Title: **Propagation of Ferns by Spore**

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Abstract:

Three propagation methods and five species of ferns were compared in a propagation trial in the Morris Arboretum Greenhouse. Spores were collected from the Fernery and the wild, and then sown. Data was collected on their germination dates, production of gametophytes, and on the initiation and growth of the asexual sporophyte generation. Some methods were shown to have higher rates of success than others and particular aspects of these methods were demonstrated to be important to successful propagation of ferns from spores. Information gathered from this project will be used to inform future propagation efforts.

TABLE OF CONTENTS

INTRODUCTION

There are multiple reasons to propagate from spores and there are many situations in which growing ferns from spores would be the preferred method of production. One of the primary motivations for producing ferns from spores relative to this project is the fact that it would allow rare species to be propagated by the Arboretum for use in the Fernery. Some of the ferns currently grown in or for the Fernery are very difficult to find and often expensive. This means that they would be difficult and costly to replace. It would be in the Arboretum's best interest to develop a propagation protocol for growing ferns from spores, as this would allow for in-house production of these rare species. Additional individuals of the many varieties could be available in the Greenhouse to replace specimens currently on exhibit or add to their numbers at any time.

A second reason to propagate ferns from spores is to preserve the genetic diversity and adaptability of species. This is especially important to projects with an element of ecological restoration, where horticultural goals can include the re-creation of plant communities formerly found on the site. According to an article in the journal Ecological Restoration, an important aspect of including elements of restoration in landscape design and horticulture is the use of genetically variable plants, not clones and cultivars (Munro 183, 184). Propagation by spores is one way to include variation and diversity in the gene pools of cultivated ferns. Pinelands Nursery, a company focused on propagation of native plants, uses spores to propagate its fern stock for this reason (Rogers).

Ferns belong to the group of plants called pteridophytes. A basic understanding of their complicated life cycle and reproductive strategies is necessary in order to understand many aspects of this research project (figure 1). Pteridophytes are seedless vascular plants that reproduce by dispersing spores. The life cycle of ferns and other pteridophytes is different from that of flowering plants in that it has alternating diploid asexual and haploid sexual generations. The sexual generation begins when spores are released from the sporangia of a mature fern.

Haploid gametophytes are produced when these spores germinate. These gametophytes mature into structures called prothalli. The underside of the mature prothallus bears male (antheridia) and female (archegonia) sexual structures. The archegonium contains one haploid egg cell. Antheridia produce flagellated haploid sperm cells. Sperm cells swim toward the egg cell after being released from the antheridia when adequate moisture is present to allow for their transit to the archegonium (Raven et al 397). These haploid gametes form a diploid zygote at fertilization, beginning the asexual generation of the pteridophyte life cycle. The embryo divides quickly, eventually growing and differentiating into an adult sporophyte. Sporophytes produce the haploid spores that germinate to become the next generation of gametophytes.

METHODS

Three propagation methods were compared in this project. The methods tested for the project were sowing of spores on clay pots, compressed peat pellets, and direct sowing onto fine germination media. Four methods were chosen initially, but one of these, the nutrient solution method, was abandoned early on in the course of the research due to impracticality of carrying it out. This method would have involved sowing spores on top of a few millimeters of nutrient solution in petri dishes and keeping them afloat until germination.

The method of sowing spores onto clay pots was designated Method 1. This technique was followed as described in Fern Growers' Manual by Barbara Joe Hoshizaki and Robbin C. Moran (Hoshizaki & Moran 77) and selected for the trial because it appears in most of the literature on propagating ferns from spores. It was set up by disinfecting 20 small clay flower pots to minimize risk of infestation by fungi or other pathogens that would have a negative effect on the spores or gametophytes after germination. The pots were then stuffed with long-fibered sphagnum moss that had been soaked in hot water to hydrate it. The pots were placed upside down on a plastic tray and spores were sown on top of them. They were then covered with plastic drink cups to create a moisture seal and provide a microclimate with very high levels of humidity. A small amount of water was poured into the tray and periodically replenished so that the sphagnum moss could keep absorbing water and humidifying the pots and their enclosures.

Method 2 involved sowing spores onto pellets of compressed peat and keeping them in humid conditions similar to those of Method 1. This method has been used to propagate ferns with some success. Peat pellets were soaked in hot water until they were fully soaked and inflated. They were placed in metal cupcake pan liners and more water was poured into the bottom, then spores were sown onto the tops of the pellets. The pan liners were placed on plastic trays and covered with plastic drink cups in the same manner as the pots in method 1. The pan liners were refilled when dry and some were eventually replaced after they rusted through. Unfiltered water from the greenhouse sink was used because it has been suggested that traces of micronutrients present in it would be beneficial to the growing ferns after germination of the spores (Hoshizaki & Moran).

The third method was the sowing of spores into plastic cells filled with a fine-textured germination media mix. The media was thoroughly watered prior to sowing in order to provide adequate moisture without displacing tiny spores or washing them away. Multiple squares were placed in plastic trays, the bottoms of the trays were filled with water, and a second tray was clipped on top of the bottom one to provide the humidity necessary to keep young gametophytes from desiccating. As with method 2, this technique has been successfully utilized in the greenhouse prior to this research project.

Five species of ferns were selected for use in this project: *Todea barbara* (L.) Moore, *Cyrtomium macrophyllum* (Mak.) Tagawa, *Dryopteris sieboldii* (Van Houtte) Kuntze, *Polypodium virginianum* L., and *Nephrolepis exaltata* 'Emina.' Two of these, *T. barbara* and *D. sieboldii*, were chosen because they are grown in the Fernery and more individuals of these species are desired. *T. barbara* is regarded as a difficult species to propagate from spore. *C. macrophyllum* was selected from the hardy fern garden adjacent to the Fernery. The parent plants were collected in the wild by Arboretum Director Paul Meyer. P. virginianum was collected from State Gameland #43 in

Chester County near French Creek State Park. The final species, *N. exaltata* 'Emina,' was chosen for this project due to the ease with which it germinates as previously observed by the supervisors of this project, in contrast with challenging species such as *T. barbara*. The idea behind these selections was to compare propagation methods across a selection of species known for multiple levels of difficulty.

Wild collection and field identification of ferns was one of the peripheral aspects of this project. Spores from two other species were collected from the same area as *P. virginianum*: *Polystichum acrostichoides* and *Dryopteris marginalis*. *Woodwardia areolata* was collected in Delhaas woods in Bucks County, and two species (*Athyrium filix-femina* and *Phegopteris hexagonaptera*) were collected from locations along Forbidden Drive in the Wissahickon section of Fairmount Park. These species were not propagated as official parts of this project due to concerns about there being insufficient quantities of spores to sow for all repetitions of the three methods. All ferns collected in the wild were identified according to dichotomous keys in the Peterson Field Guide to Ferns of the Northeastern United States.

