Pollen Germination of Rhizoma Peanut cv. Florigraze[1]

W. L. Niles and K. H. Quesenberry[2*]

ABSTRACT

Assessing pollen germination is fundamental to investigating infertility in plants. A potential cause of poor seed production in Florigraze (*Arachis glabrata* Benth.), rhizomatous peanut, was investigated by incubating pollen on *in vitro* germination media. The optimum sucrose and boron concentrations for pollen germination was delineated in a series of factorial experiments. Pollen germinability was assessed four times during the growing season. Flowers were collected at 2 h intervals spanning 30 h of development from bud to wilted flower. The optimum sucrose concentration was 100 g kg^{-1} but there were no differences in germination for B concentrations between 50 and 1,000 mg kg^{-1}. Up to 78% pollen germination was obtained in a solution consisting of 100 g kg^{-1} sucrose, 100 mg kg^{-1} H_3BO_3, 250 mg kg^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg kg^{-1} $MgSO_4 \cdot 7H_2O$ and 100 mg kg^{-1} KNO_3 in deionized water. Repeatable estimates of germinability were obtained in incubations of less than 30 min at 35 C. Florigraze pollen collected from developing buds as early as 2200 h the night before anthesis germinated *in vitro*. Peak germination extended from 2400 h to 1200 h the morning of anthesis. Under cool, dry conditions, the pollen collected 2 d after anthesis remained germinable. These results suggested poor pollen germinability was not the basis of low seed production in rhizomatous peanut. Pollen with high *in vitro* germination can dependably be collected from Florigraze flowers throughout the growing season during the first 6 h following anthesis, usually between sunrise to noon.

Key Words: Florigraze, rhizoma peanut, pollen, perennial peanut, *in vitro* germination

Florigraze, rhizoma peanut, (*Arachis glabrata* Benth.) is a warm season perennial forage legume valued as a productive, high-quality grazing crop which thrives in the well-drained sandy soils of the humid subtropics (7). Although it flowers profusely, Florigraze rarely produces seed. This study was conducted to determine if the lack of pollen germination contributed to this low seed set.

Pollination in cultivated peanut (*Arachis hypogea* L.) occurs before anthesis (8, 9). The anthers dehisce placing pollen on the stigma within the keel. Oaks (6) found this pollen to be less than 20% *in vitro* germinable. Faucette and Emery (2) measured up to 50% *in vitro* germination but considered 10% satisfactory for seed production. Variability in peanut pollen *in vitro* germination from flower to flower and plant to plant has been reported (2, 10).

Poor pollen germination should be ruled out when investigating sources of infertility. The ultimate test of pollen vigor is its fertilizing capacity as measured by seed set (10). Pollen germination *in vitro* is a direct and easy method of assessing pollen viability (4), but it may be sensitive to the media used. For many plant species, *in vitro* germination media should contain a carbohydrate supply, B, Ca, Mg, K, and be adjusted for osmolarity and pH (10). Faucette and Emery (2) reported that a 100 g kg^{-1} sucrose solution maximized peanut pollen tube growth while 150 g kg^{-1} induced maximum pollen germination.

Oaks (6) investigated the effect of temperature on peanut pollen and pollen tube elongation *in vitro*. Germination only occurred between 18.3 and 35 C while peak tube growth took place at 32.2 C, declining as temperature further increased. He concluded that a large range in diurnal temperatures may be detrimental to pollen germination *in vivo* particularly when the time interval between temperature extremes is very short.

Faucette and Emery (2) observed peak peanut pollen *in vitro* germinability from collections made between 0300 h and 0800 h. Similarly, Oaks (6) observed optimum *in vitro* germination in pollen collected at 0500 h. They recommended that large numbers of flowers be collected over extended periods of time to properly assess the range of peanut pollen germinability.

The general objective of this investigation was to determine if poor seed production in Florigraze could be due to the absence of pollen germination. Specific objectives were (a) to develop an optimum pollen germination solution and (b) to characterize the diurnal and seasonal changes in pollen *in vitro* germinability.

Materials and Methods

The same collecting and germinating procedures were used in all following studies except where indicated. Pollen was sampled from a 2-yr-old Florigraze stand located on the Agronomy Farm on the University of Florida campus in Gainesville, FL, during 1987 and 1988. Ten or more flowers were collected per treatment in a sampling transect of the field. Pollen from all flowers was extracted, mixed together, divided into subsamples, and placed on germinating media within 15 min of collection.

[1]Contribution from the Florida Agric. Exp. Stn., Journal Series No. R-02174. The research reported herein was supported in part by USDA special grant 86-CRSR-2-2846 administered by the Caribbean Basin Advisory Group.

[2]Dept. of Agronomy, Univ. of Florida, Gainesville, FL 32611.

*Corresponding author.

