Post-heat Stress Respiration Pattern of Tulip Bulbs in Storage

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Abstract

Dutch-grown tulip bulbs are shipped around the world via ocean freight. Normal transport temperatures are 17 to 20°C, but bulbs can be improperly subjected to high temperature stress due to equipment failure or mishandling. To understand effects of heat stress on internal metabolism of tulip bulbs, we determined the responses to heat treatment of a heat sensitive cultivar ('Apeldoorn') and a heat-resistant cultivar ('Sevilla'). In addition, we tested the hypothesis that heat-induced injuries are related to endogenous ethylene production by treating bulbs with an inhibitor of ethylene perception, 1-methylcyclopropene (1-MCP), prior to heat treatment. Bulbs, either untreated or treated with 1-MCP, were exposed to 35°C for 1 to 7d, and then respirations rates at 17°C were assessed daily. For non-heated bulbs, respiration rates of the heat-resistant cultivar 'Sevilla' were much higher than 'Apeldoorn'. For heated bulbs, respiration rates generally increased as the length of heat stress increased, and patterns of change were similar for both cultivars. Highest rates of respiration over time were found at 2 or 3 days after removal from heat. Effects of 1-MCP were significant, but depended on the stress level.

INTRODUCTION

Dutch-produced tulip bulbs are shipped by sea freight to worldwide customers from August to December. During the 2 to 5 weeks of transport, bulbs may encounter high stress temperatures that reduce their forcing qualities. The phenomenon of 'heat in transit' or *blindstoken* is well documented and its effects on forcing quality include flower blasting (abortion), anther damage, increased daughter bulbs, shorter shoot lengths, and faster flowering rate (Toyoda and Nishi, 1957; Rees, 1973; Rees and Briggs, 1976). However, the mechanism of heat stress in causing these symptoms such as flower abortion is not well understood. In preliminary work of cultivar screening for heat sensitivity using 4d exposures to 35°C, cultivars that are 'resistant' to heat, having 0% flower abortion, and 'sensitive' cultivars with near 100% flower abortion have been identified (Liou and Miller, unpublished data).

Respiration rates of plant organs increase with increasing temperature and they may be used as an indicator of metabolic activity. Since heat-induced injuries in bulbs are not immediately visible, respiration patterns of heated tissues may indicate the level of metabolic changes that are taking place and may be correlated to the damage that is eventually observed. Also, Rees (1973) suggested that high temperatures can induce elevated endogenous ethylene production by the anthers, which lead to flower damage and abortion. De Munk (1973) reported synergetic effects of ethylene and high storage temperature on ethylene-induced flower abortion. Furthermore, exogenous ethylene application also increased respiration of unheated bulbs (de Wild et al., 2002). During our cultivar screening for heat stress, we also investigated responses of these bulbs to ethylene stress (Liou and Miller, unpublished data). A significant number of cultivars showed similar responses to each stress. Collectively these reports indicate that responses to heat and ethylene stresses may be related; high temperature stress may induce elevated endogenous ethylene production, which results in higher respiration causing damage and flower abortion. If this relationship exists, then pre-heat application of 1-MCP (1-methylcyclopropene) may be able to prevent heat injury in bulbs. 1-MCP blocks the ethylene-binding sites preventing ethylene perception (Sisler and Serek, 1997) and has

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been used extensively on a wide variety of horticultural products (Watkins and Miller, 2004).

We have used ‘Sevilla’ and ‘Apeldoorn’ as representatives of heat resistant and susceptible groups, respectively, to examine the relationship between heat tolerance and respiration. A pre-heat stress application of 1-MCP was included to investigate the role of ethylene in heat-induced respiration.

MATERIALS AND METHODS

Plant Material & Experimental Design

Dutch tulip bulbs size 12+ (*Tulipa gesneriana* L. cvs. Apeldoorn and Sevilla), that had been stored at 17°C were used for these experiments. A complete randomized design with two factors, a pre-heat application of 1-MCP (yes/no) and heat stress (35°C) duration (0 to 7d) were used. There were three replications for each treatment and 5 bulbs per replication. Treatments began on 6 Nov and ended on 20 Nov.

