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Floral Competence of Primocane-fruiting Blackberries Prime-Jan and Prime-Jim Grown at Three Temperature Regimens

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***Abstract*. We investigated the responses of staminate and pistillate floral components of Prime-Jan and Prime-Jim primocane-fruiting blackberry (*Rubus* L. subgenus *Rubus* Watson) to three different growth chamber temperature regimens, 35.0/23.9 °C (HT), 29.4/18.3 °C (MT), and 23.9/12.8 °C (LT). Temperature was negatively related to flower size, and morphologically abnormal floral structures were evident in 41% and 98% of the MT- and HT-grown plants, respectively. Anthers of LT- and MT-grown plants dehisced. The viability of pollen (as deduced through staining) from Prime-Jan grown at LT or MT exceeded 70%, whereas that of Prime-Jim pollen was significantly reduced (<40%) by the MT regimen. In vitro pollen germinability (typically <50%) was negatively influenced by temperature but was unaffected by cultivar. Pollen useful life was diminished under HT conditions; LT-grown pollen held at 23.9 °C retained 63% of its original germinability over a 32-h period, while the germinability of that held at 35.0 °C for 16 hours decreased by 97%. Virtually all flowers cultured under HT conditions were male sterile, exhibiting structural or sporogenous class abnormalities including petaloidy and malformation of tapetal cells or microspores; HT anthers that were present, failed to dehisce. Stigma receptivity, pistil density, and drupelet set were also negatively influenced by increased temperature; values for these parameters of floral competency among control plants were reduced by 51%, 39%, and 76%, respectively, in flowers cultured under HT conditions. In this study, flowering and fruiting parameters, and presumably the yield potential of Prime-Jan and Prime-Jim, were adversely affected by increased temperature. However, their adaptive response to heat stress under field conditions awaits assessment.**

Blackberries, collectively known as *Rubus* L. subgenus *Rubus* Watson, are a group of taxonomically complex plants grown for their succulent aggregate fruits. Blackberry production in the United States has risen dramatically within the last decade, in part because of their healthful phytochemical content (Cho et al., 2004; Siriwoharn et al., 2004). They are an increasingly popular component of small farms across the United States because they are of high value and can be sold successfully using a variety of marketing strategies. However, a major challenge to increasing traditional, Midwest blackberry production is the plant's lack of winterhardiness. Winter temperatures below –18 °C reduce yield; as low temperatures approach –28 °C, flower buds for the following season's crop are virtually destroyed (Funt et al., 2000; R.C. Funt, unpublished data). Because canes can be managed as annuals, the two recently released primocane-fruiting (PF) cultivars from the University of Arkansas' fruit breeding program, Prime-Jan and Prime-Jim (Clark et al., 2005), might help producers minimize annual yield fluctuations and improve the commercial potential of blackberry in U.S. Department of Agriculture climate zones 6 and lower.

To determine if PF blackberries offer economic and production advantages, trial plantings of Prime-Jan and Prime-Jim have been established in several production regions. Although these cultivars have the potential to be widely adapted and to fruit over an extended period (Clark et al., 2005), current evidence suggests that their yield potential may not be realized in areas with early fall frosts or extreme midsummer temperatures (Stanton, 2005). For instance, early frosts significantly shortened the first fall harvest season of Prime-Jan and Prime-Jim planted in trial at Geneva, N.Y. (C.A. Weber, personal communication). In Arkansas, Prime-Jan and Prime-Jim flowered and fruited when average daily high temperatures were typically 30–35 °C. In these trials, a reduction in or damage to flowering and fruiting was observed when daytime highs were 29.4 °C or higher for 5 to 8 d in a row (Clark et al., 2005; J.R. Clark, unpublished data). In contrast, fruit yields were substantially higher in Oregon, where average daily high temperatures during flowering were 28 °C or less.

Little information has been published concerning the effects of temperature on blackberry floral competence. In our preliminary observations, growth chamber grown PF blackberries flowered and fruited normally under a regimen of 23.9/12.8 °C day/night temperatures (LT). Primocanes grown at 29.4/18.3 °C (MT) were shorter, more numerous, and had more nodes and laterals per cane. In addition, primocanes of plants grown at MT had fewer flowering nodes and set ≈20% fewer fruits than those of their LT counterparts. Primocanes grown at 35.0/23.9 °C (HT) produced almost ten times the laterals per plant, more nodes per plant, and more nodes per cane but exhibited a lower percent of flowering nodes and did not set fruit. Flowers of this latter group were visually distinct: flower diameter was smaller, filaments and styles were shorter, and some flowers had noticeably fewer anthers (Stanton, 2005).

