POLLINATION BIOLOGY OF NORTHERN RED AND BLACK OAK

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Abstract: Pistillate flower abortion in northern red oak and black oak was evaluated in relation to pollination and fertilization. The presence, position, and characteristics of the pollen grains, pollen tubes, and ovules were determined with bright field and fluorescence microscopy. Flower survival counts were made weekly, from late April to mid-September. Both species have rudimentary ovules and small locules during the first growing season. Pollen tubes cease growth during the first growing season in mid-May at the level of the distal end of the perianth and juncture of the three stigmas. In the next growing season, meiosis in the mature ovules may trigger the advance of the pollen tubes into the locules. Fertilization occurred in mid-June for northern red oak and late June in black oak.

INTRODUCTION

Bumper crops of acorns are considered to be sporadic or episodic. Sork and others (1993) found that the size of a given acorn crop was determined by both flower abundance and survival of the flowers to maturation. Most of the potential seed crop is lost when the pistillate flowers abort before fertilization (Cecich 1991). As members of the subgenus Erythrobalanus, northern red oak (*Quercus rubra* L.) and black oak (*Q. velutina* Lam.) require two growing seasons between pollination and seed maturation; only pollination occurs in the first season. However, it is during the 6-8 weeks after pollination in that first season that most of the abortion occurs. In northern red and white oak (*Q. alba* L.), up to 100 percent of the flowers may abort during that 6-8 week period (Cecich 1991). The possible role of insects, especially the treehoppers (Membracidae), as factors in oak flower abortion has been previously addressed (Cecich 1993).

Because of the time that most of the flower abortion occurs, the pollination process should be examined. The success of the acorn crop can be related to pollen dispersal. Emergence of the catkins and shedding of pollen increase with rising temperatures (Romashov 1957). Rainy weather, often associated with decreased temperature, can reduce pollen dispersal. Sharp and Chisman (1961) concluded from field observations that pollen dispersal occurred when relative humidity dropped and remained below 45 percent for several hours, but they did not mention the success of the acorn crop. Sharp and Sprague (1967) found no correlation with relative humidity and acorn yields in their field studies and concluded that temperature was a primary factor in determining acorn crops. Using growth chambers, Wolgast (1972) and Wolgast and Stout (1977) demonstrated experimentally that relative humidity at the time of pollen shed and stigma receptivity can limit the size of an acorn crop in *Q. ilicifolia* Wangenh. No acorns matured when relative humidity exceeded 61 percent, but about half the flowers matured into acorns when relative humidity was lower.

Past observations of *Quercus* pollen tube growth are conflicting and incomplete. Benson (1894) first observed pollen tubes in *Q. robur* L., a member of the Lepidobalanus (white oak) subgenus, in the locules, just before fertilization. She did not see them in the stigma or style. Jovanovic and Tucovic (1975) observed that pollen germination in *Q. robur* was completed within 24 hours, but fertilization occurred 6-7 weeks later, and speculated that pollen tube growth did not proceed until the ovule had completed development. In contrast, Allard (1932) noted that "When pollen reaches the stigma of members of the white oak group, the growth of the pollen tube containing the male cells follows an uninterrupted advance into the tissues of the style until the ovules are fertilized." Allard (1932) also found that in the red oak group the pollen tubes ceased growth at the base of the style until the following spring when fertilization occurred. Unfortunately, no details of pollen tube growth were documented in any of these reports. Therefore, in this study, we provide the first details of the pollination biology of a Missouri population of northern red oak and black

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oak during the first and second growing seasons. Pollen tube growth is examined in relation to the time of pollen shed, fertilization, and flower survival.

METHODS

The trees in this study were located along a forest road in a closed stand on the Thomas S. Baskett Wildlife Research and Education Area, Ashland, MO (38°N, 92°W); operated by the University of Missouri in cooperation with the Missouri Department of Conservation. Sampling was done from a hydraulic lift truck equipped with a 40-foot boom and reaching a height of 50 feet.

