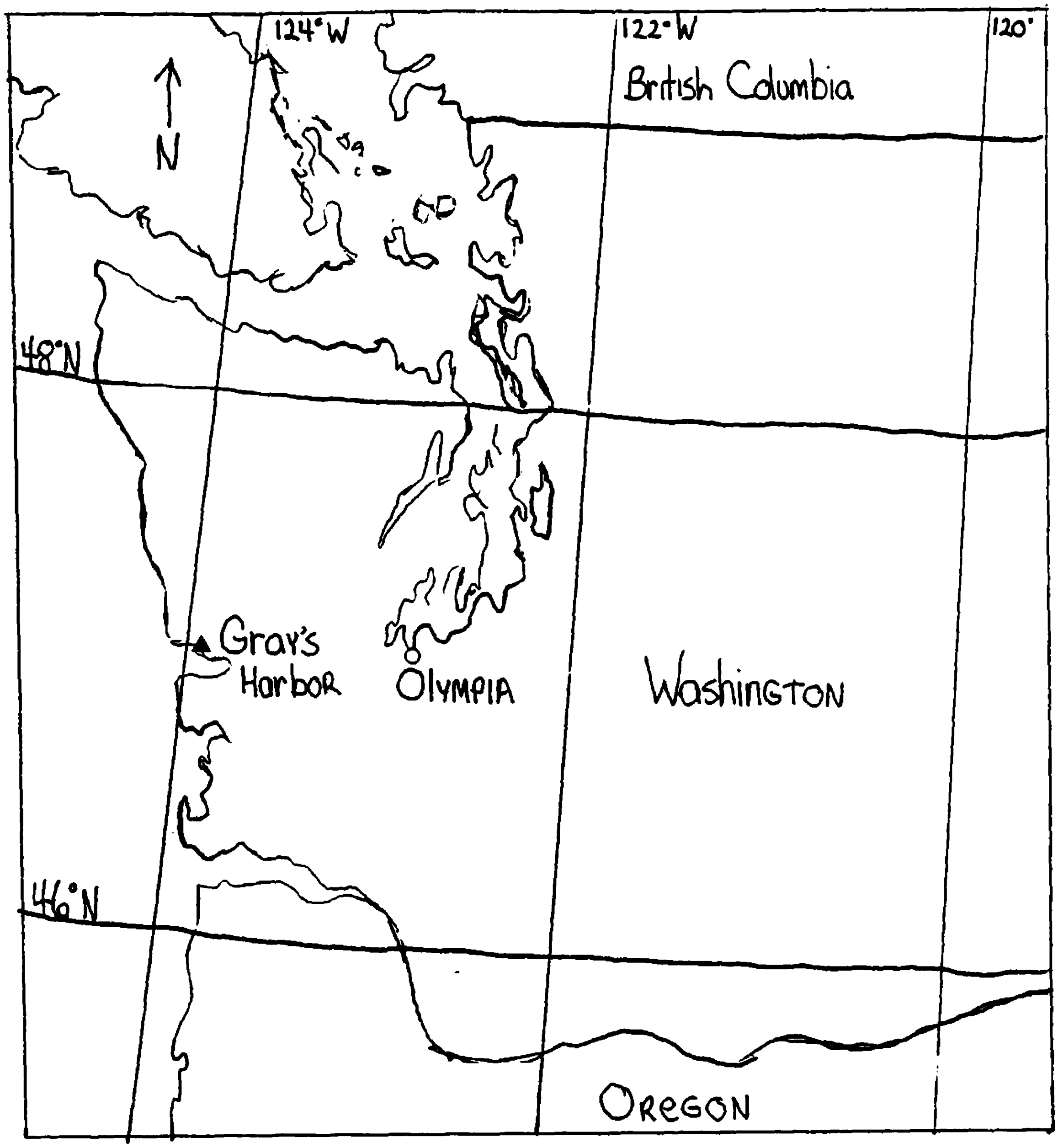
FIGURE 1.



Equisetum Study Sites in and near Missoula, Montana

- ■ Waterworks Hill
- △ Clark Fork River
- ▲ Jacob's Island
- ● Hellgate Canyon

Figure 2



E. telmateia Study Sites

Figure 3

1cm = 29.8km

stage of the strobilus. Shoots and buds were split longitudinally with a scalpel to expose the apex, which was then observed through a Spencer dissecting microscope at various magnifications. Length of stem, the condition of the apex, and developmental stage of the strobilus were all recorded. Chronological stages of strobilus development were: developing sporangiophores, developing sporangia, meiosis or tetrads, spores, and spore release. Many apices were conical, suggesting fertility, but these were recorded as sterile/conical because fertility could not be defined.

Meiosis and spore development were observed through a Reichert compound microscope. Wet mounts of sporangia and their contents were stained with a 0.05% solution of Toluidine Blue. The material to be examined was placed on a slide and a cover slip was placed on top. Pressure was then applied with a fingertip or the blunt end of a dissecting tool. Material was often macerated with a sewing needle to observe individual and small groups of cells, and to cut the sporangial wall.

Photographs of dissected and intact buds, and of stages of strobili development were taken through the dissecting microscope; tetrads and spores were photographed using the compound microscope.

To test for viability, spores of E. laevigatum, E. arvense, and E. fluviatile were cultured on Bold's Basal pH 6.6 growth medium, solidified with 2% agar, (Nichols and Bold 1965), also known as Bristol solution.

Six individual stock solutions are made of the macronutrients and 10ml of each are added to 940ml of H_2O to make 1 liter.

1.	$NaNO_3$	10g/400ml
2.	$CaCl_2 \cdot 2H_2O$	1g/400ml
3.	$MgSO_4 \cdot 7H_2O$	3g/400ml
4.	K_2HPO_4	3g/400ml
5.	KH_2PO_4	7g/400ml
6.	NaCl	1g/400ml

Three individual stocks are made of minor constituents and 1ml of each is added to the 1 liter os macronutrients and H_2O.

7.	EDTA-	
	EDTA	50g/l
	KOH	31g/l
8.	Iron-	
	$FeSO_4 \cdot 7H_2O$	4.98g/l
	H_2SO_4	1.0ml/l
9.	Boron	
	H_3BO_3	11.42g/l

One ml/l of this micronutrient solution is then added:

10.	$ZnSO_4 \cdot 7H_2O$	8.82g/l
	$MnCl_2 \cdot 4H_2O$	1.44g/l
	$NaMoO_3$	0.0024g/l
	$CuSO_4 \cdot 5H_2O$	1.57g/l
	$Co(NO_3)_2 \cdot 6H_2O$	.49g/l

Spores were taken from indehiscent sporangia which had been sterilized in a 10.0% solution of Clorox, or were shaken directly from dehiscent sporangia onto the growth medium.

The spores were kept at room temperature. The percentage of germinated spores was recorded.

To test for germination, spores of E. arvense and E. sylvaticum were put on moist sand from their habitats in a Petri dish and kept at room temperature.

Vernalization experiments were conducted to test for effects of cold temperature on fertility. E. laevigatum rhizomes with attached buds and aerial stems were collected from site 4b on September 29, 1984 and October 10, 1984. Twenty buds were dissected. Each apex was conical. All buds were planted in Jiffy/Peat 2/1 in 6" clay or plastic pots. Thirty five buds were placed in the greenhouse as a control. On October 14, 1984, 140 buds, (4 pots with 35 buds each) were placed in the ground of an open yard so that the rim of the pots was level with the soil surface. On October 11, 144 buds, (3 pots with 35 buds, 1 with 39) were placed in a coldroom at 6.5°. Pots of buds were transferred from the ground or coldroom to the greenhouse at intervals of approximately one month.

On October 24, 1984, E. laevigatum rhizomes with basal buds and aerial stems were collected from site 4a, then refrigerated for 1 week at 0°C. On October 31, 1984, the tops of the 2-3 outermost nodes and tops of leaves were removed to expose the apices of sixteen buds. Sixteen other buds were used as controls. All 32 were planted in plastic pots with Jiffy Peat, Vermiculite, and soil mix, and then kept in a coldroom at 6.5°C until November 30, 1984.

When buds from these experiments expanded they were dissected and examined for fertility. Differences in the number of strobili between experimental and control groups were computed.

Results

Equisetum arvense, (after Hauke, 1978), "the field horsetail", subgenus *Equisetum*, has annual, dimorphic stems. Vegetative stems persist throughout the growing season and are branched, 2-100cm tall, 0.8-4.5mm in diameter, with 4 to 14 ridges. The central canal makes up 1/3-2/3 the stem diameter and vallecular canals are large. The first basal internode is 4-14mm long, longer than the sheath (1-10mm), with short (1.03.5mm), narrow, dark teeth. The shorter fertile stems die following spore release in the spring. They are unbranched, non-chlorophyllous, pinkish-tan, and are terminated by a 5-35mm strobilus atop a 22-55mm peduncle. Rhizomes are dark brown to black, occasionally with tubers.

Both stem types originated from buds on the rhizome at the bases of sterile stems. These buds were initiated during the previous growing season, overwintered at or 1-5cm beneath ground level, and elongated the following spring (Figure 6).

Sterile buds and buds with conical apices were found in July, 1983 (Chart 2). Presence of many buds less than 0.5cm long indicated that initiation was in progress.

Sterile buds were found throughout the growing season in 1984 (Figure 4, Charts 7,8, and 9). Buds found on the rhizome during spring were less than 0.25cm long, appeared recently initiated, and were present while vegetative stems were elongating (Charts 8 and 9). Small buds in July indicated ongoing bud initiation (Figure 4, Charts 7,8, and 9).

Sterile buds are differentiated from those with conical apices by the presence of whorls of green leaves. Those with conical apices have few leaf whorls.

In 1983, fertile buds were first seen during late June, (Charts 2 and 3), and spores were present by late July. In late June, two fertile stems elongated (this was just after the flood receded) but there was no further elongation until September when some fertile buds elongated to or just beyond ground level. This fertile bud elongation was only seen at Jacob"s Island (site 2a).

Unexpanded fertile buds with elater bearing spores were found during spring of 1984. They appeared to have overwintered (Chart 5). No buds were seen on the rhizomes from April 3 until July 7, when fertile buds with spores were present at Jacob's Island (Figure 4, Chart 7, Plate 2). Premeiotic strobili were also found. Meiosis had recently occurred and tetrads were present. One fertile bud had elongated to 6cm and approximately 20 fertile buds elongated and released spores by late July (Figures 4,5 and Charts 5,6). One stem had both a strobilus and green branches (Plate 1). All fertile buds that released spores in summer died by September.

Meiotic anaphase was seen in late July and premeiotic strobili were found in late September at Jacob's Island, (Chart 7).

Other populations formed strobili during summer and fall and had spores with elaters by the end of the growing season (Charts 8,9).

In spring of 1983 and 1984 (Charts 3,4,5, and 6) the fertile buds elongated and released spores. The central stalk of the strobilus elongated, causing the sporangiophore whorls to separate vertically. Sporangiophore stalk curved upward, tilting the sporangia away from the central strobilus axis. The sporangia then split and released spores. Fertile stalks died following spore release in late April and early May of both years.

The vegetative buds elongated slightly after the fertile buds and were fully expanded by mid to late May (Figure 7). Broken vegetative stems often resprouted and grew for the remainder of the growing season.

No gametophytes were found. Spores cultured in Bold's Basal appeared normal, remained green for 60 days, but did not germinate. Spores placed on sand from Jacob's Island remained green for one month but did not germinate.

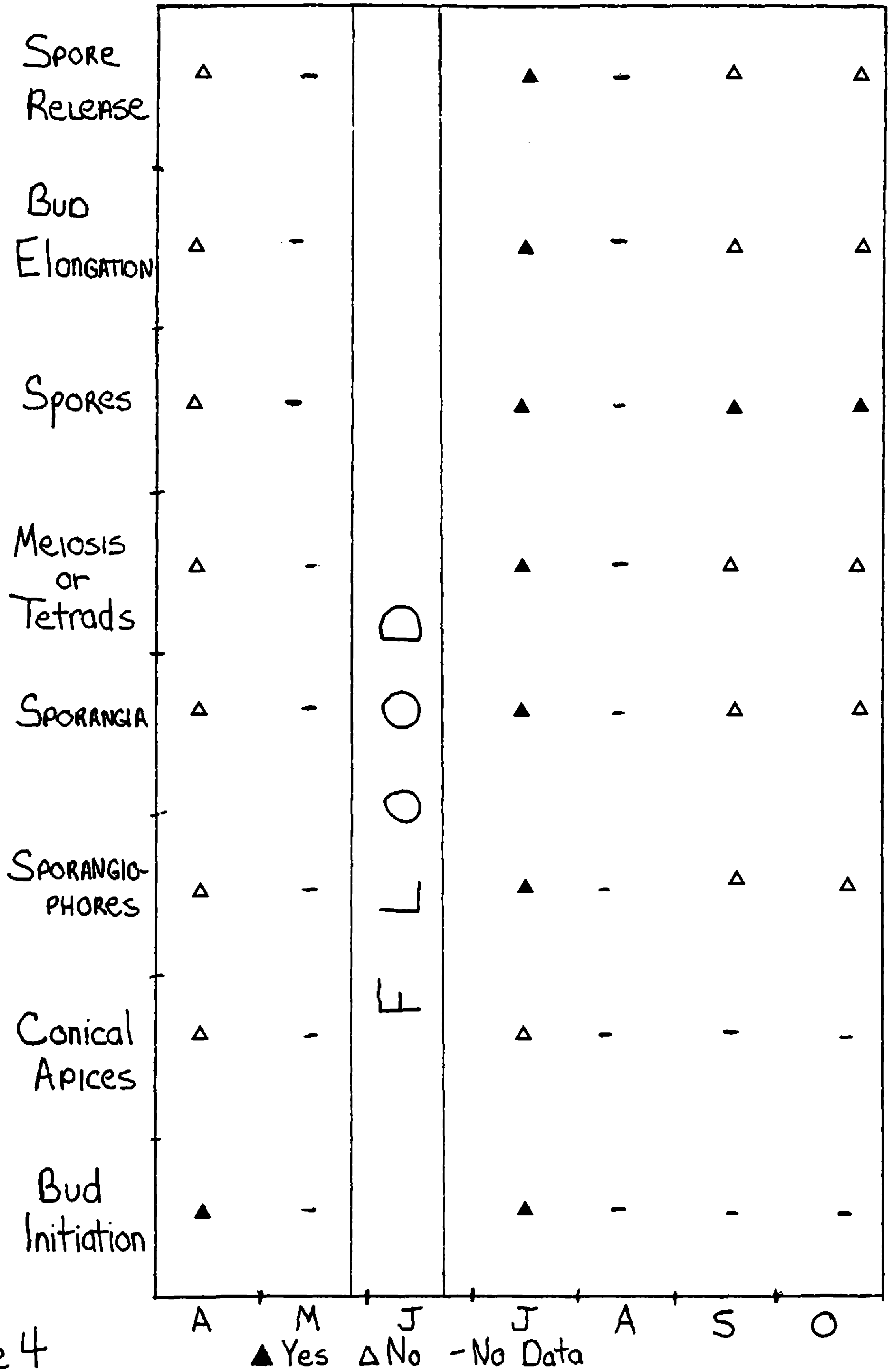
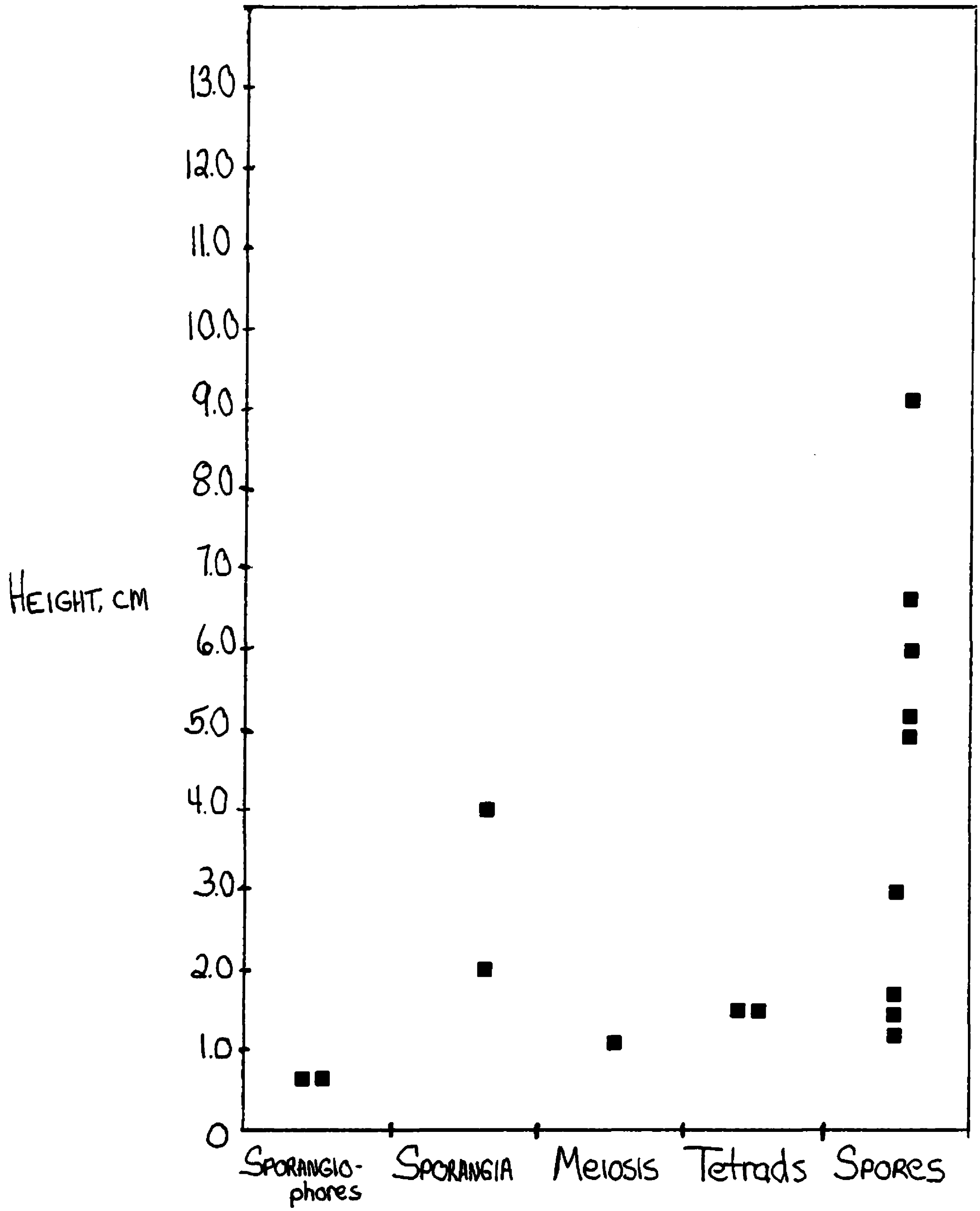