Pollen germinability was determined by placing three to five drops of germinating solution on a plastic petri dish and inoculating the drops with a sample of pollen. The germination solutions were prepared by adding sucrose and H_3BO_3 in varying concentrations to a foundation solution consisting of 250 mg kg^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg kg^{-1} $MgSO_4 \cdot 7H_2O$ and 100 mg kg^{-1} KNO_3 in deionized water. The inoculated drops were incubated 30 min at 35 C by floating the dishes on the surface of a water bath in a tightly closed 30 L styrofoam chest. The closed chest maintained a high humidity which was essential for good germination.

Immediately after incubation, pollen *in vitro* germinability was determined by viewing several areas of each drop under a microscope at 40X or 100X magnification and assigning a germination score to the sample. A pollen grain was considered to have germinated when a tube emerged to a length exceeding the grain's diameter. A qualitative scoring method was adopted after discovering that the time required to count individual grains introduced substantial time-associated errors of media evaporation and pollen deterioration. Little and Hills (5) recommend using the arcsine transformation on counts expressed as percentages when the range exceeds 40%. A pretransformed score ranging from 0 to 5 corresponding to 0, 10, 35, 65, 90, and 100% pollen germination was used in all of the germination studies. For presentation of results, the means and standard error were converted back to their corresponding percentages.

Incubation Temperature and Time

While developing a protocol for *in vitro* germination, it was observed that the percentage germination of a given pollen sample increased with incubation time and leveled off at peak germination. The time required to reach this limit was inversely proportional to the temperature of the incubation media. The shortest possible incubation periods were desired to expedite the germination studies. The temperature of incubation therefore had to be as high as possible. However, it was not known if the higher temperatures would result in a decrease in peak germination percentage. Florigraze pollen was killed by incubations exceeding 40 C. An experiment was conducted to test the hypothesis that pollen samples incubated at temperatures ranging from 20 to 35 C would all attain the same peak germination and that this peak would be reached earlier at the higher temperatures. The experiment was repeated for pollen at three stages of development (collection times) to determine if pollen maturity interacted with incubation temperature and duration.

A 4 x 3 factorial of incubation temperatures (20, 25, 30, and 35 C) and incubation periods (30, 60, and 120 min) with three replications was used. The experiment was repeated for two collection times (2400 and 0400 h) the morning of anthesis. Each set of temperature-time combinations was evaluated immediately after collection. A single germination solution was used consisting of 80 g kg^{-1} sucrose and 120 mg kg^{-1} B in the foundation solution. Three drops of media were placed on each of 12 petri dishes required for the incubation temperature x period treatments. Four baths were used to allow simultaneous incubation of the pollen samples at the four temperatures. At the end of each incubation period, one dish was collected from each bath and the drops randomly scored for germination.

Pollen Germination Solutions

The optimum concentration of sucrose and B was investigated in a 5 x 5 factorial consisting of five concentrations of sucrose (s/b 60, 90, 120, 150, 180 g kg^{-1}) and B (60, 100, 140, 180, and 220 mg kg^{-1}) in the foundation solution. A response curve to sucrose was obtained, but not for B. A peak response to B was sought in a second study consisting of sucrose at 100 g kg^{-1} and B at 0, 60, 120, 250, 500, and 1000 mg kg^{-1} in the foundation solution.

Diurnal and Seasonal Variations in Pollen Germinability

Four 24 h studies were conducted to sample pollen development over a 30 h period and at three times during the summer growing season. Flowers were collected from 14 h before to 16 h after anthesis, which occurred shortly after sunrise. The studies were conducted 5 Aug 1987, 2 June 1988, 13 July 1988, and 10 Aug 1988 enabling the effects of a variety of prevailing meteorological, edaphic, and physiological conditions on pollen germination to be observed.

Twenty flowers, including 10 buds and 10 in anthesis, were collected at 2 h intervals for 24 h beginning at 1200 h. Pollen germination was determined during the 2 h interval between collections. Ambient temperature was measured at the canopy level during each collection.

Results and Discussion

Incubation Temperature and Time

Incubations as brief as 30 min at 35 C were as effective ($p < 0.05$) for inducing maximum *in vitro* germination of Florigraze pollen as other incubation regimes regardless of temperature (Table 1). Significant differences were not obtained for the 1100 h collections due to poor germinability of the pollen. The data suggest that even shorter incubation periods are feasible with no decline in peak germination. Subsequently, incubations as brief as 15 min (data not shown) were found to give reliable peak germination scores. In preliminary tests, 40 C was lethal to Florigraze pollen and 38 C produced a decline in *in vitro* germination. Oaks (6) found no *in vitro* germination in common peanut pollen above 35 C.

This study presents evidence to confirm the hypothesis that pollen samples incubated at temperatures ranging from 20 to 35 C would all attain similar peak germination percentages and that this peak would be reached earlier at the higher temperatures.

