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Determining critical pre- and post-anthesis periods and physiological processes in *Lycopersicon esculentum* Mill. exposed to moderately elevated temperatures

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Abstract

To determine the thermosensitive periods and physiological processes in tomato flowers exposed to moderately elevated temperatures, tomato plants (*Lycopersicon esculentum* Mill., cv. NC 8288) were grown at 28/22 °C or 32/26 °C day/night temperature regimes and then transferred to the opposite regime for 0–15 d before or 0–24 h after anthesis. For plants initially grown at 28/22 °C, moderate temperature stress before anthesis decreased the percentage of fruit set per plant, but did not clarify the thermosensitive period. The same level of stress did not significantly reduce fruit set when applied immediately after anthesis. For plants initially grown at 32/26 °C, fruit set was completely prevented unless a relief period of more than 5 d was provided before anthesis. The same level of stress relief for 3–24 h after anthesis also increased fruit set. Plants were most sensitive to 32/26 °C temperatures 7–15 d before anthesis. Microscopic investigation of anthers in plants grown continuously at high temperature indicated disruption of development in the pollen, endothecium, epidermis, and stomium. This disruption was reduced, but still observable in plants relieved from high temperature for 10 d before anthesis.

Key words: Anther, fruit set, global warming, heat tolerance, male reproductive structures, microsporogenesis, pollen, temperature-sensitive period, thermotolerance.

Introduction

For many species, reproductive processes appear to be much more sensitive to temperature stress than vegetative growth. For example, in rice, plants grown continuously at 34 °C accumulate biomass and leaf area at a faster rate than plants grown at 28 °C, but grain yield declines by about 10% for each 1 °C rise above 26 °C (Baker *et al.*, 1992; Baker and Allen, 1993). The optimal temperature for net assimilation rate in tomato is between 25–30 °C (Khavari-Nejad, 1980), but optimal daily mean temperatures for fruit set of tomato have been reported as 21–24 °C (Geisenberg and Stewart, 1986) and 22–25 °C (Peet and Bartholomew, 1996). Differential temperature sensitivity for reproductive and vegetative growth have also been reported in soybean (Baker *et al.*, 1989), and wheat (Mitchell *et al.*, 1993). Because of this sensitivity of reproductive processes, predicted temperature increases of 0.8–5.8 °C (IPCC, 2001) could reduce yields of important grains and vegetables. The cause of this enhanced or hypersensitivity of reproductive development to even moderate levels of high temperature stress is not well understood.

In many crops, not only is the reproductive process more sensitive to high temperature stress than vegetative growth, but within the development of an individual flower, certain stages are also more sensitive. In groundnut, the number of pegs and pods per plant were significantly reduced by high temperature, by duration of exposure, and by timing of exposure. In tomato, it was reported that meiosis in both mega- and microspore mother cells, which took place 8–9 d before anthesis, was very sensitive to high temperature (Iwahori, 1965, 1966).

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Iwahori also reported that ovules subjected to a temperature of 40 °C 18 h after pollination aborted, perhaps due to inhibition of pollen tube growth and endosperm degeneration (Iwahori, 1966). His experiments, however, lasted for only 3 h and took place at 40 °C, which is far above the optimal growth temperature for tomato plants, and for a much shorter period than high temperature stress typically encountered in the field. In wheat, the period of microsporogenesis is also sensitive to other environmental stresses, such as drought (Saini and Aspinall, 1981; Sheoran and Saini, 1996; Lalonde *et al.*, 1997).

In experiments with male-sterile tomatoes, it was demonstrated that microsporogenesis was more sensitive to moderately elevated temperatures than megasporogenesis and post-anthesis processes (Peet *et al.*, 1998). Pre-anthesis high temperature treatments to *Brassica napus* was also shown to reduce pollen fertility (Morrison, 1991). A recent experiment with five tomato cultivars differing in temperature sensitivity indicated that when tomato plants grown at 32/26 °C were placed at 28/22 °C for 10 d before anthesis, the number of pollen grains released and germinated increased, which resulted in more fruit set than when plants remained in the high temperature treatment (32/26 °C, Sato *et al.*, 2000). The duration and timing of the sensitive period during microsporogenesis and immediately after pollination was not reported in either of these studies, however. Also, the developmental process leading to the failure of pollen release and the reduction of pollen viability was not described.

The purpose of the present experiments was to determine the critical duration and timing of moderately elevated temperature stress which is likely to be experienced under global warming and to analyse anther tissue microscopically to determine whether critical periods of sensitivity to moderately elevated temperature stress were correlated with particular developmental lesions during microsporogenesis. Another question was whether plants react similarly to the imposition and relief of the stress. As well as contributing to a better understanding of the effects of increased global warming on both managed and unmanaged ecosystems, this information will allow better evaluation of stress-tolerant materials developed through both classical breeding and genetic engineering.