Here we report subsequent research quantifying the effects of increased temperature on male and female floral competence in growth chamber grown Prime-Jan and Prime-Jim PF blackberry plants. We assessed male floral competence by gauging the relative viability and germinability of pollen collected from flowers cultivated at the three temperature regimens designated above and by calculating the effective lifespan or longevity (germinability over time) of dehisced pollen held at 23.9 and 35.0 °C. We evaluated female floral competence as affected by temperature by comparing duration of stigmatic receptivity in LT-, MT-, and HT-grown

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Use of trade names (Prime-Jan and Prime-Jim) does not imply endorsement of the products named or criticism of similar products not named.
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flowers. We measured the pistil density per receptacle and the drupelet set within receptacles of hand-pollinated flowers. LT- and HT-developed floral buds were also examined by light microscopy for morphological abnormalities.

Materials and Methods

Plant materials. Dormant root cuttings from Prime-Jan and Prime-Jim plants were obtained from the University of Arkansas in Winter 2003. Cuttings were planted in flats containing a soilless mix (Scotts Metromix 510, The Scotts Co., Marysville, Ohio) and then chilled. Flats were transferred to a cool greenhouse to stimulate shoot growth. The resultant young plants were transferred to 11.3-L containers in the same medium and then grown in the outdoor nursery for the season once the danger of frost was past. Plants were inspected in Spring 2003 for raspberry bushy dwarf virus, tobacco streak virus, and tomato ringspot virus (Robert Martin, USDA-ARS, Corvallis, Ore.). After several hard frosts in Autumn 2003, plants were pruned and brought indoors to receive a 1000-h chilling treatment at 4.0 °C. In Winter 2004 after chilling, plants were first brought into the greenhouse and then grown outdoors for the 2004 season. In late Fall 2004, plants were pruned to the ground and given a 1000-h chilling as before.

Experimental design and conditions. In Jan. 2005, 60 plants (five plants of each cultivar per chamber) were allowed to come to room temperature for 24 h; they were then placed in six growth chambers (Conviron model BDR-16, The Conviron Co., Winnipeg, Manitoba, Canada) at three different temperature regimens (HT, MT, and LT, as described above) in a completely random design in two replicates. Chambers were lighted with a combination of metal halide bulbs (Sylvania Metalarc, OSRAM Sylvania Products, Manchester, N.H.) and high-pressure sodium bulbs (Philips Ceramalux, Philips Lighting Company, Somerset, N.J.), providing a photosynthetic photon flux (*PPF*) averaging 738 $\mu mol \cdot m^{-2} \cdot s^{-1}$at canopy height (LI-COR LI-190SA Quantum Light Sensor, LI-COR Biosciences, Lincoln, Nebr.). Chambers were programmed to provide a daily photoperiod of 16 h and to gradually increase or decrease temperatures over a 2-h period between light and dark phases. Plants were kept well-watered and were fertigated with a 100 $mg \cdot L^{-1}$ solution containing fertilizers of 20N–8.7P–16.6K and 20N–13.1P–16.6K in equal parts (Peters Professional and Blossom Booster, respectively, from The Scotts Co.). Humidity was held at a minimum of 75%. Insects were not introduced into the growth chambers. Once plants were actively growing, they were thinned to three or four vigorous canes per plant; canes were trained to grow in tomato cages (OS model 4424, Hummert Int'l., Earth City, Mo.). Plants were not pruned outside of cane thinning. They remained in the growth chambers for 19 weeks from January to May. Midway through this study, leaf samples were collected, dried, ground, digested in perchloric acid, and then analyzed for levels of B, Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn using an ICP spectrophotometer (model PS 2000, Leeman Laboratories, Hudson, N.H.) at the Service Testing and Research Laboratory, OARDC, Wooster, Ohio. Midmorning on 9 May 2005, two young, fully expanded leaves per plant were examined for photosynthetic activity using an IR gas analyzer equipped with a 6.25 cm^2 Parkinson leaf chamber (model LCA2 Analytical Developmental Co., Hoddesdon, U.K.).

Male floral competence. A sample of 140 flowers (15–22 flowers per cultivar collected randomly from five representative plants in each chamber) in mid-dehiscence was used to measure pollen viability associated with LT- and MT-grown plants. Pollen was tapped with a paintbrush onto a standard microscope slide to which was added two to three drops of lactophenol cotton blue (methyl blue) stain (Sigma-Aldrich Co., St. Louis). Samples were covered with a cover slip and allowed to stain for at least 10 min before reading. They were then examined microscopically at 100× or 200× (Olympus BH-2 light microscope, Olympus America, Inc., Melville, N.Y.). Three counts of 100 grains each were taken per sample. Pollen was classed as either viable (normally formed, fully stained) or nonviable (uninfused, lightly stained, or abnormally shaped). From among the 140 flowers, a subset of 48 randomly selected flowers (six per cultivar per chamber) was simultaneously examined for pollen germinability. Pollen from 10–15 anthers per flower was tapped onto the center of a petri dish containing sterile Brewbaker and Kwack (BK) medium (Brewbaker and Kwack, 1964) modified to include 20 $g \cdot L^{-1}$ agar (Difco Bacto, Becton, Dickenson Co., Sparks, Md.) and 0.58 $M \cdot L^{-1}$ sucrose. The petri dish was then closed and placed in a chamber and incubated at 26.5 °C, a temperature previously determined to be within the optimum range of pollen germination for these cultivars (Stanton, 2005). After incubation, one to three drops of lactophenol cotton blue was added to the center of the dish to cover and stain pollen. Percentage germination was determined from three readings of 100 grains per plate; pollen was counted as germinated if the pollen tube was at least as long as the diameter of the grain itself.

