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# Breeding Systems in *Spiranthes magnicamporum* Sheviak

Christopher D. Loyet

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Author

Breeding systems in *Spiranthes magnicamporum* Sheviak.

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(TITLE)

BY

Christopher D. Loyet

**THESIS**

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CHARLESTON, ILLINOIS

1993  
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING  
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

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ABSTRACT: A study of the ecology, breeding systems, and pollinators of Spiranthes magnicamporum Sheviak was undertaken in fall 1992. An initial survey of three glacial drift hill prairies revealed sexual activity in this species via removed pollinia. Two hill prairie populations were used for pollination study. In each population, five individuals were randomly selected and crossed using autogamic, geitonogamic, and xenogamic methods. In addition, ten individuals were fitted with pollinator-exclusion bags. After capsule formation, all plants were harvested. They were examined in the lab for general morphology, the presence or absence of embryo formation, and the prescence of sexual or asexual characteristics. Samples were stained in order to determine the condition of the embryo(s) present. Asexual taxa of S. magnicamporum display adventitious polyembryony (agamospermy) while sexual taxa (autogamy) produce monoembryonic seeds. It was determined in both populations that for the most part, the breeding system exhibited was autogamy. Although no direct observations were made, it must be assumed that some pollination must be taking place due to the presence of removed pollinia.

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## INTRODUCTION

Spiranthes magnicamporum Sheviak occurs on dry prairies and related grasslands from Northwestern Indiana onto the great plains. Disjunct populations exist on the prairies of Western Ohio and the black belt of Mississippi and Alabama. It ranges from Texas north to at least North Dakota (Sheviak, 1973). The name "magnicamporum" is a direct translation of "of the great plains". The Illinois population occurs almost entirely in the western half of the state in hill prairie.\* Other common Illinois members of this Genus include: Spiranthes cernua (L.) Rich., S. gracilis (Bigel) Beck, S. lacera Raf., S. lucida (H. H. Eaton) Ames., S. ovalis Lindl., S. tuberosa Raf., and S. vernalis Engelm. & Gray. All are commonly known as "ladies tresses" orchids. They occupy a wide range of habitats from woodlands to wet areas to open areas. All flower in either late summer or early to mid-fall and have sizeable, disjunct populations in Illinois (Mohlenbrock, 1971).

The name Spiranthes is a combination of two greek words meaning "coil" and "flower" in allusion to the typically spiral arrangement of flowers in the spicate inflourescence of many of the species (Figures 1 & 2). This polymorphic genus of about 300 species is widely dispersed throughout the temperate zones of both hemispheres. It occurs in tropical Asia to Australia, Tasmania, and New Zealand, and in the Americas extends from Canada to Chile.

FIG. 1 -->



FIG. 2 -->



FIGURES 1 & 2 : Spiranthes magnicamporum Sheviak in its natural habitat.



The species are found primarily in fields, savannahs, meadows, forests, swamps, dry open waste places, thickets, rocky barrens, and sandy places in intensely acid to calcareous soils (Correll, 1950).

This study was conducted in an effort to learn more about Spiranthes magnicamporum, its breeding systems, pollinators, and general biology. Similar experiments have been performed by Catling (1982; 1983) on various Spiranthes species including S. magnicamporum. Aside from pollinator observation, all of Catling's work was performed with greenhouse specimens. Excluding lab work, all data in the present study was obtained using populations of individuals occurring in hill prairies. Catling (1982) determined that Spiranthes magnicamporum was essentially agamospermic through part of its range, but at times, particularly in the southern part of its range (including central Illinois), was sexual. The present study was undertaken to examine the breeding systems of this species in central Illinois, where numerous individuals with very fragrant flowers occur on glacial drift hill prairies along the Illinois River drainage. These populations appear to be sexual, with typical pollinators (i.e. Euglossine bees) existing in the area.

## LITERATURE REVIEW

### MORPHOLOGY OF Spiranthes magnicamporum Sheviak.

Spiranthes magnicamporum is described by Sheviak (1973) as an erect herb 15-17 cm tall, glabrous below, pubescent above with copious capitate trichomes; roots tuberous, to 8mm in diameter; leaves alternate, fugaceous, linear-lanceolate, base cuneate, apex acute, about 12 mm wide and 14cm long; stem 1.5-7 mm in diameter; inflourescence a dense spike, 15-28 mm in diameter, 4-18 cm long, the phyllotaxy 1/3; floral bracts ovate, apex attenuate to acuminate, 10-30 mm long; flowers creamy white, strongly aromatic with a coumarin odor, the perianth segments densely pubescent, dorsal sepal lanceolate, acute, 7-11 mm long, 1.5-3 mm wide, lateral sepals loosely incurved, linear lanceolate to linear oblong, acuminate to obtuse, 7-11 mm long, 1-2 mm wide; petals fused to the dorsal sepal, linear, acuminate, 7-10 mm long, 1 mm thick; the labellum oblong-ovate to rhombic-ovate to rarely triangular, apex abruptly reflexed, basally dialated with median constrictions, margins entire, or the apex slightly dentate, crisp, 7-11 mm long, 3.5-6 mm wide, the prominent basal callus pubescent (Fig. 3).