The number of pistillate flowers, their survival and anatomical development, and the time of pollen shed were determined from 1990 to 1994. Data sets for 12 black oaks (BO) and 4 northern red oaks (NRO) from the growing seasons of 1990-1991, 1991-1992, and 1992-1993 were selected to demonstrate the variation in flowering and survival. Pistillate flowers, from the upper and middle crown regions, were counted on the current year's growth of five 2nd-year (one-year-old) branches per tree. Pistillate flowers are found in the axils of current year's leaves. The average number of flowers per 2nd-year branch ranged from about 3-63, depending on the tree and the year. These same trees and branches were used to count the 2nd-year flowers that matured into acorns. For microscopic examination, ten pistillate flowers were collected weekly from one BO and one NRO, during mid-April to Mid-September, from 1991 to 1994. In the spring of 1993 and 1994, two additional black oaks were sampled. Estimates of the dates of pollen shed were done on the same trees as the flower counts, but during the years of 1991-1994.

Flowers were fixed in FAA (formalin : acetic acid : alcohol), embedded in Paraplast-Plus, cut on a rotary microtome, and sections, both longitudinal and transverse, were attached to slides with Haupt's adhesive. For observation by bright field microscopy, sections were cut 10-µm-thick, stained in safranin-fast green, and coverslips mounted in Permount. For fluorescence microscopy, sections were cut 20-µm-thick and stained with 0.005% aniline blue in 0.15M K_2HPO_4 at pH 8.2 for 10-30 minutes to locate callose, a 1,4 ß-glucan polysaccharide that fluoresces a bright yellow-green, in the pollen tube wall (Currier 1957). Coverslips were mounted in the staining solution. Sections were examined with a Zeiss Universal microscope, illuminated by an HBO 200 W mercury lamp, in conjunction with the exciter filters UG-1 and BG-38 and the barrier filters 44, 47, and 53. Photomicrographs were taken with a Wild-Heerbrugg MKa4 Photoautomat camera using Kodak Ektachrome 400 HC film.

RESULTS

Mean survival curves of NRO and BO pistillate flowers, from 1990 to 1993, are shown in Figures 1-3. In NRO, most of the flower loss occurred in the first 6-8 weeks after pollination. However, additional loss was recorded in conjunction with the overwintering period of the pollinated flower. Since observations were not made between September and April of the following year, we could not determine the timing or cause of this loss. Thus, the loss that occurred overwinter (between September and April) is expressed as the increment between April and May in those Figures.

Staminate flowers that produce the pollen are found on inflorescences or catkins. Catkins are found in buds that only produce catkins ("male buds") or in "mixed buds" (buds that contain vegetative shoots and catkins, or buds that contain vegetative shoots, pistillate flowers and catkins). Depending on the year, pollen of NRO is usually shed during the week of April 20 at the central Missouri site, and about one week later in BO (Table 1). Pistillate or female flowers are found in the axils of newly emerging leaves of the terminal bud or its subtending lateral buds. In NRO and BO, the pistillate flowers (Figure 4) tend to be distributed among the leaves of the central and basipetal portion of a branch. Based upon our weekly sampling schedule, the stigmas of the pistillate flowers (Davis 1966) seemed to be receptive for no more than one week. Our assessment of receptivity was based on the

flexibility and bright red coloration of the stigmas. Once the stigmas turned brownish in color and became rigid, we felt that receptivity had passed. Oak stigmas are classified as dry (Heslop-Harrison and Shivanna 1977), meaning that they do not produce visible secretions on the stigmatic tip.

Table 1. Annual variation in the beginning date of pollen shed. The duration of pollen shed for a species in the stand seemed to be less than one week.

YEAR	RED OAK	BLACK OAK
1991	0*	1 May
1992	24 April	1 May
1993	1 May	7 May
1994	22 April*	29 April

