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Adaptive Responses of Walnut Pollen Germination to Temperature during Pollen Development

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Abstract. **Experiments were conducted to determine if differential responses of walnut pollen germination to temperature, previously observed to occur among genotypes, were genetically fixed or expressions of phenotypic plasticity representing adaptive responses to temperatures experienced during pollen development. Individual branches of a single walnut *(Juglans regia* L. cv. Serr) tree were warmed above ambient conditions during the final stages of pollen differentiation by directing a stream of moist, heated air into polyethylene enclosures, each containing an individual branch unit. Pollen was collected at staminate anthesis and incubated in germination medium on a temperature gradient apparatus. Model curves fitted to the in vitro pollen germination data were used to determine optimum germination temperatures. We found adaptive responses of pollen germination to temperatures experienced during pollen development. The optimum temperature for in vitro germination for pollen from branches maintained under ambient conditions was lower than that of pollen from branches with elevated temperature, and optimum germination temperature increased as a log function of integrated daily temperature (degree-days) experienced during pollen development.**

Temperature ranges and optima for pollen germination and pollen tube growth vary widely among species. In a study of two walnut species *(Juglans regia* and *J. nigra)* Luza et al. (1987) found that a variable response to temperature occurs within these species and that optimum temperatures for in vitro pollen germination correlated with the relative staminate bloom date for a given clone or cultivar. Pollen from early blooming clones had lower temperature optima for in vitro germination than did pollen from clones that bloom later in the season. No such differences, either between or within the two *Juglans* species, were evident for pollen tube growth rates. A similar relationship was noted between *Prunus dulcis* (almond) and *P. persica* (peach), two closely related species, but no intraspecies variation in temperature optima for in vitro pollen germination was evident within these species irrespective of anthesis date (Weinbaum et al., 1984).

About 60% of the genes expressed in the sporophyte of flowering plants may be expressed in the male gametophyte (Mulcahy et al., 1979; Tanksley et al., 1981; Willing and Mascarenhas, 1984). However, environmental conditions experienced by the parental sporophyte, e.g., nutrient status (Young and Stanton, 1990), can influence competitive fitness of pollen. This relationship raises the question of whether the differences in temperature responses noted within the *Juglans* species are manifestations of genetically fixed responses by the male gametophytes to temperature conditions likely to be prevalent at the time of pollen dispersal, or if these differences are due to plasticity of the gametophytic phenotype in response to prevailing temperatures during pollen development. To address this question we warmed individual branch units above ambient to determine whether temperatures experienced during final pollen development affect subsequent responses to temperature during pollen germination.

Materials and Methods

A single 'Serr' walnut tree at the Univ. of Calif., Davis, campus was used for all experiments. Three branch units, each having numerous catkin buds, were selected to be heated above ambient temperature. A fourth branch was selected as an untreated control and maintained at ambient temperatures. Exposure of the first branch unit to warmed, humidified air began on 2 Feb. The capacity for bud growth on branches removed from the orchard to a warm environment indicated that the chilling requirement for this cultivar had been met by 2 Feb. (unpublished data). Warming of the other branch units began on 16 and 23 Feb. On 2 Feb., the staminate flowers contained within catkin buds had anthers with sporogenous tissue present but lacked fully differentiated microsporocytes. By 16 Feb., microsporocytes were present. On 23 Feb., catkin buds had begun to expand and the anthers contained microspore tetrads to early pollen grains. Stages of catkin bud differentiation were determined from hand sections of buds from untreated branches and follow Luza and Polito (1988).

A mechanical device (Fig. 1) was used to elevate temperature above ambient for each of the branch units from the starting date until the first anthers began to dehisce. This device contained a fan, which ran continuously, to move incoming air over a series of water-saturated sponges. This moist air flowed through three ducts, each of which terminated in a large vented plastic bag made of clear 0.25 mm (10 roil) polyethylene that enclosed the branch unit being treated. Each of the three air ducts contained a 300-W heating element regulated by a thermostat with a temperature sensor placed near the catkin buds in the plastic enclosure supplied by the duct. Individual branch units were not enclosed in the polyethylene until the time heating was begun for that branch. Separate thermostats for each air supply were adjusted to maintain minimum temperatures of ≈16 to 17C within the enclosure (Fig. 2). Temperatures were monitored by thermocouples placed within 2 cm of the catkin buds. Temperatures were recorded at 30-min. intervals with a Campbell model CR-21 datalogger (Campbell Scientific, Pullman, Wash.) and degree-days > 4C were calculated by integration (Zalom et al., 1983).