Prior to 1973, Spiranthes magnicamporum was thought to be a variety of S. cernua. S. magnicamporum differs from S. cernua in several ways (Sheviak, 1973). Flowers of well developed plants of S. magnicamporum are larger than S. cernua. The perianth often exceeds 1 cm in length. The lip

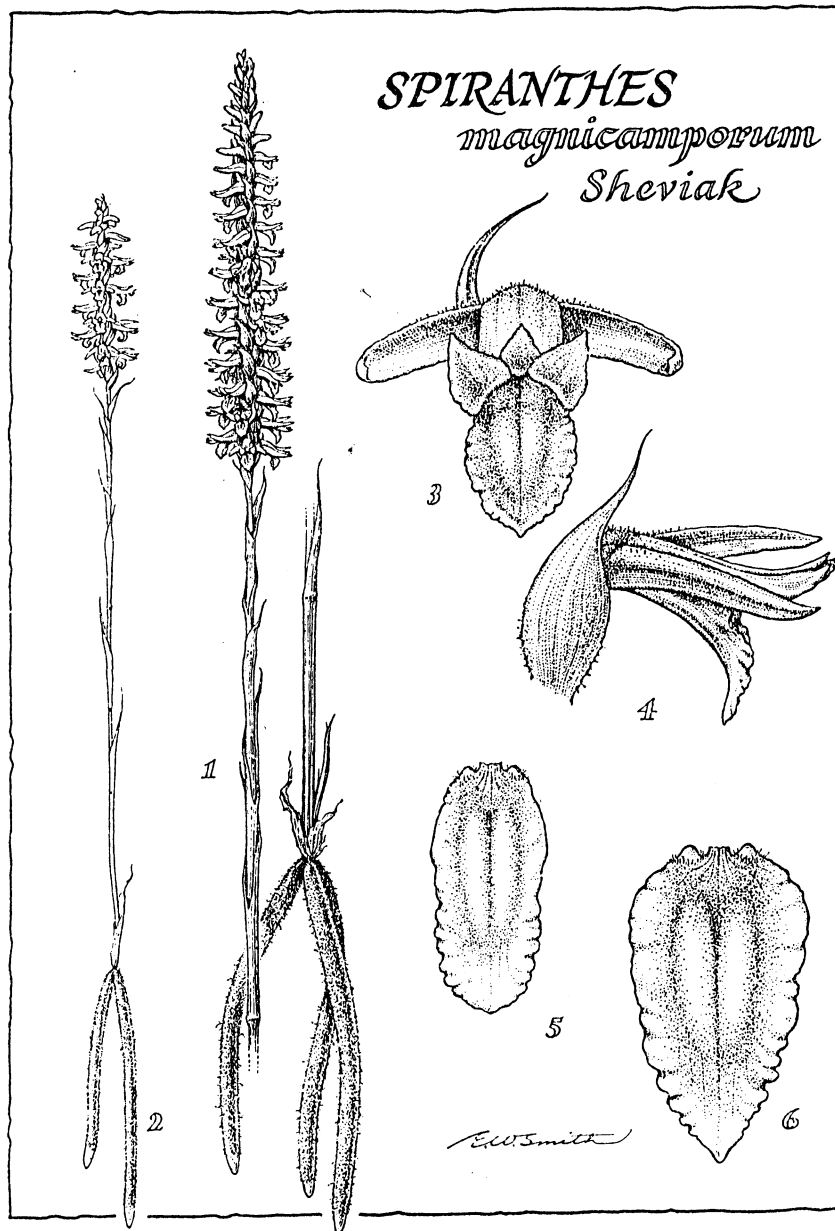


FIGURE 3: Spiranthes magnicamporum as drawn by Elmer W. Smith

of S. magnicamporum is uniformly oblong-ovate to rhombic-ovate, characteristically without the dialated base typically present in S. cernua. In S. cernua, the apical portion of the lip is also occasionally dialated, thus producing a lip with a median construction. These dialations are almost always absent in typical S. magnicamporum. The central portion of the lip in S. magnicamporum always supports a prominent thickening that is prominent even in boiled herbarium material. This characteristic is variable in S. cernua and often is absent. Crisping of the lip often is less marked in S. magnicamporum than in S. cernua, and the laceration of the lip is suppressed, with the margin often quite entire or the apical quarter nearly dentate. Lateral sepals are free and widely spreading to rarely loosely incurved or often ascending, unlike those of S. cernua, which closely parallel the petals and join to form a hood. The flowers of S. magnicamporum are often more nearly cream colored than the white usual in S. cernua, and the central thickening of the lip is usually yellow. They are strongly fragrant with the scent of coumarin (this fragrance was very evident in the field, as often plants were first located by odor), in contrast to the flowers of S. cernua, which in the geographical range of contact in the Midwest, at least, are generally scentless, or, when aromatic, exhibit only a faint fragrance strikingly like the scent of fresh Cypripedium sp. roots. In well developed specimens of the two taxa, the

inflourescences are strikingly different due to differences in the orientation and shape of the flowers. The nodding of S. cernua is accentuated by a general curvature of the dorsal sepal, whereas in S. magnicamporum this segment is arcuate only near the base. The orientation of the lip also varies: in S. magnicamporum it is rather uniformly arcuate along its length, although the apex is often abruptly reflexed, whereas in S. cernua the lip is often rather geniculate at about the middle. In S. cernua, the general impression is of an ornate inflourescence, an impression often accentuated by the reflexed tips of the sepals and petals. In S. magnicamporum, the more linearly organized flowers produce a strikingly scalariform inflourescence.

Several salient vegetative differences exist between Spiranthes magnicamporum and Spiranthes cernua. A feature of S. magnicamporum is the absence of leaves at anthesis. These senesce two to several weeks before the inflourescence appears. In S. cernua, however, they persist during anthesis or, under adverse conditions, senesce as the inflourescence develops. This behavior is genetically determined and cannot be altered under cultivation. Roots of S. magnicamporum also tend to differ from those of S. cernua in their greater tuberosity (Sheviak, 1973).