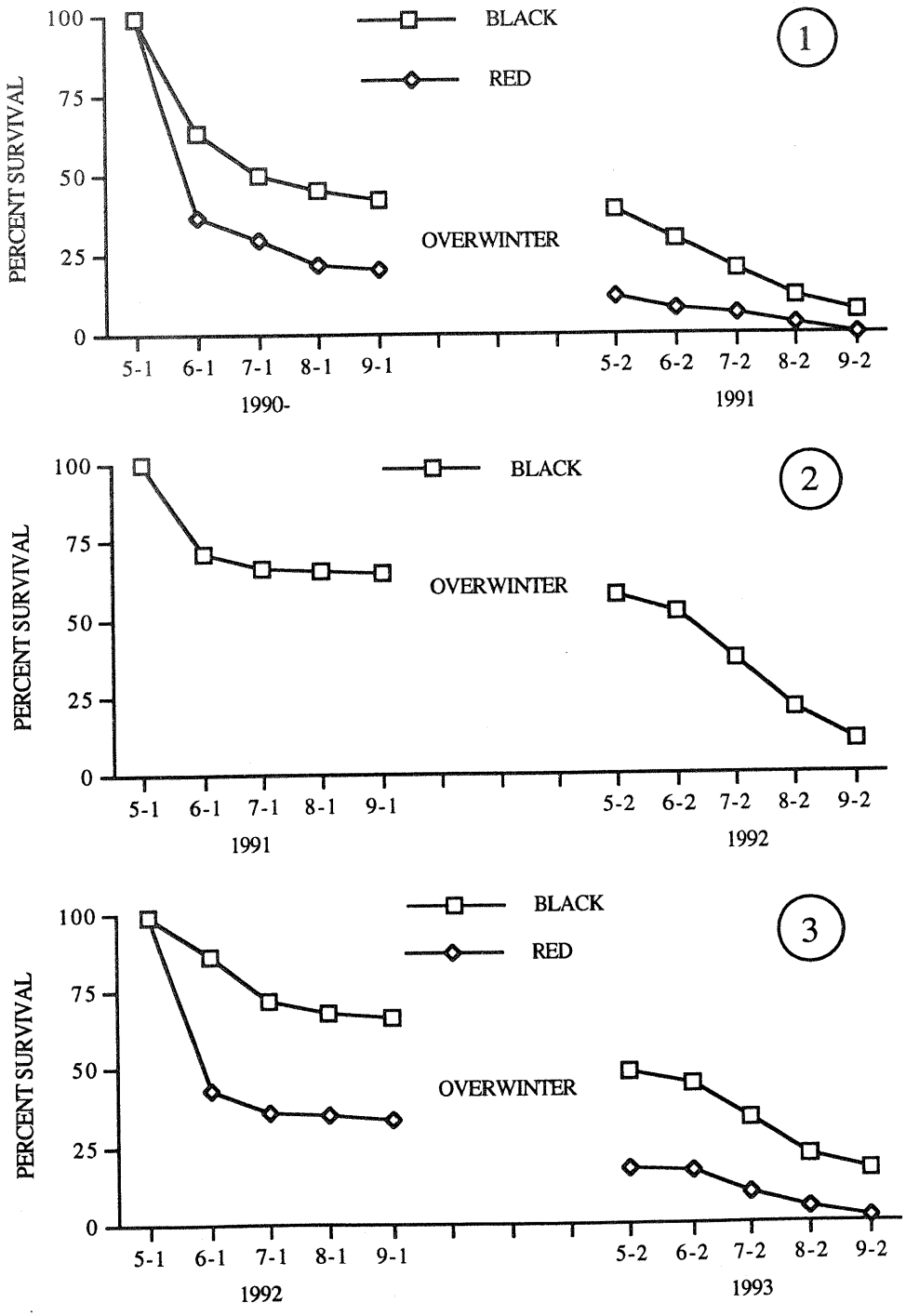
*No pistillate flowers in 1991 and few to none in 1994.

Pollen grains covered all surface tissues of the flowers, but it was only those that landed on the stigma surfaces that affected pollination (Figure 5). Heslop-Harrison and Heslop-Harrison (1985) have reviewed pollen-stigma interactions that specifically promote the germination and penetration of the pollen tube into the stigmas. Germination of oak pollen has been shown to occur within 24 hours of landing on the stigma (Jovanovic and Tucovic 1975). Our weekly collections did not permit verification of that timing. Pollen tubes were so numerous in some styles that accurate counts were not possible.