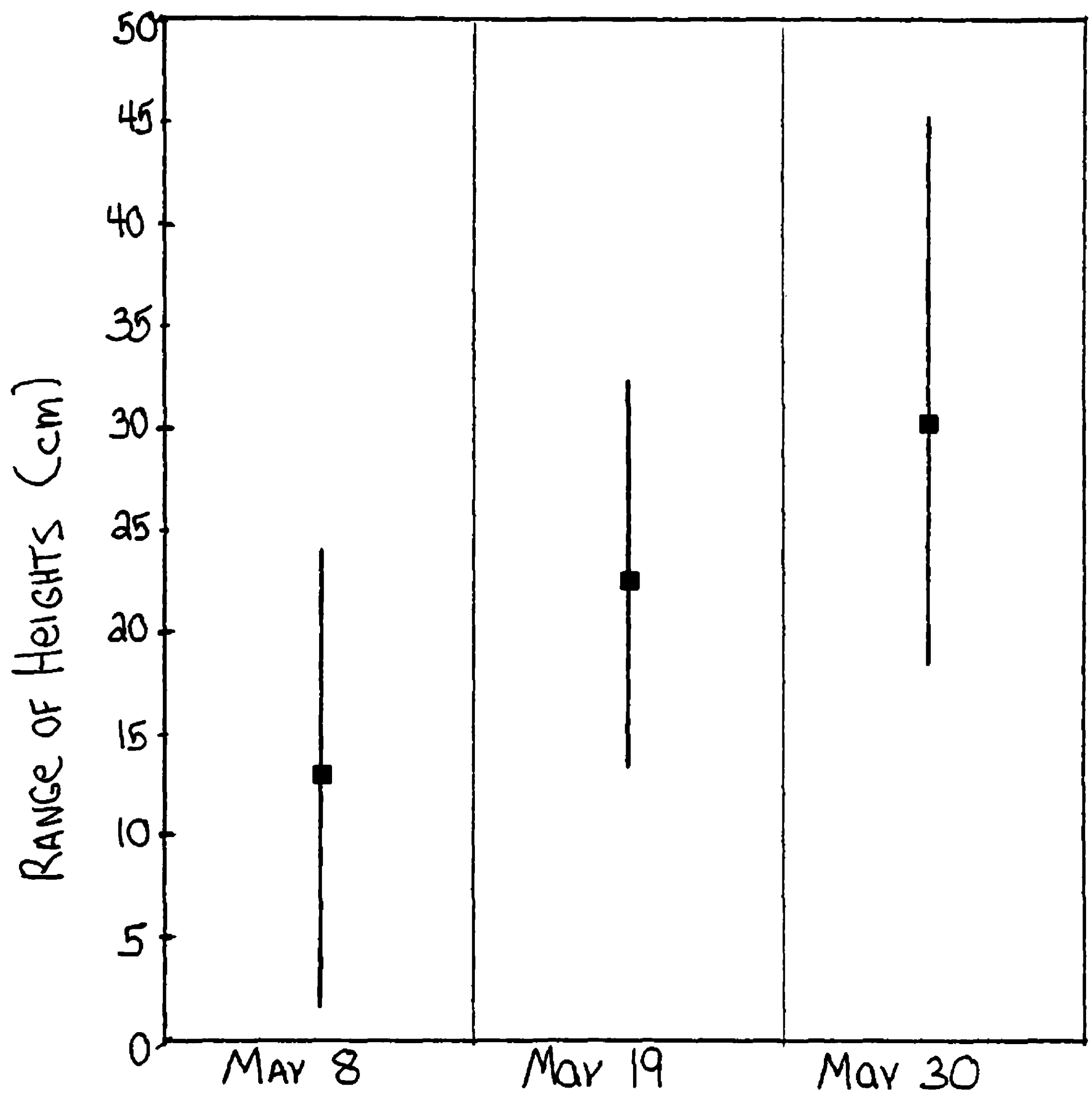
Figure 4

1984 - E. arvense Strobilus Development, Jacob's Island



E. arvense Fertile Bud Heights, July 1984
Jacob's Island

Figure 5



E. arvense Sterile Stem Elongation, 1983
Waterworks Hill

Figure 6

Plate 1

Equisetum arvense

1. top, center
   Fertile bud elongating, July, 1984, Jacob's Island.

2. lower left
   Fertile bud with sporangia, July 1984 Jacob's Island

3. lower right
   Expanded fertile stem with vegetative branches, July 1984, Jacob's Island.

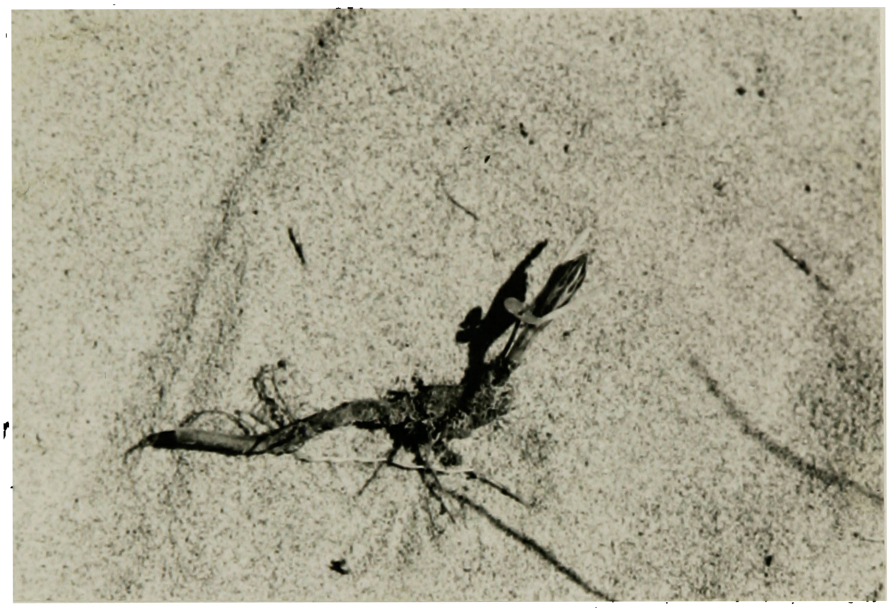
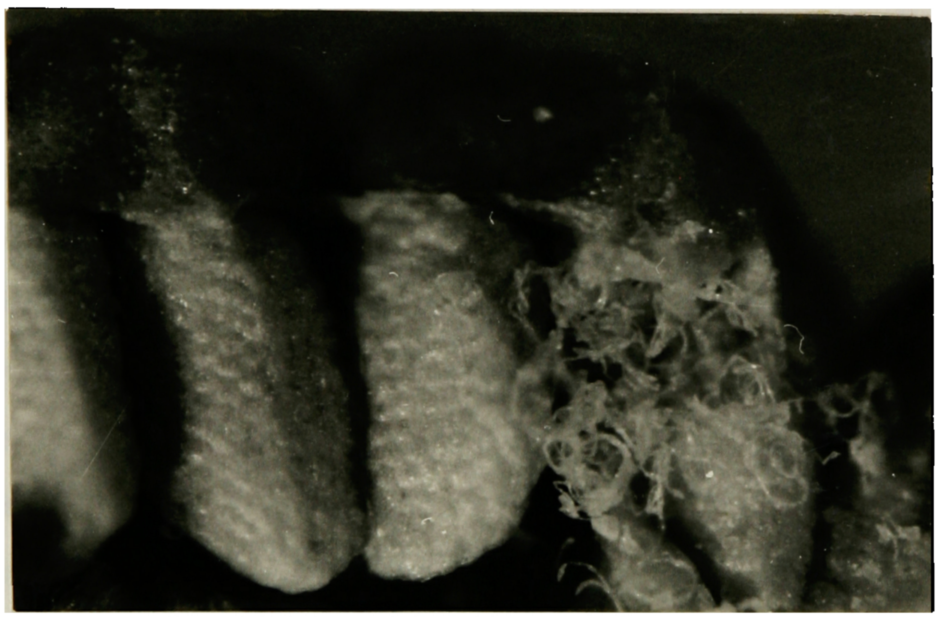


Plate 2

Equisetum arvense

Strobilus with spores from fertile, unelongated bud, collected July, 1984, Jacob's Island.



Equisetum telmateia subsp. *braunii*, (after Hauke, 1978) "the giant horsetail," is in the subgenus *Equisetum*. This inhabitant of the Pacific Coastal region of North America has dimorphic annual stems and rough, dark brown rhizomes with occasional tubers.

The branching vegetative stems persist throughout the growing season. They are 30-100cm tall, and 4-10mm in diameter with 14-30 ridges. The first branch internode is shorter than the sheath which is 5-12mm long. Teeth are 3-10mm long, green below, blackish above and connate into groups of two to four. The central canal comprises 2/3-3/4 the stem diameter and vallecular canals are prominent.

The ephemeral fertile stems die after spore release, which occurs in the spring. They are unbranched, white to tan, and shorter but wider than the vegetative stems. The apical strobilus is 4-10cm long atop a 22-55mm peduncle.

Both stem types began growth in spring from buds on the rhizome, (Plate 3). These buds were initiated during the previous growing season at the base of that season's aerial stems. In longitudinal section, sterile buds had many leaf whorls surrounding the apex. The strobilus was surrounded by approximately six whorls of leaves in fertile buds. The internodes elongated so that the innermost whorls of bud leaves were uppermost in elongated stems.

Premeiotic fertile buds collected in July, 1983 had sporangiophores or sporangia, (Plate 4, Chart 10), Vegetative stems were completely elongated.

Presumably, these buds overwintered and elongated in the spring. Fertile stems released spores and unexpanded strobili contained spores in March. Elongating vegetative stems were approximately one foot tall and not fully branched in March.

No gametophytes were found.

Plate 3

<u>E. telmateia</u> fertile stems and expanding vegetative stems, Gray's Harbor, Washington, March, 1984. Above.

Plate 4

<u>E. telmateia</u> basal bud with developing sporangiophores, Oregon, early July, 1983. Following page.

Equisetum sylvaticum, subgenus *Equisetum* (after Hauke, 1978), has annual, dimorphic stems that intergrade between the branching, photosynthetic, vegetative form and the unbranching, nonphotosynthetic fertile form. Vegetative stems are 25-70cm tall, 1.5-3mm in diameter, with 8-18 flattened ridges. The central canal is 1/2-2/3 the stem diameter and vallecular canals are large. The reddish brown sheaths are 4-6mm long, 2.5-6mm wide, have 3-10mm long teeth, and form 3-4 coherent groups at each node. Branches arch and rebranch. Their 5-9mm long first internodes are longer than the subtending stem sheaths. Strobili are 5-30mm long. Fertile stems can branch after spore release. Rhizomes are shiny, light brown and smooth. They occasionally have tubers.

Spore release occurred in June (Chart 11), and was complete by late July (Plates 5,6, and 7).

Elongated stems from the same rhizome showed a gradation from completely vegetative, vegetative with a strobilus, fertile with some green branches, to fertile with no branching (Plate 5). All stem types were less than 30cm apart. Fertile stalks without branches senesced following spore release.

Basal buds had formed by late July and were unexpanded. Some were fertile and premeiotic or had conical apices. Fertile, unexpanded buds from British Columbia had spores in August (Chart 11, Plates 8 and 9).

No gametophytes were found. Attempts to germinate spores in moist soil were unsuccessful.

Plate 5

Equisetum sylvaticum

1. above
   Expanding fertile stem with vegetative branches,
   June 4, 1984, Marshall Creek.

2. below
   Variation in *E. sylvaticum* stems collected
   June 4, 1984, Marshall Creek.

Plate 6

Equisetum sylvaticum

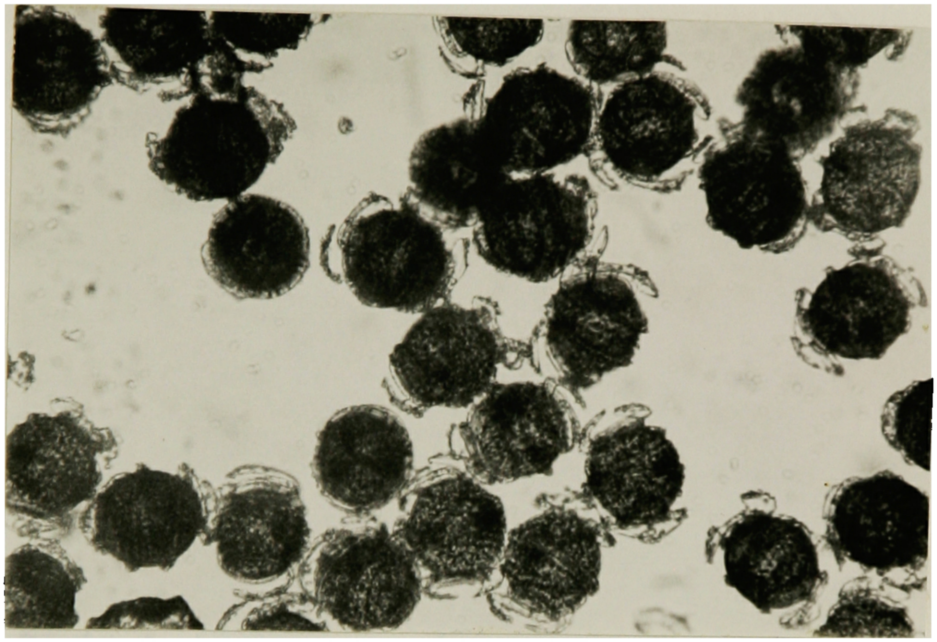
Fertile stem with indehiscent sporangia and vegetative branches, collected June 4, 1984, Marshall Creek.

Plate 7

Equisetum sylvaticum

1. above
   Spores with coiled elaters,
   collected June 4, 1984, Marshall Creek.

2. below
   Spores with uncoiled elaters,
   collected June 4, 1984, Marshall Creek.



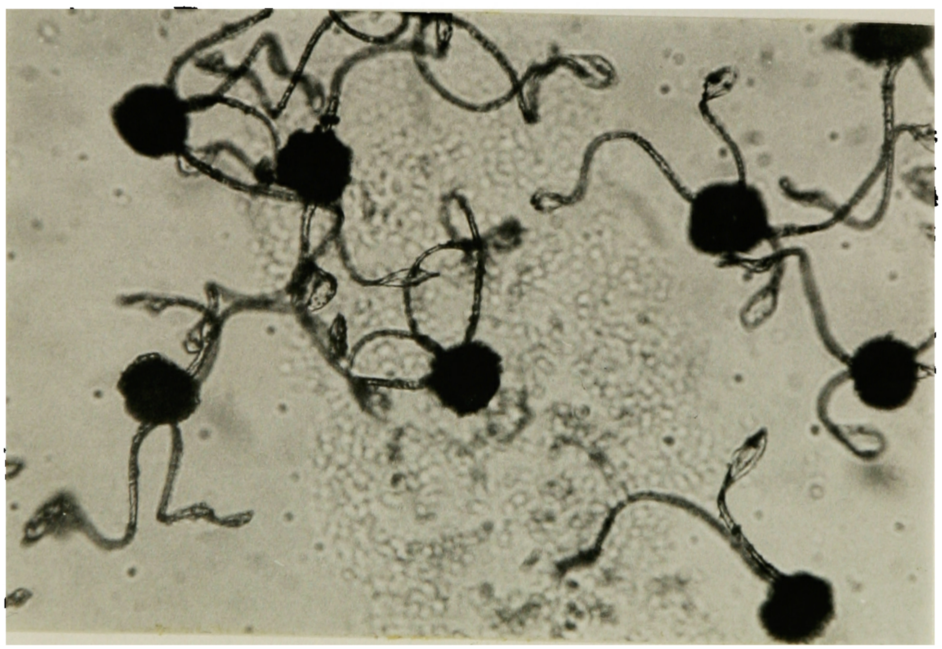


Plate 8

Equisetum sylvaticum

Strobilus and leaves of unexpanded, fertile bud. Collected in early August, 1984 in British Columbia.

Plate 9

Equisetum sylvaticum

Strobilus with spores from unexpanded, fertile bud, collected early August, 1984, British Columbia.

Equisetum pratense, (after Hauke, 1978), is a circumboreal species of the subgenus Equisetum that inhabits moist woods or meadows. Its annual, dimorphic stems have 8-18 ridges, are 16-53cm tall and 1-3mm in diameter. The central canal comprises 1/6-1/3 the diameter and vallecular canals are well developed. Sheaths are 3-5mm long, 2-4.5mm wide and the narrow teeth are 1.5-4.0mm long with dark centers and white margins. Rhizomes are dull and black. Branches are horizontal to drooping and arise from the upper internodes. The first branch internode is 2-5mm long, which nearly equals the sheaths of lower whorls but is longer than sheaths of the upper whorls. Cones are 20-25mm long. Vegetative stems are branched. Fertile stems are not green or branched initially, but become green and branched after spore release.

No evidence of fertility was found during the two visits to the Flathead River population.

Aerial stem dieback was complete and sterile basal buds were present in November, 1983.

Vegetative stems had elongated and sterile basal buds were found on the rhizome in July, 1984 (Plate 10).

Plate 10

Equisetum pratense

1. above
   Vegetative stems and rhizome,
   collected July 1, 1984 at Blankenship Bridge

2. below
   Basal bud on vegetative stem
   collected July 1, 1984 at Blankenship Bridge


LIBERTY

Equisetum fluviatile, (after Hauke, 1978), "the water horsetail", is an emergent aquatic in the subgenus *Equisetum*. The annual, monomorphic stems are 35-113cm tall and 2.5-9mm in diameter with 12 to 24 smoothed ridges. The central canal is noticeably large, about 9/10 the stem diameter. Vallecular canals are absent. Stems are branched or unbranched. Branches arise from the middle nodes, are spreading, and the first internode is shoter or equal to the stem sheath, which is squarish with short (2-3mm), narrow, dark teeth. The rhizome is smooth, glabrous and reddish brown with black roots.

Buds were found at the bases of aerial stems in the fall of 1983 and 1984 (Plate 12, Chart 12). In 1983, autumn buds had conical apices or were sterile. One apex was slightly whorled with faint lines that were possibly the beginnings of sporangiophores. Small buds indicated bud initiation. In 1984, basal buds from Salmon Lake (site 6a) were fertile and premeiotic. This was the only observation of autumn strobilus development.

Aerial stems died back in October, and the basal buds overwintered 1-5cm beneath the ground, which is in turn underwater. The apex was surrounded by whorls of telescoped internodes and was innermost in the bud, so it terminated the expanded stem.

Stem elongated occurred in May and June. Strobili developed as the buds elongated and meiosis probably occurred in spring, (Plate 11).