SEED GERMINATION AND MYCORRHIZAL SYMBIOSIS  
OF Spiranthes magnicamporum Sheviak

Seeds of Spiranthes magnicamporum are extremely small, appearing more like dust or pollen particles than mature ovules. In general, orchid seeds are minute, the largest weighing only 14 ug (Harley & Smith, 1983). Coincident with their small size, is a lack of food reserves. The testa is a fine lattice-like network covering an undifferentiated embryo. All orchids, whether they are chlorophyllous or achlorophyllous as adults, pass through a prolonged seedling stage during which they are unable to photosynthesize. In most seed plants the homologous period is passed through at the expense of reserves stored in the seed, but orchid seeds contain very limited food reserves (Arditti, 1979).

Orchid seeds first develop into protocorms, unorganized masses of cells in which there is little tissue differentiation. Ultimately these give rise to the seedling and subsequent plantlet. Little if any growth will proceed to the protocorm stage unless an alternative source of nutrition is available. According to Harley and Smith (1983), if seeds are spread on a moist substratum the embryos, undifferentiated except for their apical meristematic regions and perhaps the rudiment of a cotyledon, absorb water, swell slightly, may burst the testa, and produce epidermal hairs. The embryo does not develop further unless it receives

at least an exogenous supply of carbohydrate, or is infected by a compatible mycorrhizal fungus. Smith (1967) and Purves and Hadley (1975), using the tracers C14 and P32, confirmed the ability of these fungi to translocate and transfer carbohydrates to the developing orchid. Orchid seedlings display similar behavior akin to rust fungi. Both will grow in culture, but in nature are obligate symbionts. Their symbiotic partners, however, can survive on their own (Harley and Smith, 1983). The fungi are capable of very rapid axenic growth upon soluble carbohydrates and upon insoluble resistant carbon polymers, such as cellulose. In addition, most produce exoenzymes which hydrolyze complex polymers such as starch, pectin, cellulose, and lignin.

A very large number of the fungal isolates were referred to the form genus Rhizactonia (Harley and Smith, 1983). Now known as Epulorhiza, these fungi infect a wide variety of orchids. In particular E. repens, has been isolated from Spiranthes magnicamporum (E. repens matures into Tulasnella calospora, a resupinate basidiomycete). In an experiment performed by Anderson (1991), seeds of Spiranthes magnicamporum were germinated in vitro on water agar, modified Knudson's medium, and oat medium with the fungal symbiont Epulorhiza repens isolated from a naturally occurring plant of the same species. Symbiotic germination using this isolate was variable, with the fungus parasitizing many of the embryos and developing protocorms, but the survivors grew well.

Asymbiotic germination has been tried with this and other species of Spiranthes with limited success (Anderson, 1991; Oliva and Arditti, 1984). The best results seem to occur by using asymbiotically germinated protocorms and then transferring them to media infected with Epulorhiza repens (Anderson, 1991).

The various stages of infection are outlined by Harley and Smith (1983). Two stages of infection occur in the life cycle of many, perhaps most, orchids: (1) primary infection of the germinating seedling, and (2) reinfection of new roots of the adult. The source of the infection may be the soil, or according to Warcup (1971), sometimes the tuber, where fungi may persist on the surface or even in tissues. After germination, if a suitable fungus is present, single fungal hyphae penetrate the wall of either the epidermal hairs or epidermal cells near the suspensor cells of the embryo. The fungal hyphae invaginate the plasmalemma of the host and becomes surrounded by host cytoplasm. Nuclei of infected orchid cells are hypertrophied. Infection spreads from cell to cell so that the basal region of the protocorm becomes extensively infected. The invaginated host plasmalemma continues to synthesize cell wall components, pectin, and cellulose, but the young fungal hypha digests them. As the fungus ages the protoplasts within the hyphae disappear as a result of activity of the host, and the fungus becomes enveloped in



a cellulosic slime layer continuous with the cell wall of the host. Hyphae invade repeatedly, probably due to the host generated degeneration of the fungus. Usually a single cortical cell will outlive several fungal colonizations. Therefore we can summarize this system as triple: 1) a external hyphal system in contact with the external substrate capable of absorbing nutrients, 2) a internal system of cells parasitized by active, living hyphae, and 3) a second type of infected cell in which the hyphae collapse, fall victim to host-digestion, and continue to reinvade.

#### BREEDING SYSTEMS AND POLLINATION IN Spiranthes magnicamporum

Agamospermy is the formation of seeds without fertilization (Schmidt and Antlefinger, 1992). This parthenogenic development can be obligate if sexual function is completely lost, but most agamospermic plants are facultative (Asker, 1979). In a study done by Maheshwari & Narayanaswami (1950) parthenogenic development of the egg (essentially agamospermy) in Spiranthes cernua was investigated. It was determined that three distinct races existed: 1) a sexual race, in which syngamy takes place normally and a single embryo is produced in every seed, 2) an agamospermic race, in which the pollen is sterile and the female gametophyte shows numerous abnormalities which render it incapable of fertilization (cells of the inner integument proliferate and give rise to two to six embryos), and 3) a

intermediate race in which some ovules of an ovary follow the first mode of development and others the second mode.

The agamospermic mechanism observed in Spiranthes species is adventitious polyembryony (Catling, 1982). The production of monoembryonic seeds was associated with sexual reproduction and polyembryonic seeds with agamospermy (Sheviak and Catling, 1980). In addition to polyembryony, agamospermy can also produce seeds with single embryos and can result in extruded embryos, free embryos, and variations in embryo shape and/or positions (Sheviak, 1973). The testa, which consists of dead cells derived from the outer integument, can develop in the absence of an embryo resulting in an empty seed coat (Catling, 1982). This condition can arise in unfertilized sexual ovules or in asexual ovules that fail to produce adventitious embryos (Schmidt and Antlefinger, 1992).