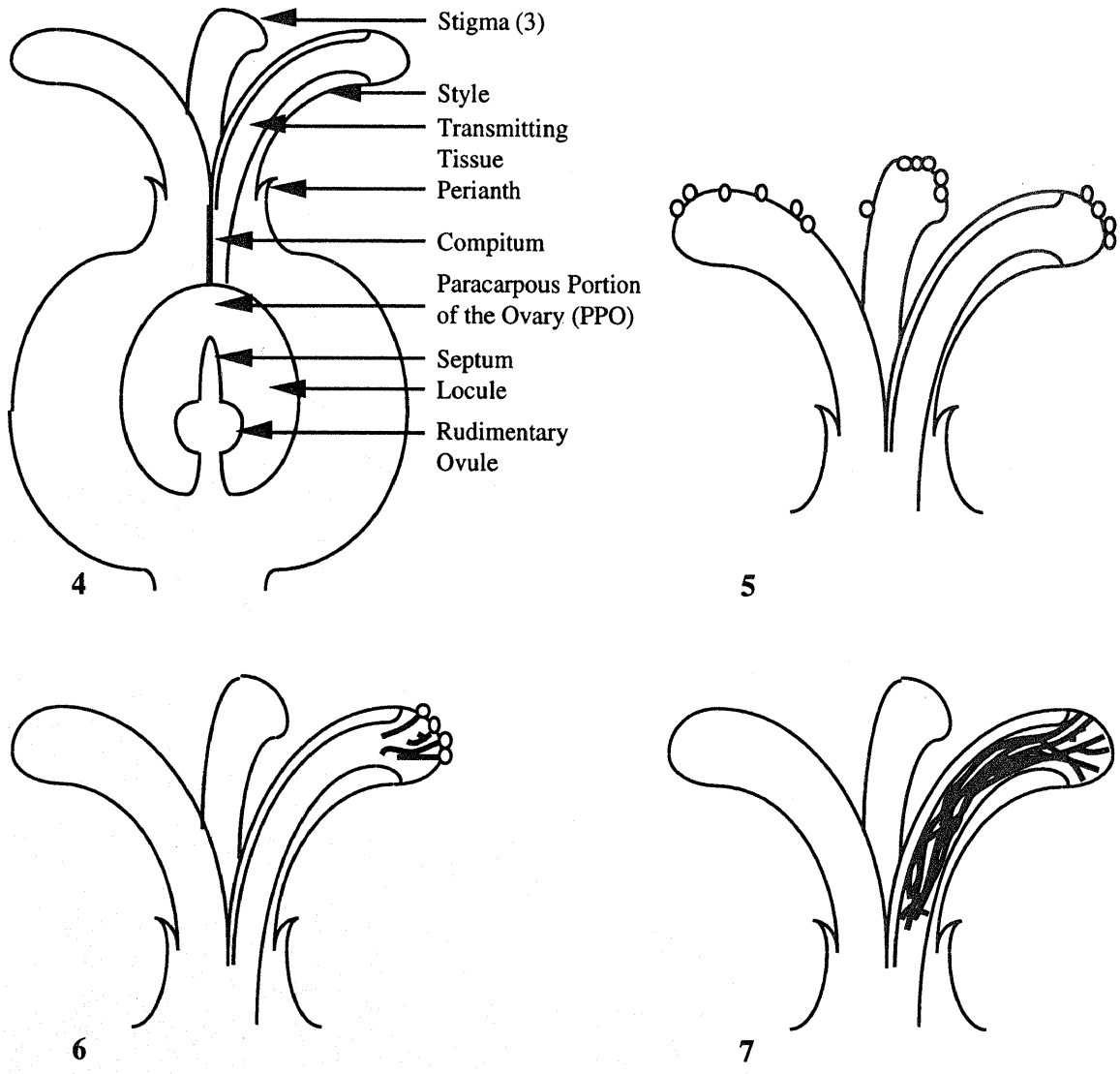
Both NRO and BO had the same pattern of pollen tube growth after the grains landed on the stigmas. The pollen tubes penetrated the stigmatic epidermis and synthesized a callose plug within the pollen tube, effectively isolating the contents of the tube from the now-empty pollen grain (Figure 6). One common feature of oak pollen tubes was the occurrence of several callose plugs within a single pollen tube. Callose plugs were generally amorphous masses. The pollen grains fell off the stigma within the week. Some pollen tubes produced numerous branches upon penetrating the stigma epidermis into the transmitting tissue of the style. Pollen tubes grew through the "central core" of the style in the parenchymatous, transmitting tissue toward the ovules (Figure 7).

