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Lowered Berry Quality due to Heat Stress at the Early Ripening Stage of Berry Growth in a Seeded Grapevine, *Vitis vinifera* L.

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Experimental Farm

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SUMMARY

1. Napa Gamay grapevines at the early ripening stage in uncooled pots were subjected to heat stress of 43℃ / 28℃ day and night temperatures inside a phytotron for 4 days. They yielded damaged clusters with shrivelled berries by heat stress. Those of cooled pots produced clusters with mostly of sound berries and some shrivelled ones. However, these sound berries were small in both berry size and weight and low in total soluble solids and acidity in comparison with non-stressed vines.
2. Heat stress for 8 days at 40℃ during the same period as above did not damage berries but decreased their size, glucose and fructose contents, acidity and malate contents. These decrements were dependent on the maturity of berries exposed to heat stress; green berries were more affected than colored ones.
3. Stomatal conductance of the stressed leaves was low during the stress, and then recovered to the initial level when returned to the green house at optimal temperatures.

INTRODUCTION

It is well known that hot seasons hasten the ripening of berries with low sugar and acid contents[1]. Forcing production of grapes in the structured conditions helps to raise farmer's income but may involve such incidents as encountering hot spell by unexpected accidents. Leaf temperatures are usually higher than air temperatures, especially on fine days and high humidity. The summer season meets these conditions.

Heat stress on grape vines bearing young berries decreased berry weight and soluble solids[2,3]. The stress near the ripening stage may have a more direct effect on berry quality than at the early development stage, since marked decreases in acids and increases in sugars occur at this time onward[1]. This study aims to clarify the effect of different stress time and the maturity of berries at the early ripening stage on berry quality in relation to changes in sugar and acid contents during ripening. Also, stomatal function was studied in related to assimilation and translocation was studied.

MATERIALS AND METHODS

Heat stress treatment: Napa Gamay grapevines of uniform size were grown in a glass house at day and night temperatures of 28℃ and 23℃, respectively. They were pruned to two shoots; each

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shoot had an inflorescence. Six vines were transferred to a phytotron maintained at 43℃ and 22℃ (day and night temperatures) and kept there from 14th April to 17th, when the berries were at the beginning of the ripening stage (veraison) (Preliminary experiment). On the second day from the start of heat stress, green pedicels became brown by sunlight. In this experiment pots were not cooled.

On 13th May six other vines were transferred again to the phytotron. Maximum soil temperatures reached 28℃ during the treatment, even though the pots were kept cool by running cold water through a coiled hose between the pots and an insulatin mat (Experiment 1).

Six other vines were exposed to 40℃ from 13th June to 20th (Experiment 2). From clusters which bloomed on 18th and 19th April, green, pink to red (red berries) and dark red (almost black) berries were chosen to clarify the difference in berry quality in terms of their age (maturity). In every experiment non-stressed (control) vines were grown in a controlled glasshouse for comparison with the heat-stressed.

Berry size, soluble solids and sugar contents, titratable acids and malate contents: Diameters of tagged berries on each vine were measured at intervals of 7 to 10 days from shortly after full bloom until harvest. In Experiment 1 berries were sampled at random from each vine on 12th, 17th May, 7th and 21th June (ripening stage), and weighed. Then soluble solid contents were determined with a refractometer, while titratable acidity was ascertained by titrating an aliquot of juice with a 0.1 N NaOH solution. Individual sugars contained in berries were determined by gas chromatography after silylation[4]. Malate content was determined enzymatically according to Kliewer's method[5].

Stomatal conductance measurement: Stomatal conductance of the abaxial surface of leaves at the node bearing clusters was measured with a diffusion porometer (Model Li 1600, Licor Inc.). Readings were taken every other day between 13:00 and 14:00. In addition, diurnal changes in stomatal conductance were determined at an interval of one hour from 6:00 to 20:00 before (19th June), during (20th June) and after (6th July) heat stress.