If a species is sexual, pollination must take place. In autogamous situations the flowers are self-pollinated, and require no vector. In xenogamic and geitonogamic situations, a pollinator is required. Terrestrial orchids are reported to be pollinator limited (Robertson & Wyatt, 1990). Flower manipulations in the orchids have been conducted in an effort to determine the effects of pollinator exclusion, agamospermy, autogamy, and outcrossing on the level of asexual seed-production (Schmidt and Antlefinger, 1992). Auto-pollination occurs in all four orchid subfamilies in North

America. Agamospermy by adventitious polyembryony has been reported only in subfamily Spiranthoideae (Catling, 1991).

The tubular, nectar-containing, protandrous and often fragrant (as is in the case of Spiranthes magnicamporum), flowers in subfamily Spiranthoideae are pollinated mostly by long tongued bees (Euglossidae) (Catling, 1991). The structure of Spiranthes flowers and the pollinia within, makes them well adapted to pollination by euglossine bees. In the more specialized orchid subfamilies, such as the Spiranthoideae, the pollen occurs in mealy or tightly packed, waxy pollinia, the grains have a porate-ulcerate aperture or are inaperturate, and the sporopollenin wall is present only on the most peripheral grains in the pollinium (Zavada, 1990). In Spiranthes magnicamporum, the anther is dorsal and the pollinia are attached to a distinct "wedge-type" viscidium which terminates the rostellum. The rostellum is two-cleft and the stigma is ventrally located on the column behind the rostellum (Catling, 1983). These pollinia can be transported from flower to flower by structures existing on euglossine bees. In these bees the flat topped galea is hinged to the stipes and is relatively elongated, extending far beyond the head when directed forward. The position of the pollinia in a freshly opened flower, with respect to the position of the insect's tongue while obtaining nectar, ensures that the pollinia will become attached by the long rigid viscidium to the dorsal surface of the galea

(Catling, 1982).

These bees are most likely attracted to the strongly fragrant flowers that exist on Spiranthes magnicamporum. In species exclusively pollinated by fragrance collecting male euglossine bees, the fragrances are primary attractants that serve both as an attractant and as a reward (Gerlach and Schill, 1991) (Fig 4).

Although the exact compounds that make up the floral fragrances observed in Spiranthes magnicamporum have not been isolated, typical orchid fragrances have been. They are monoterpenes or simple aromatic compounds. The following is a list of aromatic fractions isolated from members of the Orchidaceae: alpha-pinene, beta-pinene, myrcene, alpha-phelandrene, cineole, ocimene, p-cymene, citronellol, methyl salicylate, nerol, 2-phenylethyl acetate, 2-phenylethanol, methyl cinnamate, eugenol, vanillin, and skatole (Williams, 1980).

Spiranthes magnicamporum has been observed to go through several developmental sexual stages, which may or may not contribute to the breeding system observed. Depending upon the developmental stage, an inflorescence may be functionally male (in early flower), female (in late flower), or bisexual (in peak flower or full anthesis), but when bisexual its older and lower functionally female flowers are visited first since the bee pollinators move from the base upward (Catling, 1983). These pollinator movements are unique, follow a specific

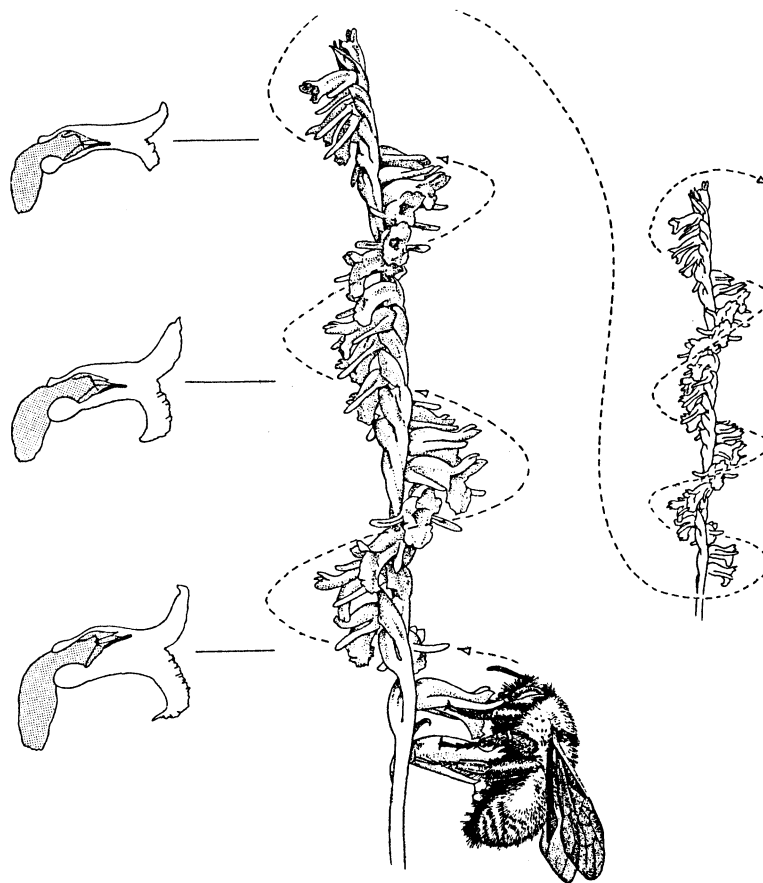


FIGURE 4: Pollination of Spiranthes cernua by Bombus sp.

pattern, and serve a particular role in the sexual races of the Spiranthoideae. The upward movement of the bee pollinator on the inflourescence is clearly essential to the enhancement of outcrossing. In the case of Spiranthes inflourescences more nectar is often in older flowers than in younger flowers. If reward per flower decreases upwardly, then by moving from the most profitable flowers to the least profitable flowers there is less waste of energy (Catling, 1983).