Eight randomly selected Prime-Jan and Prime-Jim flowers grown at LT were used to determine the effect of temperature on the useful lifespan (longevity) of pollen. These flowers were harvested, placed on petri dishes lined with moistened filter paper (grade #1, Whatman PLC, Brentford, Middlesex, U.K.), covered, and held in chambers at either 23.9 or 35.0 °C (four flowers per cultivar per temperature treatment). At 0, 8, 16, and 32 h after harvest, four to six anthers were removed from each flower, and pollen from these anthers was incubated on BK medium and scored for percentage germination as described above.

For observations of gross morphological abnormalities in HT-grown anthers, a few putatively mature anthers were harvested and then squashed and stained with the same protocol as used for pollen viability experiments. Eleven randomly chosen flower buds (two to three of each cultivar) at three sizes—2, 5, and 10 mm—were also harvested from plants grown at both HT and LT; they were vacuum-infiltrated with a glutaraldehyde-based fixative, paraffin-embedded, sectioned, and stained with safranin-O (Sigma-Aldrich) and astra blue (Sigma-Aldrich) to examine the anther, tapetum, pollen grains, or sporogenous tissues. Slides were microscopically examined at 200–600× (Leica model DM IRB, Leica Microsystems, Wetzlar, Germany); and digitally imaged (Optronics digital camera, Optronics, Goleta, Calif.; MagnaFire software, Olympus America Inc., Melville, N.Y.).

Female floral competence. To test the length, pattern, and spread of stigmatic receptivity, 36 flowers produced under LT, MT, and HT regimens (5–10 randomly selected flowers per cultivar per treatment) were visually divided into three concentric rings reminiscent of a dartboard. Testing began with the first visual indication of possible receptivity (i.e., adherent pollen grains, relative pistil position). A guaiacol staining procedure based on the peroxidase assay of Miller et al. (1987) was devised to indicate stigmatic receptivity. With the assistance of a 10× hand lens, ≈1 µL of a solution containing 10 mL of 0.12 M guaiacol, 3.0 mL of hydrogen peroxide, and 10 mL of phosphate buffer at pH 6.5 was applied with a 10-µL syringe (Hamilton Co., Reno, Nev.) to the stigmatic surface of three to five stigmas within each of the concentric rings. The appearance of a distinctive reddish brown color on the stigmatic surface within 2 minutes of application indicated the presence of peroxidase and putative receptivity (Galen and Plowright, 1987). Individual flowers were tested every 24 h until stigmas stopped indicating receptivity or until stigmas visibly senesced, as evidenced by a withering of the stigma and style.

Flowers were hand-pollinated to determine the effects of temperature on total number of ovules and on relative fruit set using the techniques of Strik et al. (1996). Flowers in all three treatments (total = 98, two to 12 per cultivar chosen randomly from five representative plants in each chamber) were pollinated with pollen collected from equal numbers of flowers of Prime-Jan and Prime-Jim grown at LT. Flowers whose stigmas appeared receptive were tagged, and fresh pollen was brushed three times across the stigmas with a soft camelhair paintbrush. Each flower was pollinated twice at 24-h intervals; flowers were not emasculated. The resultant fruit was harvested at the red-green stage; harvested fruits were individually placed in 50 mL centrifuge tubes, (Corning Life Sciences, Inc., Acton, Mass.) labeled, covered with 90% ethanol, and refrigerated until examination. Berries

were partitioned under a dissecting scope (model M2 95, Leica Microsystems, Wetzlar, Germany) at 7–10×. Uncolored drupelets smaller than 1 mm in diameter were considered to have been aborted; unset drupelets and aborted drupelets were counted together as unset. The sum of set and unset drupelets was used to estimate the number of pistils per receptacle.