At the beginning of anther dehiscence, the plastic enclosures were removed and catkins were collected. Pollen was collected from catkins that completed development within each of the three heated branch units and from untreated catkins. Catkins were spread over paper and the anthers allowed to dehisce overnight. Released pollen was passed through a 100-µm mesh screen

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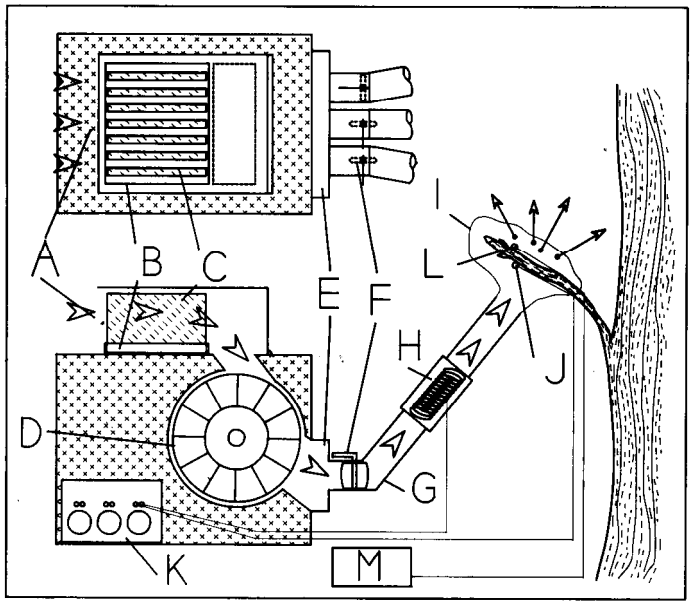

Fig. 1. Diagram of the device used to heat selected branch units durirrg walnut catkin and pollen development. Air flow (arrow heads) moves from the air intake **(A)** over a pan of water **(B)** and around several water-saturated sponges **(C).** Air movement is driven by a squirrel-cage fan **(D)** through a manifold **(E)** with three outlets regulated by err/off air-flow valves **(F)** into ducts **(G).** Each of the three ducts (only one shown here) passes the humidified air through a heating element **(H)** and into a vented polyethylene enclosure **(I)** containing the branch unit. A temperature sensor **(J)** connected to a thermostatic controller **(K),** which regulates the heat, is located within each enclosure in close proximity to the catkin buds. A thermocouple **(L)** within each enclosure is connected to a data logger **(M)** to record temperature. Separate thermostatic controllers, thermocouples, and temperature sensors are present in each of the three enclosures.

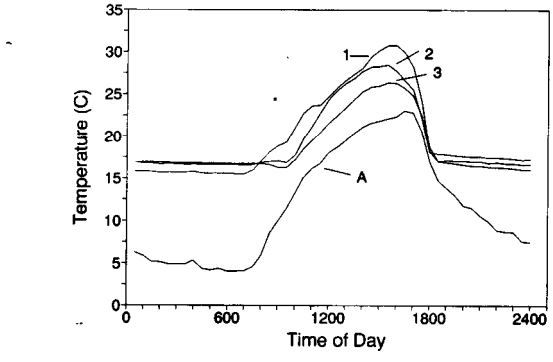

Fig. 2. Temperature readings from thermocouples in each of the three enclosures **(1,2,3)** and at ambient **(A)** conditions near an unheated catkin bud. These data are from 24 Feb. 1988, the first full day that all three heated enclosures were in position and operating.

to remove debris. Pollen from each sample was incubated for 24 h on solid medium in three petri plates at each of 10 temperatures. Incubation temperatures were maintained at 2 to 3C intervals from 14 to 38C using a temperature-gradient bar (Luza et al., 1987). After 24 h, germination percentages were determined for 100 pollen grains from each of five regions in each plate. Detailed procedures for pollen collection, pollen germination, control of temperature during pollen incubation, and data analysis are described in Luza et al. (1987). Normalized germination data for pollen from each treatment were fitted to a polynomial regression by the least squares method; quartic equations provided the best fit in all cases. Optimum germination temperatures were determined as the temperature at which the first derivative of each fitted polynomial equaled zero.