1-MCP Application

1-MCP (SmartFresh™, AgroFresh, Springhouse, PA) was applied at 1 ppm, in 227 liter plastic bins, where the Smart Fresh powder was mixed with distilled water in an Erlenmeyer flask. Immediately after mixing, the bin was covered and sealed. The containers remained closed for 16 hr at 17°C, and the bulbs then removed for heat-treatments.

Heat Stress & Respiration Rate Measurement

Following 1-MCP application, untreated and 1-MCP treated bulbs were exposed to 35°C for 1 to 7d. Control unheated bulbs remained at 17°C. After heat treatment, bulbs were transferred to a ventilated, dark, 17°C chamber. Respiration rates were determined daily by placing each rep of 5 bulbs in a sealed 0.95 liter glass jar, with a septum added to the lid. CO_2 production was measured by removing 1 mL of the headspace atmosphere and injecting into a gas partitioner (Fisher, model 1200, Fisher Scientific, Springfield, NJ).

Statistical Analysis

Data were analyzed with SAS 8.02 (SAS Institute Inc, Cary, NC, USA), with proc GLM to generate ANOVA tables. Comparisons between treatments were made with Tukey’s Studentized Range Test, alpha level = 0.05.

RESULTS

Separate analyses were made for each cultivar because of highly significant interactions between cultivar and heat treatment effects. Average respiration rates were calculated by averaging over all days after removal from heat for each treatment. The relative response ratios were calculated by dividing the maximum respiration rate (usually found at 2d or 3d after removal from heat) by the control.

Post-stress Respiration Patterns

Heat-treated bulbs of both cultivars, whether treated or untreated with 1-MCP, showed similar post-heat stress respiration patterns (Fig. 1). By 1d after removal from heat treatment, respiration rates had increased significantly, but the extent of increase was independent of the length of the heat treatment. Maximum respiration rates generally occurred at 2d post-heat for ‘Apeldoorn’ and 2 or 3d for ‘Sevilla’, depending on the stress level. For both cultivars, the increased respiration rate decreased gradually after the maximum. By the end of the week, only respiration rates of bulbs heated for 1d of both cultivars returned to those of non-heated bulbs while others remained high.

Differences in control (no heat) respiration rates between ‘Apeldoorn’ and ‘Sevilla’ are shown in Table 1. With or without 1-MCP, ‘Sevilla’ had a significantly higher respiration rate than ‘Apeldoorn’.

The Heat-stress Susceptible 'Apeldoorn'

In general, the average and maximum respiration rates as well as the relative response ratios all increased with increasing duration of high temperature stress (Table 2). The highest average respiration was induced by 3, 4 and 7 days of heating in bulbs without 1-MCP treatment and by 4 days in bulbs treated with 1-MCP. Maximum respiration rates were highest in bulbs heat stressed for 3 to 7 days in bulbs treated with and without 1-MCP. Relative response ratios were peaked in 1-MCP bulbs treated with 7 days of heat and in no 1-MCP bulbs treated with 4 days of heat. In general, 1-MCP did not depress the average or maximum respiration rates except those given 3 and 7 days of heat stress. Average and maximum respiration rates of 1-MCP treated bulbs heated for 3d and 7d (average only) were significantly lower than bulbs not treated with 1-MCP. In all heat treatments, the relative response ratios of 1-MCP bulbs appeared to be less than those without 1-MCP.