Statistical analyses. Pollen viability, pollen germinability, pollen useful lifespan, duration of stigma receptivity, pistil density, and percent drupelet set data were analyzed using SAS protocols (PROC GLM, PROC MEANS, and PROC CORR; SAS Institute, Cary, N.C.). Pollen viability, pollen germinability, pistil density, and percent drupelet set subsample values within cultivars were averaged to provide replicate values used in statistical analyses.

Results and Discussion

Before experimentation, PF blackberry plants tested negative for raspberry bushy dwarf virus, tobacco streak virus, and tomato ringspot virus (Robert Martin, personal communication). Plant health remained adequate throughout the study. Leaf samples collected in all treatment regimens contained levels of B, Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn that were within normal ranges according to Funt et al. (2000, data not shown). Midmorning photosynthetic activities were adequate (11.01 ± 0.77 $\mu mol \cdot m^{-2} \cdot s^{-1}$ CO_2) and comparable to those found by Stafne et al. (2001) for 'Arapaho' blackberry grown at similar temperatures.

Flowering and floral phenotypes. Flowering characteristics were affected by temperature. Plants grown at LT flowered 6 weeks after placement in growth chambers, whereas plants grown at the warmer two temperatures flowered within 3–4 weeks. Similar behavior with respect to temperature was encountered during the preliminary trial in 2004 (Stanton, 2005). Lockshin and Elfving (1981) found that 'Heritage' red raspberry (*Rubus ideaus* L.) plants grown at 29/24 °C flowered 2 weeks earlier than those grown at 25.5/20 °C, although both plant groups flowered after having reached the same number of nodes. Outside of the genus *Rubus*, flowering responses to heat-unit accumulations have been documented in many crop species (Summerfield et al., 1991).

In this study, temperature also affected floral morphology. Flower diameter appeared to be negatively associated with increased temperature, with typical flowers produced at MT and HT measuring 85% and 62% as wide, respectively, as those produced at LT. Flowers grown at LT exhibited a characteristic rosaceous phenotype; each had five petals and five sepals. In contrast, supernormal numbers of petals and sepals (i.e., 6 to 9) were evident in 41.2% and 98.2% of the flowers of MT- and HT-grown plants, respectively, with Prime-Jim apparently sensitive to heat-induced changes at a lower threshold than Prime-Jan (Stanton, 2005). Those flowers with six or eight petals appeared to have two rings of three or four petals each. Temperature-induced floral abnormalities also included radial sepal–petal chimeras, pigment irregularities, and long, tongue-like petals or leafy-looking sepals. In some flowers produced under HT conditions, whorls or partial whorls were replaced or substituted (e.g., petal-like structures replaced stamens). In addition, some HT flowers also manifested distinct abnormalities in petal color (off-white, ecru, light tan) or thickness; others persisted after senescence. Lohar and Peat (1998) found that temperatures at or above 26.0/20.0 °C during meiosis in tomato (*Solanum lycopersicum* L.) flowers (7–8 d before anthesis) caused floral aberrations such as empty flowers (no gynoecium or androecium), whorl and calyx malformation, and flowers that did not abscise; a subsequent reduction in yield was observed.

Male floral competence of LT- and MT-grown plants. The majority of flowers produced at the two lower temperature regimens (LT and MT) had normal-appearing anthers that dehisced regularly over 24–48 h and produced pollen abundantly. The viability of pollen grains as indicated by a heavily-stained, dark-blue appearance was significantly influenced by cultivar and temperature regimen (Table 1). Pollen from Prime-Jan grown at either temperature regimen displayed high viability, while the percent viability of pollen from Prime-Jim was lower and more sensitive to increased temperature (Fig. 1A). Nybom (1986) reported average pollen viabilities (as determined by staining) among tetraploid blackberries ranging from 41% to 82%.

Pollen germination percentages for Prime-Jan and Prime-Jim were not significantly different (Table 1). Pollen from LT-grown flowers displayed a relatively low mean germination rate, although it was twice that of pollen from flowers grown at MT. In contrast, Perry and Moore (1985) found that pollen of field-grown 'Cheyenne' and 'Cherokee', two sibling Arkansas cultivars, germinated at rates of ≈ 90%. Turemis and Derin (2000) reported pollen germination rates of four commercial blackberry cultivars to vary from ≈ 48% to 79%. In their study, pollen germination rates exhibited among and within several cultivars were influenced greatly by the composition of the germination media used. Although we experimentally optimized the composition of BK medium and the corresponding germination temperature before initiating this study, we did not conduct an exhaustive survey of potential germination media and environments. If alternatives had been employed, we might have realized higher germination rates.