Pollen Germination Solutions

Collections were made between 0800 and 1000 h for both germination solution studies. The pollen response to sucrose concentration was curvilinear and significant ($P<0.05$) (Fig. 1). The first derivative of the response function ($Y = 13.27 + 11.24X - .53X^2$) placed peak germination at 106 g kg^{-1} sucrose concentration. This concurs with Brewbaker and Kwack (1) who found 100 g kg^{-1} optimal for many pollen species. Faucette and Emery (2) obtained maximum peanut pollen *in vitro* germination at 150 g kg^{-1} sucrose. Response to B concentration was not significant.

A peak pollen germination response to B was sought using sucrose at 100 g kg^{-1} and B in a broad range of concentrations (0, 60, 120, 250, 500, and 1000 mg kg^{-1}). No differences (Fig. 2) were detected between any of the solutions containing B. However, the control which lacked B had a lower germination percentage ($P<0.05$). Evidently, while a concentration of B greater than 0 but less than 50 mg kg^{-1} is sufficient for *in vitro* germination, a narrow optimum inducing maximum germination does not exist. In a similar fashion, Brewbaker and Kwack (1) obtained 'excellent' pollen germination in a diverse array of plant species using Ca concentrations between 50 and 5000 mg kg^{-1}.

In summary, differences in Florigraze pollen *in vitro* germination were detected only in response to relatively large changes in sucrose concentration and to the complete absence of B. After no peak response to B was obtained, no

Table 1. Florigraze pollen *in vitro* germination response to temperature by incubation period combinations at three pollen collection times.

Incubation		Collection time*	
Temp	Time	2400 h	0400 h
C	Min	-- Percentage germination --	
35	60	70.7 a	72.4 a
35	30	66.6 a	66.6 ab
20	120	62.6 a	62.6 ab
25	120	60.1 ab	62.6 ab
35	120	60.1 ab	62.6 ab
25	60	57.5 ab	58.3 ab
30	120	57.5 ab	58.3 ab
30	30	49.7 ab	49.7 b
30	60	36.8 b	49.7 b
20	60	4.7 c	11.5 c
25	30	2.9 c	6.7 c
20	30	0.0 c	0.0 c

*Means in the same column followed by same letter are not different ($P < 0.05$).

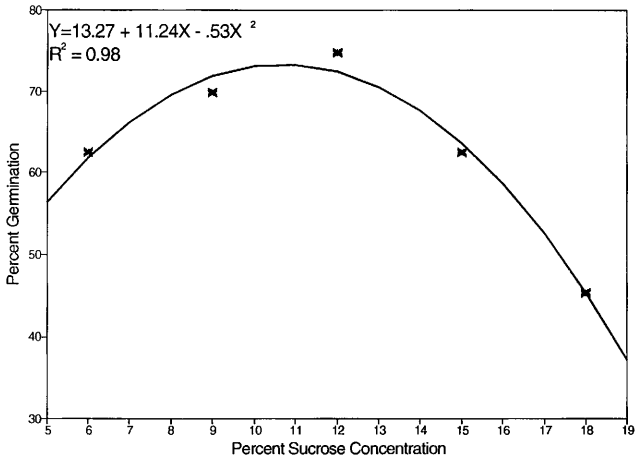

Fig. 1. *In vitro* germination response of Florigraze pollen to media sucrose concentration. Means of five replications of a 5 x 5 factorial of sucrose and B. Response to B was not significant.

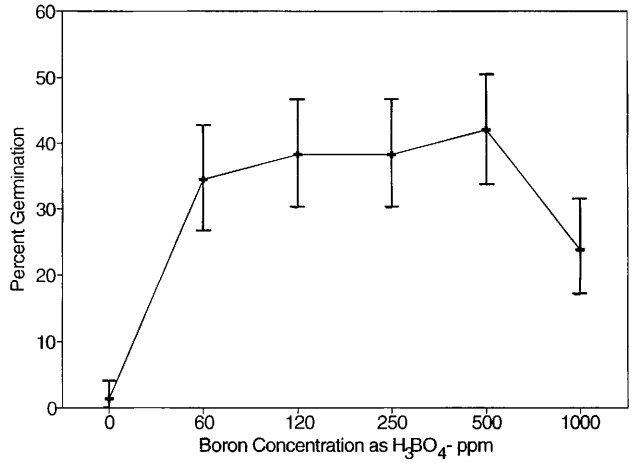

Fig. 2. Pollen *in vitro* germination response to increasing concentrations of B. Bars indicate LSD at P < 0.05.

additional concentration studies were conducted for other media constituents.