The Heat-stress Resistant 'Sevilla'

The average, maximum and relative response ratios of 'Sevilla' increased with elevated heat stress, similar to 'Apeldoorn' (Table 3). The highest average respiration rates were induced by 3 to 4 days of heat stress, independent of 1-MCP treatments. In + and – 1-MCP bulbs, respiration maxima were reached with 3 to 4 days and 2 to 4 days of heat stress, respectively. The highest relative response ratios of both 1-MCP treatments were induced by 3 to 4 days of high temperature. When heat-treated for 7d, 'Sevilla' interestingly showed a significant respiration decrease down to the level of 2d heat stress, in its average, maximum respiration rates and relative response ratios. These decreases were not found in 'Apeldoorn'. 1-MCP did not reduce the average or maximum respiration rates except for bulbs treated with 2 to 3d of heat where the average respiration rates were significantly lower in 1-MCP bulbs.

DISCUSSION

Harvest times and storage conditions were similar for 'Sevilla' and 'Apeldoorn', and thus by early October, the flowers of both cultivars were highly developed, well past the G stage (De Hertogh and Le Nard, 1993). Higher basal respiration rates of 'Sevilla' may be an indication of higher metabolism, which may be related to its resistance to high-temperature induced flower abortion (Liou and Miller, unpublished data). Furthermore, the relative response ratios of all treatments of 'Sevilla' were higher than those of 'Apeldoorn', indicating greater response to increasing heat stress. These two properties of 'Sevilla', higher basal respiration rate and greater relative response, may be correlated to its resistance to heat stress. However, the linkage between post heat stress respiration and susceptibility to flower abortion is still unclear and requires further investigation. It is important, to note differences in cultivar response to factors such as heat. In our investigation into heat stress response of tulips, cultivar variations are not only significant, but could become misleading if only one or two cultivars are tested.

The respiration patterns described for 'Apeldoorn' and 'Sevilla' are similar to those of heated hyacinth bulbs (van der Hulst and de Munk, 1992). After 2 wks at 38°C and 3d at 44°C, hyacinth respiration rate initially dropped during the first 6 hours as bulbs were returned to 30°C, followed by a large increase in respiration rate, with maximum rate observed after 60 hr or 2.5d (van der Hulst and de Munk, 1992). In heated tulip bulbs, this delayed respiration peak appeared 2 to 3d after removal from heat (Fig. 1). It was suggested that the hyacinth respiration peak at 2.5d reflects an active metabolic activity that results in membrane repair and the relative CO_2 rise is indicative of degree of reversible damage caused by high temperature (van der Hulst and de Munk, 1992). For heated tulips, the average and maximum respiration rates of both cultivars increased with increasing duration of heat stress. Thus it is possible to infer that for heated tulips, high temperature does cause damage (presence of post-stress respiratory peak) and that increased duration at high temperature promoted additional damage, as reflected by increased respiration averages and maxima. 'Sevilla' bulb heated for 7d presents a dilemma to this argument, because average and maximum respiration rates decreased to the level of bulbs heated for 2d. Reason for this drop is unclear as this has not been reported by others.

In the study by De Wild et al. (2002) exogenous ethylene induced a temporary rise in respiration in ‘Apeldoorn’ bulbs and pre-application of 1-MCP prevented this effect. In our experiment, 1-MCP decreased heat-induced respiration rates for both cultivars but its effect was dependent on the level of heat stress in each cultivar. However, evidence from forcing of ethylene treated ‘Apeldoorn’ and ‘Sevilla’ bulbs showed only ‘Sevilla’ to be insensitive to ethylene (Liou and Miller, unpublished data). Thus further investigation is needed to understand how 1-MCP, an ethylene perception inhibitor, could reduce respiration of heated bulbs and the role/source of ethylene in heated bulbs.

CONCLUSIONS

In this experiment, we have shown that tulips bulbs given a heat stress (35°C) for up to 4d, have higher respiration rates. Bulbs treated with 7d of 35°C either respired less or equally as the level at 4d of heat, depending on the cultivar. Given that respiration rate has been suggested to have a strong correlation with the relative amount of reversible damage, respiration rate may possibly be used as a short-term indicator of heat damage. Post heat stress respiration patterns were similar for heat sensitive and resistant cultivars of tulip. Significant differences were found between respiration rates for the heat sensitive and insensitive cultivar, which highlights the importance of cultivar variations. 1-MCP was effective in reducing heat-induced respiration in both cultivars, but dependent on stress level. Thus one cannot rule out role of ethylene in heat-induced respiration response. However, understanding the importance and mechanism of the role of ethylene in the heat response requires further work.