Unlike the experimentally imposed, constant growing temperature regimens used in this study, primocane-fruiting blackberries in commercial production fields may experience fluctuations in growing temperatures. Figure 2 demonstrates the potential effect of a sudden rise in daily high temperatures on useful lifespan of functional pollen produced at more moderate temperatures. Prime-Jan and Prime-Jim pollen produced under LT conditions and then kept at 23.9 °C after dehiscence showed a gradual reduction in relative germinability but still demonstrated at least 50% of its original functionality at 32 h. Under these conditions, pollen from Prime-Jim lost significantly less of its initial efficacy than did pollen from Prime-Jan. Pollen kept at 35.0 °C, by contrast, decreased rapidly in functionality, with less than ideal relative germination percentages after 8 h, and only negligible activity at 16 h. Perry and Moore (1985) found a similar relationship between pollen storage temperature and its useful lifespan for blackberry breeding purposes.

Male sterility of HT-grown plants. While flowers produced at the two lower treatment temperatures varied in the measurements of male competency, virtually all of the flowers produced at HT developed irregularities that resulted in dysfunctional male organs. Kaul (1988) described various types of phenotypic abnormalities that interfere with male floral competence as forms of male sterility. Three of Kaul's classes were observed here: 1) structural male sterility, wherein male organs were completely absent or deformed; 2) sporogenous male sterility, wherein stamens developed normally but pollen was absent or malformed; and 3) functional male sterility, wherein viable pollen was formed but other barriers (such as indehiscence) prevented pollination or fertilization. All flowers of Prime-Jan or Prime-Jim that we examined were found to be structurally, sporogenously, or functionally male-sterile. Both structural and sporogenous class abnormalities are also found in tomato and pea (*Pisum sativum* L.), and in both species these abnormalities can be heat-induced (Kaul, 1988).

Under HT conditions, some flowers of Prime-Jan and Prime-Jim opened, but their anther filaments very quickly atrophied or their anthers prematurely senesced, obviating the possibility of dehiscence (Fig. 3A). However, the most notable structure-based form of male sterility in these cultivars was that of petaloid sterility, wherein anther tissue was supplanted by miniature petal-like structures (Fig. 3B). Of 490 HT-grown flowers observed, 115 flowers exhibited petaloid sterility in whole or in part (i.e., 10% to 100% anther substitution) (Table 2). The expression of the petaloid trait was inconsistent within plants; no plants were observed to produce petaloid flowers entirely, while other plants grown at HT never expressed this trait. Flowers grown at LT did not express this trait, and at MT only one flower out of 180 examined exhibited any degree of petaloidy. In carrots (*Daucus carota* L.), the petaloid trait can be stable, controlled by interactive nuclear and cytoplasmic genes (Wolyn and Chahal, 1998), or environmentally induced. Eisa and Wallace (1969) found that within known lines of petaloid male-sterile carrots, the degree of petaloidy varied within plants, within umbels, and within individual flowers and increased as temperature increased.

Table 1. Main and interactive effects of cultivar and growing temperature on measures of male and female floral competence in Prime-Jan and Prime Jim PF blackberries.

Factor	Pollen viability[z] (%)	Pollen germinability[y] (%)	Receptivity[x] (d/flower)	Pistil density[w] (no./flower)	Relative drupelet set[v] (%)
Cultivar (CV)					
Prime-Jan	72.2 a	37.5	3.9	103 a	40.0
Prime-Jim	51.9 b	27.4	3.4	125 b	33.6
CV main effect (*P*)	0.0001	NS	NS	0.0146	NS
Growing temperature (GT)					
23.9/12.8 °C	68.9 a	43.2 a	5.5 a	142 a	55.3 a
29.4/18.3 °C	55.2 b	21.8 b	3.1 b	113 b	57.5 a
35.0/23.9 °C	NA	NA	2.7 c	87 c	13.4 b
GT main effect (*P*)	0.0002	0.0485	0.0001	0.0023	0.0011
CV × GT interactive effects (*P*)	0.0004	NS	0.0001	NS	NS

[z]Mean pollen viability was determined using 140 flowers (15–22 flowers per cultivar per chamber). Pollen was considered viable if it stained dark blue when exposed to cotton blue (lactophenol).
[y]Mean pollen germinability was determined using 48 flowers (six flowers per cultivar per chamber). Pollen was germinated at 26.5 °C for 12 h on modified BK medium.
[x]Duration of stigma receptivity within a flower was determined using 36 flowers (5–10 flowers per cultivar per treatment). An individual stigma was considered receptive if it stained brown when exposed to a solution containing guaiacol and hydrogen peroxide in phosphate buffer (pH 6.5).
[w]Mean pistil density was determined using 98 flowers (2–12 flowers per cultivar per chamber).
[v]Relative drupelet set using 98 flowers (2–12 flowers per cultivar per chamber) following the technique of Strik et al. (1996).
NS = Nonsignificant; $P > 0.0500$.
NA = not available; indehiscent anthers.