Materials and methods

General procedures

Tomato plants (*Lycopersicon esculentum* Mill.) cv. NC8288 (Gardner, 1993) were used. Fruit set in this cultivar is reduced by mean daily temperatures exceeding 25 °C (Peet *et al.*, 1997, 1998). NC8288 produces male-fertile (MF) and male-sterile (MS) plants, which can be distinguished by hypocotyl colour (purple in MF plants and green in MS plants). Both MS and MF plants of this particular cultivar were used in earlier experiments (Peet *et al.*, 1997, 1998; Sato *et al.*, 2000) and in Experiment 2, so the timing of pollination could be determined precisely and also to separate high temperature effects on male and female reproductive structures. In Experiments 1 and 3, however, only male-fertiles were used. Experiments were performed in the North Carolina State University unit of the Southeastern Plant Environmental Laboratory (Phytotron). Plants were seeded in a gravel : peat-lite mix (1 : 1 by vol.), and placed in a germination room maintained at a constant temperature of 22 °C. Seedlings were transplanted 15 d after sowing (DAS) to 6 cm diameter pots (207 ml in volume) filled with gravel : peat-lite mix. The transplants were placed in a Phytotron greenhouse maintained at 26/22 °C day/night temperature. Natural light was supplemented by incandescent lights from 23.00 h to 02.00 h. The long-day regime with a 3 h dark period interruption was standard procedure in the Phytotron of the North Carolina State University. The irradiance during the dark period interruption was 11–12 μmol m^{-2} s^{-1}. The effectiveness of this interruption in controlling flowering in a wide range of short- and long-day plants was established by HB Borthwick's Laboratory at Beltsville, MD, USA (Borthwick *et al.*, 1952).

Seedlings were transplanted to 20 cm diameter pots (4.0 l in volume) at 25 DAS, and moved into growth chambers (2.97 m^2, vertical clearance 2.13 m) set at 28/22 °C day/night temperature or 32/26 °C day/night temperature. Light intensity at pot surface level was set at 550 μE m^{-2} s^{-1} by a combination of fluorescent and incandescent lights in both chambers. Relative humidity was not controlled, but was similar (86–90% midday) in both treatments. Day temperatures and light regimes began at 07.00 h and ended at 19.00 h. Phytotron standard nutrient solution (modified half-strength Hoagland's solution; Thomas and Downs, 1991) was applied to all treatments twice a day.

Experiment 1

This experiment was conducted to investigate the effect of moderately elevated temperature on male reproductive development during the pre-anthesis period. At 25 DAS (second transplanting), male-fertile plants in the 28/22 °C and 32/26 °C were randomly assigned to experimental groups of eight plants each designated as: 0 (HTC), no relief, representing continuous high temperature stress, 0 (LTC) low temperature controls, representing no high temperature stress; and treatment periods of 5, 10, and 15 d pre-anthesis. Flowers were examined daily from 45 DAS to determine when they were 5 d before anthesis. When buds in most of the plants assigned to a particular treatment period were at stage iii–iv (Sawhney and Bhadula, 1988), plants were moved into treatment chambers. Plants assigned to the 5, 10 and 15 d treatments were moved from the 32/26 °C and 28/22 °C chambers for high temperature imposition (HTI, 28/22 to 32/26 °C) or relief (HTR, 32/26 to 28/22 °C). For example, plants assigned to HTI for 15 d were moved from the original growth chamber (28/22 °C) to the treatment chamber (32/26 °C) 5 d before anthesis of the first flower on the first cluster, grown under high temperature for 15 days, and then moved back to the original 28/22 °C growth chamber. Plants assigned to HTR for 15 d were moved from the original chamber (32/26 °C) to the treatment chamber (28/22 °C) 5 d before anthesis of the first flower on the first cluster, kept under optimal temperature for 15 d, then moved back to 32/26 °C. Control (HTC and LTC) plants remained in the original chambers. Two additional chambers set at 28/22 °C and 32/26 °C for the HTI/HTR treatment were used to avoid exposing control plants to temperature swings while plants were being moved between treatments. In all treatments, when a fully opened flower was found, a pollinating rod was applied to the flower cluster for a few seconds to assist pollination, as is

standard practice in controlled environment tomato production (Picken, 1984). The flower was then tagged by date. An average of three flowers were pollinated and tagged on each plant during the course of the experiment. Since tomatoes flower sequentially within a single inflorescence and continue to produce inflorescences as they develop, a single temperature exposure treatment affects flowers at different developmental stages. All pollinated flowers were tagged on the day of anthesis, and the dates of the start of each temperature treatment were known, so it was possible to determine the developmental stage at which that particular flower had been exposed to high temperature relief or imposition. Plants assigned as 0 d imposition (LTC)/relief (HTC) were kept in the original growth chamber for the entire experiment as controls. At 98 DAS, all tagged flowers, pedicels and fruit were counted. Fruit containing at least one visible seed in medial cross-section were recorded as set.