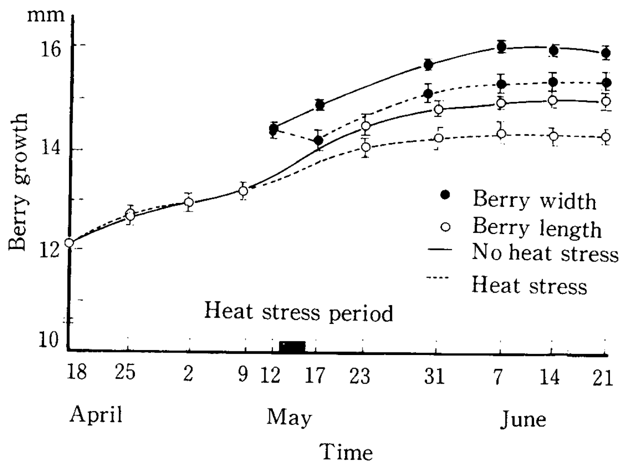

Fig. 1. Growth curves based on length and width of Napa Gamay berries on grapevines heat-stressed at the veraison stage compared non-stressed grapevines.
Bars represent SD at P : 0.05

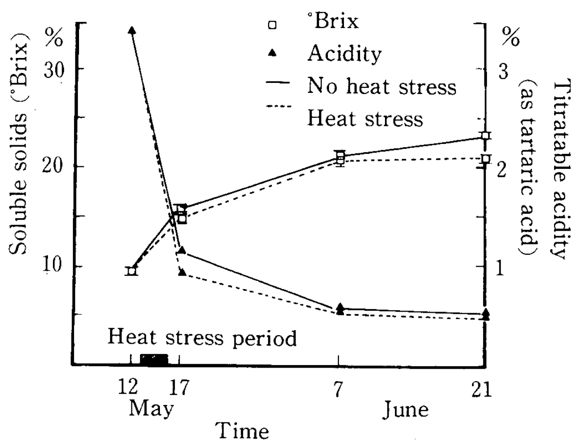

Fig. 2. Seasonal changes in titratable acidity and total soluble solids contents of Napa Gamay berries after heat stress.
Bars represent SD at P : 0.05.

RESULTS

Sunburn of cluster and berry growth

In the preliminary heat-stress experiment of the grapevines at 43℃ in uncooled pots, berries on all of them were shrivelled after 2 days and rachises of clusters were injured by heat. When pots were cooled in Experiment 1, all clusters of two vines were injured by heat. However, most clusters of the other vines remained and mainly produced sound berries and a few injured ones. In spite of the 8-day

Table 1. Effect of 8-day heat stress at the beginning stage of ripening (veraison) on increments of berry size and total soluble solids (Brix) in Napa Gamay grapevines.

Berry Maturity	Increment of berry width (cm)			Brix (%)			
	Green[z]	Red[z]	Black[z]	Green[z]	Red[z]	Black[z]	Parth.[z]
No heat stress (Control, C)	1.73	1.50	.92	23.4	24.4	25.0	24.0
Heat stress (H)	2.08	.51	.03	20.7	22.2	23.0	21.3
H/C (%)	120.1	34.2	3.1	88.5	91.0	92.0	88.8

Analysis of variance

	d.f.	m.s,	F_o	d.f.	m.s.	F_o
Heat stress	1	2.341	29.3**	1	6759.25	283.8**
Maturity	2	3.988	49.9**	3	866.69	36.4**
Interaction	2	3.925	49.1**	3	657.51	27.6**
Error	30	.079		40	23.81	

z Green, Red and Black indicate the respective skin color of the berries stressed at the treatment. Parth. indicates parthenocarpic berries.
* * Significant at the 1 % level.

Table 2. Effect of 8-day heat stress at the beginning stage of ripening on glucose and fructose contents in berry juice in Napa grapevines.