Protandry is to be expected in all North American Spiranthes species that display the appropriate morphology (Catling, 1983). The mechanisms of protandry exhibited by Spiranthes was defined by Catling (1983), and both the lip and the column are involved. Comparison of tissues in adjacent flowers of different ages suggests that there is an elongation of the cells on the lower side of the column making it straighter and removing the curve so that the stigmatic surface assumes a more upright position. This may be brought about partly by elongation of the basal claw of the lip and the resulting pushing of the basal calli against the sharp distal curve of the column.

#### ILLINOIS HABITAT OF Spiranthes magnicamporum

Spiranthes magnicamporum is mostly restricted to the loess hill prairies of western Illinois. Hill prairies in Illinois have a dunic nature of both sand and loess deposits derived from adjacent valleys after Wisconsin glaciation. The

soil normally has a very calcareous nature and has a specific biota. Typically associated plants are listed in table 1 (Evers, 1955; Schwegman, no date).

It appears that Spiranthes magnicamporum is a western prairie counterpart of Spiranthes cernua, although not allopatric (S. cernua occurs farther west than S. magnicamporum in several areas). S. magnicamporum is the more prevalent in the West and is better adapted to the droughty conditions of the area than is S. cernua, which often relies on local abundance of moisture for its survival. The two species are however, truly sympatric only on rare occasions, for even when occurring at the same area these species are spatially isolated by ecological factors. Of the two ecotypes occupied by S. cernua in the Illinois region, one occurring principally in moist sand prairie, the other in dry open woods and old fields on finer textured soils, usually only the former occurs with S. magnicamporum. At such areas S. magnicamporum occupies the dry ridge tops and S. cernua the wetter swales. Both of these taxa occur in calcareous hanging fens, but S. magnicamporum does so very rarely (Sheviak, 1973).

TABLE 1 : PLANTS ASSOCIATED WITH Spiranthes magnicamporum

ACANTHACEAE:	<u>Ruellia humilis</u> Nutt.
ANACARDIACEAE:	<u>Rhus glabra</u> L.
ASCLEPIDACEAE:	<u>Asclepias verticillata</u> L., <u>A. viridiflora</u> Raf., <u>A. amplexicaulis</u> Sm.
ASTERACEAE:	<u>Silphium integrifolium</u> Michx., <u>Heliopsis helianthoides</u> (L.) Sweet, <u>Rudbeckia hirta</u> L., <u>Echinacea pallida</u> (Nutt.) Nutt., <u>Helianthus rigidus</u> (Cass.) Desf., <u>Coreopsis palmata</u> Nutt., <u>Heterotheca villosa</u> (Pursh) Shinner, <u>Solidago rigida</u> L., <u>S. speciosa</u> Nutt., <u>S. nemoralis</u> Alt., <u>Aster azureus</u> Lindl. <u>A. oblongifolius</u> Nutt., <u>A. sericeus</u> Vent., <u>A. ptarmicoides</u> (Nees) Torr. & Gray, <u>A. pilosus</u> Willd., <u>A. ericoides</u> L., <u>Erigeron strigosus</u> Muhl., <u>Eupatorium altissimum</u> L., <u>Brickellia eupatoroides</u> (L.) shinnners, <u>Liatris aspera</u> Michx., <u>Veronia missurica</u> Raf., <u>Microceris cuspidata</u> (Pursh) Sch.-Dip., <u>Tragopogon dubius</u> Scop.
BORAGINACEAE:	<u>Lithospermum incisum</u> Lehm., <u>L. canescens</u> (Michx.) Lehm. <u>Onosmodium occidentale</u> Mack.
CAMPANULACEAE:	<u>Lobelia spicata</u> Lam.
CYPERACEAE:	<u>Cyperus filiculmis</u> Vahl., <u>Carex pensylvanica</u> Lam., <u>Carex meadii</u> Dewey.
EQUISETACEAE:	<u>Equisetum laevigatum</u> A. Br.
EUPHORBIACEAE:	<u>Euphorbia corollata</u> L.
IRIDACEAE:	<u>Sisyrinchium albidum</u> Raf., <u>S. campestre</u> Bickn.
LABIATAE:	<u>Monarda fistulosa</u> L., <u>Physostegia virginiana</u> (L.) Benth.
LEGUMINOSAE:	<u>Amorpha canescens</u> Pursh., <u>Psoralea tenuiflora</u> Pursh., <u>Petalostemum purpureum</u> (Vent.) Rydb., <u>Melilotus alba</u> Desr., <u>Lespedeza capitata</u> Michx.
LINACEAE:	<u>Linum sulcatum</u> Riddell.
OROBANCHACEAE:	<u>Orobanche fasciculata</u> Nutt.



TABLE 1 (continued)

OXALIDACEAE:	<u>Oxalis violacea</u> L.
POACEAE:	<u>Sorghastrum nutans</u> (L.) Nash., <u>Andropogon gerardi</u> Vitman. <u>Elyms canadensis</u> L., <u>Schizachyrium scoparium</u> (Michx.), <u>Eragrostis spectabilis</u> (Pursh) Steud., <u>Tridens flavus</u> (L.) Hitchcock, <u>Bouteloua curtipendula</u> (Michx.) Torr.
POLEMONIACEAE:	<u>Phlox bifida</u> Beck.
ROSACEAE:	<u>Rosa carolina</u> L.
SALICACEAE:	<u>Populus deltoides</u> Marsh. ( A notably large one exists in the middle of Panther Creek.).
SCROPHULARIACEAE:	<u>Penstemon pallidus</u> Small., <u>Pedicularis canadensis</u> L., <u>Gerardia aspera</u> Doug.
VERBENACEAE:	<u>Verbena stricta</u> Vent.