The first step in testing these propagation methods was to collect spores. This was accomplished by examining the ferns for mature sori. The species studied in the propagation trial bear sori on the undersides of their pinnae and do not produce separate fertile fronds. Sections of sporulating fronds were removed from the fern, placed in folded envelopes of heavy bond paper, marked with collection information, and brought back to the Arboretum. The folded paper provided a repository for spores from which they were sown. The fertile fronds were allowed to dry out and release spores for a period of two weeks to one month, with the exception of *T. barbara.* Unlike the other species of ferns utilized in this study, the spores of *T. barbara* are only viable for a short period of time after collection (Fern Society of Victoria). It was necessary to sow them as soon after collection as possible to ensure germination. In addition, the spores of this species are only ripe on the plant for a short amount of time. The mature sori of many fern species display shades of brown, but the spores of *T. barbara* are green while ripe (Hoshizaki & Moran 74). Brown sori indicate that the spores are no longer viable. These constraints on timing were addressed by careful monitoring of the parent plant for ripe spores and prompt sowing once the spores were collected and adequately prepared.

Multiple methods have been employed successfully for sowing spores. Spores may be isolated and sterilized before being sown on agar for tissue culture or scientific research. Because of their minute size, it is not possible to count and separate fern spores with the naked eye. For this project, a simple method of sowing them was employed that allowed the spores to reach the substrate on which they would germinate without use of special equipment. After the fertile parts of fern fronds were collected, they were placed in folded bond paper and allowed to dry slightly. During this time, the spores were released from the ferns' sori onto the paper (Fern Society of Victoria). Upon opening the paper envelopes, spores were visible on the sheets as a fine dust of variable color. Especially notable were spores of *P. virginianum* and *T. barbara,* which were saffron orange and deep green respectively. The dried fern fronds and pinnae were removed from the envelopes in order to prevent contamination of the substrate with broken fern parts or other impurities that could rot or harbor pathogens. The paper was held at a low angle to the substrate and tapped four times over each repetition of the three methods for each species. The bench surface

was disinfected prior to sowing the spores and was cleaned between sowings of each species to try to keep the repetitions of different species as separate and uncontaminated as possible.

Care of the sown spores was relatively simple. During the period of data collection, repetitions of every method were kept in the propagation room of the Morris Arboretum Greenhouse. This room is kept very warm and is equipped with a fog machine to maintain high levels of humidity. Each repetition was checked regularly to insure that the plastic lids and covers were in place and maintaining a suitably humid microclimate. The bottoms of cupcake wrappers and plastic trays were monitored and regularly refilled with water to prevent desiccation. High levels of environmental moisture in both the propagation room and the trays of sown spores ensured that the repetitions never became dry to the point of damage to the delicate early stages of growth of young ferns after germination.

The period of data recording began when the first spores germinated. The repetitions were carefully monitored every week and germination dates were recorded in a table that appears later on in this report (figure 2). Collection of data on populations of gametophytes began after germination was confirmed and growth was visible to the point at which assessment was possible. Population data was not collected for the first few weeks after germination due to the difficulty and inaccuracy of counting tiny (<.5 mm) gametophytes against a background of dark media. The methods of data collection for gametophyte populations focused on estimating the percentage of the surface of each repetition that was covered by mature or immature prothalli at each collection period.

The exceptions to this were the repetitions of Method 1, in which gametophytes were spaced far enough and their numbers were low enough to allow for them to be counted individually. Population data collection began on December 11th, 2009 and continued weekly until February 1st, 2010. Some adjustments were made to the timing of data collection due to the interruption of the winter vacation break.

Although the initial focus of this study was to compare the germination and production of gametophytes of five fern species across three methods of propagation, the sporophyte stage became included in trial data as well. Sporophyte populations were initially low enough to be counted as individuals. During data collection, each sporophyte leaf in a repetition was counted. Because of this, the numbers that appear in population graphs reflect the number of sporophyte leaves more than the actual number of sporophytes produced, and serve to monitor the growth of older sporophytes as well as production of new individuals. Data on sporophyte growth was recorded simultaneously with that on gametophyte populations from January 4th, 2010, to February 1st, 2010.

RESULTS

The earliest data collected for this project was the germination dates of spores in each repetition of each method. Most germination occurred about a month after the spores were sown. The earliest date at which germination was confirmed was November 20th, 2009. All but five repetitions of Method 2 and all but two repetitions of Method 3 were recorded as germinated on November 30th, 2009. In contrast, only two repetitions of Method 1 germinated in 2009. Method 1 also had the largest number (three) of repetitions that did not germinate during the recording period. One repetition of Method 2 did not germinate during this time. Every repetition of Method 3 germinated by December 11th, 2009. A graph of germination dates is included as figure 2 in this report. Some species of ferns germinated faster than others in patterns that are visible across all three methods. *C. macrophyllum* and *N. exaltata* 'Emina' were the fastest to germinate, while *T. barbara* lagged behind the other four species.

After germination, gametophyte populations were monitored and similar patterns were found in both the rate of population growth and the number of gametophytes produced. The maximum number present at this time was close to 60. *N. exaltata* 'Emina' and *P. virginianum* exhibited the best response to this method and produced the largest populations of gametophytes. Methods 2 and 3 produced sufficiently sized populations to have their numbers recorded as the percentage of the surface covered by gametophytes.

The populations produced by repetitions of Method 3 were largest and had the fastest rates of growth (figures 13-17). Method 2 produced moderate numbers of gametophytes on average, and the growth of the populations occurred at a fast rate (figures 8-12). Most populations were close to their final sizes by the third week of the recording period. Method 1, in contrast produced very few gametophytes. The numbers were low enough to be recorded as individuals instead of percent cover (figures 3-7). Populations grew slowly and did not appear until relatively late in the recording period. Some repetitions of Method 1 had only two gametophytes at the end of the trial.

The sizes and growth rates of gametophyte populations varied by species as well. The largest numbers of gametophytes were produced by *C. macrophyllum*, *N. exaltata* 'Emina', and *P. virginianum*. These species did extremely well in repetitions of Methods 2 and 3, and *N. exaltata* 'Emina', and *P. virginianum* had some of the strongest responses to Method 1. In some repetitions, especially of Method 3, the entire surface of the propagation media became covered with gametophytes. *P. virginianum* and *N. exaltata* 'Emina' achieved close to 100% coverage very early on. *D. sieboldii* produced both large and small populations, with a great deal of variation present across repetitions of a single method. As with the other species in this study, the largest populations were present on repetitions of Method 3. *T. barbara* was not especially slow to germinate, but had the smallest populations of all the species, regardless of propagation method.