Berry maturity	Glucose (%)			Fructose (%)			Total (%)		
	Control	Heat	H/C	Control	Heat	H/C	Control	Heat	H/C
Green[z]	14.1	13.9	99.0	11.0	9.3	84.7	25.1	23.3	92.8
Red[z]	14.9	14.8	99.2	11.5	9.6	84.1	26.4	24.4	92.6
Black[z]	16.2	15.1	93.4	11.8	10.3	88.1	28.0	25.5	91.1
Parth[z]	15.1	13.6	90.1	11.3	9.0	80.1	26.5	22.7	85.8

Analysis of variance

	d.f.	m.s.	F_o	m.s.	F_o	m.s.	F_o
Heat stress	1	5.95	8.09*	38.62	167.6 **	74.90	50.8**
Maturity	3	6.20	8.42*	2.24	9.75**	15.80	10.7**
Interaction	3	1.42	1.93	.36	1.59	2.32	1.58
Error	40	.73		.23		1.47	

z The same as in Table 1.
* Significant at the 5% level.
** Significant at the 1% level.

Table 3. Effects of 8-day heat stress at the beginning stage of ripening on acidity of berry juice in Napa Gamay grapevines.

Berry Maturity	Titratable acids[z] (mg/100ml)				Malate (mg/100ml)			
	Green[y]	Red[y]	Black[y]	Parth.[y]	Green[y]	Red[y]	Black[y]	Parth[y]
Control (C)	875	801	716	892	399	439	553	314
Heat stress(H)	580	665	670	742	347	311	253	224
H/C (%)	66.3	83.0	93.5	83.1	86.9	70.7	45.8	71.3

Analysis of variance

	d.f.	m.s.	F_0	d.f.	m.s.	F_0
Heat stress	1	295788	98.0**	1.	244245	98.5**
Maturity	3	33414	11.0**	3	41919	16.9**
Interaction	3	32981	10.9	3	35766	14.4**
Error	40	3018		40	2479	

z As tartaric acid

y The same as in Table 1.

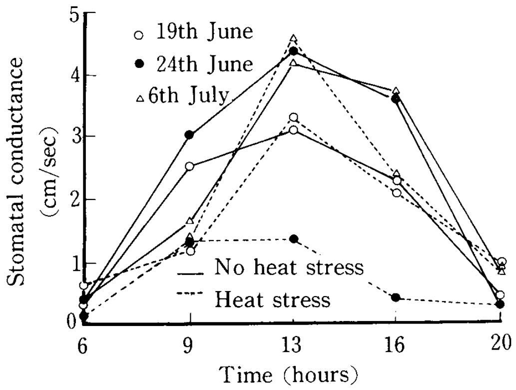

Fig. 3. Diurnal fluctuation of stomatal conductance of Napa Gamay grapevines before (○), during (●), and after(△) heat stress compared with non-stressed grapevines.

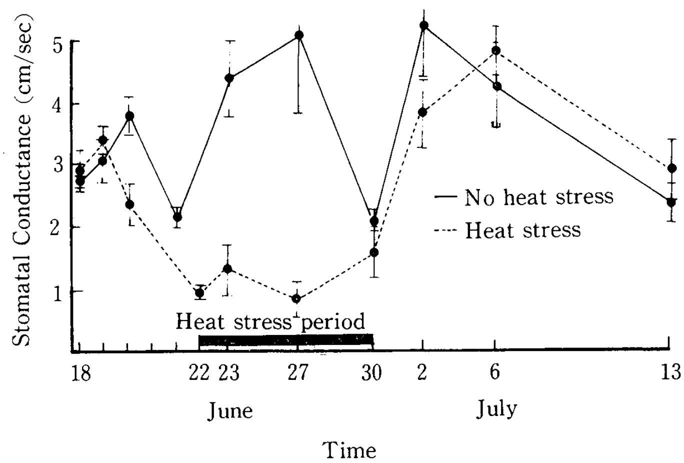

Fig. 4. Recovery of stomatal conductance in stressed grapevines after heat stress treatment. Bars represent SD at P : 0.05.

heat stress period, other vines in Experiment 2 showed no apparent damage since the intended air temperature for heat stress was low (40℃).