-Nomenclature follows Jones (1963)

## MATERIALS AND METHODS

An initial survey of three glacial hill prairies was made in the fall of 1992. They were: 1) Panther Creek Nature Preserve, Cass Co., Illinois where a large number of Spiranthes magnicamporum was found scattered through the prairie, 2) Roadside Prarie, Cass Co., Illinois where Spiranthes magnicamporum was found along the adjacent road, in lowland-ditch areas, and on the hill prairie itself, and 3) Revis Hill Prairie Nature Preserve, Mason Co., Illinois where Spiranthes magnicamporum was found in abundance along the bluffs and ridges (Figs. 5 & 6).

During the initial survey, three specimens per study site were collected, checked for removed pollinia, and dried for herbarium specimens. Only Panther Creek and Roadside Hill Prairie were suitable for a population and observation study.

### Panther Creek Nature Preserve (Fig. 7)

Five specimens were randomly chosen for pollination studies. The first three of these plants were manipulated in the following manner. Starting at the base of the spike, the first flower was left undisturbed; the second flower was self-pollinated; the third, following emasculation, was fertilized with an intact pollinia from the same plant; the fourth, following emasculation, was fertilized with an intact pollinia from a neighboring plant. This sequence was repeated on four other sequential flowers on the spike for a total of

FIG 5 -->



FIG 6 -->



FIGURES 5 & 6: Revis Hill Prairie Nature Preserve, Mason Co., Illinois.

eight flowers per spike. Suitable space between the two groups of flowers was allowed as all unmanipulated flowers were removed from the plant. A diagram of each specimen was made to ensure proper identification of the individual flowers. The remaining two plants were treated in the same fashion except only four flowers per plant were used due to their diminutive size. All five plants were fitted with cheesecloth pollinator exclusion bags and tagged for identification. Their exact locations were recorded and mapped. To facilitate easy location on the somewhat vast hill prairie, red tipped marker flags were used which also provided support to the somewhat fragile inflorescences.

An additional population of ten random individuals were left intact and fitted with pollinator exclusion bags in an effort to check for self-pollination and/or agamospermy. They were tagged and mapped (Figs. 9 & 10).

Of the remaining individuals, five specimens were decapitated at the base of the spike and placed in vials containing 80% ethanol. These specimens were checked for morphology. Other specimens were left undisturbed to facilitate pollinator observation. In addition, a pitfall trap was erected at the base of a randomly selected individual to collect pollinators. Only those pollinators collected with conspicuous pollinia attachment to their mouthparts via the viscidium (Catling, 1983) were counted.



FIGURE 7: Panther Creek Nature Preserve Cass Co., Illinois.

### Roadside Prairie (Fig 8)

Five individuals were randomly selected for pollination study. They were treated in the same fashion as the Panther Creek plants with the following exceptions:

1. All plants within this population were somewhat diminutive, thereby warranting that only four flowers per plant should be used instead of eight.
2. On specimens 3, 4, and 5, the pollinia from the third and fourth flowers were interchanged. In the previously mentioned procedure, the third and fourth flowers recieved pollinia from flowers on the same plant, and a neighboring plant, respectively. This was done due to the diminutive size of most individuals in question and the relatively low population density.

As an alternative to the use of pollinator exclusion bags, 10 unmanipulated individuls and their associated soil were dug up and placed in plastic pots. These individuals then were transported back to the university to serve as greenhouse specimens.

All specimens from both study sites were allowed to mature until capsule formation was observed. Plants were



FIGURE 8:           Roadside Hill Prairie Cass Co., Illinois.



FIG. 9 -->



FIG. 10 -->



FIGURES 9 & 10: Specimens following the bagging procedure.



then harvested by collecting the entire inflorescence. The mature spikes were deposited into appropriately labeled manilla envelopes and stored at 0 degrees C.

#### Laboratory Work

All capsules were checked for maturity, and results were recorded for the number of viable capsules in the unmanipulated exclusion and greenhouse specimens. The manipulated specimens were checked for capsule formation. All manipulated capsules were transferred to labeled vials for later use.

A random sample of approximately 100-300 seeds was taken from each one of the unmanipulated plants and checked for seed color and general morphology. Approximately 100-200 of these seeds were transferred to a clean microscope slide and stained (Owczarzak, 1951). Several batches of glycerol jelly were prepared by dissolving 40g of gelatin in 100 ml of water. 60ml of glycerol and 2 g of phenol crystals were incorporated into the heated jelly. The jelly was decanted in 50 ml portions into glass jars. When ready for use, a portion of the jelly was reheated. To it was added 2.5 ml of methyl green and 2.0 ml of concentrated phloxine B. The stained jelly was then decanted into a glass jar. Small portions were used as needed since excessive melting and remelting breaks down the gelatinous nature of the jelly.

The transferred seed sample was washed twice with 75% Ethanol. Excess debris was washed off and formed a "smudge

ring" around the sample. This excess was removed by means of a Kim-wipe. After transferring the slide to a warmer, 1-2 drops of heated jelly mixture was placed directly on the sample. Seeds were stirred with a clean needle to ensure even distribution. The slide was covered with a glass cover slip and allowed to cool.

Slides were prepared from samples from each of the unmanipulated plants, in addition from each capsule of the manipulated plants. Slides were observed in the following manner: The entire slide was scanned under the 10X objective. The first variable examined was seed fertility. If a high number of empty seed coats existed, a random fertility count of the first 100 seeds observed was performed. Of those seeds found to be positive, the percentage of polyembryonic and monoembryonic seeds was determined. In slides exhibiting a low number of empty seed coats (approximately 100% fertility), the percent of monoembryony and polyembryony was determined.

## RESULTS AND DISCUSSION

Pollination: The results of specimens examined for intact pollinia are listed in table 2. Specimens from Panther Creek Hill Prarie had the lowest percent of intact pollinia, indicating that pollinators were active at this site. Intact pollinia in specimens ranged from 100 to 71.8 %, with an average of 88.1 %. Of the three sites studied in this initial survey, Panther creek was closest to an adjacent woodland area, and had the highest species diversity.