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Tables

Table 1. Control respiration rates (ml CO_2/kg FW/hr) of 'Apeldoorn' and 'Sevilla' where bulbs remained at 17°C. Averages represents 6 day measurements with 3 rep each.

	Without MCP (ml CO_2/kg FW/hr)[1]	With MCP (ml CO_2/kg FW/hr)
'Apeldoorn'	7.3[a]	8.1[a]
'Sevilla'	18.6[c]	15.3[b]

[1]Data with a letter in common are not significantly different at p=0.05%, using Tukey's Studentized Range Test

Table 2. Average and maximum respiration rates (ml CO_2/kg FW/hr) of 'Apeldoorn' bulbs heated for 1-7d at 35°C.

Days at 35°C (days)	Average Respiration Rate[1] (ml CO_2/kg FW/hr)		Maximum Respiration Rate[1] (ml CO_2/kg FW/hr)		Relative Response Ratio[2]	
	– MCP	+ MCP	– MCP	+ MCP	– MCP	+ MCP
Control	7.3[a]	8.1[b]	9.6[a]	9.9[a]	–	–
1	12.2[cd]	10.3[bc]	15.8[abc]	13.4[ab]	1.7	1.4
2	13.3[de]	12.0[cd]	20.9[cdef]	18.7[bcd]	2.2	1.9
3	18.4[f]	14.4[de]	27.0[fg]	19.8[cde]	2.8	2.0
4	20.7[f]	19.2[f]	27.0[fg]	25.1[efg]	2.8	2.5
7	18.2[f]	15.5[e]	28.7[g]	24.9[defg]	3.0	2.5

[1]Data with a letter in common are not significantly different at p=0.05%, using Tukey's Studentized Range Test
[2]Relative response ratios are calculated by dividing maximum respiration rate by control

Table 3. Average and maximum respiration rates (ml CO_2/kg FW/hr) of 'Sevilla' bulbs heated for 1-7d at 35°C.

Days at 35°C (days)	Average Respiration Rate[1] (ml CO_2/kg FW/hr)		Maximum Respiration Rate[1] (ml CO_2/kg FW/hr)		Relative Response Ratio[2]	
	– MCP	+ MCP	– MCP	+ MCP	– MCP	+ MCP
Control	18.6[a]	15.3[a]	22.9[b]	17.7[a]	–	–
1	30.1[cd]	30.3[cd]	40.3[c]	40.2[c]	2.5	2.8
2	37.8[e]	33.4[d]	49.3[de]	41.3[cd]	3.3	3.1
3	45.7[g]	40.3[ef]	52.4[e]	52.3[e]	3.5	3.9
4	42.7[fg]	43.7[fg]	52.2[e]	49.9[e]	4.5	3.7
7	25.5[b]	28.0[bc]	35.3[c]	39.2[c]	2.3	2.9

[1]Data with a letter in common are not significantly different at p=0.05%, using Tukey's Studentized Range Test
[2]Relative response ratios are calculated by dividing maximum respiration rate by control