Results and Discussion

Experiments similar to those described here were conducted the previous year. At that time, however, air flow to the catkins was not humidified, and pollen from these treatments failed to germinate at any temperature. We assumed that this was a consequence of excessive drying during pollen development caused by the flow of dry, heated air. This assumption is supported by "the recovery of terminable pollen from all treatments when the air was humidified.

Ambient temperatures and temperatures within the three heated enclosures for a representative day (24 Feb.) are shown in Fig. 2. Note that night temperatures were maintained in the 16 to 17C range in all three enclosures. Day temperatures were more variable, ranging ≈4 to 8C above ambient. The heating ele-. ments were not activated during the day, because solar radiation was sufficient to warm the air within the polyethylene enclosures above ambient temperatures. Differences in daytime temperatures among the three enclosures probably were caused by positions of the enclosures in relation to the direction of solar radiation and partial shading of the enclosures.

Temperature data were accumulated from the time the first branch unit was placed in its enclosure and heating of that branch commenced. At ambient temperature, catkins required 38 days from that time to the dehiscence of the first anthers. Staminate buds that were heated beginning 2 Feb. had first anther dehiscence after only 23 days. Heat units were accumulated at the rate of 8.1 degree-days/day under ambient conditions and 15.5 degree-days/day in the heated enclosure. Anther dehiscence occurred 4 days later in catkins that were heated beginning 16 Feb. These received 286 degree-days to dehiscence, 14 days at the rate of 7.2 degree-days/day, and an additional 12 days at 15.4 degree-days/day. Those heated beginning 23 Feb., when catkin buds showed perceptible swelling, had the first anthers dehisce on day 29 after exposure to 22 days at 7.7 degree-days/day and an additional 7 days at 14.7 degree-days/day.

Accumulated heat units and times to several readily identifiable developmental stages for each of the treatments are tabulated in Table 1. The stages noted here are the first indication of catkin bud swelling, opening of the bud scales enclosing the mixed terminal bud (budbreak), emergence of leaves and expansion of leaflets from the terminal bud, expansion of catkins to reveal the individual staminate flowers, opening of the individual flowers, and dehiscence of the first anthers. We continued to record temperatures after the enclosures were removed and have noted times to two events that follow anther dehiscence in this protandrous cultivar: emergence of distillate flowers from the terminal bud (visible flowers), and growth of the stigmas out of the distillate flowers) (red stigma tips visible) (Table 1). Several of these stages are related to accumulated heat units.

Normalized germination percentages as functions of temperature were fitted to quartic equations (r^2 = 0.987,0.961,0.961, 0.990, for branch units enclosed on 2, 16, and 23 Feb. and the untreated control, respectively), and temperature optima were calculated from the first derivatives of the fitted curves. The optimum temperature for pollen germination increased with temperature prevailing during the period of pollen development. Calculated temperature optima were: 25.6C for pollen that developed under ambient conditions, 26. lC for pollen from branches heated beginning 23 Feb., 26.lC for branches heated beginning

Table 1. Timing of developmental events for 'Serr' walnut branch units under ambient conditions and in heated enclosures. Data are presented as accumulated degree-days with Julian date in parentheses. Accumulated degree-days for all treatments are calculated from the time the first branch unit was placed in a heated enclosure.

