WALNUT FLOWER BIOLOGY STUDIES -- 1983
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ABSTRACT

Pollen from several cultivars was stored at -20°C (-4°F, freezer storage) and -196°C (-321°F, liquid nitrogen storage). Relative humidity of the freezer storage environment was regulated by maintaining the pollen in closed vessels over saturated solutions selected to provide a range of relative humidities from 8% to 84%. Results indicated that survival in storage varied among the varieties but that for all varieties a relative humidity of approximately 30% provided the optimum storage environment; survival after storage where RH was not maintained at or near 30% was uniformly low for all varieties. Similar variation was noted among varieties held at -196°C and, those varieties that responded well under one storage regime did not necessarily respond well in the other. 'Chandler' pollen, for example, showed the lowest percent germination (6.2%) after -20°C, 30% RH storage but was among the best following liquid nitrogen storage.

Pistillate flower development was followed in several cultivars not previously examined with particular attention paid to protogynous varieties in order to elucidate a developmental basis for dichogamy in walnut. Results indicated that development in all of the protogynous varieties examined had proceeded at least as far as the initiation of sepals by the time of the onset of dormancy in the fall. In some cases development had proceeded beyond that stage. By contrast, sepal initiation had not begun in any of the floral primordia examined from protandrous varieties. Thus, it appears that dichogamy in walnut is a function of the degree of development of the pistillate flowers that must be completed in the weeks prior to bloom in the spring.

OBJECTIVES

Several aspects of walnut flower biology were under continuing investigation during 1983:

Pollen viability, storage and growth in vitro. Work was continued on development of a system for in vitro pollen germination and pollen tube growth, development of tests for pollen viability, and evaluation of pollen storage methods to provide a tool for determining how long pollen remains viable under field and storage conditions and for further refinement of the ELISA testing for blackline-virus infection.

Pollen development and pollen tube growth in vivo. An ongoing investigation of pollen development is intended to determine the basic developmental information that is lacking in the literature regarding the timing of and structural events associated with pollen development. Studies of pollen tube growth in pistillate flower tissues are designed to determine the duration and path of pollen tube growth in the female flower and the possible pathways for pollen transmission of blackline virus.
Pistillate flower development. Two aspects of pistillate flower development are under investigation: floral organogenesis during the months prior to bloom and details of ovule development as it proceeds during the weeks immediately prior to bloom. One objective is to extend previous studies of the timing of key events in flower development to additional varieties, particularly recent releases and protogynous varieties, and to elucidate the developmental basis for protogyny. Another is to understand, in detail, critical cellular events associated with determinants of flower quality and fruit set.

Pistillate flower receptivity and ovule quality. Histological and cytochemical parameters affecting pistillate flower receptivity and ovule strength are being examined. Our objectives are to describe events associated with fertilization and normal set and how these may differ in ovules which undergo post-pollination abortion.

PROCEDURES

Pollen storage and pollen tube growth in vitro. Pollen was collected from representative varieties several days prior to anther dehiscence and at the time the first staminate flowers of the catkin had begun to shed pollen. Pollen was tested for initial germination percentage on artificial medium at the time of collection.

Pollen was stored at -20°C over saturated salt solutions selected to provide a range of relative humidity (RH) environments for storage. Salts used and approximate RH at -20°C for each were: LiCl·2H₂O (18%), MeCOOK·1.5H₂O (28%), MgCl₂·6H₂O (33%), CaBr₂·6H₂O (30%), NaI·2H₂O (48%), NaBr·2H₂O (70%), and NH₄Cl (84%). NaOH·H₂O was also used to provide a low RH (ca. 8%) as well. Samples were removed and assayed for viability after 3 and 8 months. Tests of liquid nitrogen storage of pollen were also done. This year a long-term liquid nitrogen facility was used. Pollen was cooled to liquid nitrogen temperatures (-196°C) at a rate of 1°C per minute and held at that temperature for 8 months. In each case a final sample will be assayed for survival in storage after one year.

Pollen development. Catkins were collected beginning 4 to 6 weeks before staminate bloom and at intervals thereafter. These samples were fixed and embedded in plastic resins for high resolution light and electron microscopy. Material was sectioned and stained for specific chemical constituents (protein, total carbohydrates, specific components of the pollen cell wall, storage lipids) as well as for general structural features of pollen development.