Experiment 2

The purpose of this experiment was to investigate the effect of relief and imposition treatments imposed immediately after anthesis on pollen germination, pollen tube growth and fertilization. Plants were divided between two growth chambers (28/22 °C and 32/26 °C) after transplanting (25 DAS), with each growth chamber containing 32 MS plants. An additional 32 MF plants were moved into a chamber set at 26/22 °C day/night temperature to provide pollen. The 32 MS plants in each growth chamber were divided into four groups consisting of eight plants each. Groups of eight plants in the 28/22 °C chamber were assigned to receive a high temperature imposition (HTI) treatment of 32/26 °C lasting 3, 6, 12 or 24 h after pollination. Groups in the 32/26 °C chamber were assigned to receive a high temperature relief (HTR) treatment of 28/22 °C at 3, 6, 12 or 24 h after pollination. These intervals were chosen based on preliminary experiments (Dunn and MM Peet, unpublished data) showing that under both 32/26 °C and 26/22 °C, pollen grains had germinated, grown through the style and penetrated the ovules within 24 h of pollination. General procedures for Experiment 2 have already been described.

Temperature treatments were started immediately after pollination of all flowers. In each treatment, pollination (procedures described below) was started at 09.00 h, finished at 09.30 h, and HTI and HTR began at 10.00 h. Thus, the 3 h and 6 h HTI and HTR treatments occurred only during the 'day' thermoperiod. The 12 h treatments took place over 9 h of the light period and 3 h of the dark period. The 24 h treatment took place over 12 h each of the light and dark periods, with the 12 h daytime treatment being split over two separate days since it started at 10.00 h of day 1 and ended at 10.00 h on day 2. The 3, 6, 12, and 24 h HTI and HTR began as soon as all plants assigned to that treatment had multiple flowers at anthesis (63, 64, 69, and 63 DAS, respectively). Thus, the chronological ages of the plants were slightly different when treatments started but all plants were at the same developmental stage.

Plants assigned to the same length of HTI and HTR were exchanged between chambers so chamber plant density remained the same (32 plants). Thus, the eight plants assigned for 3 h of HTI in the 28/22 °C chamber were exchanged with the eight plants assigned for 3 h HTR in the 32/26 °C chamber after pollination. Plants were returned to the original growing chamber at the end of the assigned treatment period.

Pollen was collected on glass slides by vibrating flower clusters from MF plants in the 26/22 °C chamber with a commercial pollinating rod. Immediately before moving MS plants into the high temperature imposition and relief treatments, the stigmas of all open MS flowers were brushed gently through pollen