Event	Heating started (February)			
	Ambient	2	16	23
	Accumulated degree-days (Julian date)			
Branch enclosed and heat started	---	0 (33)	90 (47)	152 (55)
Catkin bud swelling	152 (55)	155 (44)	161 (52)	152 (55)
Terminal budbreak	229 (63)	232 (49)	222 (56)	232 (60)
Leaf emergence from terminal bud	283 (69)	278 (52)	255 (58)	272 (63)
Catkins expanded	283 (69)	278 (52)	270 (59)	259 (62)
First staminate flowers open	292 (70)	310 (54)	286 (60)	273 (63)
First anther dehiscence	299 (71)	340 (56)	286 (60)	288 (64)
First pistillate flowers visible	359 (78)	356 (57)	377 (70)	363 (72)
Red stigma tips visible	---	394 (60)	390 (71)	390 (76)

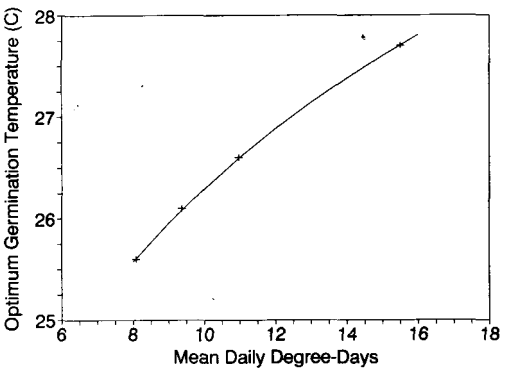

Fig. 3. Optimum temperature for pollen germination as a function of mean daily degree-days experienced during the period from 2 Feb. to anther dehiscence. Data points represent optimum temperatures as determined from the first derivatives of quartic equations fitted to pollen germination data using the least-squares method. The curve represents the regression $T = a \ln (d) + b$, where T = optimum germination temperature, d = mean daily degree-days; $a = 3.227$, $b = 18.859$, and $r^2 = 0.996$.

16 Feb., and 27.7C for pollen from catkins that were heated for the longest period. A highly significant regression ($r^2 = 0.996$) exists between optimum germination temperature and the natural log of mean daily degree-days from the start of the experiments to anther dehiscence over the range of conditions for these experiments (Fig. 3).

Jansson and Warrington (1988) noted a somewhat similar response in pollen from kiwifruit *(Actinidia deliciosa)* vines. They grew 'Matua' kiwifruit vines at five temperature regimes ranging from cool (13/7C) to warm (21/17C) conditions. The maximum percentage of pollen germinated in vitro at 22 to 28C for that from plants grown at the highest temperatures and over the broader range of 16 to 28C for pollen from plants grown under the cool conditions.

In a previous investigation of temperature and walnut pollen germination, Luza et al. (1987) used the same methodology to determine optimum germination temperatures for pollen of 10 *J. regia* clones, selected to cover the full range of staminate bloom date within the species, following development under ambient conditions. These optima ranged from 25.8 to 30. lC and were correlated with mean staminate bloom date, such that later-blooming clones showed higher temperature optima. The lowest optimum in the earlier study was with 'Serr,' the cultivar used here, and the clone having the earliest staminate bloom of the 10 examined. The 25 .8C optimum determined at that time compares well with the 25 .6C optimum for the control sample from this experiment. It is interesting that the highest optimum temperature obtained here is at the approximate midpoint of the range determined earlier for the 10 *J. regia* clones.

Phenotypic plasticity describes the ability of a plant to alter its developmental program in response to the external environment (Bradshaw, 1965). Plant response to environmental stimuli is considered adaptive if it enhances fitness (Schlichting, 1986). That is, organisms that are better adapted to the variability inherent in the environment will tend to leave more offspring that proliferate within the population over time relative to less adapted individuals (McDonald, 1983). It would appear that the plastic responses of crop plants have great practical significance, i.e., the greater the range of phenotypes a genotype can produce, the less vulnerable is productivity to environmental extremes. The results presented here partially answer the question of whether the observed response of pollen germination to temperature is a genetically fixed adaptation correlated with sporophyte phenology or a plastic response to environmental conditions during pollen development. Clearly, there is a component of the in vitro pollen germination–temperature response that is at least partially affected by the environment during the developmental period. The range of response covers about half of that exhibited by several clones in the species, however. Thus, it appears that the range of this plastic component of the phenotype may be limited within a given genotype.

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