The pollen tubes of both species penetrated downward, through the transmitting tissue until they reached the level of the perianth, just above the juncture of the three stigma/styles, on or about May 15. Thus, both species ceased penetrating on the same date, even though NRO began pollen tube growth at least one week before BO. No further pollen tube growth occurred during the first growing season. The ovary tissues of NRO and BO pistillate flowers are not well-developed in the first growing season. The three locules are small and contain a few hairs that surround the rudimentary ovules. From mid-May until the following spring, there were no observable changes in the structure of the pistillate flower or the position of the pollen tubes.

During the second growing season, pollen tubes of NRO resumed elongation on or about the May 21 collections. By that date, the ovary tissues had greatly enlarged. The ovules became organized into a nucellus that was surrounded by inner and outer micropyles. The locules enlarged and the number of hairs in the locules increased. In the next specimen collection (about May 28), pollen tubes were seen in the compitum (Carr and Carr 1961), the narrow "cylinder" of empty space that separates the three styles within the flower (Figure 8). After leaving the basipetal end of the compitum, the pollen tubes entered the paracarpous portion of the ovary (PPO) (Carr and Carr 1961). The central axis of the ovary is formed by the fusion of three septae. Each septum gives rise to a pair of rudimentary ovules. The septae are incompletely developed toward the top of the locules, creating a free space (the PPO) that connects each locule to the other two. Thus, as pollen tubes enter the compitum and PPO, they can fertilize any of the six ovules. Pollen tubes were randomly oriented and interwoven in the PPO.



Figures 1-3. Survival of pistillate flowers in the first growing season and developing acorns in the second growing season. Fig. 1. 1990-1991 growing seasons. Fig. 2. 1991-1992 growing season. There were no flowers on the red oak in 1991. Fig. 3. 1992-1993 growing season. For purposes of plotting data, we have used an estimated value for the first day of each month, not the weekly data.



Figures 4-7. Line drawings of the pistillate flower and pollen tube growth. Fig. 4. A median-longitudinal section of an oak pistillate flower with pertinent parts labelled. Fig. 5. Pollen grains on the three stigmas. Fig. 6. Pollen tubes grow through the stigma epidermis and produce a callose plug. Branches may form on the tubes. Fig. 7. Pollen tubes cease growth in mid-May at the level of the perianth.

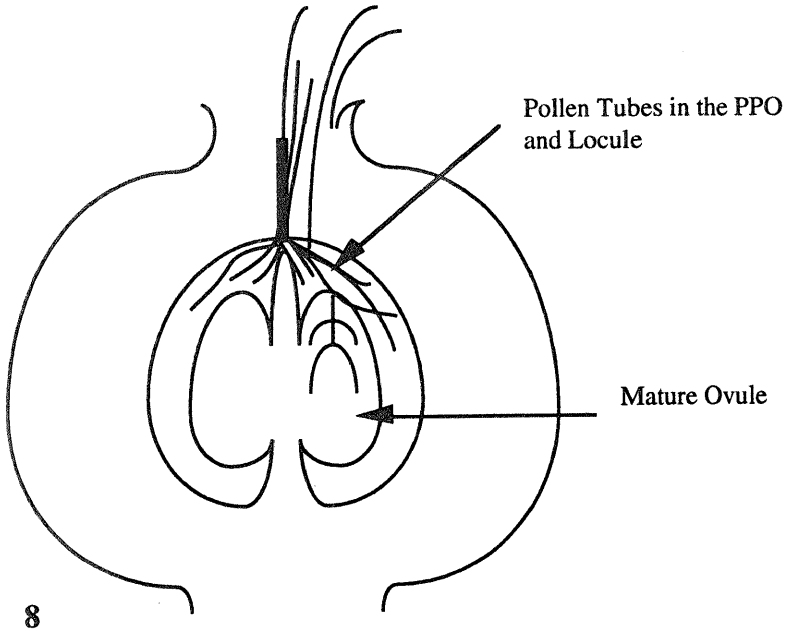


Figure 8. When pollen tubes resume growth, they leave the transmitting tissue and enter the compitum and paracarpous portion of the ovary (PPO), where they can randomly enter any of the three locules and fertilize any of the six ovules and their eggs. See Fig. 4 for additional labelling of flower parts.