The specimens obtained from Revis Hill Prairie Nature Preserve had a greater number of intact pollinia than Panther Creek, ranging from 93.5 to 85.7%, with an average of 88.7%. This area was not used as a study site following this initial survey since upon the return visit to the site, it was entirely devoid of flowering specimens of Spiranthes magnicamporum.

Specimens initially obtained from Roadside Hill Prairie had the highest number of intact pollinia, ranging from 100.0 to 96.6%, with an average of 98.9%. Flora in this area is not as dense as the others studied. A lack of forage could mean a lack of available pollinators, and few pollinia were removed.

Pollinators: With more than forty hours spent in the field, no pollinator was seen approaching Spiranthes magnicamporum. The pitfall trap that was erected at the base of a randomly chosen individual plant failed to give any hard

evidence either. The insects present in the trap were members of Coleoptera, Lepidoptera, and Orthoptera. None of these specimens had the attached pollinia necessary to qualify them as potential pollinators of Spiranthes magnicamporum. The pollinator groups observed by Catling (1983) were never seen or located. It must be assumed however, that at least in the Panther Creek population, pollinators had been visiting flowers. The pollinia are not easily removed. The lower lip of the flower must be brushed up against in a manner typical of euglossine bees. As the lip is depressed, the viscidium adheres to the mouthparts of the bee, and is consequently removed.

The following explanations are possible for the observations. First, the pollinator for this species is crepuscular and was not active during the times observation was taking place (no earlier than 9:00 A.M. and no later than 5:00 P.M.). This explanation would preclude the most commonly observed pollinator group (euglossine bees) as they are typically diurnal. Second, the pollinator for this species was active only prior to early to middle September when the initial survey was conducted. Third, the pollinator(s) in question consisted of a very small population of individuals, or were the primary pollinators of a more available, herbaceous species. This explanation appears to be the most likely scenario, but no definite answer exists.

Roadside Hill Prairie Breeding Study: All bagged but

TABLE 2: EXTENT OF POLLINIA REMOVED FROM SPECIMENS AT THE  
THREE STUDY SITES IN WEST-CENTRAL ILLINOIS

LOCATION	SPECIMEN	POLLINIA PRESENT/ FLOWERS/PLANT	PERCENT INTACT POLLINIA
Panther Creek	A	23/32	71.8
	B	38/41	92.7
	C	22/22	100.0
			----- X = 88.1
Roadside Prarie	A	28/29	96.6
	B	21/21	100.0
	C	13/13	100.0
			----- X = 98.9
Revis Preserve	A	43/46	93.5
	B	20/23	87.0
	C	18/21	85.7
			----- X = 88.7

unmanipulated specimens from Roadside Prairie, except for three, exhibited capsule formation. Maturation took place within the greenhouse. None of the manipulated specimens from this prairie exhibited capsule formation. Due to time constraints, the manipulations had to be conducted at a later date than those at Panther Creek Hill Prairie. Most likely, pollen tubes did not have appropriate time to reach the ovules and/or meiotic development was cut short by frost.

Slides made from the capsules of the unmanipulated plants at Roadside Prairie exhibited no polyembryony. Therefore we can assume that the breeding system involved in this population is autogamy. As determined by Schmidt & Antlefinger (1992), the mechanism observed in agamospermic populations of Spiranthes is adventitious embryony. This condition was not observed in this population. During the initial survey, an average 98.9% pollinia were left intact. This observation all but excludes xenogamic and geitonogamic breeding systems via pollinators.

Panther Creek Hill Prairie Breeding Studies: Results from Panther Creek Hill Prairie are similar to those of Roadside Hill Prairie. All bagged but unmanipulated plants produced capsules. Upon examination of slides under the microscope, no evidence of adventitious polyembryony was found among these plants. Since these plants were fitted with pollinator exclusion bags, it must be assumed that the mechanism involved is autogamy.

The manipulated plants produced somewhat varied results (Table 3). Plant A produced 100% monoembryony (Fig. 11) with the exception of flower A-4, which after being cross pollinated with a pollinia from a neighboring plant failed to develop a capsule.

Plant B produced the most varied results. Flower B-4A did not develop a capsule. Flowers B-2 and B-2A, both self-pollinated exhibited 5% and 4% polyembryony, respectively. Flower B-3, a xenogamic flower, exhibited 2% polyembryony (Fig. 12), and occasional empty seed coats (Fig. 13). Although polyembryonic, the ovules in question displayed a side by side pattern not typical of adventitious polyembryony. The data are negligible and not an indication of agamospermy.

Plant C failed to produce any capsules. The reasons for this lack of capsules are unknown as the procedure was carried out exactly as before.

Plants D & E with the exception of flower E-2 exhibited 100% monoembryony. In flower E-2 less than 25 intact embryos were found. Very few seeds were in the capsule, which was greatly shrunken.

Given these data we can assume that the race of Spiranthes magnicamporum in Central Illinois is, in general, autogamously sexual. Since adventitious polyembryony is the sole mechanism responsible for agamospermy in S. magnicamporum and was not found in these populations, they cannot be agamospermic. Geitonogamic or xenogamic breeding systems also

may be present, but due to the lack of evidence concerning pollinators, this activity must be assumed to be rare.



FIG. 11: A typical monoembryonic seed observed in this study (specimen obtained from Panther Creek Nature Preserve).