Spore release occurred in early July. Spores appeared normal and remained green in culture for approximately 14 days but did not germinate.

No gametophytes were seen.

Plate 11

Equisetum fluviatile

Strobilus with sporangiophores from expanding stem. Collected May 24, 1984 at the Flathead River.
Strobilus approximately 2mm. long.

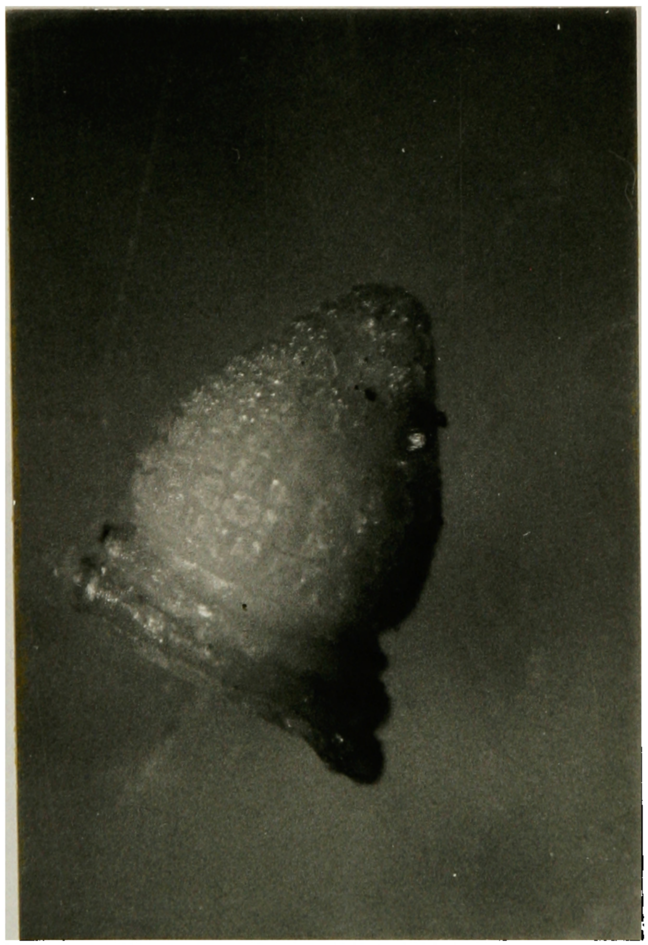


Plate 12

Equisetum fluviatile

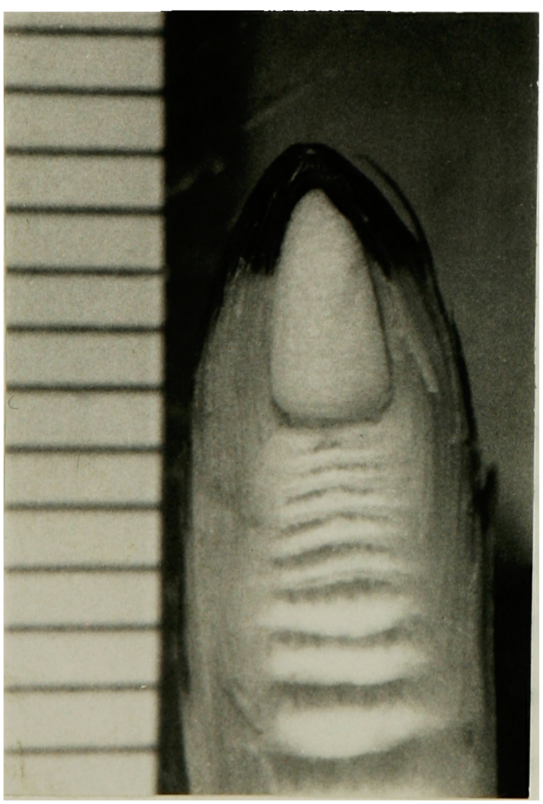
1. upper left
   Developing strobilus in elongating stem, collected May 24, 1984 at the Flathead River.

2. upper right
   Unelongated basal bud, collected November 5, 1983, Flathead Lake.

3. lower left
   Unelongated, fertile basal bud.
   Scale in millimeters, strobilus 4.0mm long, sporangiophores present.
   Collected October 28, 1984 at Salmon Lake.

4. lower right
   Strobilus with sporangiophores, millimeter scale.
   From unexpanded basal bud collected October 28, 1984, at Salmon Lake






Equisetum laevigatum, (after Hauke, 1963), subgenus *Hippochaete*, is found in western and northwestern North America along rivers, ditches and railroad embankments, and in open, moist places such as meadows and prairies. The annual, monomorphic stems are unbranched, 21.5-152.5cm tall, and have 10-32 rounded ridges that cause the stems to be smooth. The central canal makes up 3/4 of the 2-7mm stem diameter; vallecular canals are prominent. Green sheaths are 7-15mm long, 3-9mm wide, and flare out slightly at the tip where there is a black band. Teeth are quickly deciduous with dark centers and white margins. Rhizomes are dull, rough, and dark brown. Strobili are yellow or brown and blunt or inconspicuously apiculate.

Underground buds at the bases of aerial stems elongated in the spring (Figure 9, Charts 17 and 18). Strobili developed as they elongated and tetrads were seen in April and May, 1984 (Plate 13, Figure 10, Charts 20, 21, and 22). Stems had completely elongated by June. Spores were released in early summer (Figure 10, Charts 20, 21, and 22). Spores appeared normal and germinated in culture, (Chart 13), but no gametophytes were found in nature. Basal buds formed in summer and overwintered underground. Many had conical apices but sterility or fertility could not be determined (Figures 7 and 8, Charts 16, 17, 18, and 19, Plate 13). Bud leaves were coriaceous and surrounded the apices and unexpanded internodes. Aerial stems died back in October of both years.

Small buds clustered around the base of a new (1984) stem were less than 0.5cm long, (Figure 8, Chart 18). They would not have overwintered. They could have been new stems for 1984, buds that would overwinter and expand during the spring, or buds that formed inside the bud that elongated to form their "parent" stem. It is also possible that they were root buds.

Although small buds were found in the spring, (Figure 8, Charts 17 and 18), most basal bud formation occurred in the summer and many buds formed during July. Coriaceous leaves began to form in late summer. No bud elongation or strobilus development occurred until the following spring.

In spring, both unelongated and elongating buds had developing strobili. Differentiation of the strobili was acropetal. A triangular apex above whorls of sporangiophores was present in strobili that had not formed all of their sporangiophores; these apices would presumably have formed the remaining whorls. Strobili began to form before the buds were above ground, and further strobilus development proceeded as buds elongated. Elongated conical apices in unexpanded buds could have been an early stage of strobilus development, (Charts 20,21, and 22).

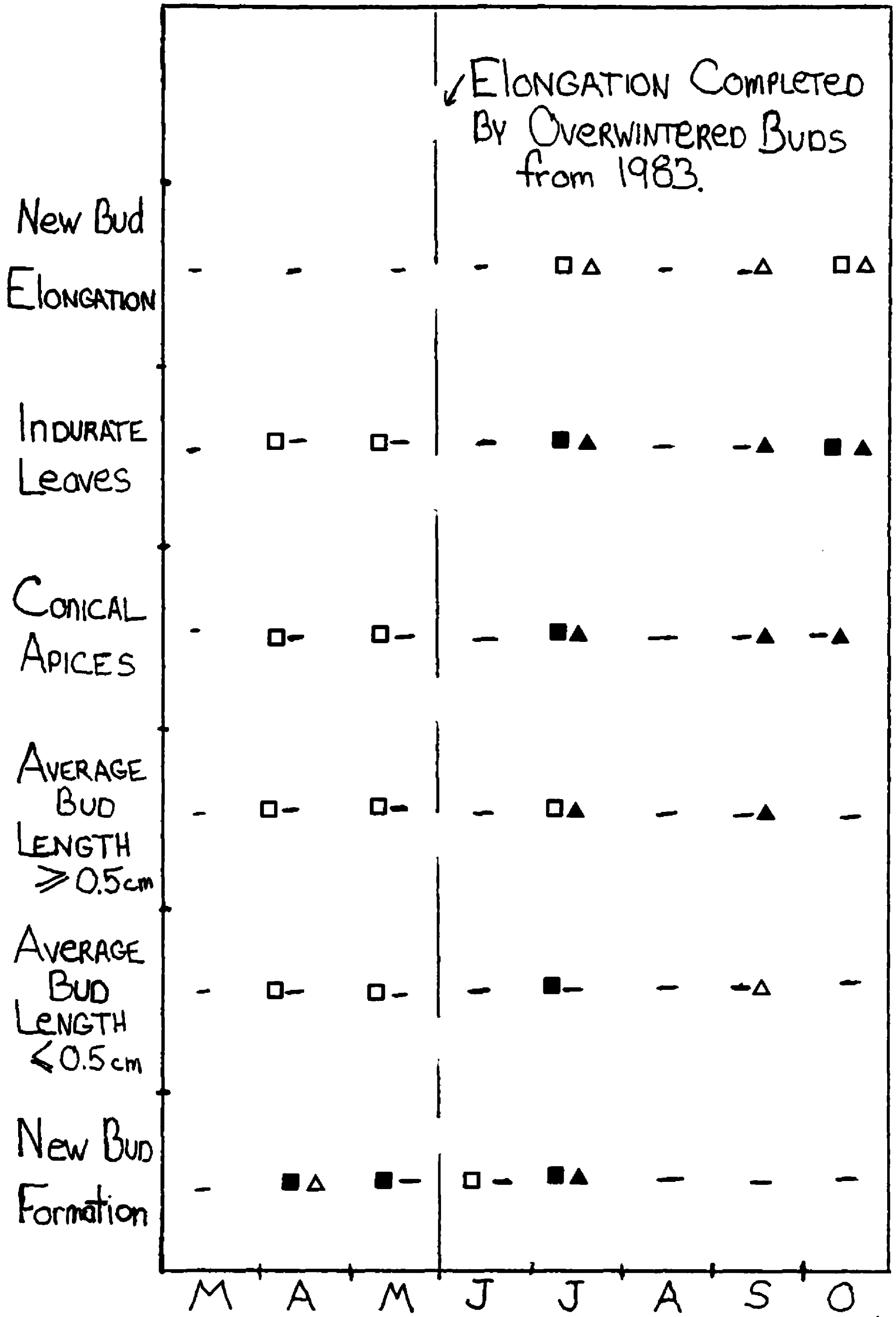
Strobilus development was completed by late May or early June and was followed by spore release.

Spores germinated in culture formed green, branched gametophytes 1-2mm in diameter within 17 days. These gametophytes remained alive for approximately 59 days but had no further growth, possibly because of contamination.

Vernalization experiments were conducted to determine whether or not extended periods of cold influence fertility, (see Materials and Methods, page 12). All buds that elongated after being placed in the greenhouse were sterile. Many of the basal buds originally planted died. The bases of the buds separated from the rhizomes. Small shoots grew from the nodes of the rhizomes. Small shoots grew from the nodes of the rhizomes. When these shoots grew almost to ground level they branched extensively: causing a proliferation of narrow stems with diameters of approximately 2.0mm. None of the narrow stems were fertile, (Chart 14).

Surviving buds did not elongated readily after being transferred from the coldroom or outdoors to the greenhouse. One bud from Coldroom 1 began to elongate in early December but proved sterile. Buds and narrow stems had begun elongation by late February, 1985, and narrow stems and two of the surviving basal buds were still elongating on April 27, 1985.

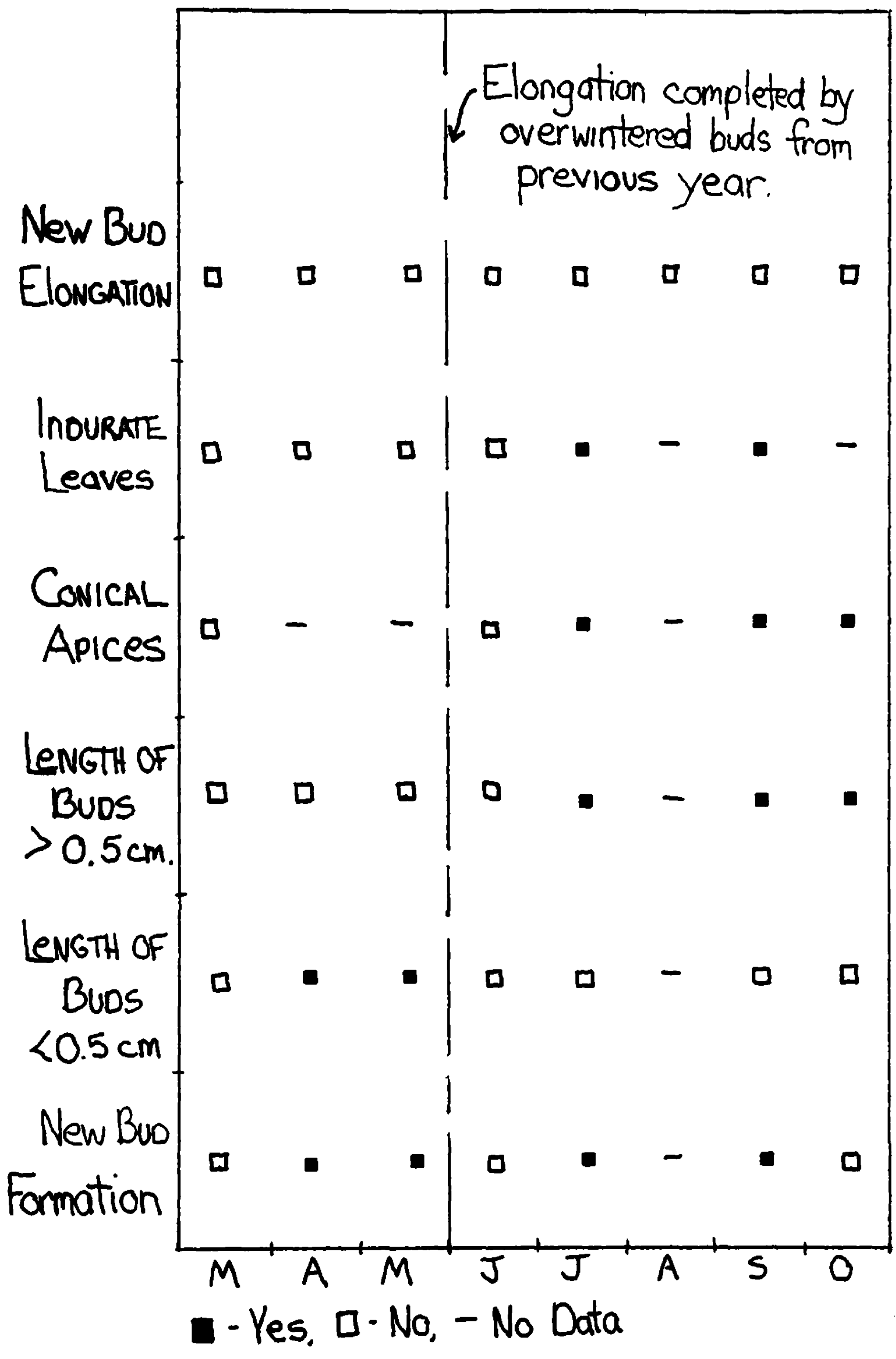
An experiment on removal of outer whorls of leaves of buds to determine any possible effects on survival and fertility yielded no conclusive results as most basal buds died and those that survived were not fertile. Narrow stems (2.0 mm in diameter) were produced when small shoots grew almost to ground level and then branched. These were similar to those produced in the vernalization experiment, (Chart 14).



□-No ■-Yes, -No Data, Clark Fork, △-No, ▲-Yes, -No Data, WH

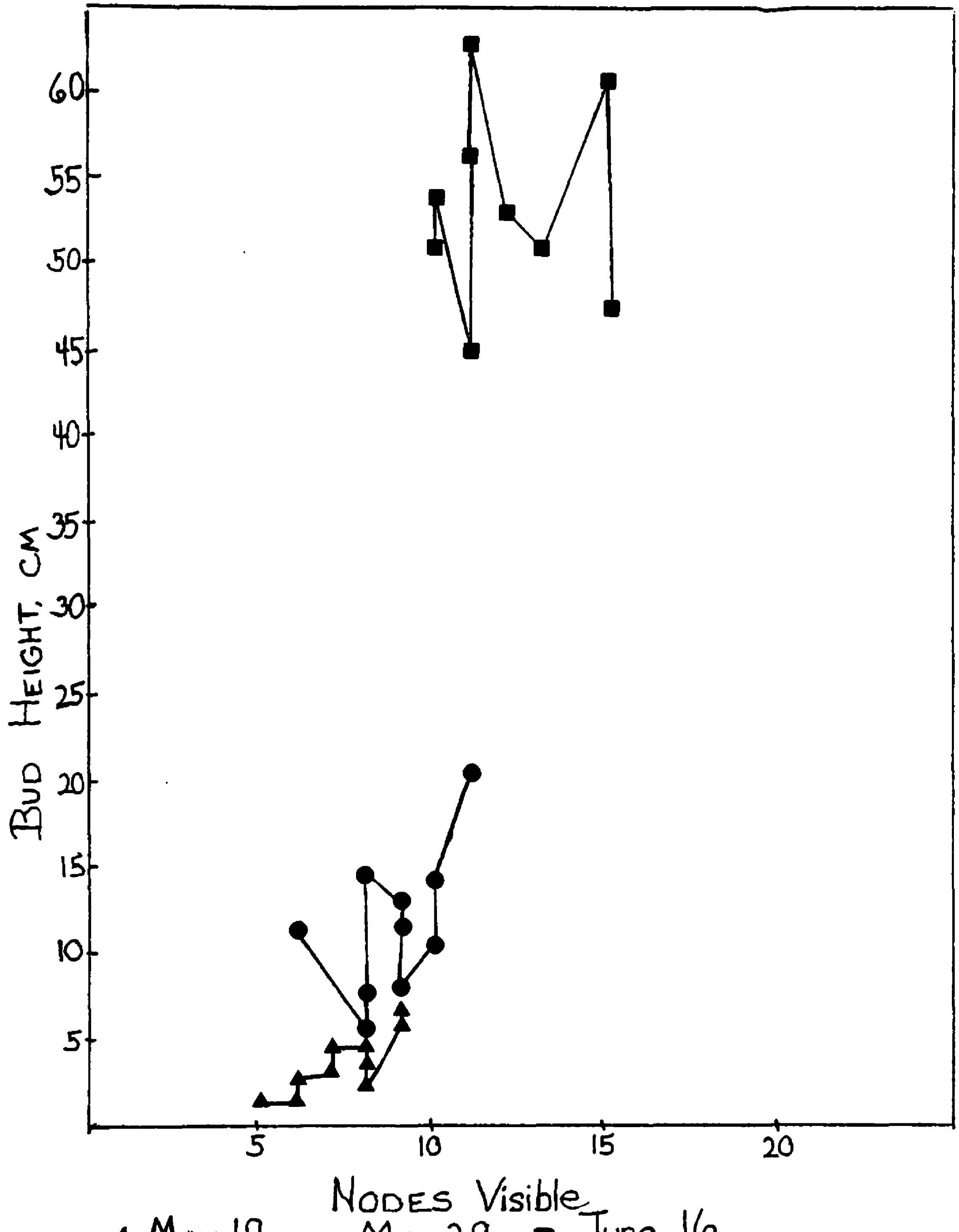
E. laevigatum Basal Buds, 1984

Figure 7.