Initially, this research was focused on documentation of germination and gametophyte production across three different propagation methods. Production of the asexual sporophyte generation began faster than expected, and data on sporophyte production and growth was added to this project. The earliest sporophytes were found in early January of 2010. Dates of sporophyte initiation were recorded starting January 4th, 2010 (figure18). Method 1 was the last to produce this generation, with two individuals appearing on two separate repetitions on the last day of the

recording period. Nine repetitions of Method 2 produced sporophytes, on dates varying from January 4th through February 1st, 2010. All but three repetitions of Method 3 produced sporophytes by the end of the recording period.

As with the gametophyte generation, sporophyte populations grew to different sizes at different rates. Method 1 produced very few individuals at a later date that did not allow for monitoring of growth rates for this method (figures 19-23). Method 2 produced sporophytes earlier, and their populations increased very quickly (figures 24-28). The fastest growth and largest populations were found in Method 3 (figures 29-33).

A great deal of variation by species was present in sporophyte populations. *C. macrophyllum* and *N. exaltata* 'Emina' developed sporophytes fastest and in great profusion. *C. macrophylum* was the only species to produce sporophytes on repetitions of Method 1 during the data recording period. *D. sieboldii* and *P. virginianum* developed fewer sporophytes, and the populations grew at a slower rate. *T. barbara* produced very few sporophytes, in keeping with the small numbers of gametophytes that were present on repetitions of this species.

DISCUSSION AND CONCLUSION

The results of this study show a clear preference for Method 3 as a propagation technique. This method produced the earliest germination and sporophyte initiation, as well as the fastest increase in populations of both generations of the fern life cycle. In addition, this method also required the least amount of care. The water supply in the bottom of the plastic cases ensured high levels of environmental humidity and did not need to be refreshed regularly. In contrast, the cupcake papers of Method 2 required frequent refilling to prevent the peat pellets from drying out. The cupcake papers also needed to be replaced frequently due to the bottoms rusting out from contact with water. The bottoms of the plastic trays employed in Method 1 also required frequent refilling.

There is a clear link between the level of moisture present in a method and how quickly ferns develop. High moisture levels are necessary in order for spores to germinate and for gametophytes to survive (Fern Society of Victoria). In addition, moisture and humidity are what allows the mature gametophyte to reproduce sexually. The release of the sperm from the antheridia is determined by the moisture present in the surrounding environment. Once released, the sperm are carried to the archegonia on drops of water to fertilize the egg inside (Raven et al 397). A lack of moisture will retard the development of the gametophytes and prevent or delay the start of the asexual generation. Given this need for plentiful moisture, it follows that the method that remained consistently wettest (Method 3) would yield the best results. Second best results were exhibited by Method 2, a propagation technique that remained moist, if not to the levels of Method 3. Method 1, the technique with the poorest results, was also notable for having comparatively low levels of humidity and minimal contact between gametophytes and sources of moisture.

Another clear result of this study is that Method 1 is not a reliable, timely, or easy method of propagating ferns from spores. Repetitions of this method lagged behind the others in terms of germination, gametophyte populations, and initiation of the sporophyte generation. Method 1 was also subject to higher levels of contamination than the other two methods studied. More algae and moss infestations were observed in the trays and on the pots of this method than on any of the other repetitions in the study. The effects of this contamination on the success of this method can be observed in the graphs showing the numbers of gametophytes (figures 3-7). The populations are shown to fall in multiple instances, representing the demise of individual gametophytes to contamination and decay. Given the small numbers of prothalli present on any repetition of this method, these losses represent an unacceptably large percentage of the total population.

Contamination is a common problem when propagating ferns from spores (Hoshizaki & Moran 72). This problem was present in a few repetitions of Methods 2 and 3, but did not damage the populations to the extent of those affecting Method 1. Some algae growth was observed in the plastic tray of Method 3. An advantage of this technique is that the pots of media can be removed from the trays and placed in other disinfected ones without harming young ferns or any part of the propagation apparatus. This is not as simple in Method 1, where great care must be taken to avoid damaging gametophytes located on the sides of the clay pots. A simple way to address contamination in Methods 2 and 3 is to regularly inspect and clean all materials that come in contact with gametophytes and their substrate. Contamination and problems from overcrowding

of gametophytes can be prevented by pricking them out once they become so crowded as to impinge on each others' growing space (Fern Society of Victoria).

A second, unexpected kind of contamination occurred in repetitions of Methods 2 and 3. Despite the fact that sowing surfaces were kept very clean and sowings of different species were separated as much as possible, spores from species of ferns other than those intended made their way into the substrates of multiple repetitions. This was not discovered until the sporophytes had developed sufficiently to be identified. *Cyrtomium macrophyllum* and *Nephrolepis exaltata* 'Emina' were found in repetitions of other species in small numbers. One individual of an as-yet unidentified species from the fernery was found growing in with *Dryopteris sieboldii.* Perhaps the most interesting example of these unexpected spores was the one found growing with the wild-collected *Polypodium virginianum.* Two sporophytes of another as-yet identified species (possibly a member of the genus *Woodsia*) were produced in a repetition of Method 3. These two individuals and the species from the fernery were pricked out into separate containers and will be grown on and possibly accessioned separately once they are identified.

The primary objective of this research project was to compare methods of fern propagation by spores and develop a better understanding of their benefits and drawbacks. Information gained from this project can be used to inform future propagation efforts and research projects. Two of the most important findings of this study were related to the success and failure of specific methods. Method 3, a simple, low-maintenance propagation technique, was found to have the best results out of the three tested. Method 1 was found to have unsatisfactory results. Based on these observations, it should be recommended that Method 3 become the primary method of propagating ferns from spores used in the greenhouse.

In addition, it is recommended that any methods of propagation used be carefully monitored for contamination and kept very wet. When propagating ferns, the cupcake papers or plastic trays in which water is kept should be checked and, if necessary, refilled, at least once per week. Enough water should be added to keep the propagation media or peat pellets wet at least until the next weekly check-in. Any cupcake papers that have rusted through should be replaced at this time. This project combined with past propagation efforts show that Method 1 is not a reliable way to propagate ferns from spores, and it should not be used by arboretum propagators in the future. With the knowledge gained from this project, future propagation efforts can be focused on methods and techniques that have been shown to work.