Figures

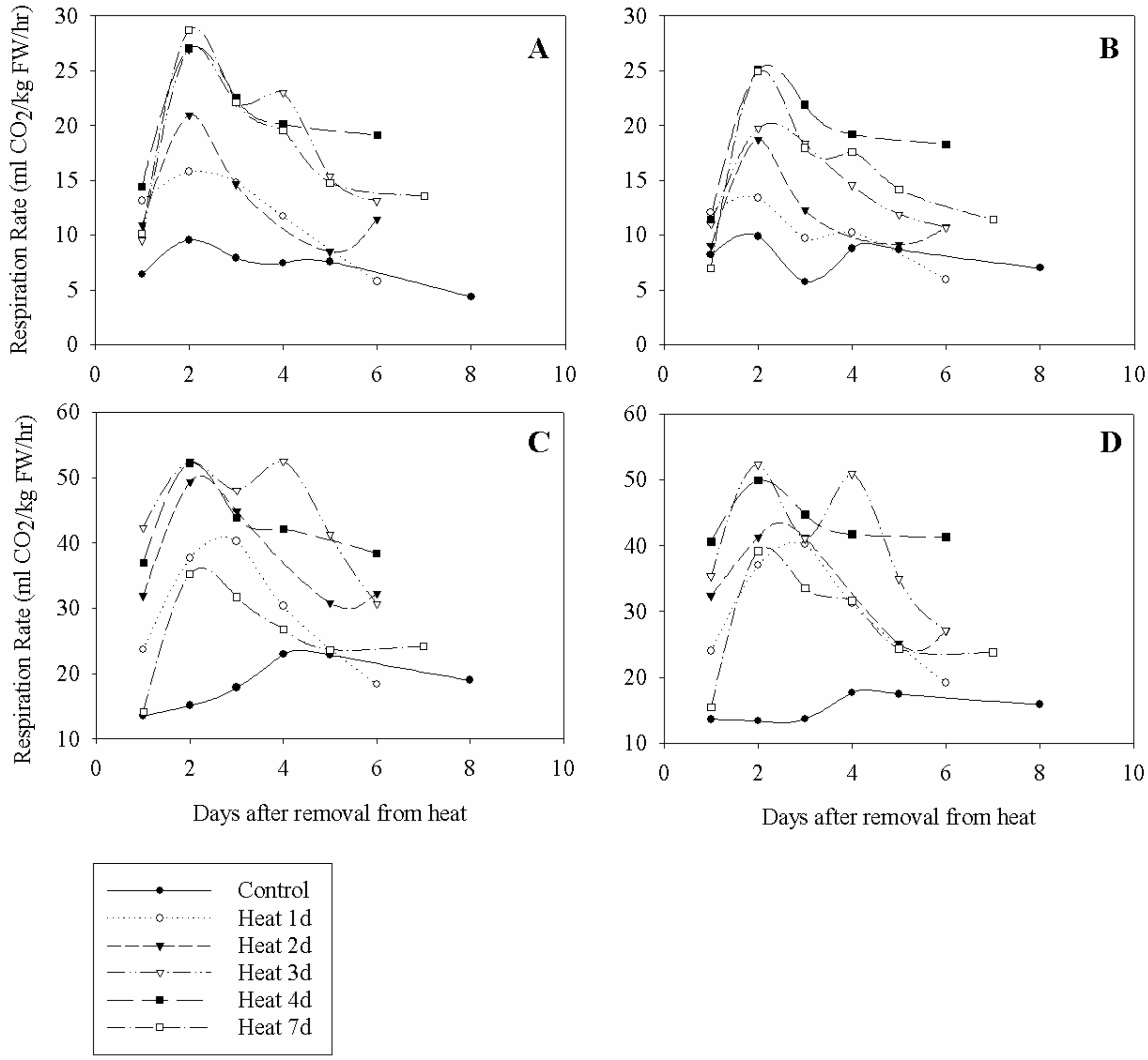

Fig. 1. Respiration patterns of ‘Apeldoorn’ and ‘Sevilla’ bulbs after 0-7d at 35°C. A and C: ‘Apeldoorn’ and ‘Sevilla’ bulbs not treated with 1-MCP, respectively. B and D: ‘Apeldoorn’ and ‘Sevilla’ bulbs pretreated with 1 ppm of 1-MCP, respectively. Each respiration rate (ml CO_2/kg FW/hr) represents the average of 3 reps of 5 bulbs each.