E. laevigatum Basal Bud Formation, 1983-84

Figure 8.



E. laevigatum Bud Growth, 1983, Hellgate Canyon

Figure 9

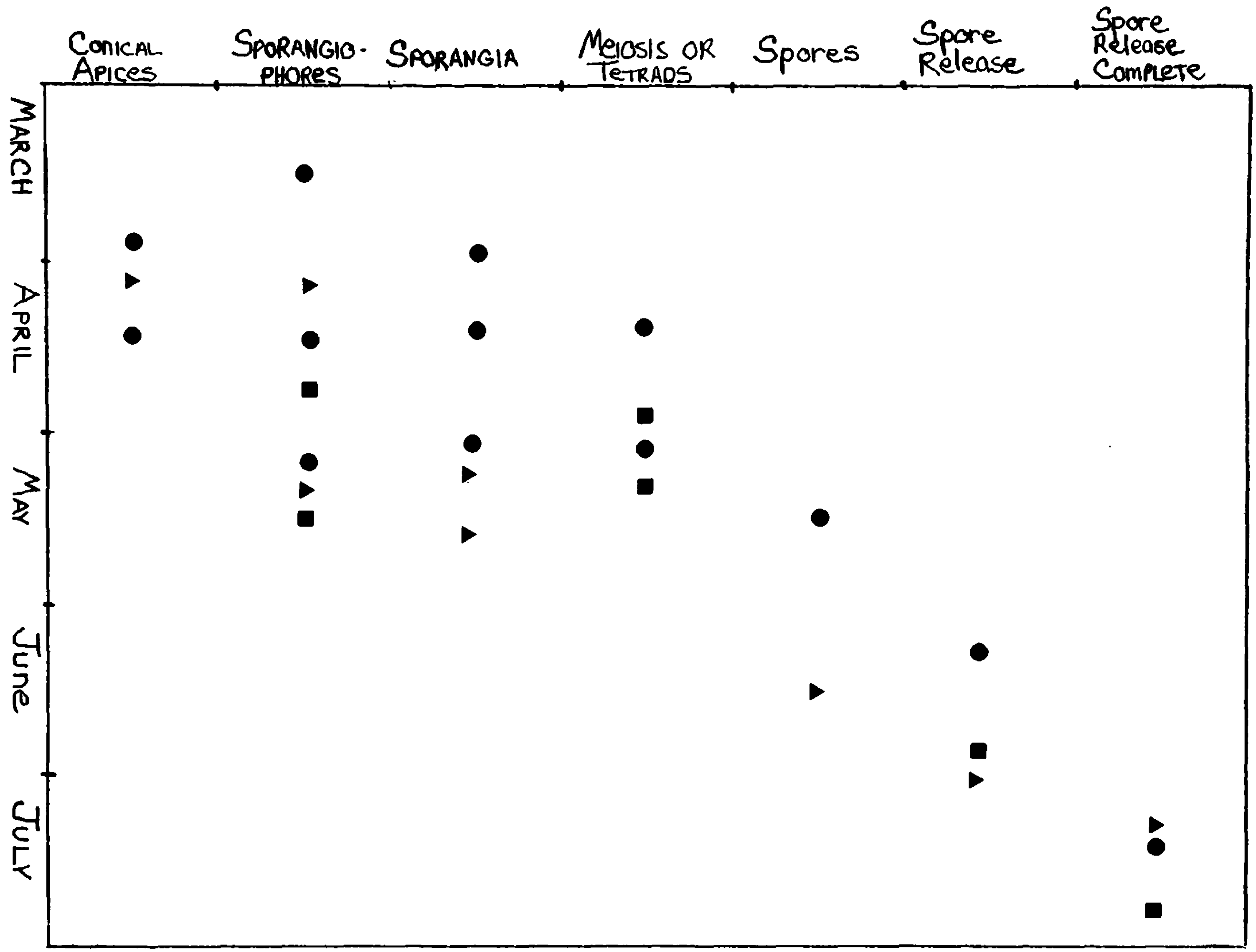


FIGURE 10

E. laevigatum Strobilus Development, 1984

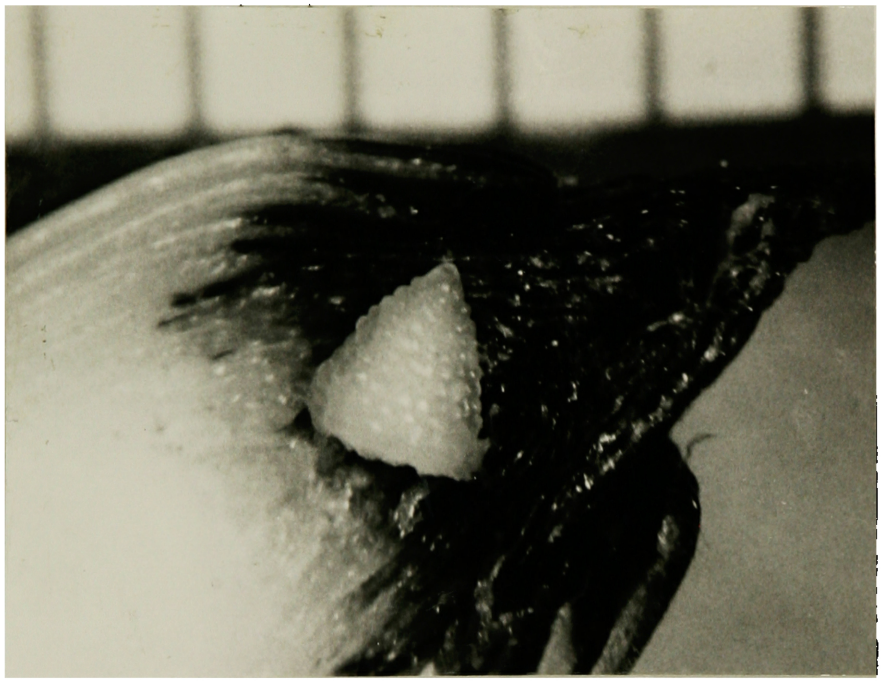
▲HC, ●WH, ■CF

Plate 13

<u>Equisetum laevigatum</u>

1. above
   Strobilus with developing sporangiophores, millimeter scale.
   Collected April 15, 1984 at Hellgate Canyon.

2. below
   Unelongated basal bud, overwintered.
   Collected March 14, 1984 at Waterworks Hill
   Millimeter scale.



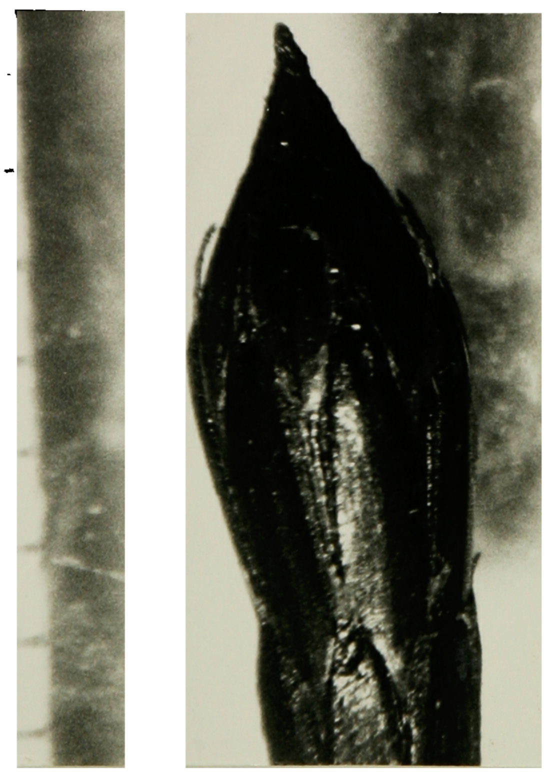
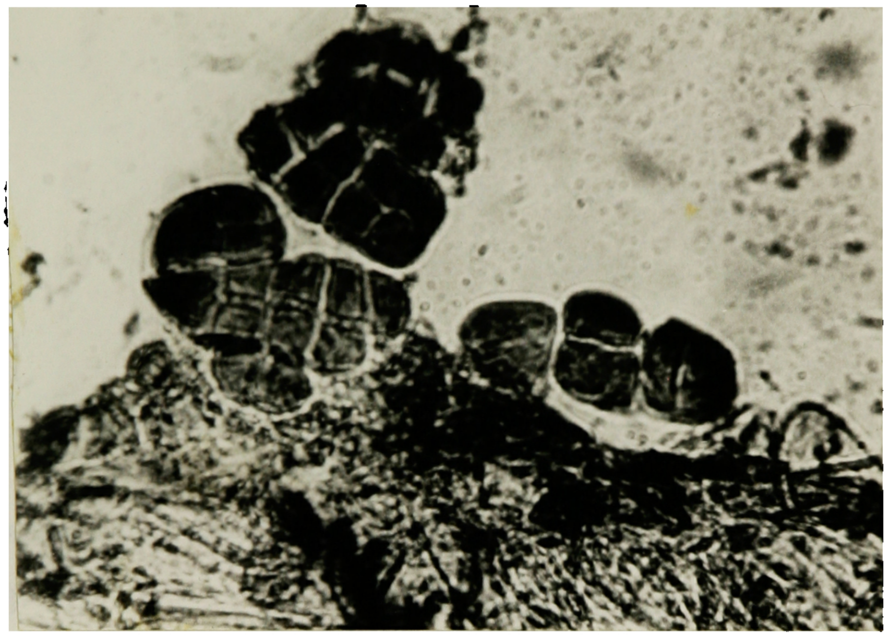


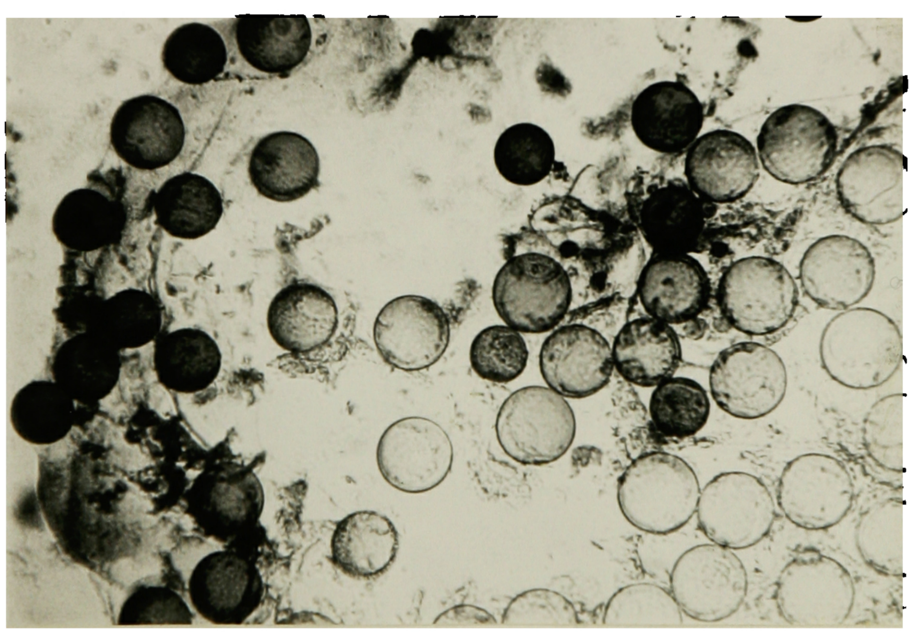
Plate 14

Equisetum laevigatum

1. above
   Meiotic tetrads,
   from strobilus collected April 22, 1984
   at the Clark Fork River.

2. below
   Spores without elaters, spores light
   yellow-green.
   from strobilus collected May 13, 1984
   at the Clark Fork River.





Equisetum palustre (after Hauke, 1978), is a North American and Eurasian member of the subgenus *Equisetum.* It inhabits swamps, marshes, and other wet places. Stems are annual, monomorphic, and branched. They are 20-80cm tall, 1-3mm in diameter with 5-10 ridges. The central canal is 1/6-1/3 the stem diameter and vallecular canals are nearly as large. The green sheaths are 4-9mm long with persistent 2-5mm long teeth that are narrow and black with white margins. The first internode of the spreading branches is 2 to 5mm long, (shorter than the subtending stem sheath). Strobili are 9-35mm long. Rhizomes are shiny and black to dark brown.

Basal buds enclosed in coriaceous leaves were observed in autumn (Chart 23). These were sterile or had conical apices. Buds overwintered at the bases of aerial stems, which died back in October (Plate 15).

Buds elongated in spring and by late June strobili were present at the apex of elongated stems. Spores were green with elaters but were not being released. Many fertile plants were mowed down before early July, when the remaining stems began to release spores. Spore release was complete by late July.

No gametophytes were found.

Plate 15

Equisetum palustre
Basal bud collected in October, 1984 at Flathead Lake.

Equisetum variegatum, (after Hauke, 1963), is an evergreen, circumboreal member of the subgenus *Hippochaete*. Monomorphic stems are small (6.5-47.5cm), thin, (0.5-3.0mm), and unbranched with 3-12 ridges. The central canal is about 1/3 the stem diameter or absent in the narrowest stems. Vallecular canals are conspicuous. Sheaths are slightly spreading, green with a black rim, and have persistent teeth that are 1-2mm long, 0.5-0.75mm wide with white margins, brown centers and deciduous, filiform, 1mm long tips. Apiculate strobili have four or more whorls of sporangiophores. Spores frequently abort in small specimens from northern regions. The rhizome is smooth, dark brown to black, and has a central canal.

Buds were initiated along the rhizome at the base of older stems throughout the 1983 and 1984 growing seasons. These elongated, and if fertile, developed strobili until the ground froze in November, (Chart 25). If they had not elongated before the soil froze, they overwintered at or within 3cm of the ground's surface and elongated in the spring.

Marked stems elongated in the late summer and early fall. Elongation slowed by early November.

Underground buds, incompletely expanded stems, and completely expanded stems overwintered in 1984. Strobili in various developmental stages overwintered within unexpanded stems and at the apices of expanded stems, (many of which were broken by ice). Stems with conical apices were often less than 13.5cm tall in fall of 1984, (Figure 11). Conical apices were noticeably large and sometimes elongated but were not visibly fertile or sterile.

Stem elongation and strobilus development began during spring, (Figure 12, Chart 24). Overwintered stems were completely elongated by mid June. The population was underwater from mid-May until mid-June in both years. Strobilus development occurred throughout the growing seasons and meiosis was seen in June, July, and November of 1984, (Figure 12). Small, noncircular spores without elaters were produced. Abortive spores were yellow or white, having no chlorophyll. Tetrads from November, 1984 were incomplete or irregularly shaped; one tetrad had three intact cells but four cell walls.

Spores were present by mid-June in both years but were not released in either year. The spores changed color from green to white and the strobili turned yellow and aborted, (Plates 16 and 17).

No gametophytes were seen.

Fertile stems died after strobilus abortion or at the growing season's end, which is also when sterile stems died. Those initiated in autumn that overwintered as buds or as unexpanded stems died the following growing season, while those initiated in spring died during the fall of the same year.