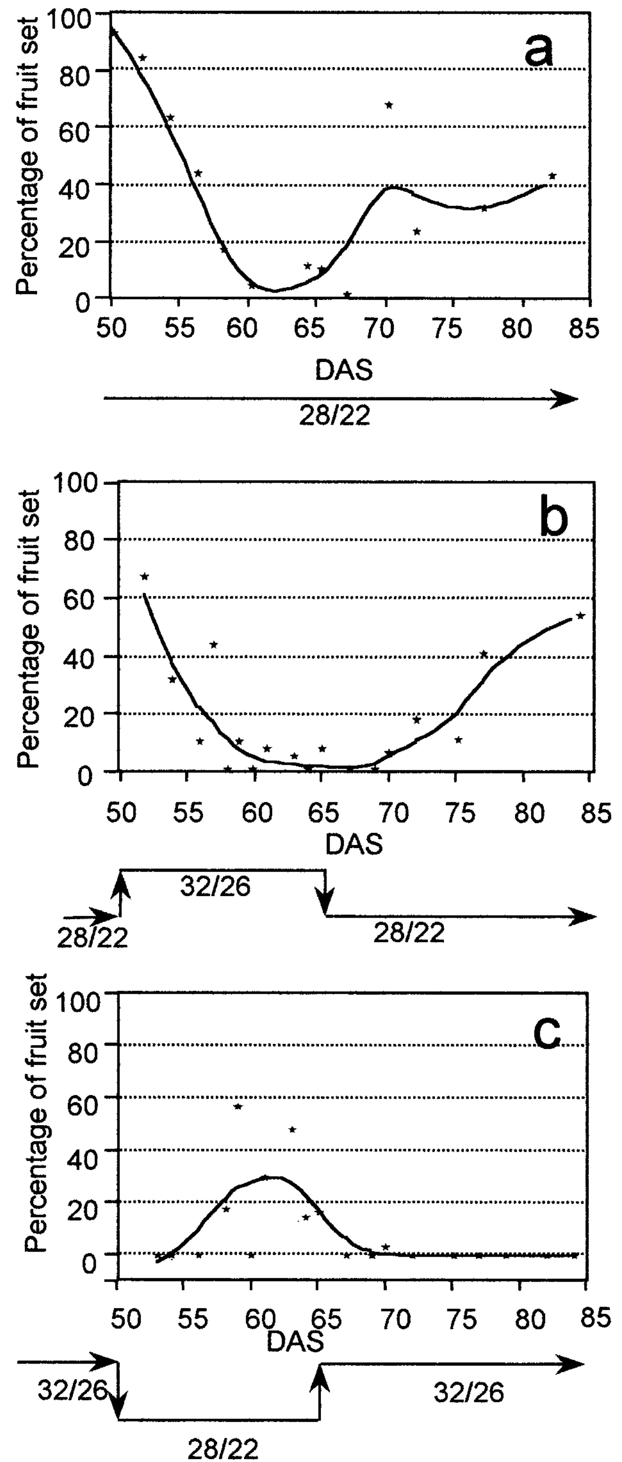

Fig. 1. Percentage of fruit set in (a) 0 d and (b) 15 d high temperature imposition (HTI) and (c) 15 d high temperature relief (HTR) treatment. Data from the 5 d and 10 d HTI and 10 d HTR treatments are not shown, as they represented intermediate points, and made interpretation of the figures more difficult. No fruit were set in the 0 d and 5 d HTR treatments, so these data are also not shown. The x-axis represents the number of days after sowing (DAS). Percentage of fruit set was calculated by dividing fruit number by pollinated flower numbers for each plant. The percentage was averaged in each treatment group (8 plants). The value of lambda was set at 10 in all analyses for smoothing spline fit.

collected on the slide and the flowers tagged by date pollinated. Plants were then moved into the imposition or relief treatments, as described above. In total, 3–7 flowers in the first and second clusters of each MS plant were pollinated and tagged at the time temperature treatments started. Later-opening flowers were not pollinated. Similar numbers of flowers (3–6) were pollinated in the first and second clusters of 0 h HTI and HTR plants. At 93 DAS, all MS plants were harvested to determine if fruit set had occurred in pollinated flowers.

Experiment 3

Experiment 3 was organized to prepare histological samples of anther tissue exposed to stress treatments for microscopic analysis. General procedures were similar to those in Experiment 1, but at transplanting, MS plants were moved into three temperature treatments, constant 28/22 °C, constant 32/26 °C, or 32/26 °C with 10 d relief before anthesis. Day/night temperatures of the relieving treatment were reduced to 28/22 °C for approximately 10 d before anthesis of the first flower, then plants were returned to the 32/26 °C growth chamber.

Flowers at anthesis were collected into vials, which were cooled and immediately transported to North Carolina State University EM Center for processing. Collected flowers were fixed for 24 h under continuous agitation (Clement and Audran, 1995). After dehydration, samples were plastic-embedded with a JB-4 embedding kit (Polysciences, Inc. Warrington, PA) overnight, then placed in embedding moulds containing polymerization solution for 3 h under −55 kPa. After polymerization, samples were sectioned with an ultramicrotome at approximately 4 μm thickness using a dry glass knife, transferred onto a water drop on a glass slide and air-dried. Sections were then stained with toluidine blue (cellulose cell walls and cytoplasm are stained blue and lignified cell walls are green/blue) and basic fuchsin (cell components are stained purple or red, depending on pH), and examined under a Nikon Bioshot light microscope (Nippon Kogaku USA Inc., Garden City, NY) at 100× magnification. Digital pictures were taken with a Pixera PVC100C (Pixera Corporation, Los Gatos, CA).

Data analysis

Data for all flower clusters on the plant were grouped and percentage of fruit set, rather than actual number of fruit, was calculated. The numbers of pollinated flowers were different for every pollination date and for each plant, so actual numbers of fruit between treatments or different days of pollination within the same treatment were not directly comparable. Percentage of fruit set was calculated by dividing fruit number by pollinated flower number for each plant. Percentage data were confirmed to be normally distributed, therefore transformation was not needed. Data were subjected to statistical analysis using the JMP statistical package (SAS Institute, Cary, NC). Statistical analysis included smoothing spline fit, ANOVA, comparing means with standard errors and linear regression. For the smoothing spline fit, the value of lambda was set at 10 in all analyses.