FIGURES AND GRAPHS

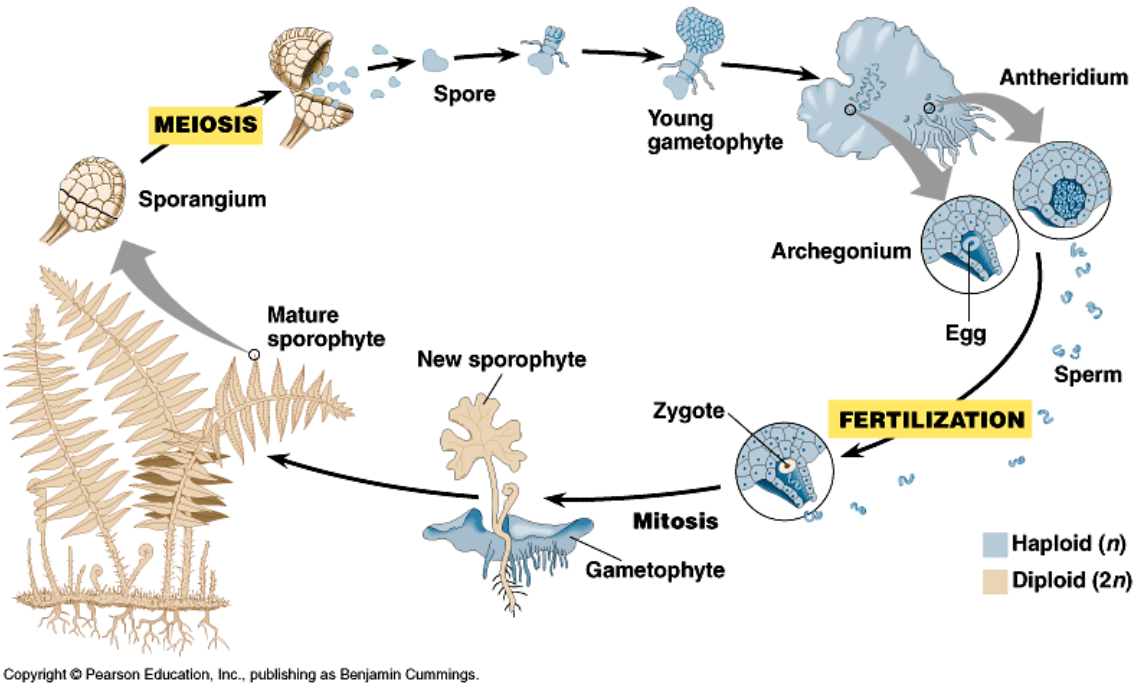

Figure 1: The life cycle of ferns

Species and repetition	Method 1	Method 2	Method 3
Cm rep 1	1/4/10	11/30/09	11/30/09
Cm rep 2	1/20/10	11/30/09	11/30/09
Cm rep 3	1/4/10	11/30/09	11/30/09
Cm rep 4	x	11/30/09	11/30/09
Ds rep 1	1/12/10	11/30/09	11/30/09
Ds rep 2	1/12/10	11/30/09	11/30/09
Ds rep 3	1/4/10	11/30/09	11/30/09
Ds rep 4	1/12/10	11/30/09	11/30/09
Ne rep 1	1/12/10	11/30/09	11/20/09
Ne rep 2	1/4/10	11/30/09	11/20/09
Ne rep 3	1/4/10	12/25/09	11/20/09
Ne rep 4	12/25/09	11/30/09	11/20/09
Pv rep 1	1/4/10	11/30/09	11/30/09
Pv rep 2	12/25/09	11/30/09	11/30/09
Pv rep 3	1/12/10	11/30/09	11/30/09
Pv rep 4	1/12/10	11/30/09	11/30/09
Tb rep 1	1/12/10	12/11/09	12/11/09
Tb rep 2	x	12/11/09	11/30/09
Tb rep 3	x	x	12/11/09
Tb rep 4	1/12/10	12/11/09	11/30/09

Figure 2: Graph of documented germination dates

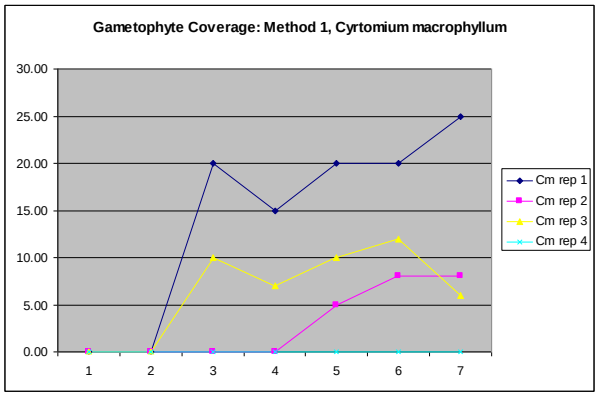

Figure 3: Gametophyte populations, Method 1, *Cyrtomium macrophyllum*

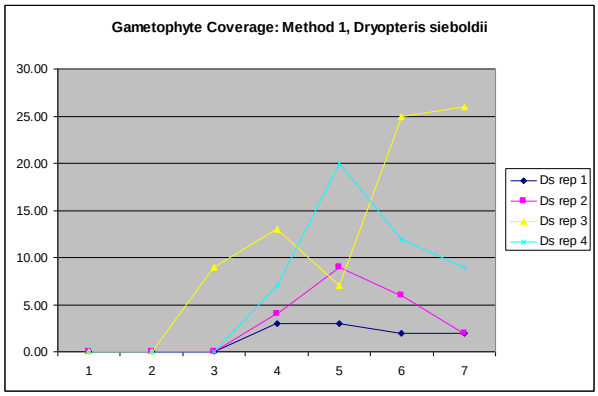

Figure 4: Gametophyte populations, Method 1, *Dryopteris sieboldii*

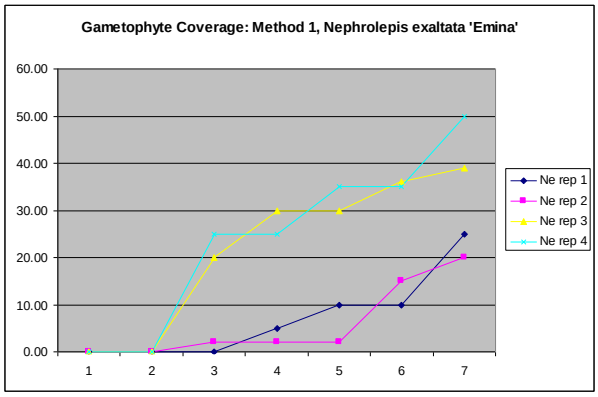

Figure 5: Gametophyte populations, Method 1, *Nephrolepis exaltata* 'Emina'