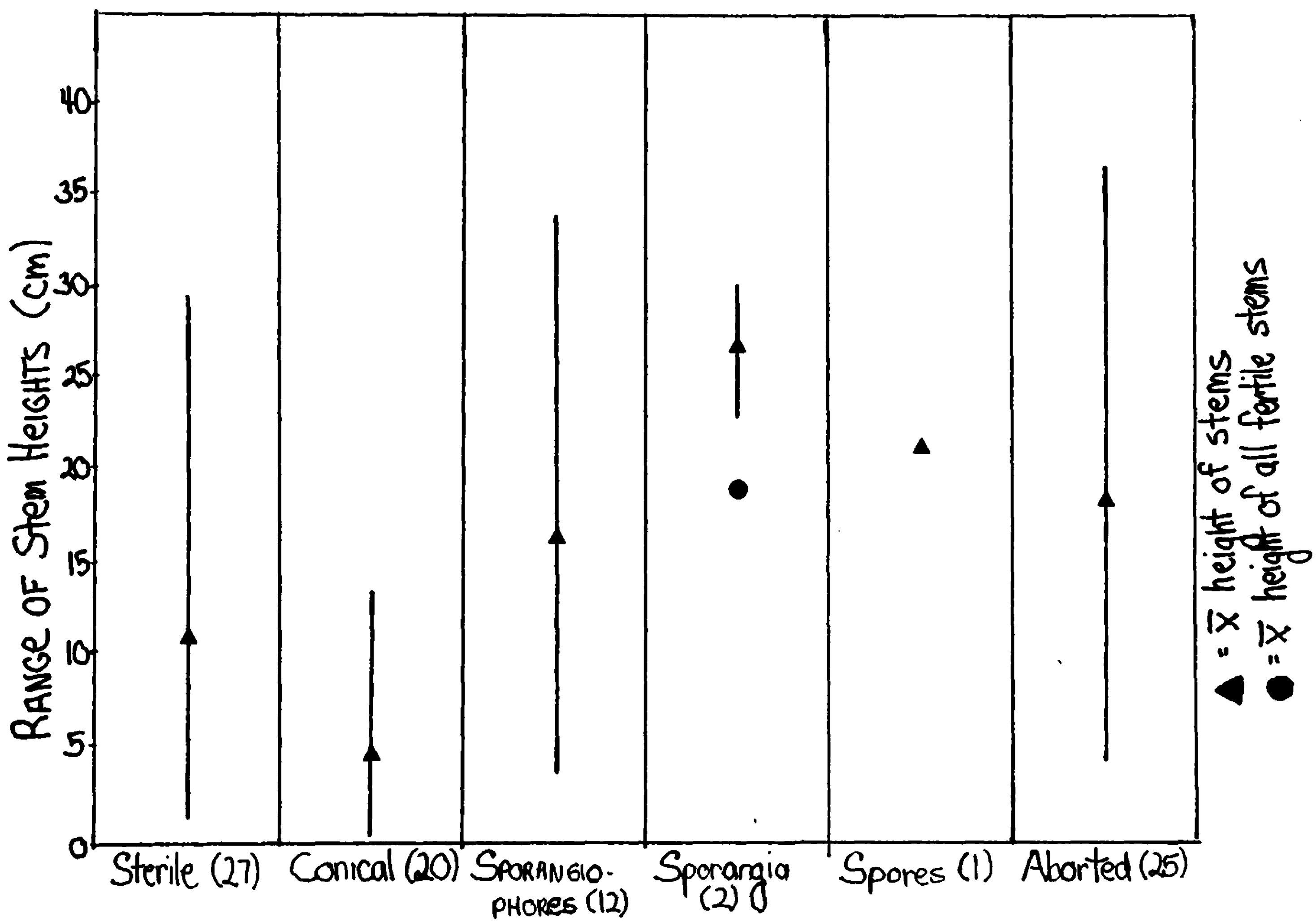


Figure 11 *E. variegatum* Stem Height and Fertility. October and November, 1984

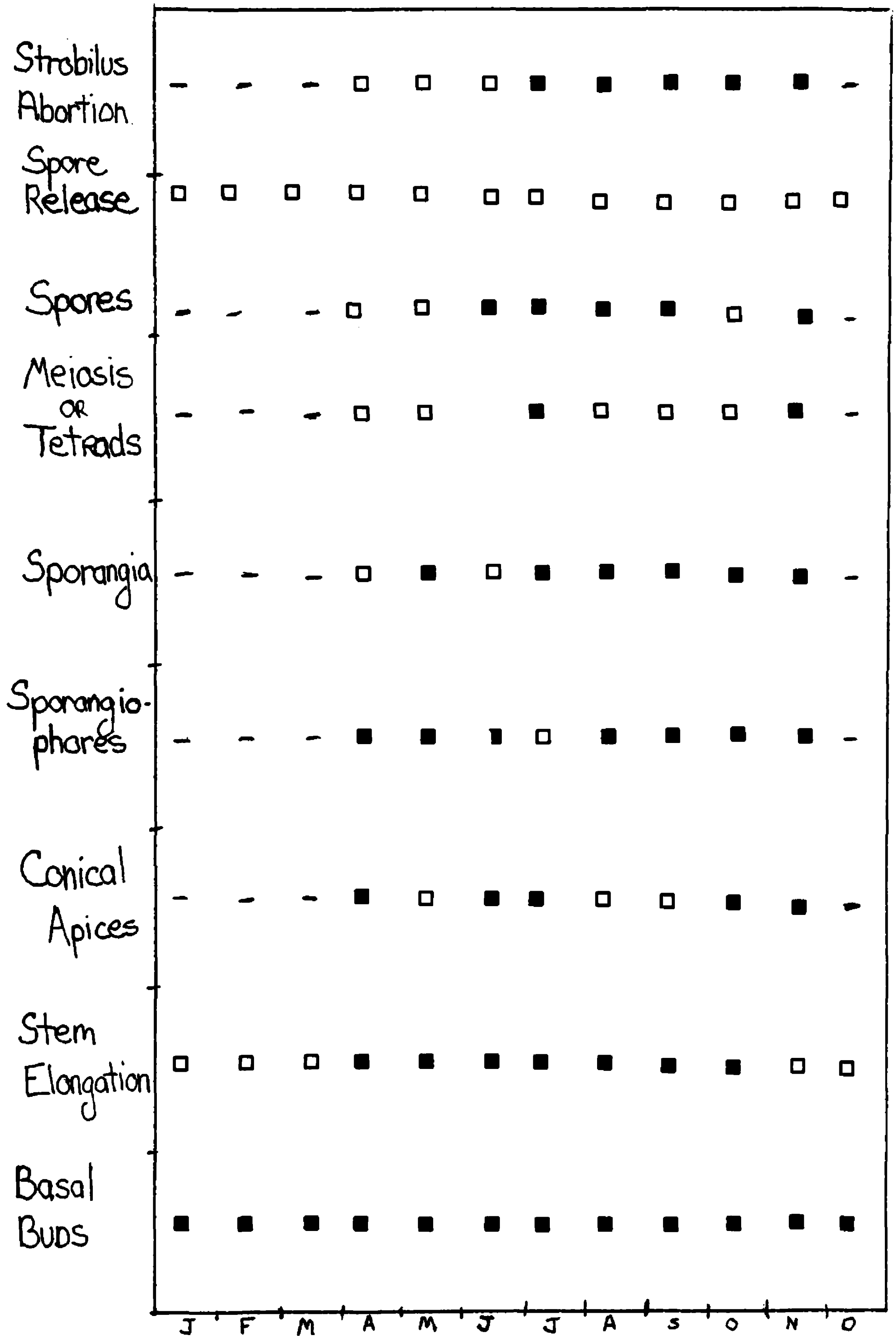


FIGURE 12 ■ Yes, □ No, – No DATA

<u>E. variegatum</u> Strobilus Development and Stem Elongation, 1984

Plate 16

Equisetum variegatum
Strobili at tips of evergreen, expanded stems.
July, 1983 at Jacob's Island.

Plate 17

Equisetum variegatum
Abortive strobili and developing lateral branches on evergreen stems at Jacob's Island in July, 1983.

Equisetum hyemale, (after Hauke, 1963), subgenus Hippochaete, lives in woods and along rivers, ditches, and railroad tracks in North America, Europe, and Asia. Evergreen, unbranched stems are terminated by 13mm long apiculate strobili if fertile. Stems are 20-95cm tall, 3-8mm in diameter, with 14-26 ridges. Vallecular canals are large and the central canal is 2/3 the stem diameter. The white sheaths are are 5.5-14.0mm long, appressed to the stem, and delineated by black rims at the top and near the bottom. Teeth are pushed off during stem elongation to form a cap. Rhizomes are dull, black, and coarse.

Strobili overwintered at the tips of the evergreen stems and buds overwintered at their bases. The strobili released their spores and the buds elongated during the following spring (Figure 13).

Basal buds formed during summer and fall of both years. They developed at the base of aerial stems, approximately halfway between the rhizome and ground level. These buds were first visible as small swellings on the stems or rhizomes during July of both years. Initiation of buds occurred until the ground became frozen in late November.

Buds had conical apices from their initiation onwards, and coriaceous leaves developed as the buds grew. Conical apices examined in late fall and winter were approximately the same shape and size as those seen in early fall, (Figures 14 and 15, Charts 26, 27, and 28).

No buds elongated immediately after being formed. All buds overwintered and elongated during the following spring.

Buds began to elongate in late April and early May of both years. This coincided with spore release. Stem elongation was complete by late July, (Plate 19, Charts 29, 30, 31, and 32).

Strobili developed as the stems elongated, (Plates 18 and 19, Figure 14, Charts 29, 30, 31, and 32). Lengthened conical apices were found before sporangiophores were seen. Strobili differentiated acropetally. Premeiotic strobili were seen in July, 1984, but some meiosis occurred by late June. Stems were completely elongated by late July and black rings were forming acropetally on the sheaths. The strobili were visible and their basal parts were enclosed in the uppermost whorls of leaves. No strobilus elongation or spore release occurred until the following spring. The strobili containing green spores with elaters overwinter.

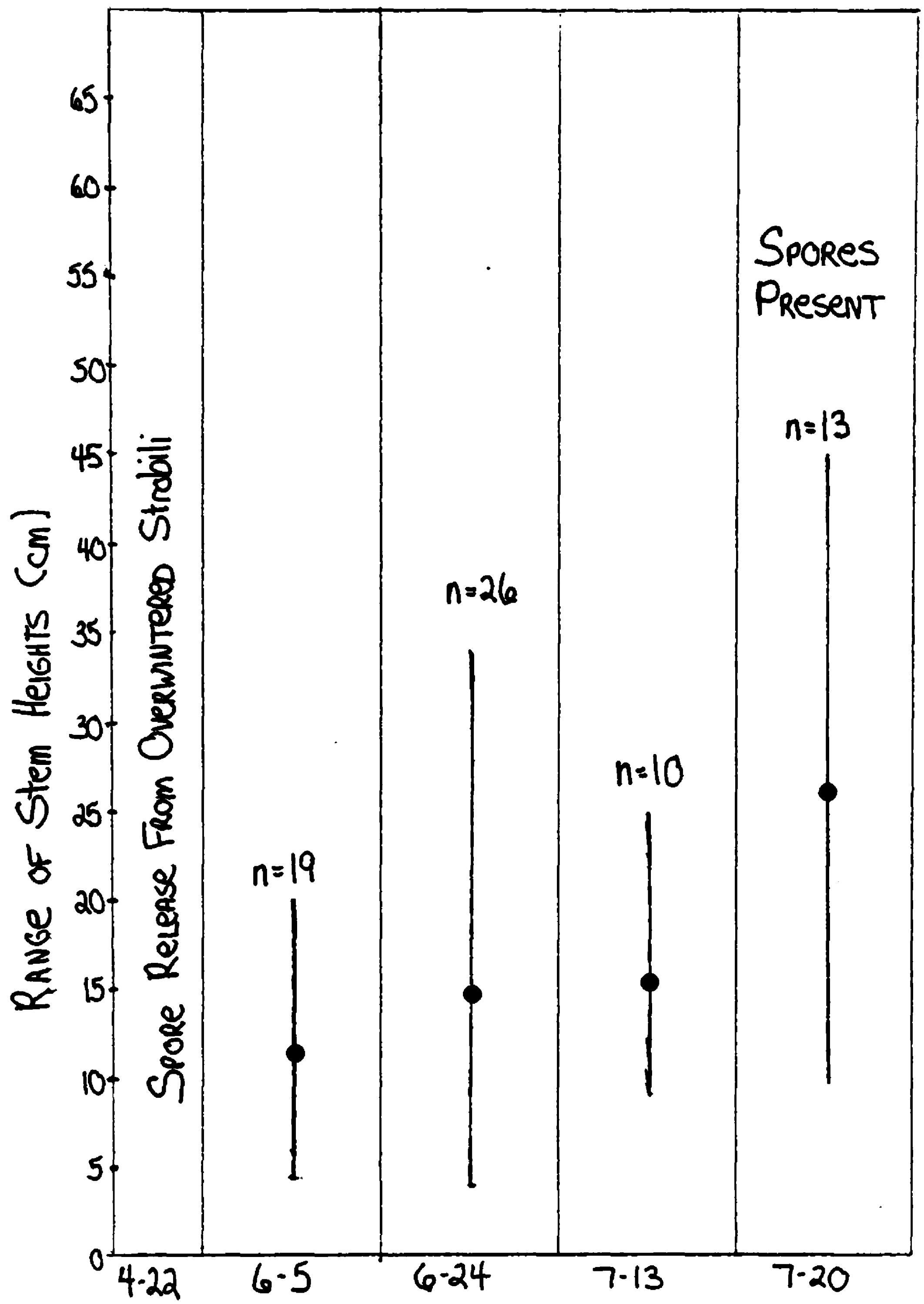
Spores were released in late April of both years. Spores were released basipetally. Apical parts of strobili that were not enclosed by leaves during the winter had green spores that appeared normal. Strobili change color from orange-green to yellow upon spore release.

No gametophytes were found.

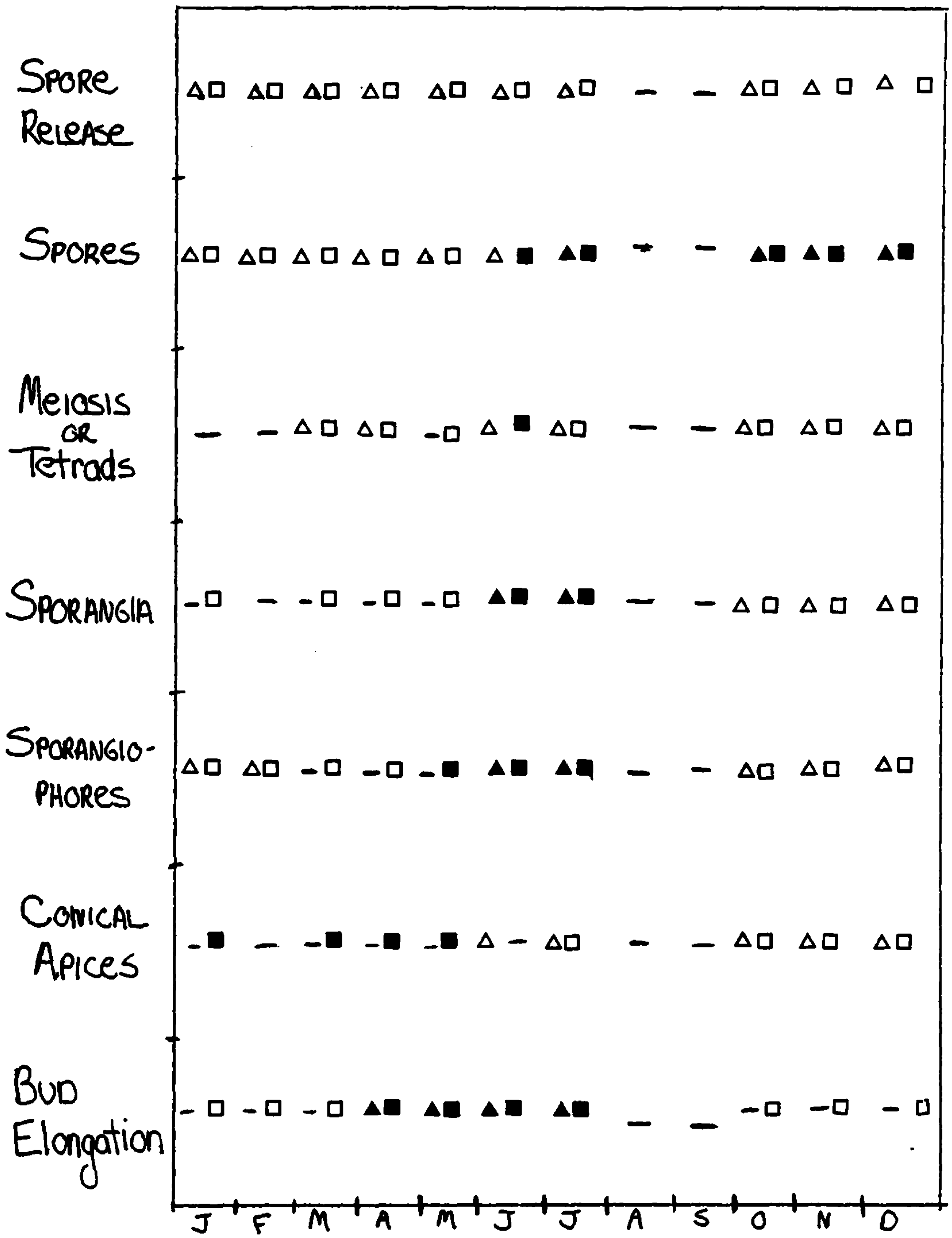
Stems usually did not die back immediately after spore release, (Chart 32). Stems that released spores from a single, terminal strobilus in 1984 and which then developed lateral, strobilus bearing branches were not dying by November, 1984. Many stems that released spores in 1984 and that did not develop side branches were dying by November, 1984.

To study the effects of heat on spore release, eleven fertile stems were placed in a clay pot in the greenhouse at 22.2 C. Ten other fertile stems were placed in a clay pot outside of the greenhouse. These stems were collected on October 6, 1984 at the Clark Fork River, (Site 5c). On October 15, 1984 none of the stems placed outside had released spores or died. All eleven of those placed in the greenhouse had turned orange. Three of the eleven were releasing spores, and an additional three had elongated strobili, but no spores were released.

A second experiment was begun on October 16, 1984 with 20 fertile stems from Jacob's Island. Three of the ten greenhouse stems released spores and none of the outside stems had by November 7, 1984.

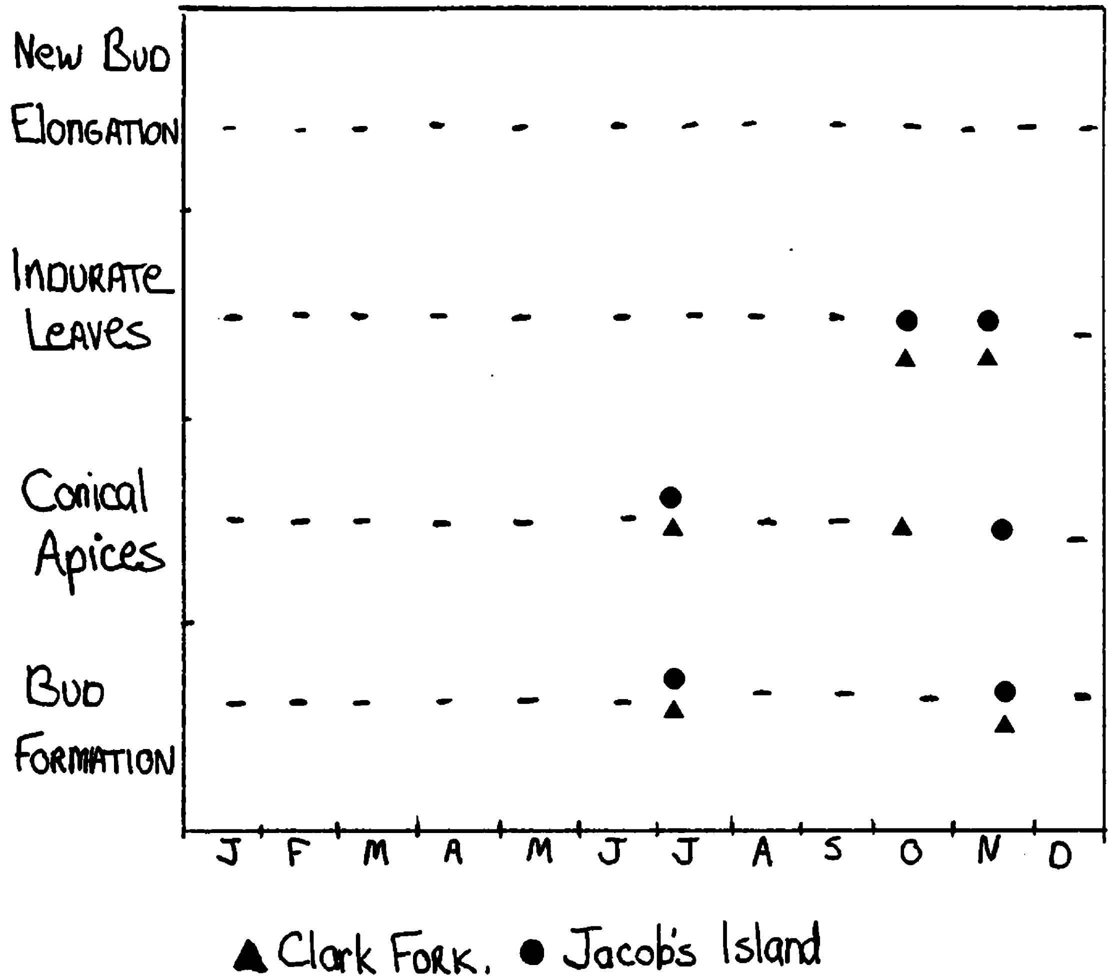


E. hyemale Bud Elongation, Jacob's Island, 1983
Figure 13



■ Yes, □ No, Clark Fork, ▲ Yes, △ No, Jacob's Island
– No Data

E. hyemale New Strobilus Development, 1984
Figure 14



E. hyemale Basal Bud Formation, 1984

Figure 15

Plate 18

<u>Equisetum hyemale</u>

1. left
   Elongating stem with strobilus and expanding internodes.
   Collected June, 1984

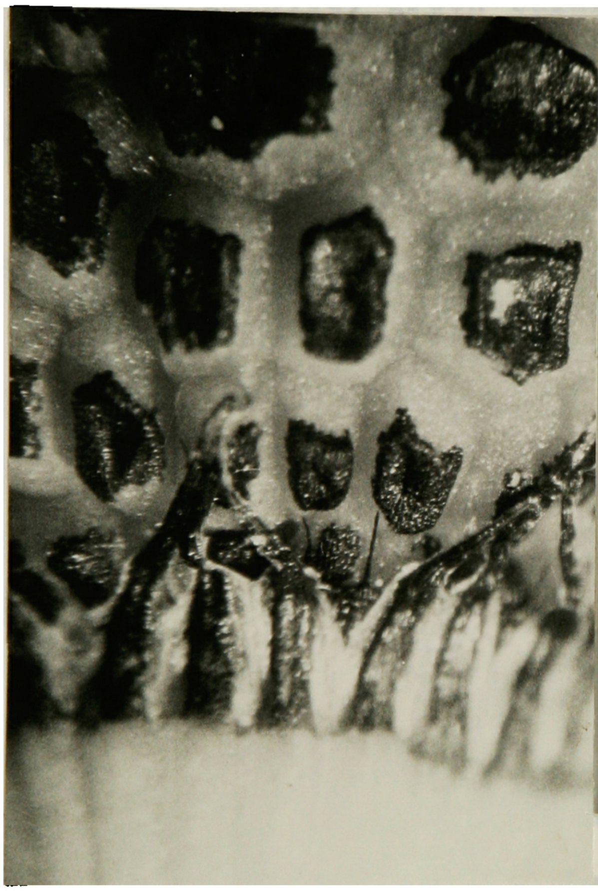
2. right
   Strobilus with developing sporangiophores
   Collected June, 1984

Plate 19

<u>Equisetum hyemale</u>

1. left
   Outside of strobilus, showing upper
   leaves and sporangiophores.
   Summer, 1984

2. right
   Elongated stem
   Summer, 1984.
   Stem at left marked with yarn.