Results

Experiment 1

The percentages of fruit set for the LTC (i.e. constant 28/22 °C) and 15 d high temperature imposition (HTI) treatments are shown in Fig. 1a and b, respectively. Data from the 5 d and 10 d HTI treatments are not shown, as they represented intermediate points, and made interpretation of the figures more difficult. Data points in the graph represent the percentage fruit set in the flowers pollinated each day during the period from 50–85 d after sowing (DAS).

In the 0 d imposition (i.e. no high temperature stress) (Fig. 1a), the percentage of fruit set was high in flowers pollinated early in the experiment (50 DAS), then gradually declined almost to less than 10% in flowers pollinated 10 d later (60 DAS). Fruit set began to recover for flowers pollinated two weeks after the start of the experiment (around 64 DAS), and stabilized at around 40% for flowers pollinated from 3–5 weeks after the start of the experiment. In the 15 d HTI treatments (Fig. 1b), the percentage of fruit set was also initially high, declined with time, then recovered. However, with 15 d at 32/26 °C, the beginning of recovery was delayed from 2 weeks after the start of the experiment to 3 weeks, and yield did not recover to initial levels until 5 weeks after the start of the experiment. Overall, there was a trend towards decreasing fruit set per plant by longer imposition of high temperatures (Fig. 2) and the linear regression of days of imposition on percentage of fruit set was significant (data not shown, $P = 0.03$).

No fruit set occurred when high temperatures were only relieved for 5 d. In the 15 d high temperature relief (HTR) treatment (Fig. 1c), flowers pollinated between 58 DAS (59% fruit set) and 70 DAS (3.5% fruit set), representing 8 d and 20 d, respectively, after the start of the relief were able to set fruit. For all pollinated flowers for the entire crop, 15 d and 10 d HTR allowed approximately 10% and 3% fruit set, respectively, compared to no fruit set for the 0 d and 5 d relief treatments (Fig. 2).

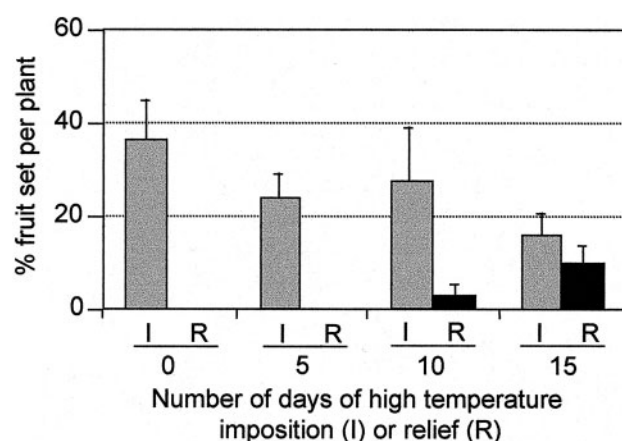

Fig. 2. Percentage of fruit set per plant in 0, 5, 10, and 15 d high temperature imposition (I) or relief (R). Percentage of fruit set was calculated by dividing fruit number by pollinated flower number for each plant. The percentage was averaged in each treatment group (8 plants). No fruit set was observed in 0 d and 5 d relieving treatments. Bars represent standard errors.

Experiment 2

On average, 5.4 flowers were pollinated per plant and no significant difference between the treatments was observed in number of flowers pollinated based on the Student *t*-test (data not shown). Although differences in flower numbers between the treatments were not significant, slightly more flowers were pollinated in the HTI than the HTR, so data are shown as the percentage fruit set and average weight per fruit rather than totals.

No fruit set were observed before high temperature relief (0 h HTR). The longer the relief period from HT, the higher the percentage of fruit set (Fig. 3). Linear regression analysis showed that the relationship between the percentage of fruit set and the length of RT after pollination was significant at $P = 0.0057$. On the other hand, there was no significant difference between the four durations of HTI, based on ANOVA, means comparison with standard errors or linear regression. Percentage fruit set was much higher in the HTI treatments (70–80%) than in the HTR treatments (0–60%).

Experiment 3

In anthers not receiving moderately elevated temperature stress, the pollen grains were round and well stained and dehiscence occurred normally (Fig. 4a). Under continuous stress, however, the stomium was still intact, precluding anther dehiscence (Fig. 4b). Other abnormalities under high temperature stress included an irregular arrangement of the epidermal cells. These cells appear to be growing outward, and the middle layers and endothecium did not form regular layers, comparable to those in Fig. 4a. Pollen grains remaining in the anther sac were flattened and irregularly shaped, rather than round.