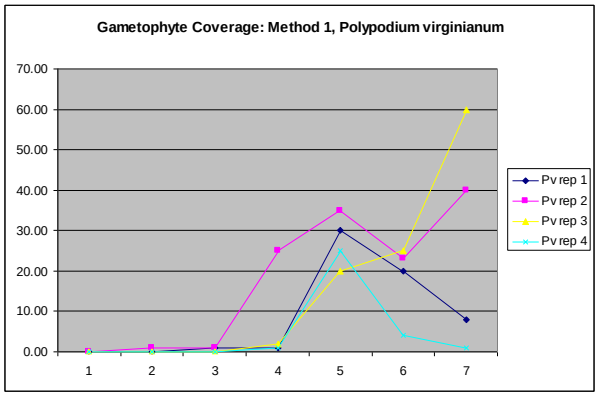

Figure 6: Gametophyte populations, Method 1, *Polypodium virginianum*

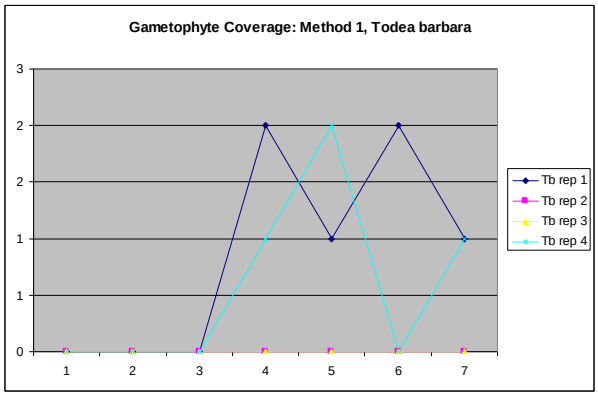

Figure 7: Gametophyte populations, Method 1, *Todea barbara*

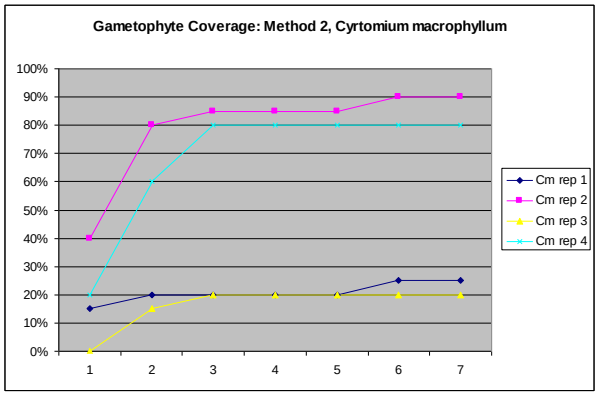

Figure 8: Gametophyte populations, Method 2, *Cyrtomium macrophyllum*

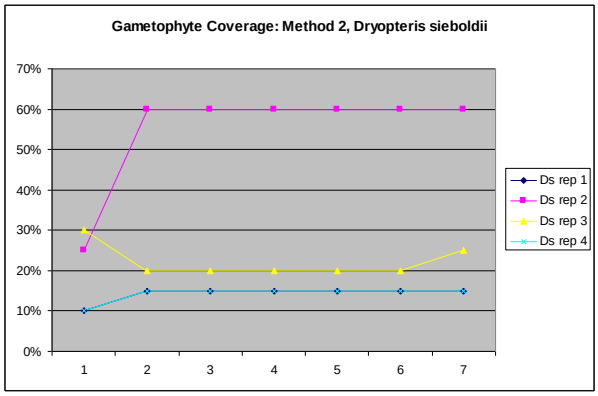

Figure 9: Gametophyte populations, Method 2, *Dryopteris sieboldii*

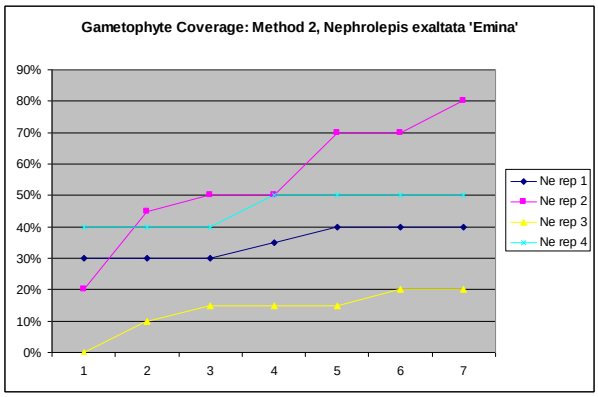

Figure 10: Gametophyte populations, Method 2, *Nephrolepis exaltata* 'Emina'