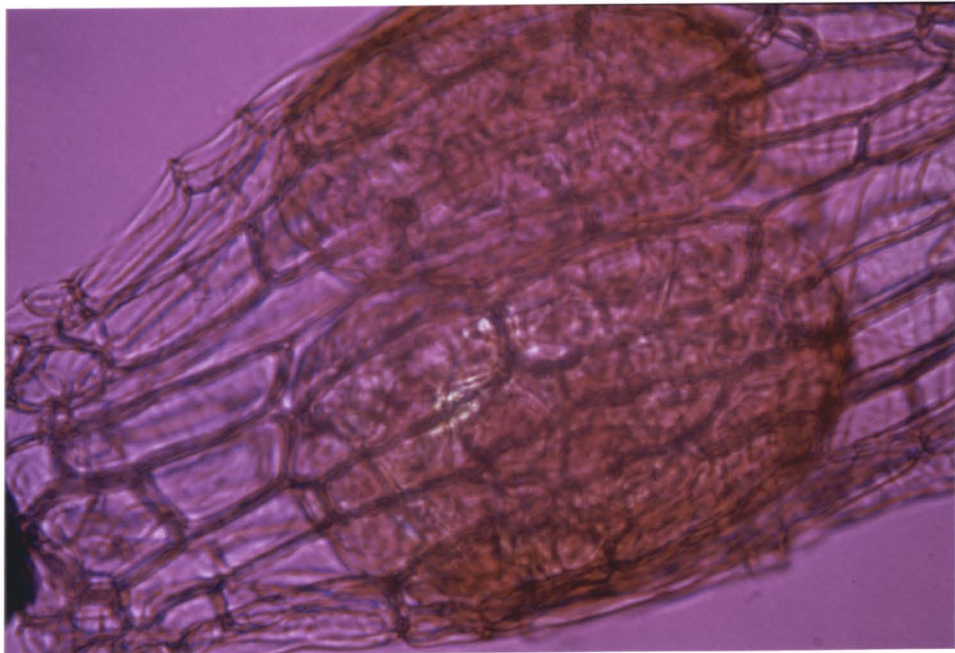


FIG 12: Polyembryony exhibited by Spiranthes magnicamporum (Specimen obtained from Panther Creek Nature Preserve).





FIGURE 13: An empty seed coat of Spiranthes magnicamporum.

TABLE 3: RESULTS OF MANIPULATED PLANTS FROM PANTHER CREEK

SPECIMEN	% MONOEMBRYONY	% POLYEMBRYONY
PLANT A		
[A-1]	100	--
[A-2]**	100	--
[A-3]	100	--
[A-4]*	--	--
[A-1A]	100	--
[A-2A]	100	--
[A-3A]	100	--
[A-4A]	100	--
PLANT B		
[B-1]	100	--
[B-2]	95	5
[B-3]	98	2
[B-4]	100	--
[B-1A]	100	--
[B-2A]	96	4
[B-3A]	100	--
[B-4A]*	--	--
PLANT C		
[C-1]*	--	--
[C-2]*	--	--
[C-3]*	--	--
[C-4]*	--	--
[C-1A]*	--	--
[C-2A]*	--	--
[C-3A]*	--	--
[C-4A]*	--	--
PLANT D		
[D-1]	100	--
[D-2]	100	--
[D-3]	100	--
[D-4]	100	--
PLANT E		
[E-1]	100	--
[E-2]***	--	--
[E-3]	100	--
[E-4]	100	--

- \* -Denotes dead specimen
- \*\* -Due to a high % of empty ovules a random fertility count was performed.  
Fertile--25%, Infertile--75%
- \*\*\* -Less than 25 intact embryos were found.  
Very few seeds were in the capsule  
itself which was greatly shrunken.
- [ ] -All specimens contained within brackets  
have the following numerical code.
  - X1 - unmanipulated
  - X2 - self-pollinated
  - X3 - cross-pollinated from the same  
plant.
  - X4 - cross-pollinated from a different  
plant.

## GLOSSARY

AGAMOSPERMY -	Asexual production of seed.
ALLOPATRIC -	When species occupy different regions with no overlap.
ARCUATE -	Curved or bent like a bow.
AUTOGAMOUS -	Self-pollinating.
AXENIC -	In pure culture.
CREPUSCULAR -	Active at twilight.
FUGACEOUS -	Falling or withering away very early; ephemeral.
GALEA -	In bees, the outer lobe of the maxilla, borne by the stipes.
GEITONOGAMIC -	Referring to being self pollinated with a different flower on the same plant.
GENICULATE -	Bent abruptly, like a knee.
HYPERTROPHIED -	Having a higher DNA content than normal.
LABELLUM -	In bees, the expanded tip of the labium or lower lip.
PARTHENOGENIC -	Developing without fertilization; apogamous.
PHYLLOTAXY -	The manner in which the leaves are arranged with regard to the axis.
PLASMALEMMA -	Outer boundary of the protoplast next to the cell wall; consists of a single membrane unit.
POLLINIA -	A mass of waxy pollen or of coherent pollen grains.
PORATE-ULCERATE -	Having an exit pore or ulcer.
PROTANDRY -	In flowers, the maturation of male parts before female parts.
ROSTELLUM -	A little beak; a slender extension from the upper edge of the stigma.

SCALARIFORM -	Having markings suggestive of a ladder.
SPOROPOLLENIOUS -	Containing sporopollenin; the tough substance of which the ekine, or outer wall, of spores and pollen grains is composed.
STIPES -	The second segment or division of a maxilla (mouthpart structure).
SYMPATRIC -	When species occupy the same or overlapping regions.
SYNGAMY -	The process by which two haploid cells fuse to form a diploid zygote; fertilization.
TESTA -	The outer seed coat. Normally hard and brittle, but in the case of <u>Spiranthes</u> sp. thin and fragile.
VISCIDIUM -	The glutinous end of the pollinial mass.
XENOGAMIC -	Cross pollinated with a flower from another plant.

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