Equisetum scirpoides, (after Hauke, 1978), subgenus *Hippochaete*, is a circumboreal inhabitant of tundra, mossy places, and moist woods. The small monomorphic stems are evergreen, clustered, and unbranched. They are somewhat twisted, up to 15.0cm tall, 0.5 to 1.0mm in diameter with 3 ridges, no central canal and 3 vallecular canals. Sheaths are three-segmented, green below, black above and 1.0-2.5mm long with a short apiculum and 2-3 whorls of sporangiophores. Rhizomes are nearly black, rough, and 0.5-1.0mm in diameter.

Buds overwintered at the base of the evergreen stems and strobili overwintered at their tips, (Charts 33 and 34).

Side branches at the bases of stem clusters and small (0.25mm) buds on the rhizome both overwintered with conical apices, (Chart 33, Plate 20). The small buds were present by mid November, 1983 but were not present in early October, 1984.

Empty, withered strobili at the Jocko River, (site 9a), in May, 1984 indicated recent spore release, (Chart 34). Since plants collected and grown in the greenhouse and plants at the Jocko River had developed strobili with spores by July, meiosis probably occurred in June or early July.

As there were no elongated strobili, spore release, or empty strobili in July or October, the strobili formed in early summer probably overwintered.

No gametophytes were found.

Fertile plants from October and November, 1983 collections released spores within 2 weeks after being placed in the greenhouse. These had been refrigerated for 7-14 days. Fertile plants placed in a house window released spores within 14 days. These developed 23 strobili from existing stems and 3 from new stems. Collected plants from October 7, 1984 did not release spores when placed outside the greenhouse, while others collected the same day and put in the greenhouse did release spores.

Plate 20

<u>Equisetum scirpoides</u>

1. above, center
   Strobilus with spores
   Collected July 22, 1984 at Jocko River.

2. below, left
   Side shoots on bases of expanded stems
   Collected October 9, 1983 at Jocko River.

3. below, right
   Strobilus with spores,
   Collected October 9, 1983 at Jocko River.

Conclusions

There are five different life cycle patterns among the ten Equisetum species studied. The life cycle patterns are defined by the timing of basal bud formation, strobilus formation, spore release, stem elongation, and stem dieback. Other factors are the presence of monomorphic stems, overwintering buds, and delays between strobilus formation and spore release.

The first life cycle pattern is characterized by the presence of dimorphic stems, strobili with spores that overwinter within basal buds, and spore release from the nonphotosynthetic fertile stems in spring or early summer. The fertile stems expand quickly at the beginning of the growing season, release their spores, and then die or can become branched and photosynthetic. Vegetative stems begin growth and expansion while the fertile stems are releasing spores. They persist throughout the growing season. Both stem types originate from basal buds that are formed the previous growing season. After fertile buds are initiated, strobilus development and then meiosis occur. The fertile and sterile buds then overwinter and elongated the following spring to complete the life cyle.

E. telmateia, E. arvense, E. sylvaticum, and E. pratense are all in the subgenus Equisetum and share this life cycle pattern.

Bierhorst (1971) states that most populations of E. arvense form strobili and spores during the summer, which then overwinter and expand the following spring. This is in agreement with my observations.

As the E. pratense population observed was sterile, its life cycle pattern can only be extrapolated from Hauke's work, which describes E. pratense as having dimorphic stems and releasing its spores in May and June.

No meiosis during summer of fertile basal buds with spores were observed in E. telmateia, but it appears that spores do overwinter, as fertile stem elongation and spore release occur in late March. One fertile bud was seen elongating in July; this could indicate some summer spore release in E. telmateia.

E. arvense followed this life cycle pattern except for some fertile bud elongation and spore release in late June and July at the Jacob's Island population. This occurred just after the spring floodwaters of the Clark Fork River receded. This was unusual because all other E. arvense populations studied released spores only in spring and because no other populations were flooded. This unique event provided an opportunity to observe a different set of conditions and compare any differences in the life cycle pattern with unflooded E. arvense populations.

Flooding was followed by warm summer days with daylengths of greater than 15 hours. This is similar to early spring when melting snow provided extra moisture and the temperature rises. This similarity could explain the two separate sets of spore release. The fertile buds expanding in summer could have overwintered, been delayed in growth, were then flooded, and expanded when the flood receded. Or, the fertile buds could be initiated while the river level is high. When the floods recede, they would be able to expand, undergo meiosis, and release spores. Other fertile buds could possibly have been initiated too late to be mature enough to expand after the flood recedes. These would remain unexpanded, develop their strobili, and undergo meiosis later in the season than the expanded fertile buds did. Since they develop later, they overwinter and release spores during the next spring when the snow melts, temperatures are warmer and daylengths are long.

No basal buds were found in E. arvense between spore release and the end of flooding when the second set of spore release occurred. This could be due to sampling difficulties. Flooding begins during the end of the first set of spore release, and the second set of fertile stems are elongating when the floods recede. This makes it difficult to determine whether the second set of fertile stems overwintered or were newly initiated. Basal buds are always found at the bases of vegetative stems. There were no fully expanded vegetative buds at the onset of flooding, so it is possibly that fertile buds are initiated during the spring flood. They could also be initiated just before the spring flood.

One of the elongated stems was branched with an apical strobilus. Another had a strobilus in the middle of the stem and branches above the strobilus. Bierhorst (1971) attributes branched strobilus bearing stems to strobili that stop growing at an earlier stage in the fall, and then elongate in the spring. As these stems were found in July, it cannot be concluded that they were formed in the fall, although the possibility exists. Perhaps the strobili that cease growth at an early stage have not converted their entire apices to strobili, allowing the stem to branch.

Basal buds form along the rhizome at the bases of vegetative stems. The rhizome is underwater during the flood, so it could not detect daylength. Daylength is possibly perceived by the fertile stems during late April and early May when the days are between 14 hours, 22 minutes, and 15 hours, 2 minutes long. The fertile stems are not flooded during these daylengths and could transfer a signal for fertile bud initiation to the rhizome.

Many fertile buds with developing sporangiophores were found in late July and late September. Because of their early stages of development, they were probably initiated after the flood. Also, as not all populations experienced the flood, it seems that the long days of approximately 15 hours trigger the beginning of fertile bud initiation, and that fertile bud initiation probably continues to occur until the end of August when days are 13 hours and 24 minutes long.

In this life cycle pattern, high moisture levels, (early spring snowmelt and late spring flooding), increasing daylength and warm temperatures probably trigger bud elongation and spore release. Long photoperiods initiate basal bud and strobilus formation.

E. fluviatile, also in the subgenus Equisetum, has the second life cycle pattern. This life cycle pattern differs from the first in having monomorphic stems and spore release later in the growing season, and because some strobili overwinter and others form in the spring while the stems are elongating. Conical apices overwinter in basal buds; this did not occur in the first life cycle pattern. If fertile, these conical apices develop into strobili in the spring when the bud elongates. Strobili that overwinter form within basal buds in October, when the aerial stems die back. Those forming in October were premeiotic, and since no meiosis was seen in the strobili that developed in spring it seems that meiosis occurs between May and July, when the spores are released.

Long daylengths (15 hours) could induce the conical apex formation. If basal buds are formed in the summer, the conical apices could also form while the daylengths are long. If long daylengths trigger fertility, the conical apices could then become fertile. Strobili form in October when the daylength is just over ten hours. If the strobili develop from a conical apex that was induced during long daylengths, it could mean that the conical apex became fertile during the long days and that the strobili develop during the remainder of the growing season.

It is not known why developing strobili were not observed in the fall in the Flathead River and Flathead Lake populations while they were in the Salmon Lake populztion. Different genotypes in the different population could influence different phenologies. Or, as the sample size was small and sampling was infrequent, the Flathead populations could possibly have developed some strobili during October and November.

E. palustre, subgenus Equisetum, and E. leavigatum, subgenus Hippochaete have the third pattern. Annual, monomorphic stems, overwintering conical apices, spring strobilus formation and summer spore release characterize this pattern. Also, basal buds of both species are presumably formed in the summer, as E. palustre rhizomes were not examined in the summer, but basal buds were present by October.

In E. laevigatum, basal bud formation begins in July when daylengths are more than 15 hours. Bud formation continues until September. Small buds seen in April and May were probably root buds. The basal buds had conical apices from their inception in July onward. The long daylengths that probably triggered basal bud formation could also have influenced the development of conical apices. The conical apices could develop by a signal that halts the initiation of leaves, leaving the rest of the apex conical and possibly fertile. Basal buds of E. palustre collected in October had strongly conical apices. Other buds collected at the same time did not. Their apices had mostly been converted to leaves. This suggests that the conical apices develop into strobili when spring arrives. Buds overwinter with coriaceous leaves that surround the conical apices and possibly protect them.

Strobilus development occurs in spring for E. palustre and E. laevigatum. In E. laevigatum, strobilus formation occurs between March and May while the stems are elongating. Meiosis occurs in April and May, when daylengths are between 13 hours, 35 minutes, and 15 hours.

Warm temperatures and extra moisture from melting snow probably act in concert to cause stem elongation. As the plant has no overwintering aerial parts, the stem elongation is probably not triggered by increasing daylength. Underground rhizomes could not detect the changes in daylength. Since development of strobili begins before the buds have elongated to ground level, presumably it is not influenced by daylength. Temperature and moisture are probably the important factors. Conical apices could already be induced to fertility be the previous summer's long days. Then the warmer temperatures and extra water trigger their development into strobili.

The experiment on removal of coriaceous leaves yielded no conclusive results because basal buds did not survive transplanting in either control or experimental groups. Disrupted plants often do not develop aerial stems. Instead the stems do not elongate above ground and secondary branches arise from the underdeveloped stems to cause clusters of slender stems (Hauke, 1963).

E. hyemale and *E. scirpoides*, subgenus *Hippochaete*, have the fourth life cycle pattern. This pattern is characterized by monomorphic, evergreen stems with apical strobili that overwinter and release their spores in spring. Basal buds are initiated in summer and overwinter with conical apices surrounded by coriaceous leaves. These buds expand in the spring and strobili devleop during their expansion. Meiosis occurs in summer.

In *E. hyemale*, basal bud elongation does not occur until the overwintered spores are released. The same is probably true of *E. scirpoides*, although no direct observation was made. Spore release could be a signal for the buds to begin elongation. Or, bud elongation could be triggered by warmer temperatures, an increase in moisture and longer daylengths, as spore release coincided with these environmental changes. The evergreen stems could possibly detect the increase in daylength and communicate this information to the buds. Daylengths are 15 hours when basal buds expand.

Strobili develop in the late spring and early summer when daylengths are greater than 15 hours. Since the temperature is high and the water level is up, these factors could also influence strobilus development.

In *E. hyemale*, basal buds form during the summer and fall. They begin to form when the daylengths are 15 hours, 31 minutes in July. Bud formation continues until the soil freezes in late November when the daylengths are 9 hours, 22 minutes. Long daylengths could trigger bud formation and then bud formation continues until it becomes too cold. Conical apices develop at the same time as basal buds do, so the same factors could influence their inception. Fertility could be determined when the buds form or the next spring when they begin to elongate. The basal buds do not begin to form strobili until they elongate above ground and are able to detect photoperiod.

In E. scirpoides, the side shoots examined in October had conical apices, but there were no basal buds until November. Even then, the basal buds were only 0.25mm in diameter. This could mean that the strobili develop the following spring from the side shoots or from the conical apices in the basal buds. The basal buds form when the daylengths are between 9 and 10 hours long, and temperatures are cold. Possibly, the cold temperatures could influence their development.

The basal buds were very small and could possibly have been root buds. They were too yound to have formed many whorls of leaves, but they did have conical apices. The conical apices in the side shoots were well developed; they could produce the next season's strobili. Visits to the population and observations were too infrequent to make any definite conclusions. Since plants placed in a house window developed strobili from both existing stems and new stems that elongated after the transplant, side shoots and buds could both produce strobili if this is similar to what happens in nature.

The fifth life cycle pattern is exhibited by E. variegatum, an evergreen member of the subgenus Hippochaete. This pattern is characterized by the complete lack of spore release in the population observed. All of its strobili aborted. Strobilus abortion and misshapen spores are common in E. variegatum from northern regions, (Hauke, 1963). Since more primitive species of Equisetum have perennial, green shoots terminated with apiculate cones, and are more common at lower latitudes, (Foster and Gifford, 1974), it seems that E. variegatum is primitive and would release its spores farther south.

Basal buds form throughout the growing season. These basal buds could actually have been secondary branches, since the primary aerial stems in E. variegatum are small and secondary branches are exactly like them (Hauke, 1963).

Strobilus development occurs throughout the growing season. Since meiosis was observed in June, July, August, and November, over a range of photoperiods from 15 hours, 52 minutes, to 9 hours, 51 minutes, meiosis can probably occur at any daylength after strobili are initiated. Growth and strobilus development ceases when the ground freezes.

All five life cycle patterns release spores when temperatures are warm, daylengths are relatively long, and moisture is abundant. E. variegatum is the exception as it does not release spores at Jacob's Island, but strobili appeared mature during summer. All species have overwintering buds or stems. Some species have overwintering strobili with spores; those that do not have conical apices that overwinter and can become fertile during the following spring.

The effects of moisture, temperature and daylength cannot be separated enough to conclude that any one factor is contributing to or causing strobilus development. Most strobilus development occurs when the daylength is long; at least 11 hours and 52 minutes. Some strobilus development occurs when the daylength is 10 hours, 14 minutes (E. fluviatile, October). As strobilus development was observed in different species from mid-March (E. laevigatum) until early November, E. variegatum, it would appear that unfrozen soil allows strobili to develop.

Basal bud and conical apex formation usually occur at the same time. Conical apices were found in buds that were formed in autumn, so long days are not necessary for conical apex formation.

The same signal that induces basal bud formation probably also induces conical apex formation. The beginning of basal bud formation during a growing season occurs when days are long for every species except E. variegatum. Bud formation is dependent on carbohydrates produced during the growing season. Once the buds begin to form, they often continue to do so until the ground becomes frozen. This suggests that once the plant perceives the signal for bud growth, it will continue to make buds even after the signal is no longer received. For example, long daylengths and warm temperatures both occur in mid-summer when much bud initiation begins. Days are short and temperatures are low in the fall, but bud initiation is still occurring.

The bud initiation-conical apex signal could also induce fertility and work in the same way. The signal for conical apex formation could be perceived during long days and cause the conical apices to be determinately fertile, but the strobili just would not develop until the climate was right.

This raises a further question. Why do some species have buds that overwinter with conical apices and other species have buds that overwinter with mature strobili? The answer seems to be that only species with dimorphic stems will overwinter with mature strobili in buds. E. fluviatile has strobili that overwinter in basal buds, but these were premeiotic.

Species with dimorphic stems would probably benefit from having the whole strobilus and stem present in one bud. This is because they release their spores soon after elongation, and usually fairly early in their growing season. The fertile stems are not as long lives as the sterile ones; fertil stems are designed for quick spore release. They usually die soon afterwards, but can become branched and photosynthetic.

Species with monomorphic stems have only one set of stems that lasts throughout the growing season. Their strobili develop from conical apices as the stem elongates, or development continues in premeiotic strobili that have overwintered. As they have only one set of stems, time must be allowed for a stem to develop enough to last throughout the whole season.

E. hyemale and E. scirpoides have strobili that overwinter at the tops of evergreen stems, and basal buds that overwinter at their bases with conical apices. In this way, strobili can release their spores early in the season and strong vegetative stems can develop.

Vernalization could also be a factor in strobilus development from conical apices. Vernalization, or prolonged cold periods, has long been known to be effective in producing flowers (Wareing and Phillips, 1981). As flowers and strobili both develop from the apices of their respective plants, vernalization could influence strobilus development. Flowers develop from apical cells (Foster and Gifford, 1974). Experiments conducted on E. laevigatum to determine the possible effects of vernalization on fertility and strobilus development yielded no conclusive results. The transplanted basal buds did not survive. Underdeveloped primary branches (aerial stems) branched to produce clusters of slender, secondary branches similar to those produced in the leaf removal experiment.