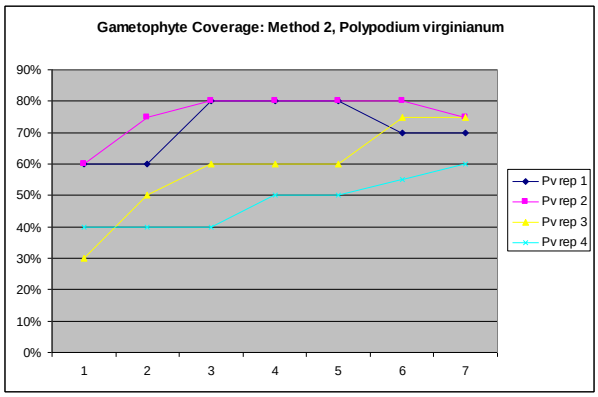

Figure 11: Gametophyte populations, Method 2, *Polypodium virginianum*

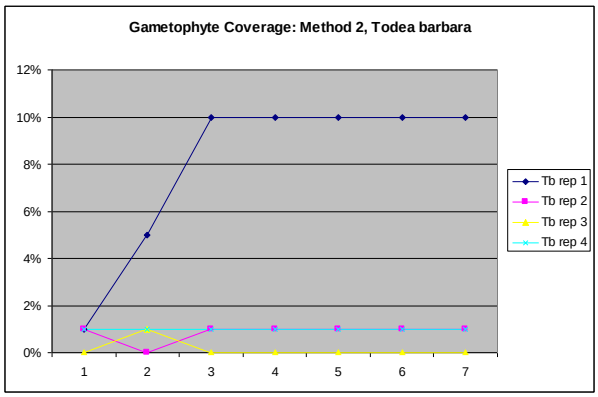

Figure 12: Gametophyte populations, Method 2, *Todea barbara*

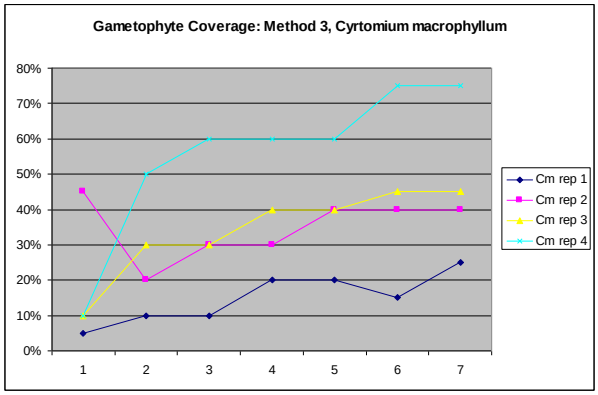

Figure 13: Gametophyte populations, Method 3, *Cyrtomium macrophyllum*

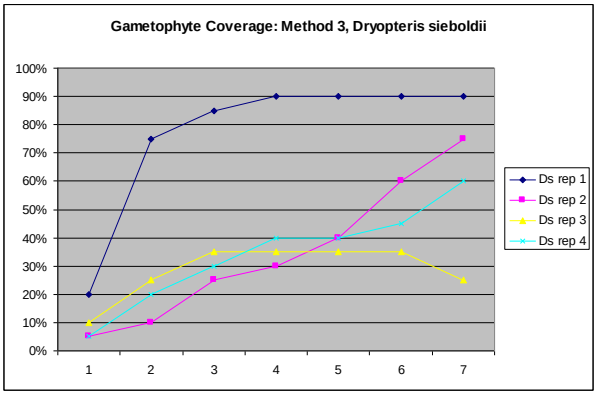

Figure 14: Gametophyte populations, Method 3, *Dryopteris sieboldii*

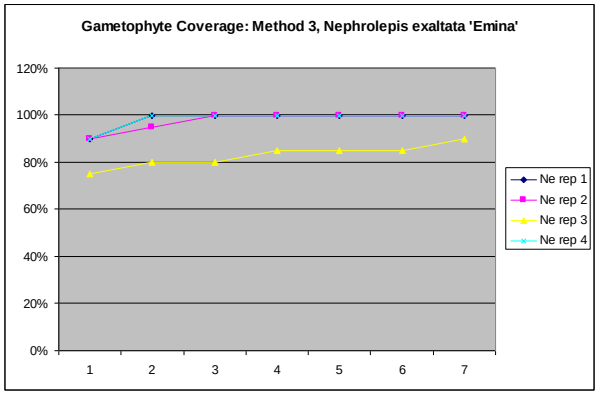

Figure 15: Gametophyte populations, Method 3, *Nephrolepis exaltata* 'Emina'

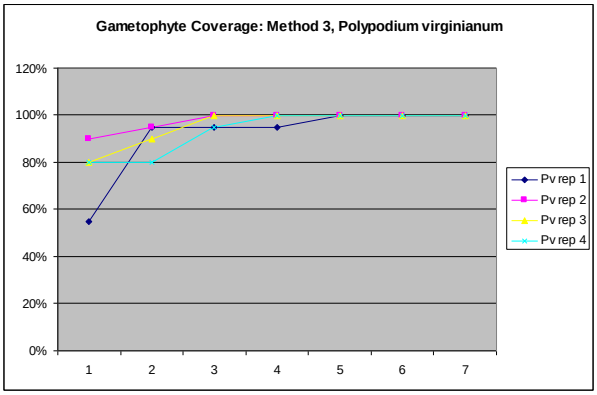

Figure 16: Gametophyte populations, Method 3, *Polypodium virginianum*

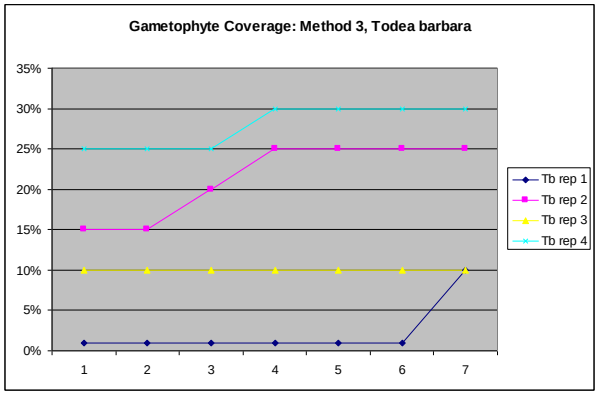

Figure 17: Gametophyte populations, Method 3, *Todea barbara*

Species and repetition	Method 1	Method 2	Method 3
Cm rep 1	2/1/10	1/4/10	1/4/10
Cm rep 2	x	1/4/10	1/4/10
Cm rep 3	2/1/10	1/12/10	1/4/10
Cm rep 4	x	1/4/10	1/4/10
Ds rep 1	x	2/1/10	2/1/10
Ds rep 2	x	2/1/10	1/12/10
Ds rep 3	x	1/20/10	2/1/10
Ds rep 4	x	2/1/10	2/1/10
Ne rep 1	x	x	1/4/10
Ne rep 2	x	2/1/10	1/12/10
Ne rep 3	x	x	1/12/10
Ne rep 4	x	x	1/12/10
Pv rep 1	x	x	1/25/10
Pv rep 2	x	x	x
Pv rep 3	x	x	1/25/10
Pv rep 4	x	x	1/12/10
Tb rep 1	x	x	1/20/10
Tb rep 2	x	x	x
Tb rep 3	x	x	1/25/10
Tb rep 4	x	x	x