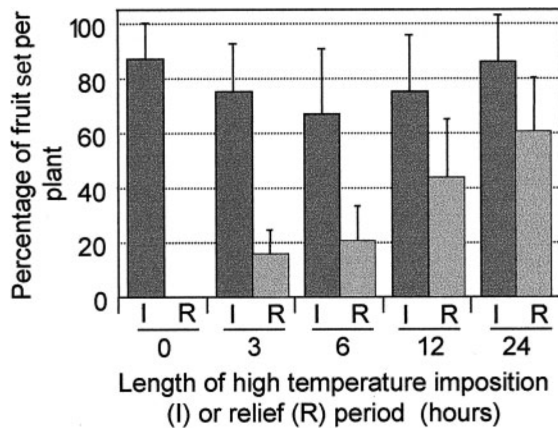

Fig. 3. Effect of duration of high temperature imposition (I) or relief (R) after anthesis on the percentage of fruit set. Mean comparison with standard error (shown as bars). Percentage of fruit set per plant was calculated by dividing the number of fruit set by the number of pollinated flowers within plant. Means and standard errors were calculated from each treatment group ($n = 8$).

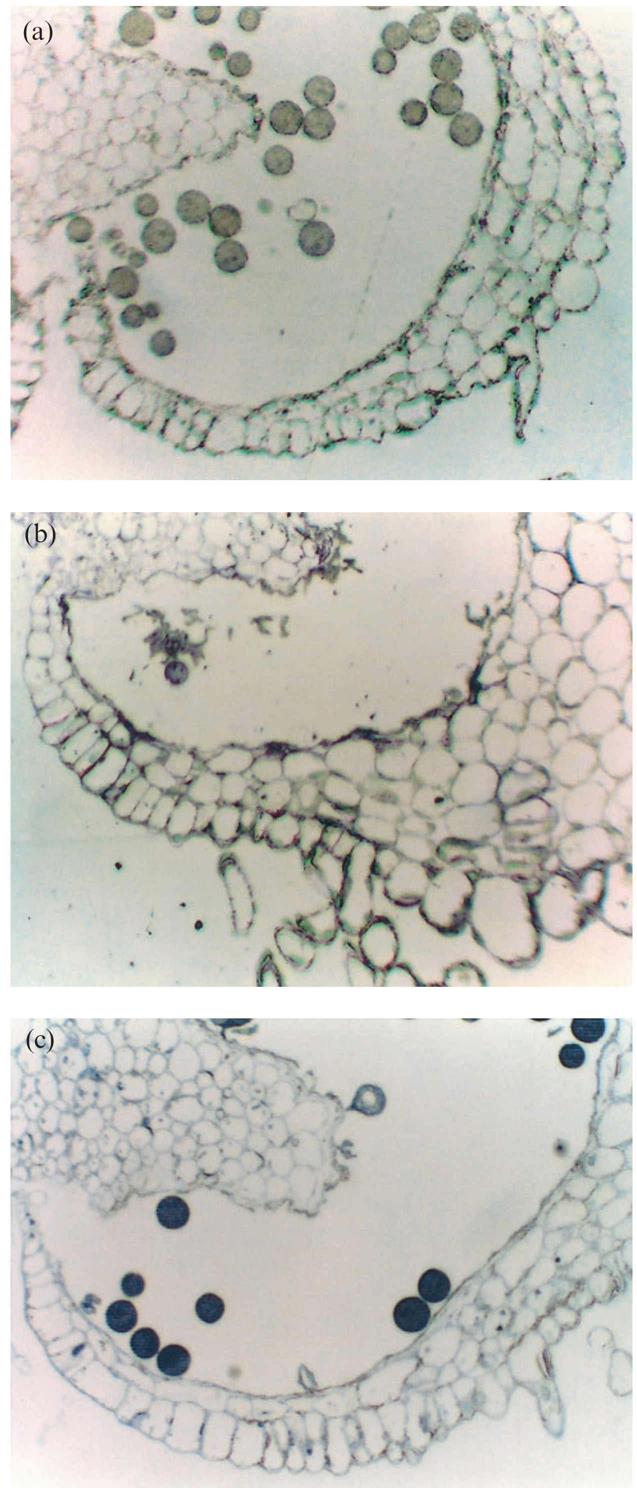

Fig. 4. Temperature effects on anther dehiscence. Magnification is ×40. Specimens were stained with toluidine blue and basic fuchsin. Samples were collected on the day of anthesis; (a) grown at 28/22 °C; (b) grown at 32/26 °C; (c) grown at 32/26 °C but stress relieved at 28/22 °C for 10 d before anthesis.

When the stress was relieved for the 10 d before anthesis (Fig. 4c), the stomium broke down normally, allowing anther dehiscence. Regularity of shape of epidermal cells and roundness and staining of pollen grains were intermediate between the treatment with no stress (Fig. 4a) and the treatment with continuous stress (Fig. 4b).