Pollen tubes of NRO resumed growth in the May 21 collections and were observed in the locules, below the level of the PPO, in the June 4 and June 11 collections, as the megaspore mother cells were undergoing meiosis. The free nuclear endosperm stage was observed on June 18, indicating that fertilization had occurred after June 11. The BO pollen tubes were first seen resuming growth as they entered the compitum on June 4, two weeks after NRO. However, a free nuclear endosperm was not seen until July 2, indicating that fertilization occurred about two weeks after it did in NRO.

DISCUSSION

Our observations suggest several possible mechanisms for the control of pollen tube growth. Since both species ceased elongation on May 15, an external or environmental factor may be involved. This relationship has been demonstrated in a number of annual plants and grasses (Lyndon 1992). Pollen of *Q. pubescens* W. requires a higher temperature for germination (38° C) than pollen of*Q. robur* (20° C). Low and erratic fertility of *Q. pubescens* in cold habitats was attributed to the prevention of pollen germination (Jicinska and Koncalava 1978). However, the control over pollen tube elongation may be sporophytic, as manifested physiologically through the incomplete development of the ovules. For instance, the pollen tubes may stop penetrating the transmitting tissue because the rudimentary ovules may not be providing the correct signal, perhaps a hormonal gradient, for the pollen tubes to continue growing. This may be the type of feedback mechanism between pollen tubes and ovules that Owens (1992) cites. In addition, pollen tube growth may have stopped on May 15 because some portion of the ovary was producing an inhibitory substance that prevented the pollen tubes from elongating past the stylar juncture, and that this product was removed or neutralized with the approach of megasporogenesis. It may only be when the ovules reach a critical or threshhold stage of development, for instance in the second growing

season, that the proper stimulus for continued pollen tube growth is produced. This is especially evident in these two red oak group species that have only rudimentary ovules in the first growing season. It was observed in this study that the pollen tubes entered the compitum and PPO just as the megaspore mother mother cells were undergoing meiosis and not before. Thus, the putative "signal" for pollen tube growth resumption could have been produced shortly before meiosis was visible.

We have found no evidence of late spring frosts at our study site. Sharp (1958) concluded that low temperatures in the spring did not affect flowering unless there was a freeze sufficient to damage shoots and leaves. Goodrum and others (1971) also concluded that the influence of low temperatures on flowering, setting of fruit and subsequent acorn yield was inconclusive. However, low air temperatures were associated with a delay in the development, but not the number, of pistillate inflorescences of *Q. robur* near Moscow (Minina 1954).

The role of various pollen sources must also be considered as factors in the success of an acorn crop. For instance, NRO is the first oak species to flower at our relatively dry ridgetop site. The fast abortion rate of the pistillate flowers in May (Fig. 1-3) could reflect a lack of available outcrossed NRO pollen or a high degree of self pollination. Oaks are relatively self incompatible (Cottam, Tucker, and Santamour 1982). Since there are few NRO trees in our stand, the chance for cross pollination is reduced. But, there is an increased opportunity for a greater proportion of self pollinated flowers. Schwarzmann and Gerhold (1991) reported that northern red oak is a highly outcrossed species with a low level of self fertilization. Their sudy was done with acorns, the product of successful fertilizations. Our results, to a large extent, deal with flowers that have a high likelihood of aborting. Thus, a comparison may not be completely valid. The black oaks in our study have a slower rate of flower abortion during the first 6-8 weeks, compared to NRO. There are many more trees in the stand, allowing a greater likelihood of cross pollination. Any selfing would occur in less proportion and lead to a higher survival rate of those flowers.

One speculated role for callose plugs is as an incompatibility reaction, either from interspecific pollen or from self pollination (Dumas and Knox 1983). Thus, the callose plugs in these oak pollen tubes could indicate that many of the pollen tubes are from selfing. However, we have occasionally observed callose plugs in pollen tubes that had reached the locules and were apparently ready to fertilize the ovules in the second growing season. We expect that a self-pollinated flower would have aborted early in the first growing season. An alternate hypothesis is that callose plugs are a regulator of turgor pressure in the pollen tube (Dumas and Knox 1983). Callose plugs are formed sequentially as the tubes grow, perhaps allowing turgor pressure to be maintained in the cytoplasm at the tip of the pollen tube and facilitating penetration. Controlled pollination experiments are planned to examine the relationship between various types of crosses and the occurrence of callose plugs in oaks.

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