Figure 18: Graph of documented sporophyte initiation dates

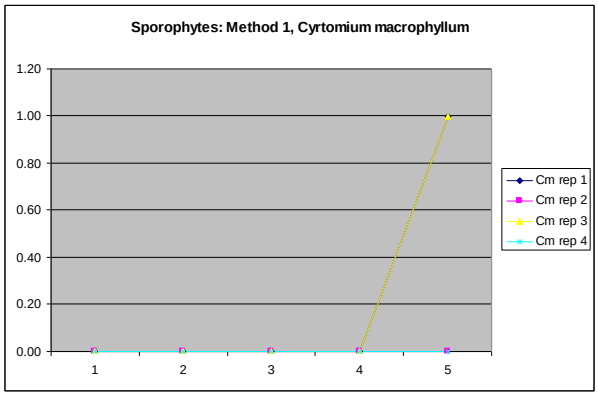

Figure 19: Sporophyte populations, Method 1, *Cyrtomium macrophyllum*

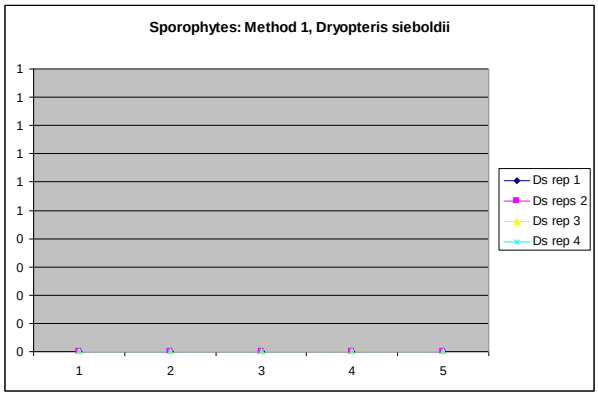

Figure 20: Sporophyte populations, Method 1, *Dryopteris sieboldii*

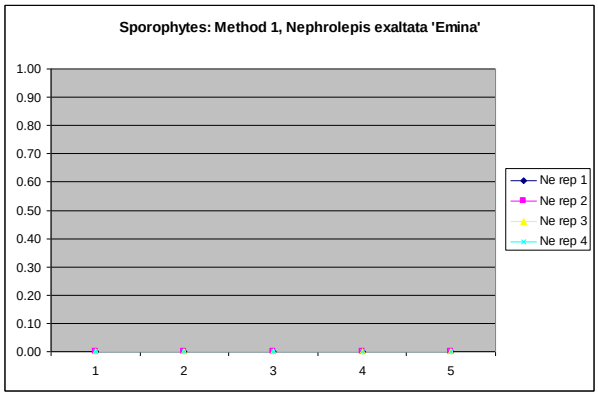

Figure 21: Sporophyte populations, Method 1, *Nephrolepis exaltata* 'Emina'

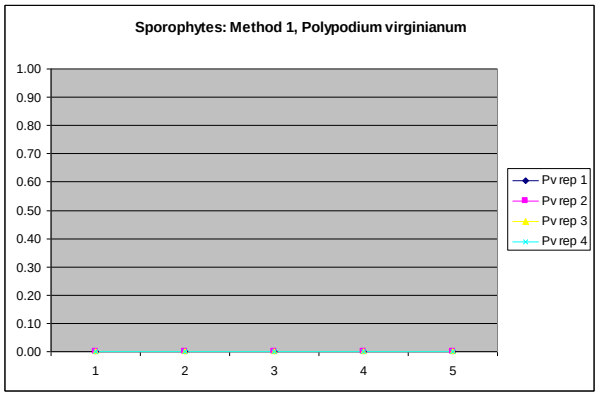

Figure 22: Sporophyte populations, Method 1, *Polypodium virginianum*

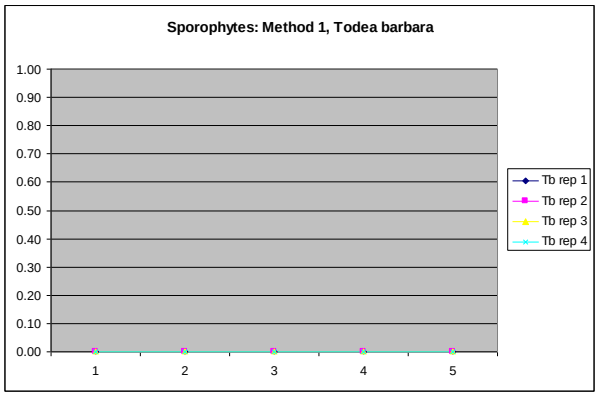

Figure 23: Sporophyte populations, Method 1, *Todea barbara*

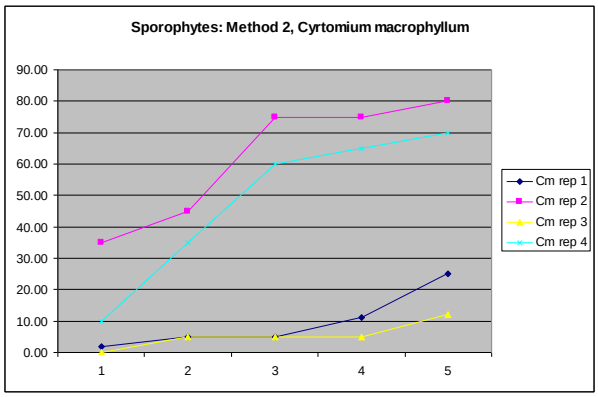

Figure 24: Sporophyte populations, Method 2, *Cyrtomium macrophyllum*

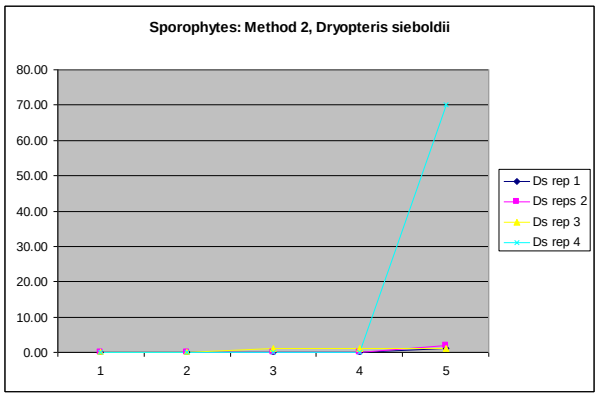

Figure 25: Sporophyte populations, Method 2, *Dryopteris sieboldii*

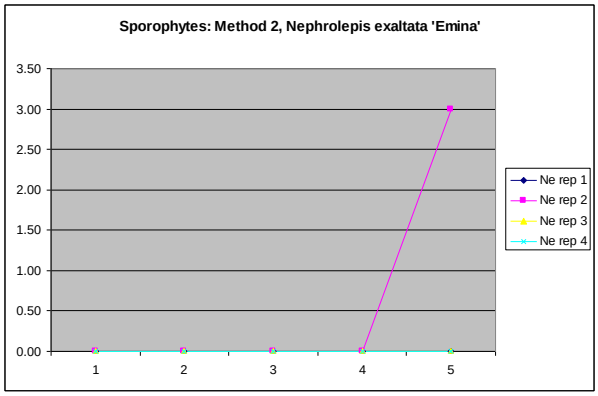

Figure 26: Sporophyte populations, Method 2, *Nephrolepis exaltata* 'Emina'