Discussion

Imposition of high temperature stress depressed fruit set per plant linearly with duration of the stress when applied pre-anthesis (Experiment 1, treatment during microsporogenesis period, Fig. 2), but not when applied immediately post-anthesis (Experiment 2, pollen germination and fertilization period, Fig. 3) to non-stressed pollen. The lack of stress imposition effects post-anthesis can be attributed both to the period of stress (up to 1 d) being short, relative to the non-stress period (50 d) and to the use of pollen grown under optimal temperatures: microsporogenesis in this cultivar is more sensitive than post-anthesis processes (Peet *et al*., 1998). Even with 15 d imposition of a temperature stress of 32/26 °C, however, some fruit set took place. By contrast, only 3 h exposure to 40 °C prevented fruit set in tomatoes (Iwahori, 1965, 1966), presumably because of the higher temperatures used.

High temperature relief, on the other hand, was effective in increasing fruit set both before and after anthesis. For this temperature range, high temperature relief before anthesis had to last more than 5 d in order for plants to produce fruit (Fig. 2). Using pollen developed under near-optimal temperature, however, as little as 3 h relief from high temperature stress after anthesis allowed some fruit set, and when relief continued for 24 h, fruit set levels approached those of non-stressed plants (0 h imposition, Fig. 3).

The timing of relief was also critical. Iwahori observed that tomato pollen was in the microsporocyte stage 10 d before anthesis and in the meiosis and tetrad stages by 9–8 d before anthesis (Iwahori, 1965). In his study, a temperature stress of 40 °C for 3 h each for two consecutive days at meiosis inhibited pollen development to a greater extent than stress at any other stages of development. In Experiment 1, fruit set occurred only in flowers pollinated more than 8 d after relief from high temperatures started and less than 5 d after the plants were returned to the stress treatment. Consequently, 5 d before anthesis to the day of anthesis can be interpreted as a relatively 'non-sensitive period'. It has also been noted that flower buds are rather tolerant to high temperature 1–3 d before anthesis (Sugiyama *et al*., 1966). Relief treatments of 10 d or longer were needed for fruit set in this experiment, suggesting 15 (5+10) to 5 d before anthesis would represent the temperature-sensitive period in this study's temperature regime. Alternatively, 13 (5+8) to 5 d before anthesis could be the temperature-sensitive period, since earliest fruit set was observed at 8 d after relief started in 15 d HTR (Fig. 1c).

This represents a much longer critical period than that of 3 h found by Iwahori at 40 °C, presumably because of the reduced severity of the stress. Extreme and moderate heat stress may affect different physiological processes (Iwahori, 1965).

The midpoint of this critical period (9–10 d before anthesis) corresponds fairly well with the critical period found in other crops. In a reciprocal transfer experiment in peanut (Vara Prasad *et al*., 1999), reproduction was found to be most sensitive to heat stress during the 6 d period before anthesis, although in their study the fate of individual flowers was not followed. Vara Prasad *et al*. also found that the number of pegs and pods per peanut plant were reduced by duration of exposure, as well as timing of exposure (Vara Prasad *et al*., 2000). Ahmed *et al*. indicated that the temperature-sensitive period of cowpea (*Vigna unguiculata*) was 9–7 d before anthesis under 33/30 °C day/night temperatures (Ahmed *et al*., 1992).

Lalonde *et al*. reported that male sterility in wheat resulted from water deficit occurring about 10 d before anthesis, the period of meiosis in microspore mother cells (Lalonde *et al*., 1997). They reported that water stress caused loss of orientation, lack of starch deposition in male reproductive cells, abnormal vacuolization in tapetal cells and abnormal starch deposition in connective tissue. In this study's experiments, plants in constant high temperature had shrunken pollen grains, failure of anther dehiscence, and the endothecium and epidermis were irregularly arranged (Experiment 3; Fig. 4b). These abnormal developments were not as pronounced if the temperature stress was relieved 10 d before anthesis (Fig. 4c). Since treatments lasted several days, multiple phases of microsporogenesis were undoubtedly affected. In barley (Sakata *et al*., 2000), at least three stages of reproductive growth were sensitive to high temperature. When plants were exposed to high temperature for 5 d at the early panicle differentiation stage, pollen grains had apparently normal exine, but no or little cytoplasm. At the pre-meiotic stage of pollen mother cells, high temperature caused short anthers possessing no pollen grains. When plants were exposed to high temperature during meiosis of pollen mother cells (the stage that was found to be most sensitive to anthesis), all pollen grains possessed exine and were swollen, but showed little starch accumulation. In these plants, the panicles at the heading stage had a normal appearance, but their seeds were virtually sterile.