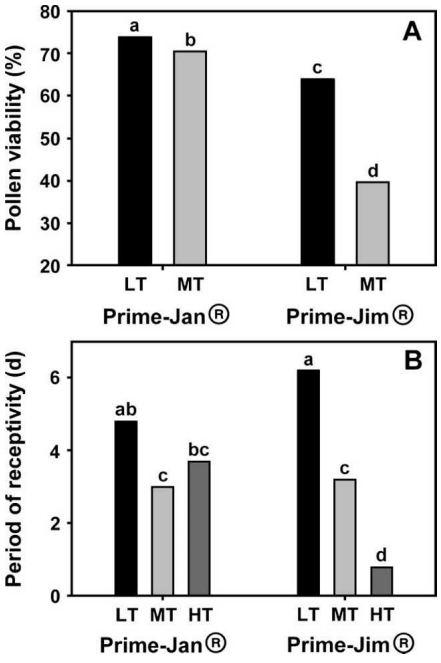

Fig. 1. Interactive effects of Prime Jan and Prime Jim PF blackberry cultivars and growth-chamber growing conditions of 35.0/23.9 °C (HT, dark gray bars), 29.4/18.3 °C (MT, light gray bars), and 23.9/12.8 °C (LT, black bars) day/night temperatures. Bars with different script designations are significantly different according to Duncan's multiple range test ($P = 0.05$) applied to interactive effects: (**A**) pollen viability (140 observations, 15–22 flowers per cultivar per chamber); (**B**) period of stigmatic receptivity (36 observations, 5–10 flowers per cultivar per treatment).

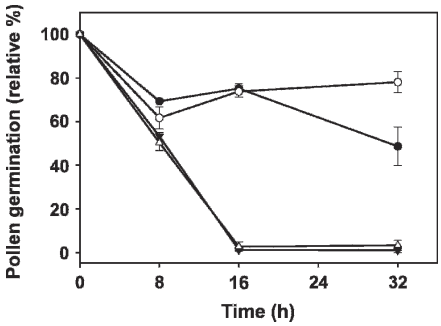

Fig. 2. Relative useful lifespan of Prime-Jan (closed markers) and Prime-Jim (open markers) PF blackberry pollen stored on dehiscent anthers within the flower at HT (35.0 °C, triangles) or LT (23.9 °C, circles). Flowers were developed under a 23.9/12.8 °C temperature regimen. For each flower, the storage data were expressed in relative percent germination (i.e., Δ germination from the initial germinability at harvest); values represent the mean ($n = 4$) decrease in germination percentage over time.

Male floral irregularities in Prime-Jan and Prime-Jim also included aborted or malformed pollen grains and other abnormal anther tissues. Preserved, sectioned, and stained floral buds from HT-grown plants revealed anther tissue without discernible pollen grains (Fig. 4A). The sampled tapeta varied in their degree of development, but none appeared to be functional. By contrast, anthers from flowers grown under LT conditions appeared normal, with clearly stained pollen grains (Fig. 4B); in the section shown, the two halves of the anther have separated, and the stomium is retracting to permit dehiscence.

Anthers present in HT-produced, open flowers rarely dehisced. When indehiscent anthers were squashed and stained on slides, they appeared to be of two types: those containing unstained and presumably malformed pollen grains, and those with pollen grains that stained readily with lactophenol cotton blue (Stanton, 2005; data not shown). When pressure was manually applied to a slide of normal-looking, non-dehiscent anthers, pollen grains were physically released, but in vivo such anthers were never observed to dehisce.

Female floral competence. Stigmas found in flowers of Prime-Jan and Prime-Jim were light green in color and papillate. The guaiacol stain procedure yielded clear positive or negative responses. However, the intensity of staining differed among tested stigmas, presumably indicating varying levels of peroxidase activity and pollen germination potential (Galen and Plowright, 1987). In some flowers, stigmas were receptive in all three areas sampled—bottom, middle, and upper rings—during at least one sampling period. In others, stigmas were receptive in only one or two areas of the flower on a given test day. Stigmatic surfaces at or near the base of the torus were generally receptive first; the pattern of receptivity then progressed toward the torus apex. The relative timing of stigma receptivity varied within flowers, with some stigmas appearing to be receptive toward the middle or end of anthesis, while others were receptive after anthers had dehisced and either shortly before or synchronous with petal abscission (Stanton, 2005). Asynchrony in stigmatic maturity has also been reported in pear (*Pyrus communis* L), where stigmas classed as immature, mature, and degenerated were found within a single flower (Sanzol et al., 2003a).