Diurnal and Seasonal Variations in Pollen Germinability

Florigraze pollen in developing flowers matured sufficiently to germinate *in vitro* between 2200 and 2400 h the day preceding anthesis. Anther dehiscence took place near dawn immediately preceding anthesis. The period of peak germinability generally lasted from 2400 to 1200 h (Fig 3). Pollen germination tended to decline as ambient temperatures rose. Cooling rains in three of the studies appeared to briefly arrest the decline of *in vitro* germinability. However, the biological decomposition of the pollen was enhanced in the hours following the rain (visual observation). The results presented in Fig. 3 from 2 June 1988 are an example of germination where no afternoon rainfall occurred. On this date, Florigraze pollen remained germinable 12 h longer than in any of the other studies (data not shown). This may have been due to the lack of rain, lower ambient temperatures, and lower relative humidity prevailing during the study.

Summary and Conclusions

Up to 78% of Florigraze pollen samples germinated *in vitro* in a solution consisting of 100 g kg^{-1} sucrose, 100 mg s/b kg^{-1} H_3BO_3, 250 mg kg^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg kg^{-1} $MgSO_4 \cdot 7H_2O$ and 100 mg kg^{-1} KNO_3 in deionized water. A reliable estimate of germinability could be obtained in 30 min or less when incubated in this media at 35 C.

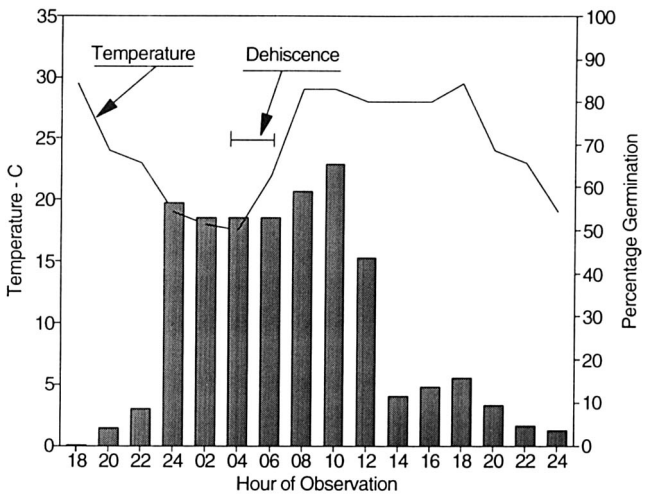

Fig. 3. Florigraze pollen *in vitro* germination behavior observed 1-2 June 1988 covering 30 h of development from immature bud to wilted flower. Temperature is ambient measured at canopy level.

The pollen developing in buds matured sufficiently to germinate *in vitro* by 2200 h the night before anthesis. It remained germinable for most of the morning of anthesis, declining as temperatures rose. Under cool dry conditions pollen in the field may remain germinable for more than 24 h after anthesis. For breeding purposes, pollen displaying good *in vitro* germination can be dependably collected from Florigraze flowers between sunrise and noon. Poor seed production in Florigraze is probably not due to the lack of pollen germination.

Literature Cited

1. Brewbaker, J. L. and B. H. Kwack. 1963. Essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. 50:747-858.
2. Faucette, D. F. and D. A. Emery. 1974. Preliminary evaluations of techniques for collecting and culturing peanut (*Arachis hypogaea* L.) pollen. Peanut Sci. 1:14-17.
3. Hammons, R. O., and D. B. Leuck. 1966. Natural cross-pollination of the peanut, *Arachis hypogaea* L., in the presence of bees and thrips. Agron. J. 58:396.
4. Heslop-Harrison, J., Y. Heslop-Harrison, and K. R. Shivanna. 1984. Evaluation of pollen quality and a further appraisal of the florochromatic (FCR) test procedure. Theor. Appl. Genet. 67:367-375.
5. Little, T. M. and F. J. Hills. 1978. Agricultural Experimentation Design and Analysis. John Wiley and Sons, New York, NY.
6. Oaks, A. J. 1958. Pollen behavior in the peanut. Agron. J. 50:387-389.
7. Prine, G. M., L. S. Dunavin, J. E. Moore, and R. D. Roush. 1981. Florigraze rhizoma peanut a perennial forage legume. University Florida, Agric. Exp. Stn. Circ. S-275.
8. Smith, B. W. 1950. *Arachis hypogaea*. Aerial flower and subterranean fruit. Am. J. Bot. 37:802-815.
9. Smith, B. W. 1954. *Arachis hypogaea*. Reproductive Efficiency. Am. J. Bot. 41:607-616.
10. Stanley, R. G. and H. F. Linskens. 1974. Pollen Biology, Biochemistry, Management. Springer-Verlag, New York.
11. Tappan, W. B. and D. W., Gorbet. 1979. Relationship of seasonal thrips populations to economics of control on Florunner peanuts in Florida. J. Econ. Entomol. 72:772-776.

Accepted August 15, 1992