Better characterization of the heat-sensitive period should help in developing more heat-tolerant cultivars both through classical and molecular techniques. Using a functional genomics approach, for example, gene

expression during the sensitive period in tolerant and resistant cultivars could be compared and genes identified as critical incorporated into transgenic plants. Temporal and spatial specific genes have been isolated in tobacco plant anthers (*Nicotiana tabacum*) (Goldberg *et al.*, 1993) and a genetically-engineered eggplant (*Solanum melongena*) which successfully set parthenocarpic fruit under environmental stress has also been reported (Rotino *et al.*, 1997).

As shown in Experiment 1, the percentage of fruit set also decreased from the start of pollination in non-stressed plants (0 d high temperature imposition, Fig. 1a). Competition between fruit in the same inflorescence (Bangerth and Ho, 1984), successive fruit clusters and progressively decreasing fruit set with plant age have been documented in several crops including tomatoes (Bertin, 1995; Hurd *et al.*, 1979) and peas (Guilioni *et al.*, 1997). Tomato fruit are the strongest sink in the plant during early development (Ho, 1996).

Under both moderately elevated temperature stress and control treatments, abortion frequency followed a consistent pattern along the stem with no abortion on the first flower clusters, a temperature-dependent abortion frequency on later flower clusters and complete abortion on the apical clusters. High temperature stress increased the tendency for younger flowers to abort when developing pods were present. Thus, competition, presumably for carbohydrates, in a sequentially-flowering crop such as tomatoes and peas, complicates investigations of high temperature stress, particularly when the stress is imposed on plants with different levels of previously set fruit. Low light can increase competition between various plant sinks and also interacts with temperature effects (Kinet and Peet, 1997). Kinet found that the detrimental effect of a short stay under insufficient light during sporogenesis was reversed by decreasing the temperature from 20 °C to 16 °C (Kinet, 1982). It is not clear if irradiances in the present experiment should be considered as low, although 550 $\mu E\ m^{-2}\ s^{-1}$ is lower than peak irradiances under natural light. It was provided for 12 h and Kinet described irradiances of only 180 $\mu E\ m^{-2}\ s^{-1}$ for 16–20 h as favourable compared to 8 h days under 90 $\mu E\ m^{-2}\ s^{-1}$ (Kinet, 1989).

Certainly, competition between plant parts does not explain all the high temperature effects observed here. In Experiment 1, no fruit set occurred in HTC (0 d relief) and 5 d HTR. Even in the 15 d HTR treatment, no fruit set took place unless flowers were relieved from high temperature for more than 8 d at a specific stage before anthesis. For these fruit, there was no carbohydrate competition among developing fruit although competition with vegetative sinks would still have been taking place. In fact, the absence of fruit made it easier to illustrate the critical period for moderately elevated temperature stress (Fig. 1c).

Carbohydrate metabolism and/or translocation may also explain high temperature effects observed during the post-anthesis period. Pollen tube elongation has a high energy requirement. It was observed that starch reserves in stylar tissue gradually disappeared and carbohydrate secretion occurred as the pollen tube passed through the pistil (Gonzalez *et al.*, 1996). This suggests pollen tube elongation relies on carbohydrate supplies secreted by the pistil. In the post-anthesis high temperature stress experiment (Experiment 2), pollen grains were collected from non-stressed MFs and applied to MSs grown under either optimal temperatures or high temperature stress. A post-pollination HTI on non-stressed plants had no effect, but HTR treatments to stressed plants increased fruit set. For MS plants experiencing poor carbohydrate availability under continuous HT, a short period of relief from high temperature stress might have significantly increased carbohydrates available for pollen tube elongation. On the other hand, if carbohydrate reserves were abundant in MS plants grown at 28/22 °C, lack of carbohydrates for a short period may not have significantly depleted these reserves.

In conclusion, in tomatoes, 8–13 d before anthesis was the most sensitive period to moderately elevated temperature stress, although stress relief for at least 3 h post-anthesis also increased fruit set, assuming pollen developed under non-stressed conditions. This critical pre-anthesis period was associated with developmental changes in the anther, most strikingly, irregularities in the epidermis and endothecium, lack of opening of the stomium, and poor pollen formation. These data suggest that, in evaluating individual flowers of either crop plants or native species for tolerance to global warming, the 2 weeks before anthesis are the most critical. It is also critical to observe whether the fruit set is seeded or seedless since parthenocarpy increases under heat stress (Foster and Tatman, 1937; Sato *et al.*, 2001). The relative position of the flower on the plant is also important, as fruit set declines with time even under favourable conditions. Thus flower abortion or lack thereof should not be used as the only indication of high temperature tolerance. To improve the ability of tomtoes to set fruit under predicted inceases in temperature, breeders and biotechnologists should focus on the period 8–13 d before anthesis.

Acknowledgements

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