The period of stigmatic receptivity can significantly impact a plant's effective pollination period (Sanzol and Herrero, 2001). The ideal length of *Rubus* stigmatic receptivity is unknown, but flowers grown at MT and HT were receptive for only 56% and 49%, respectively, as long as those of LT-grown plants (Table 1). Although cultivar main effect means were not significantly different, Prime-Jan stigmas were affected less by increasing temperature than those of Prime-Jim (Fig. 1B). Three of the five HT-grown Prime-Jim flowers sampled failed to show cytochemical evidence of stigma receptivity in any test period, resulting in the relatively low mean receptive period (<1 d) for this group. Similar effects of temperature were found in sweet cherry (*Prunus avium* L.), peach [*Prunus persica* (L.) Batsch], and

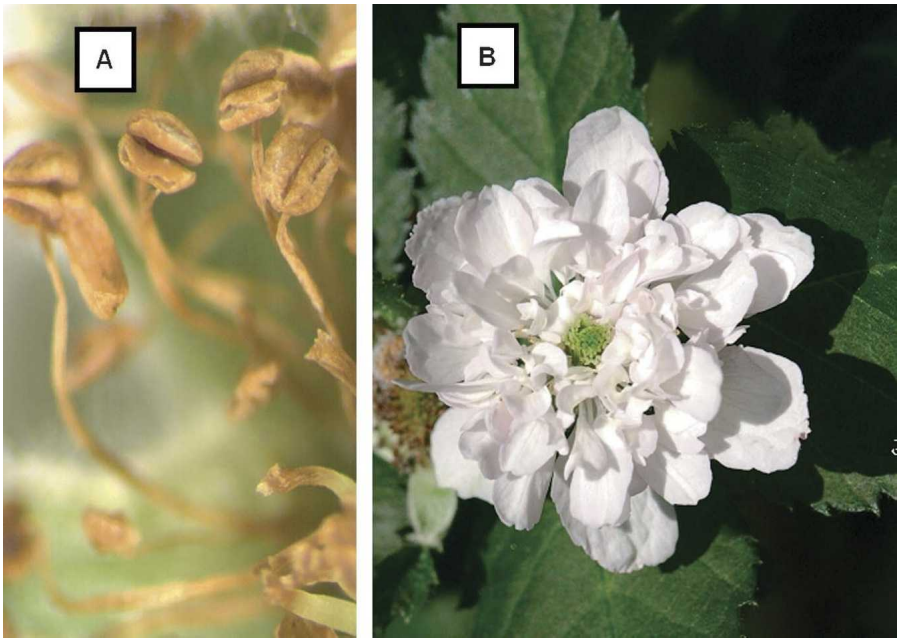

Fig. 3. Male sterility in Prime-Jan and Prime-Jim PF blackberry cultivars, growth chamber-grown under HT conditions (35.0/23.9 °C day/night temperatures): (**A**) indehiscent anthers withered in place; (**B**) petaloid male-sterile flower (*photograph by Kenneth Chamberlain, OSU CommTech*).

Table 2. Expression of petaloidy as a percent of total anthers observed in 490 flowers of Prime-Jan and Prime Jim grown at 35.0/23.9 °C.

Expression of petaloid (% anther substitution)	Proportion of flowers expressing trait (%)	
	Prime-Jan	Prime-Jim
None	77.2	74.0
10–25	12.3	20.2
26–50	3.2	4.6
51–75	3.4	0
76–100	3.9	0.9

pear, where pollen grain adherence to stigmatic surfaces declined significantly as temperatures increased from 10, 20, and 30 °C, respectively (Hedhly et al., 2003, 2005; Sanzol et al., 2003b).

Within blackberry genotypes, fruit mass is significantly correlated with the number of drupelets commonly comprising their ripe fruit (Strik et al., 1996). Therefore, the number of potential ovaries (i.e., pistil density) present in the aggregation and the percentage of drupelets set are additional measures of female competence. Pistil density was significantly greater in Prime-Jim than in Prime-Jan (Table 1). However, in both cultivars, this trait was most substantially affected by growing temperature regimens, with fruit from HT-grown plants exhibiting only 61% of the potential of those produced by plants in the LT temperature regimen. The percentage of developing drupelets was statistically similar in LT- and MT-grown fruit, but at HT, relative drupelet set was significantly compromised. When averaged over the LT and MT regimens, values for pistil density (127) and relative drupelet set (56.4%) in Prime-Jan and Prime-Jim agreed with average reports for three floricane-fruiting cultivars from the Arkansas breeding program grown in Oregon (i.e., 124% and 59.1%, respectively; Strik et al., 1996).