Pistillate flower development. Shoots of selected protandrous varieties ('Ashley', 'Chandler', 'Howard') and the five protogynous varieties available at Davis ('Chico', 'Amigo', 'Sharkey', 'XXX Mayette', 'Meylan') were collected at two-week intervals during the period of pistillate flower development. Terminal and lateral (where appropriate) buds were dissected from the shoots, prepared for light and electron microscopy, and examined for development of the floral organs. Samples of pistillate flowers were collected daily for the two weeks prior to bloom and ovules and stigmatic tissue were prepared for light and electron microscopy.
Pollen tube growth, fertilization and fruit set. Pistillate flowers of 'Chico' were pollinated with 'Serr' pollen and collected at two day intervals for the next two weeks. Flowers were fixed and embedded in paraffin for microscopic analysis of pollen tube path and details of ovule development and fertilization.

RESULTS AND CONCLUSIONS

Pollen storage and pollen tube growth in vitro. Controlling the relative humidity of the storage environment greatly enhanced the storage life of pollen from all varieties. Optimum RH for storage was in the range of 30% which provided significant improvement over freezing without regulation of RH. While varieties with high retention of viability under uncontrolled storage did improve greatly, the effect was most dramatic in varieties that failed to germinate at all after a short time in storage. 'Chandler' and 'Tehama' were two such varieties going from 0 to 6.2% in the case of 'Chandler' and 23.6% in the case of 'Tehama'. We tentatively conclude (pending one-year testing) that storage at -20°C (= -4°F) should be possible if the relative humidity of the storage environment is maintained at approximately 30% by using a saturated solution of MeCOOK·1.5H2O (potassium acetate), CaBr2·6H2O or MgCl2·6H2O. Even under these conditions viability in storage will vary among cultivars and, in the case of some cultivars (eg. 'Chandler'), will be insufficient for effective use in a field situation. However, where the objective of pollen storage is to have material for hand pollinations, as in the walnut breeding program, it seems likely that the method described should be useful. We further conclude that under storage without controlled RH the likelihood of having a high percentage of viable pollen is low.

Our results for liquid nitrogen storage also indicated variability among the varieties tested. Interestingly, 'Chandler' pollen, which had the poorest response to storage at -20°C, was extremely responsive to storage at -196°C.

Pistillate flower development. This year's investigation has extended previous work to several new varieties. As noted in previous studies, the time of initiation of a floral meristem varies among varieties and there is no clear correlation with earliness of bloom or protogyny. Protogyny does appear to have its basis in developmental events occurring during the season prior to bloom. In the case of each of the protogynous varieties significantly greater development had occurred prior to the onset of dormancy in the fall. Typically, development in the protogynous varieties had proceeded to the stage of sepal initiation, and in many cases, subsequent stages were attained by that time. By contrast, in none of the protandrous varieties examined this year, or in previous studies, had floral primordia developed beyond the initiation of the involucral ring and the emergence of bracts and bracteoles. Thus, when development resumes in the spring the pistillate flowers of protogynous varieties have completed all early organogenesis; all that remains is the completion of pistil formation. Protandrous varieties must initiate and complete the development of the calyx and early stages of pistil before they are at the same stage protogynous varieties had attained by mid-November. The time required to complete this development would account for the delay of pistillate bloom to some time after pollen shedding.
A second factor examined was the extent of differences between terminally and laterally borne flowers. Little difference was noted in the timing of development between the terminal and the first few lateral buds. However, proceeding down the shoot to more basal lateral buds in strong lateral bearers such as 'Chandler' and 'Howard' we noted that development is delayed relative to the terminal and more apical lateral buds. The effect of this phenomenon is that in these lateral bearing varieties there is substantially more variation in stages of pistillate flower development at any given time during the summer growing season.

Pollen tube growth and fertilization. Since the work of German plant morphologists at the turn of the century, the pollen tube growth path in J. regia has been considered to be chalazogamous. That is, rather than pollen tubes entering the ovule through the micropyle as is typical for nearly all flowering plants, the pollen tubes are supposed to grow around to the basal (chalazal) end of the ovule, through the vascular tissue supplying the ovule and enter the ovule through the side opposite the micropyle. This view of pollen tube growth is so well entrenched in the literature on floral biology that J. regia is typically listed in textbooks as the prime example of chalazogamous fertilization. We, as well as others interested in the transmission of blackline virus in walnut, have assumed that this is the case and have designed our analyses based on this assumption. Over the past two years we have come to believe that this is not the case after all. We believe that we have identified the artifact that led earlier workers to suppose that the species is chalazogamous and have reasonably good evidence that the usual mode of fertilization, entry of the pollen tube through the micropyle, is the case in walnut.