Equisetum strobilus formation follows a definite, ordered sequence of events. First, a conical apex inside of a basal bud or in an elongating stem lengthens. The lengthened apex has the outline of an elongated triangle, while the unlengthened apex has the outline of an equilateral triangle.

After the apex lengthens, sporangiophores begin to form acropetally. Sporangiophores first appear as whorls of circular lines around the apex. Circular swellings then form between the whorls of lines. The base of a strobilus can have whorls of developing sporangiophores, and its apex can be an elongated cone of undifferentiated tissue. The sporangiophores become hexagonal and flattened as they develop.

Sporangia first appear as small swellings on the inside of the sporangiophore. One sporangium develops at each corner of the hexagonal sporangiophore. Sporangia are premeiotic at this stage. After they enlarge, meiosis occurs. Following meiosis, the developing spores are in tetrads. The tetrads separate and then the elaters form.

After the spores develop, the strobilus will wither release its spores during the same growing season or will overwinter without releasing its spores.

Spore release occurs when the axis of the strobilus lengthens, separating the hexagonal sporangiophores. The hexagons were appressed to each other, barring spore release. The sporangia then dehisce along a longitudinal slit and the sporangial walls turn inside out. The spores disperse and are either carried in the air and eventually fall to the ground or fall to the ground immediately. Equisetum spores do not float, but do remain green in water for approximately 48 hours. As spores are released during the times of abundant moisture, it would seem beneficial to spore survival if spores could survive in water for a time.

Some species of *Equisetum* release spores soon after the strobilus is formed while others are dormant through a cold period that occurs between spore formation and release. *E. laevigatum*, *E. palustre*, and *E. fluviatile* release spores soon after formation, while *E. hyemale* and *E. scirpoides* spores last through the winter before being released. *E. variegatum* would probably have immediate spore release since its strobili are not hardened as they are in *E. hyemale* and *E. scirpoides*. There is not enough data on *E. sylvaticum* or *E. telmateia*, but it seems that they do not release their spores immediately after formation because no fertile bud elongation was seen except for one *E. telmateia* bud.

What factor could cause the delay between spore formation and spore release? Members of both subgenera had both rapid and delayed spore release. The causative factor is unknown. Tightly sealed strobili and strobili that overwinter inside buds probably both function to delay spore release until the following spring. All species with these characteristics release spores early in the growing season, with the exception of *E. fluviatile* which has premeiotic overwintering strobili. Annual, monomorphic species without overwintering strobili release their spores in the same season as their formation. This occurs in *E. laevigatum*, *E. palustre*, and probably in *E. fluviatile*, depending on when it undergoes meiosis.

Since spores are formed in mid-summer in species with overwintering spores, daylength probably does not induce the dormancy of the strobilus. The delayed release of spores could be due to varying concentrations of hormones (Wareing and Phillips, 1981).

Equisetum is very proficient at vegetative reproduction because of its rhizomes, tuberous underground stems, and intercalary meristems. Gametophytes are rare in nature and were never found during this study. This suggests that asexual reproduction is much more common than sexual. _Equisetum_ is also believed to be one of the oldest extant genera of vascular plants. This could be because it usually reproduces asexually and thereby preserves existing, successful genotypes. Sexual reproduction remains possible to allow new genotypes to arise and become established by asexual reproduction. This limited sexual reproduction could explain why the genus has existed for so long. It undergoes little change but has the capacity to do so. Strobilus production and spore production occur often, so _Equisetum_ obviously invests energy in the possibility of sexual reproduction. Spores germinate readily in culture. It is not known what environmental conditions allow gametophytes to form or what factors promote sexual reproduction. It may be that _Equisetum_ gametophytes are more common than is believed (they could just be hard to find), or it could be that spores will only germinate in nature under a set of conditions with very narrow parameters. There is definitely not a shortage of conditions in which strobili and spores will be produced.

As so many of the species grow in somewhat similar habitats, it is difficult to correlate habitat with phenology. E. fluviatile is the only aquatic species, and E. sylvaticum is called the woodland horsetail, but the habitats of the other species seem to intergrade. E. hyemale seems to grow in soils that are drier and rockier than does E. laevigatum, so E. hyemale's evergreen stems could allow it to inhabit what seem to be more harsh environments. However, E. scirpoides lives in poorly drained floodplains and it has evergreen stems. Morphology and phylogeny influence phenology, but habitat and environment must have some effect or the plants could not survive in their habitats and their probably would not be so many different phenologies within such a small area.

Perhaps differences in the amounts of water and the timing of such seasonal events as floods influence the phenologies of the different species. Careful environmental measurement with respect to phenologies could be the basis for another project.

This study suggests several possibilities for further work on Equisetum phenology. Separation of environmental factors to determine which factors effect phenology and which factors effect different stages of the life cycles is one project. The problems of delayed spore release, delayed strobilus formation from conical apices, and possible fertility of conical apices should be pursued.

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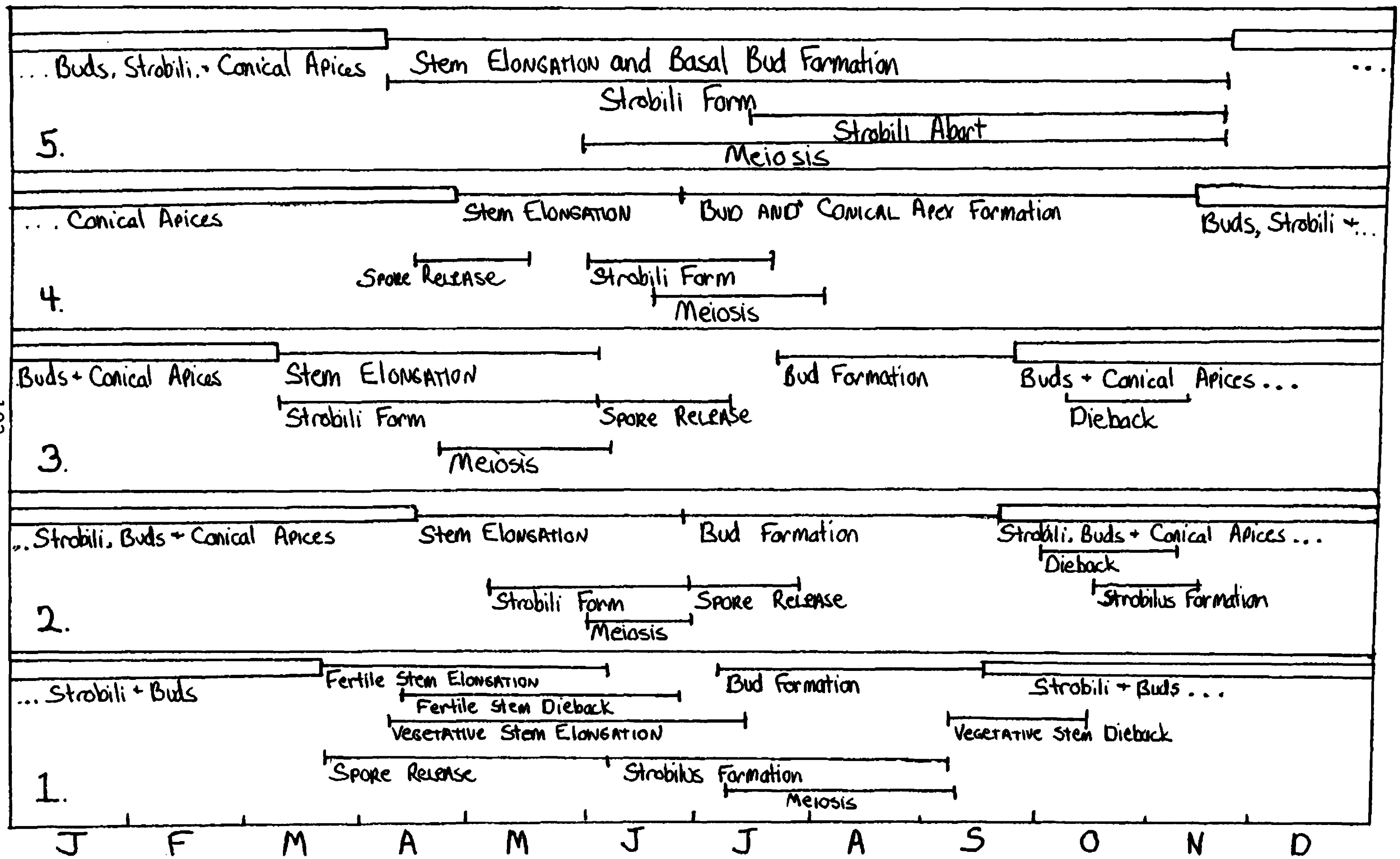
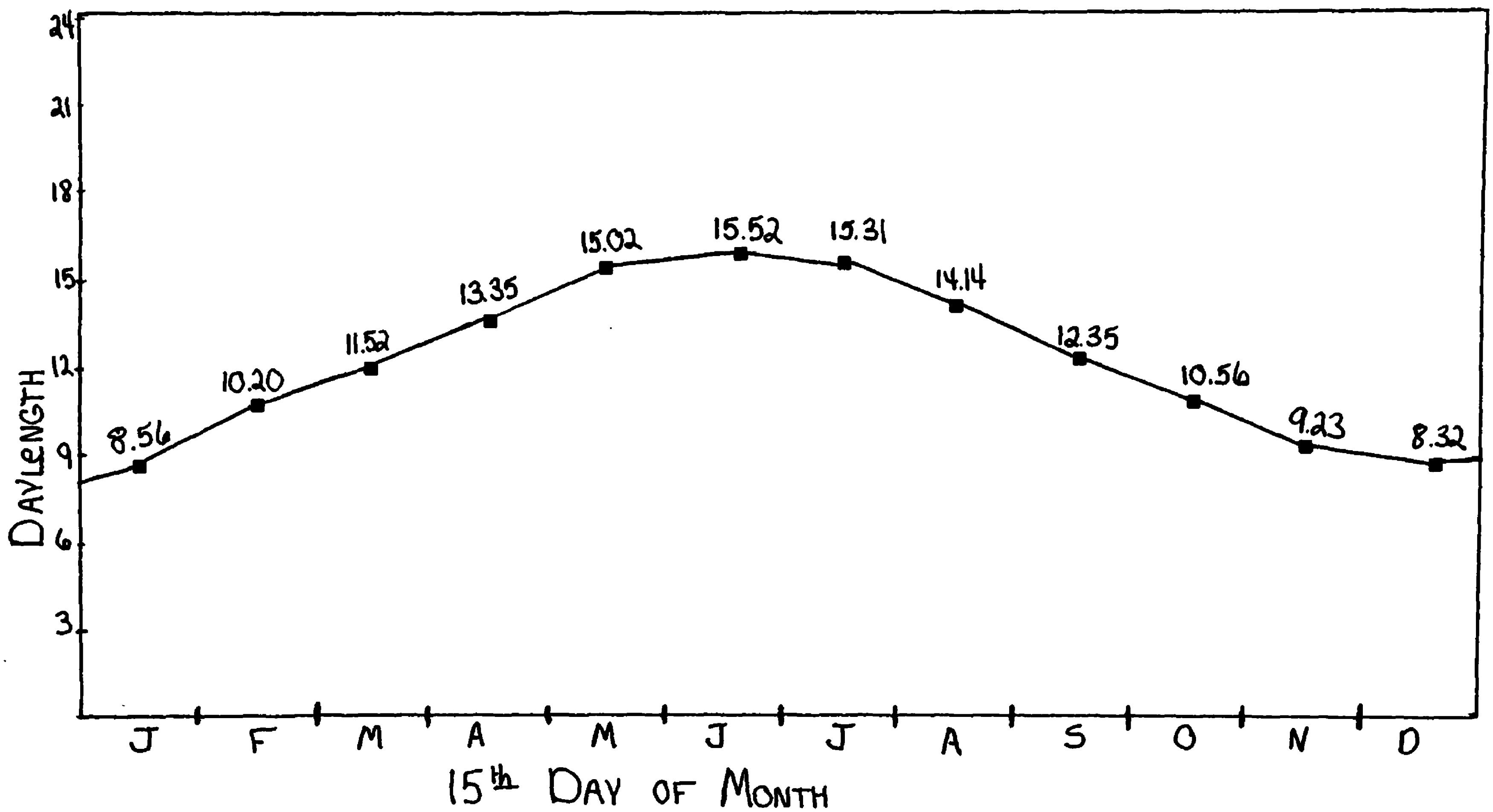


Appendix 1 Five Phenological Patterns in *Equisetum*



APPENDIX 2: DAYLENGTHS AT MISSOULA, MONTANA, 46° 52" N.

Appendix 3

Summary Charts

Species	Subgenus	Dimorphic Stems	Spore Release	Strobilus Formation
E. telmateia	*Equisetum*	Dimorphic	late March	Summer
E. arvense	*Equisetum*	Dimorphic	April, May (some in June)	Summer
E. sylvaticum	*Equisetum*	Dimorphic	June	Summer
E. pratense	*Equisetum*	Dimorphic	?	?
E. fluviatile	*Equisetum*	Monomorphic, Annual.	early July	May-June, October
E. palustre	*Equisetum*	Monomorphic, Annual	July	Between November and June.
E. laevigatum	*Hippochaete*	Monomorphic, Annual.	June	March-May
E. hyemale	*Hippochaete*	Evergreen	Spring, late April	May-June
E. scirpoides	*Hippochaete*	Evergreen	Spring, before May 24	Summer

CHART 1: *EQUISETUM* LIFE CYCLE PATTERNS

Species	Meiosis	Bud Formation	Overwintering Structures	Stem Dieback
E. telmateia	Summer?	Summer	Strobili in basal buds	Autumn?
E. arvense	Summer	Summer	Strobili in basal buds	Autumn
E. sylvaticum	Summer	Summer	Strobili in basal buds	Autumn?
E. pratense	?	Summer	Basal buds	Autumn
E. fluviatile	June?	Summer. by October.	Basal buds with conical apices or premeiotic strobili.	By November
E. palustre	before June 24	Summer	Conical apices in buds	By November
E. laevigatum	April, May	Summer	Conical apices in buds	By November
E. variegatum	June, July, August, November	All season	Conical apices in buds and incompletely expanded stems, premeiotic strobili in unexpanded stems, and strobili with spores in expanded stems.	Evergreen
E. hyemale and E. scirpoides	June, July	Summer, Fall	Evergreen stems with apical strobili and spores Conical apices in basal buds	Evergreen

Date	Location	Information
6-27	Jacob's Island.	2 fertile buds elongating, Sterile buds elongating. Flood receded in late June.
7-18	JI	Fertile buds above ground level, one fully expanded. Others unexpanded. Many fertile buds along rhizome. Sterile buds present.
7-20	JI	Fertile buds wtih green spores with elaters. 23 buds dissected had conical apices, apices less than 1.0mm.
9-8	JI	Fertile buds elongated to ground level.
9-27	JI	Fertile buds with green, elater bearing spores. 2 fertile buds elongated to 1.0cm above ground level.
10-12	JI	No fertile buds completely expanded, some approximately 1.0cm above ground level.

7-21	Waterworks Hill	Fertile buds present, unexpanded. Below moss layer. Conical apices in 5 sterile buds. Buds being initiated (approximately 2.0mm long)

CHART 2: <u>E. arvense</u> BASAL BUD FORMATION, 1983

Chart 3: *E. arvense* Spore Release and Stem Elongation, 1983

Jacob's Island

Date	Information
4-22	Fertile stems expanded. Spore release in progress. No sterile stems elongating.
5-1	Spore release in progress.
5-19	Fertile stems senescing. Spore release nearing completion. Sterile stems expanding, average length of 22 stems was 7.5cm.
5-19 until 6-26	River level was high, population underwater.
6-27	2 fertile stems elongating; breaking ground. New sterile stalks elongating.
7-18	1 fertile stem elongated.
9-8	No sterile stem dieback.
10-12	Sterile stem dieback beginning.

Chart 4: _E. arvense_ Spore Release and Stem Elongation, 1983

Date	Location	Information
4-22	Waterworks Hill	Fertile stems expanded. Spore release. Sterile stems beginning expansion.
5-8	WH	Sterile stems expanding, green or dark reddish brown when elongation begins. Average height 12.9cm.
5-19	WH	Fertile stalks senescing, spore release nearly complete. Sterile stems expanding and branching. Average height 22.3cm.
5-30	WH	Sterile stems fully expanded with average height of 27.6cm.
7-21	WH	Sterile stems beginning to die back.
9-27	WH	Large patches of sterile stems have died back.

Chart 5: E. arvense Spore Release and Stem Elongation, 1984

Jacob's Island

Date	Information
4-3	Fertile buds just above ground level. No expansion or spore release.
4-30	Spore release beginning. Fertile stems elongating. Some strobili releasing spores at ground level.
5-13	Sterile stalks elongating, spore release complete.
6-12	Sterile stalks expanded. Fertile stalks have died back.
6-23	Population underwater, river level rose.
7-7	1 fertile stem elongated to 6cm.
7-22	Approximately 20 fertile stems releasing spores.

<u>E. arvense</u> Spore Release and Stem Elongation, 1984

CHART 6

Date	Location	Information
3-4	WH	Fertile buds just above ground level. Holes punched in fallen leaves indicated some expansion after leaves fell.
4-12	WH	Fertile stems elongated. No strobilus expansion.
5-1	WH	Spore release complete. Fertile stalks senescent. Sterile shoots elongating.
5-17	WH	Sterile stems elongated.
10-24	WH	Sterile stem dieback nearing completion.

Date	Location	Information
4-22	Clark Fork River	Spore release in progress. Vegetative stems elongating.
5-19	CF	Spore release nearing completion. Fertile stems senescing. Sterile stems elongating, approximately 5.0cm tall.
5-26	CF	Sterile stems fully expanded and branched.
11-10	CF	Sterile stem dieback complete.

Chart 7: <u>E. arvense</u> Basal Bud and Strobilus Formation, 1984

Jacob's Island

Date	Information
4-30	No buds on rhizome. River rising.
6-23	Jacob's Island population underwater.
7-7	Fertile buds present. Of 13 fertile buds dissected: 3 had sporangiophores, 5 had premeiotic sporangia, 5 had spores. Tetrads present. Average fertile bud length was 1.1cm, one fertile bud elongated to 6.0cm. 11 of 24 buds collected were sterile. Buds less than 0.25cm were present.
7-22	Approximately 20 fertile buds expanded and released spores. One stem had vegetative branches and an apical strobilus. Buds are on rhizome at vegetative stem base. 8 of 20 buds dissected were sterile. Of 12 fertile buds, 1 had sporangiophores, 1 had premeiotic sporangia, 2 had tetrads, and 6 had spores and elongated stems.
7-27	9 of 16 buds were sterile. Of 7 fertile buds: 1 with sporangiophores, 1 with premeiotic sporangia, 1 in meiotic anaphase, 4 with spores.
9-29	Fertile buds that release spores in July have died. Unelongated fertile buds had green spores. Average fertile bud length was 1.26cm.
10-20	No fertile buds above ground level, buds at ground level to 3.0cm below.