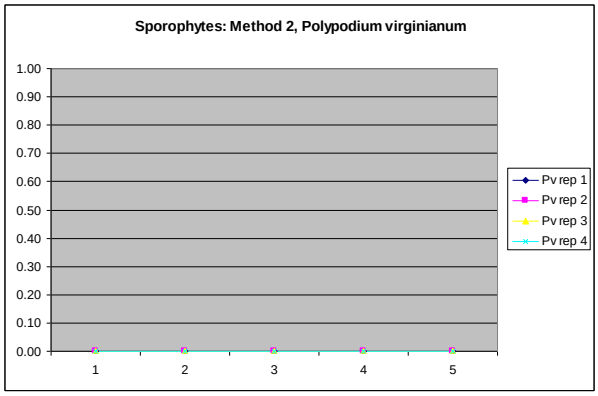

Figure 27: Sporophyte populations, Method 2, *Polypodium virginianum*

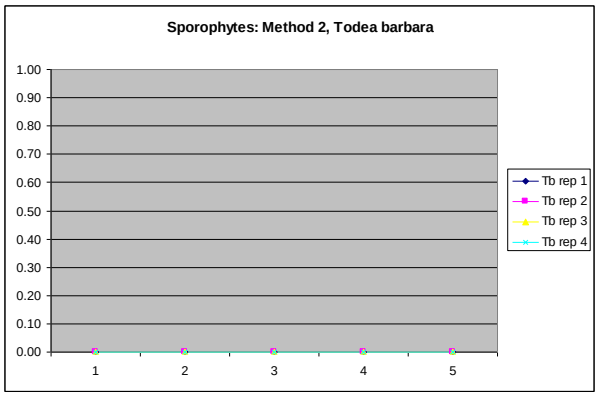

Figure 28: Sporophyte populations, Method 2, *Todea barbara*

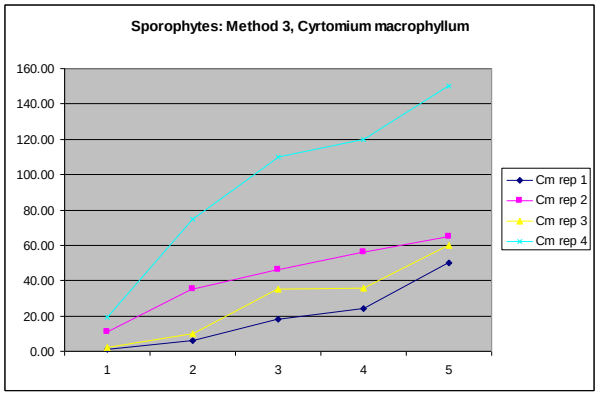

Figure 29: Sporophyte populations, Method 3, *Cyrtomium macrophyllum*

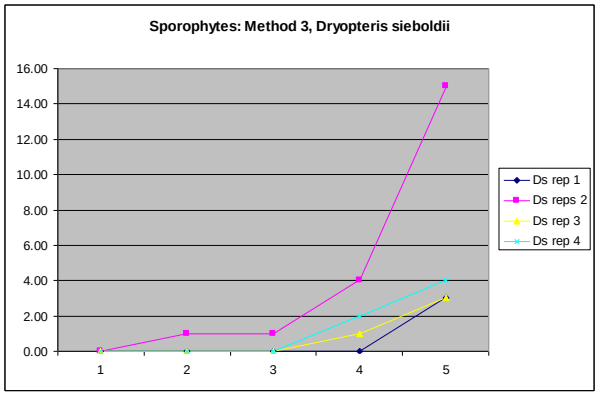

Figure 30: Sporophyte populations, Method 3, *Dryopteris sieboldii*

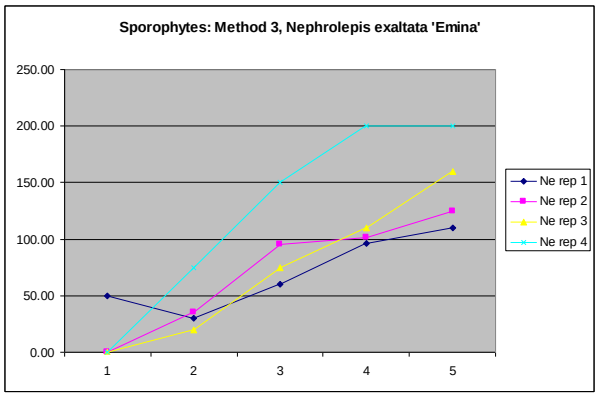

Figure 31: Sporophyte populations, Method 3, *Nephrolepis exaltata* 'Emina'

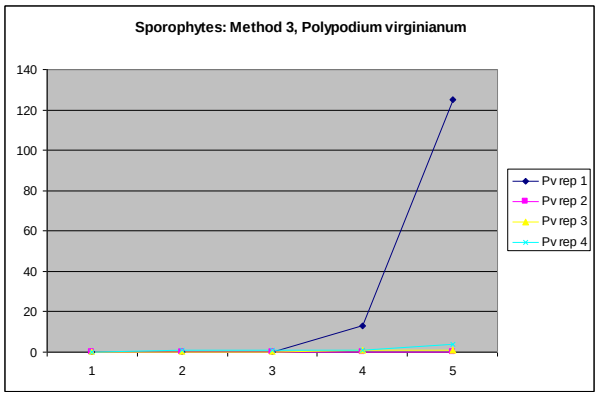

Figure 32: Sporophyte populations, Method 3, *Polypodium virginianum*

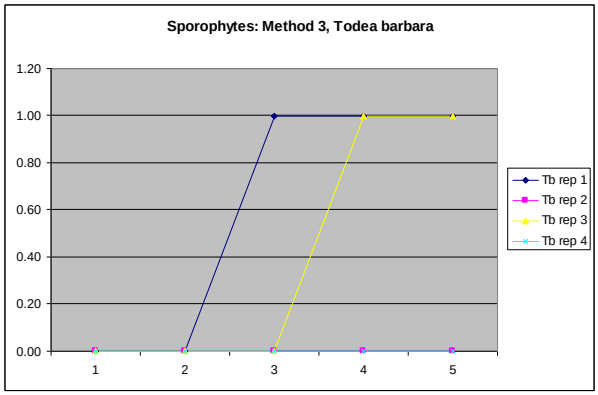

Figure 33: Sporophyte populations, Method 3, *Todea barbara*

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