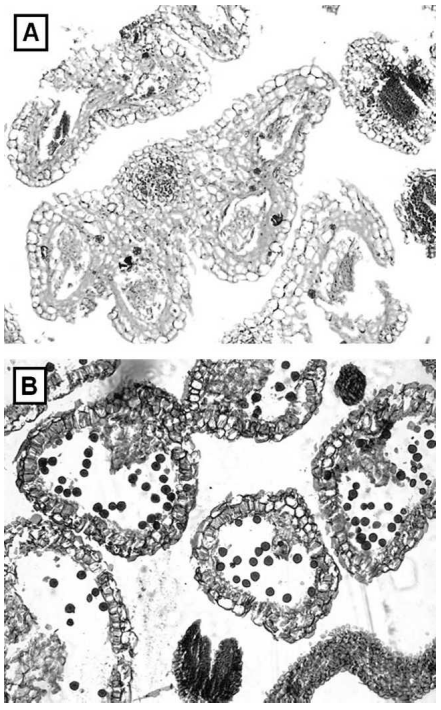

Fig. 4. Stained sections of anthers from Prime-Jan and Prime-Jim: (**A**) abnormal development in anthers from developing flower buds grown at HT (35.0/23.9 °C day/night temperatures); (**B**) normal development in anthers from developing flower buds grown at LT (23.9/12.8 °C day/night temperatures).

High temperatures have been shown to reduce fruit set in other crops. For instance, the delivery of abundant, viable pollen was not sufficient to ensure adequate fruit set in heat-stressed tomatoes (Peet et al., 1997). When *Brassica napus* (L.) plants were exposed to short periods of high temperature and then pollinated with control-temperature pollen, the seed and pod set were both reduced (Young et al., 2004). Cytological evidence in the *Brassica* study indicated that pollen tube growth and fertilization were unaffected by heat stress, but heat stress during the first 4 d after fertilization caused failure of fruit and seed development through a cessation of development occurring in the megagametophytes or carpels. In our study, detailed cytological examinations of pollen germinability at the stigmatic surface, pollen tube growth, or of fertilization and post-fertilization events were not practical. However, stained sections of primocane-fruiting blackberry floral buds failed to reveal obvious temperature-induced irregularities in ovule tissue (Stanton, 2005).

Temperature and PF blackberry floral development. Herein we have reported floral competence to be compromised when Prime-Jan and Prime-Jim blackberries are produced in growth chambers at high growing temperatures. Among these effects, male sterility may be of specific concern to blackberry producers. Although polyploid *Rubus* spp. can be apomictic, many are pseudogamous; functional pollen is required to stimulate viable endosperm or embryonic growth (Nybom, 1988). Independent of male sterility, high temperatures affect the ability of Prime-Jan and Prime-Jim to form ovaries as well as to fertilize those that are formed. The preliminary nature of our study precluded an exploration of the underlying physiological mechanisms by which heat stress may have compromised the floral competence of PF blackberries. However, structural whorl anomalies could have been the result of temperature-induced changes in MADS-box gene expression (Lozano et al., 1998). Studies also report male sterility in plants results from alterations in the levels of one or more plant growth substances (Sawhney and Shukla, 1994), which are perhaps influenced by temperature. Although Prime-Jan or Prime-Jim may have produced floral-specific heat-shock proteins when grown at high temperatures, we did not examine floral tissues for their presence. Heat-shock proteins have been found in floral tissues of other heat stressed plants (Hernandez and Vierling, 1993; Sanmiya et al., 2005; Young et al., 2004), and have been implicated as important factors controlling floral development in heat-stressed *Arabidopsis thaliana* (L.) Heynh. (Tsukaya et al., 1993).

Although our results are explicit, the constant growing conditions imposed in this study limit extrapolation of the data to predict the behavior of field-grown plants. Plants under production are likely to experience high-temperature conditions for short periods; the timing and duration of high-temperature

periods may occur at different developmental stages in floral ontogeny, and thus may greatly affect plant response (Kim et al., 2001; Young et al., 2004). There may also be optimal temperatures for individual reproductive processes (Sato et al., 2004), and floral response to heat stress may be more dependent on mean daily temperature than on daytime highs alone (Peet et al., 1997). High night temperatures may negatively affect floral competence as well (Vara Prasad et al., 1999). Moreover, high humidity levels may exacerbate the detrimental effects of high temperatures (Kim et al., 2001; Peet et al., 2003). Humidity levels could affect the geographic adaptation of these cultivars as well as the optimization of greenhouse cultivation. Prime-Jan and Prime-Jim cultivated in drier western regions with greater diurnal fluctuations might tolerate higher daytime temperatures than those produced in the Southeast. In wheat (*Triticum aestivum* L. subsp. *aestivum*), male sterility may be induced not only by heat stress but also through water deficit via the same developmental pattern (Saini et al., 1984). Producers in particular might find soil moisture easier to control than other environmental factors. In conclusion, extensive field experimentation will be necessary to ascertain the full adaptive response of Prime-Jan and Prime-Jim to a variety of growing conditions. The information reported herein and that developed in subsequent studies may also be useful to breeders developing future generations of improved PF blackberry cultivars.

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