Chart 8: *E. arvense* Basal Bud and Strobilus Formation, 1984, Waterworks Hill

Date	Information
5-1	Buds on rhizome with conical apices.
5-17	Buds on rhizome were sterile.
6-26	Buds were being initiated.
7-7	Buds on bases of expanded vegetative stems had conical apices.
8-14	4 sterile and 4 fertile buds collected: 1 with sporangia, 3 with spores. Strobili 0.5cm, 0.4cm, and 0.6cm. Indurate leaves present.
10-24	No fertile buds at ground level. Sterile buds at ground level.

Chart 9: *E. arvense* Basal Bud and Strobilus Formation, 1984, Clark Fork River

Date	Information
4-29	Small (less than 0.25cm) vegetative buds on rhizome.
7-27	12 buds dissected; 10 were sterile, 2 were fertile. Both fertile buds premeiotic, one with sporangiophores, one with sporangia.
11-10	15 of 16 buds were sterile. Fertile bud had spores, bud length 2.2cm. Buds had indurate leaves.

Chart 10: *E. telmateia* Basal Bud and Strobilus Formation, 1983-84

Date	Location	Information
3-83	Olympia, WA	Spore release in progress.
3-84	Gray's Harbor, WA	Spore release in progress. Green spores with elaters present in unexpanded strobili. Vegetative stems elongating. Small buds (less than 1.0cm long) on rhizome with whorls of new leaves and conical apices. Appeared vegetative, fertility possible.
7-83	Eugene, Ore.	17 (80%) of 21 buds were fertile. Fertile buds were premeiotic with sporangiophores or sporangia. One fertile bud elongated above ground level. Vegetative stems fully elongated.

Chart 11: E. sylvaticum Basal Bud and Strobilus Formation, 1984

Date	Location	Information
6-4	Marshall Creek	Spores were being released. Spores from indehiscent sporangia were green with elaters. Unelongated fertile stalks and unexpanded strobili present.
7-28	Marshall Creek	Spore release complete. Of 14 unexpanded basal buds: 11 were sterile, 1 had a conical apex and was possibly fertile, 2 were fertile and premeiotic with developing sporangia.
August	British Columbia	Three unexpanded buds were fertile with spores. Bud lengths were 1.7cm, 1.3cm, and 1.4cm. Strobilus lengths were 1.1cm, 0.8cm, and 0.9cm, respectively.

Chart 12: _E. fluviatile_ Basal Bud and Strobilus Formation

Date	Location	Information
10-9-83	Flathead River	Basal buds approximately 2.0cm long. Leaves reddish orange, not indurate. Of 11 buds, 3 were sterile, 8 had conical apices. Small buds indicated recent bud initiation.
5-24-84	Flathead River	Buds elongated above water level; buds approximately 15 cm tall. Of 8 buds, 7 were premeiotic, (3 had sporangiophores, 4 had sporangia, and 1 was possibly meiotic.)
11-5-83	Flathead Lake	2 of 9 buds were sterile, remaining 7 had conical apices.
10-28-84	Salmon Lake	Basal bud length ranged from 1.6-4.9cm. Of 15 buds, 7 were sterile, 1 had a conical apex, 7 were fertile. Of these, all were premeiotic, 2 had sporangiophores, 5 had sporangia.

Chart 13

E. laevigatum Spores Cultured in Bold's Basal, pH 6.6

	Germinated	Ungerminated	Percent Germiantion
Petri Dish 1	55	25	69.0
Petri Dish 2	56	49	53.0
Petri Dish 3	60	129	32.0
Average	57.6	67.6	51.3

Chart 14: *E. laevigatum* Vernalization Experiment

	Days in Cold	Elongated Buds	Unelongated Buds	Narrow Stems	Fertile Stems
Control	0	0	0	27	0
Coldroom					
1	28	3	0	1	0
2	46	6	0	1	0
3	62	0	3	6	0
4	85	0	0	70	0
Outside					
1	31	1	2	19	0
2	59	4	0	11	0
3	90	1	0	16	0
4	194	---------------plants destroyed-----------------------			

Chart 15: *E. laevigatum* Leaf Removal Experiment

	Cold Days	Elongated Basal Buds	Narrow Stems	Fertile Stems
Control	31	3	30	0
Leaves Removed	31	3	41	0

Chart 16: *E. laevigatum* Basal Buds, 1983

Date	Site	Apices	Comments
7-21	WH	6 conical, 7 buds dissected	No new buds were breaking ground.
9-27	WH	18 conical, 18 dissected.	Buds 1cm long and underground.
7-24	HC		Basal buds present, no new stems elongating.
9-20	HC	6 conical, 11 buds dissected.	Buds 1cm long and underground.
11-5	HC	16 conical, 17 dissected.	Indurate leaves. Buds etiolated inside.

Chart 17: <u>E. laevigatum</u> Basal Buds, 1984, Waterworks Hill

Date	Buds
3-14	Overwintered buds beginning to elongate. Buds underground. Strobili developing.
4-12	Overwintering buds elongated. 1mm swelling on rhizome was possibly a new bud.
5-12	Swellings at rhizome nodes possibly new buds.
6-9	No new basal buds seen. All overwintered buds elongated.
7-7	7 of 27 stems had new basal buds. All buds had conical apices. Apices were 0.2mm long. Average bud length was less than 0.5cm. Indurate leaves were forming.

Chart 18: *E. laevigatum* Basal Buds, 1984, Hellgate Canyon

Date	Buds
4-1	Overwintered buds unelongated. Conical apices.
7-6	13 of 33 stems had new basal buds. Often greater than 1 bud per stem. 0.5cm average bud length. Indurate leaves forming. All buds had conical apices. Apices were 0.2mm long.
9-29	Average length of 10 buds was 0.61cm. Indurate leaves present. All buds had conical apices. One apex appeared slightly whorled.
10-10	10 buds had conical apices. Indurate leaves present.

Chart 19: *E. laevigatum* Basal Buds, 1984, Clark Fork River

Date	Buds
4-29	Small buds clustered at base of stem that elongated in 1984.
7-27	New basal buds present. Conical apices were less than 1.0mm long. 20 buds had an average length of 0.79cm.

Chart 20: <u>E. laevigatum</u> Strobilus Development, Waterworks Hill, 1984

Date	Stems	Sterile	Conical Apex	Fertile
3-14	28	3	0	25; Premeiotic with sporangiophores, 6 of 7 unelongated buds fertile, 19 of 21 elongated buds fertile.
3-29	17	2	2 (apices elongate)	13: Premeiotic sporangiophores and sporangia. Stems not above moss layer.
4-12	42	9	2	31; Meiotic 20 with sporangiophores, 11 with sporangia - 6 of these with tetrads.
5-1	35	9	0	26; Meiotic 5 with sporangiophores, 21 with sporangia, 5 of these had tetrads or spores. Stems elongated.
5-17				Strobili changing from green to yellow-green.
6-9				Spore release beginning, strobili elongating.
7-7				Spore release complete. Strobili withering.

Chart 21: *E. laevigatum* Strobilus Development, Hellgate Canyon, 1984

Date	Stems	Sterile	Conical Apex	Fertile	Comments
4-1	14	0	13	1; Premeiotic Sporangiophores forming.	Buds unelongated.
4-15	37	21	0	16; Premeiotic Sporangiophores forming.	Buds elongating.
5-5	33	6	0	27; Premeiotic 15 with sporangiophores, 12 with sporangia.	Stems elongating.
5-20	19	1	0	18; Premeiotic 1 with sporangiophores 17 with sporangia	Stems elongated.
6-12				Spores present, elaters forming.	
6-23				Spore release beginning. Peduncles and axes of many strobili not yet elongated.	
7-6				Spore release nearing completion.	

Chart 22: <u>E. laevigatum</u> Strobilus Development, Clark Fork River, 1984

Date	Information
4-22	Of 9 stems, 2 were sterile, 7 were fertile: 1 with sporangiophores 6 with sporangia and tetrads. Strobili evident in tallest stems.
5-19	Spores present with and without elaters.
5-26	Strobili changing from green to yellow-green.
6-23	Spore release beginning. Strobili peduncles and axes elongating.
7-27	Spore release complete.

Chart 23: *E. palustre* Basal Bud Formation and Spore Release

Flathead Lake

Date	Information
11-5-83	All 6 buds dissected had conical apices. No buds had strobili. Indurate leaves present.
10-7-84	Basal buds present with indurate leaves. Average bud length was 1.5cm. Of 13 buds, 5 were sterile and 8 had conical apices.
6-24-84	Strobili on tips of elongated stems. Green spores with elaters present. No spore release.
7-8-84	One strobilus was releasing spores. Other strobili were expanding.
7-22-84	Spore release complete.

Chart 24: E. variegatum Strobilus Development, Jacob's Island, 1984

Date	Information
3-4	No elongation in overwintering basal buds and incompletely elongated stems.
4-3	Basal buds elongating. One fully expanded stem (17.6cm) was premeiotic with sporangia. One unexpanded stem (9.5cm) was premeiotic with sporangiophores. 10 of 18 unexpanded stems had conical apices. 14 of 15 basal buds had conical apices.
4-9	4 of 21 unelongated stems were fertile and premeiotic with sporangiophores. Remaining 7 shoots had conical apices.
4-30	Basal buds elongating. Of 55 unexpanded stems (less than 8.0cm tall), 24 were fertile and premeiotic with sporangiophores. 9 had conical apices. 22 were sterile.
5-4	5 of 12 unelongated stems were fertile with sporangiophores.
5-13	Overwintered strobili present on overwintered expanded and incompletely expanded stems. Basal buds elongating. Of 25 stems (less than 10cm tall), 12 were sterile and 13 fertile. 6 of the fertile stems had sporangiophores, 6 had sporangia. Two of the fertile stems had overwintered.
6-12	Strobili evident as apical swellings on expanding stems. Of 13 elongating stems, 4 were sterile, 1 had a conical apex, and 8 were fertile. Of the fertile stems, 2 had sporangiophores, 6 had spores without elaters
7-7	Of 66 overwintered stems, 41 were sterile, 25 were fertile. Of the fertile stems, 19 had aborted, white spores,

Chart 24, Continued

7-7 4 had small, stunted spores that were lens shaped without elaters.
1 had normal appearing spores.

Of 5 stems elongating in 1984, 4 were premeiotic and 1 had normal tetrads.

7-22 Of 25 elongating stems, 23 were sterile and 2 were fertile with premeiotic sporangia.

8-14 Of 37 expanding stems, 29 were sterile and 8 were fertile.
Of the fertile stems, 3 overwintered as unexpanded stems.
5 strobili were from stems expanding in 1984.
4 had sporangiophores,
2 had sporangia,
2 had spores.

9-29 Of 6 fertile stems, 3 had aborted strobili (on stems elongating in 1984)
2 had sporangiophores,
1 had premeiotic sporangia.

10-3 Of 23 stems elongating in 1984, 16 were sterile, 3 had conical apices, 4 were fertile. Of the fertile stems, 2 had premeiotic sporangia and 2 had aborted strobili.

10-20 New buds elongating.
Stems developing strobili before July, 1984 were dying back.
Of 42 stems, 22 were sterile and 11 had conical apices, (6 conical apices from buds less than 1.5cm), and 9 were fertile.
Of the fertile stems, 5 had sporangiophores, 1 had premeiotic sporangia and 3 had white, aborted spores.

11-5 Of 39 stems, 15 were sterile, 14 had aborted apices, 9 had conical apices, and 1 was fertile with sporangiophores. Strobilus 0.8mm. Conical apices 2-3mm long

Chart 24, Continued

11-11 Incompletely expanded stems green.
Basal buds present.
Of 27 incompletely expanded stems dissected,
19 were sterile,
8 were fertile.
Of the fertile stems,
5 had sporangiophores,
2 had premeiotic sporangia,
1 had tetrads.
Tetrads were irregularly shaped and sometimes did not have all 4 cells present.

Equisetum variegatum Stem Height and Fertility, Jacob's Island during November, 1983

Chart 25

Stem Height					
Less than 6.0cm			Greater than 6.0cm		
Sterile	Conical Apex	Fertile	Sterile	Conical Apex	Fertile
7	20	3	6	15	0

Totals: 13 sterile, 35 conical apices, 3 fertile.

Chart 26: *E. hyemale* New Basal Bud Development, 1983

Date	Site	Buds Present	Condition
7-24	JI	Yes	All 4 buds dissected had conical apices. Buds being initiated. No new stems breaking ground.
9-10	JI	Yes	All 6 buds dissected had conical apices. Indurate leaves present.
12-12	JI	Yes	4 of 9 buds had conical apices. Indurate leaves present. 0.25cm diameter bud appeared to be recently initiated.
9-27	WH	Yes	All 6 buds dissected had conical apices.
11-4	CF	Yes	12 of 13 buds had conical apices. These buds at bases of fertile stems. Indurate leaves.

Chart 27: *E. hyemale* New Basal Bud Formation, 1984, Jacob's Island

Date	Information
7-7	3 of 27 stems had basal buds; 2 were on 1983 stem bases, 1 was on 1984 base. 1 3.0mm long bud had a conical apex.
10-16	Buds had indurate leaves.
11-11	Buds had indurate leaves. 18 of 20 buds had conical apices. All conical apices were less than 0.5mm. 4 stems had 2 basal buds, 1 stem had 3 basal buds. Average length of 20 buds was 0.5cm, range was 0.3-1.0cm. 2 buds appeared as swellings on the rhizome and were less than 1.0mm in diameter. They appeared to have been recently initiated.

Chart 28: *E. hyemale* New Basal Bud Formation, 1984, Clark Fork River

Date	Information
7-7	1 of 17 stems had a basal bud.
7-27	4 of 9 buds had conical apices. Conical apices were less than 0.5mm long. Average bud length was 0.64cm. Buds present on new fertile, sterile, and broken stems.
10-5	20 buds dissected had conical apices. Average bud length was 0.7cm, lengths ranged from 0.4cm-1.5cm. 3 of 26 pre-1984 stems had buds, 23 of 30 new fertile stems had buds. Indurate leaves present.
11-14	Indurate leaves present.

Chart 29: <u>E. hyemale</u> Strobilus Development and Bud Elongation, 1983, Waterworks Hill

Date	Information
4-22	Spore release from overwintered strobili.
5-8	Spore release complete.
5-29	Bud elongation beginning. Average bud length was 4.14cm (for 16 buds). 19 of 20 buds were sterile.
6-24	Strobili visible terminating elongated stems. Sporangiophores tightly appressed.
7-7	Spores present in newly formed strobili. 10 of 23 new stems were fertile.
9-27	Stems that released spores in April, 1983 were dying back.

Chart 30: <u>E. hyemale</u> Strobilus Development and Bud Elongation, 1983, Jacob's Island

Date	Information
4-27	Spore release from overwintering strobili.
5-24	Average length of 32 elongating buds was 4.14cm.
6-5	Average length of 19 elongating buds was 11.7cm.
6-24	Average length of 26 elongating buds was 15.0cm.
7-13	New buds breaking ground. Average length of 10 elongating buds was 15.9cm. Stems that released spores in 1983 were still green.
7-20	Spores present in fully elongated stems. Average length of 13 buds was 17.3cm.

Chart 31: E. hyemale Strobilus Development and Bud Elongation, 1984, Clark Fork River

Date	Information
1-31	2 unelongated basal buds had conical apices.
3-12	31 unelongated basal buds had conical apices.
4-3	10 unelongated basal buds had conical apices.
4-15	10 unelongated basal buds had conical apices. Apices elongating. Overwintered strobilus beginning to expand.
4-22	Spore release.
4-29	Spore release nearing completion. Buds elongating; none above ground level.
5-13	Spore release complete. 4 buds elongated above ground level.
5-19	Buds above ground level. Of 13 buds, 10 were sterile (9 of these had conical apices), and 3 were fertile with developing sporangiophores.
6-5	Of 14 stems, 3 were sterile, 11 were fertile and premeiotic with sporangiophores Stem length ranged from 5.0-12.5cm.
6-23	Of 26 fertile stems, 9 had sporangiophores, 12 had sporangia, 8 had spores or tetrads.
7-7	Buds elongated and strobili visible at stem tips. Green spores with elaters present. Of 11 premeiotic stems, 5 had sporangiophores, 6 had sporangia.
7-27	18 fertile stems - 4 with sporangia, 14 with spores.
10-5	Green spores with elaters present. Sporangial walls green.

Chart 32: *E. hyemale* Strobilus Development and Bud Elongation, 1984, Jacob's Island

Date	Information
4-29	Spore release from overwintered strobili in progress. Basal buds elongating, none above ground.
5-13	Buds elongated above ground.
6-12	Strobili evident on elongating stems. No new buds breaking ground. 20 fertile stems were premeiotic: 10 with sporangiophores, 10 had sporangia.
6-23	Strobili visible on tips of elongated stems. Tallest new stems were 35.8 and 31.0cm. Of 29 premeiotic stems, 19 had sporangiophores, 10 had sporangia.
7-7	Green spores with elaters present. Meiosis late June, early July. Of 20 premeiotic strobili, 14 had sporangiophores, 6 had sporangia.
7-22	9 fertile stems - 4 had sporangiophores, 1 had sporangia, 4 had spores.
10-16	Green spores with elaters present.
11-11	Upper 2-3 nodes of 1983 and 1984 spore releasing stems dying back. Green spores with elaters present. No spore release or strobilus elongation in strobili that formed in 1984.

Chart 33: E. scirpoides Basal Bud Formation, Jocko River

Date	Information
10-9-83	Side branches (approximately 1cm long) present at bases of older stems. Two side branches had conical apices. No buds on rhizome.
11-15-83	Small (0.25mm) reddish brown buds at rhizome nodes, recently formed. All 33 buds dissected had conical apices.
5-24-84	No new basal buds present.
7-22-84	No new basal buds present.
10-7-84	No new basal buds present. Three side branches and one shoot on rhizome had conical apices.

Chart 34: E. scirpoides Stroiblus Development, 1983-84

Date	Information
10-9-83	Strobili containing green spores with elaters present. No strobilus elongation, spore release, or empty strobili were seen.
11-15-83	Strobili contained green spores. No spore release, strobilus elongation, or empty strobili were seen.
5-24-84	Empty, withered strobili indicated recent spore release. No strobili with spores or that were meiotic were present. One strobilus with sporangiophores was found.
7-6-84	In greenhouse - Plants from Jocko developed strobili, spores with elaters.
7-22-84	New strobili with spores present on stems elongating in 1984.