Heat stress of 4 days retarded the rapid growth after veraison(Fig. 1) and reduced berry weight (1.97g) at harvest compared with non-stressed vines (2.15 g). Growth inhibition was still observed by the 8-day stress treatment at 40℃ in spite of no visual damage (Table 1).

Berry quality

Total soluble solids of berries on 4-day stressed vines were lower at every measurement time than those of the control (Fig. 2). Heat stress of 8 days decreased significantly fructose and glucose contents in juice of seeded and parthenocarpic seedless berries, although the latter berries showed the lowest levels (Table 2) in accordance with soluble solid contents (Table 1). Table 2 also indicates that sugar contents were dependent on berry maturity at the stressed time. Fructose accumulation was more retarded than glucose, causing elavations in the ratios of glucose to fructose.

Acidity decreased significantly just after 4-day heat stress but not significantly at harvest time (Fig. 2). Decrease in acidity was marked by 8-day heat stress; green berries gave the lowest acidity. Malate contents were also lower in the stressed berries than those of the non-stressed, although green berries contained the most (Table 3).

Stomatal conductance

Stomatal conductance was low at dawn and high at noon, followed by gradual decreases till sunset. This diurnal fluctuation was found even in the vines before, during and after stress; during heat stress the stomatal conductance values were markedly lower than before and after stress(Fig. 3). Fig. 4 also shows that lowered stomatal conductance of the stressed vines recovered to the initial levels 2 days after stress.

DISCUSSION

Sunburn is a popular injury occurring in grapevines subjected to high temperature. Kobayashi[6] reported the same injury of berries kept in an incubator at 42℃ and its shortened incidence time and increased rates with increasing temperature. Compared with results from Experiment 1 ans 2, heat stress at 43℃ for 4 days was very harmful, but that of 40℃ for 8 days was not so severe. In the case of Napa Gamay, the critical temperature for visible injury seems to range between 40 and 43℃. Soil temperatures around roots affected the severity of the injury. The age of berries also affected the severity as shown in Table 1 in which berries with increasing maturity became subject to stress and were more sensitive than stressed immature berries with no visual damage[2].

It is obvious that, even with no visual damage, exposure to high temperatures of a given duration reduced growth of not only nearly mature berries but also immature ones on Napa Gamay[2] and Thompson Seedless[3] grapevines, which showed decreased levels of soluble solids and titratable acids as well. Thus, heat summation markedly influences rates of developmental changes in sugar accumulation and decreases in acidity. Kliewer et al[17] reported that Cardinal and Pinot Noir grapevines grown at 30℃ from one or two weeks before veraison decreased berry weight, total acidity and malate contents compared with those at 20℃. Decreased soluble solids and total acidity in Tokay grapevines grown in 35℃ were also found[8]. High night temperatures are also inhibitory. Kobayashi et al[9] reported reduced berry weights of Muscat of Alexandria grapevines accompanied with reduced soluble solids and titratable acids.

Heat stress during stage II also decreased fructose, glucose and malate contents. Decreased amounts of these sugars were also found in Chardonnay grapevines by Sepulveda et al[10]. More decreases in fructose than in glucose might be attributed to its lowered translocation from leaves. This might be partially evidenced by findings of Kliewer that relative amounts of 14C in glucose from green berries near the early ripening stage were greatly increased at high temperature (37℃), whereas fructose had corresponding decreases in acidity.

The highest amounts of malate and tartrate at the veraison period decreased rapidly during ripening, depending on air temperature and varieties; high temperatures decreased both acids[7,8] and the decrements of malate were greater than that of tartrate[12]. As shown in Table 3, heat stress also decreases malate contents. No translocation of acids during the ripening stage[11] suggests that the translocated acids from leaves at the young berry stage could be respired more in heat-stressed berries.

Possible factors explaining the lower berry quality are : 1) The decreased photosynthetic activity of leaves. 2) The changed sink−source relationship mediated by plant hormones. Recovery of stomatal conductance after the stress period indicates that the photosynthetic activity of leaves was not damaged by heat. Sepulveda et al[13] found from their results of administration of 14C to leaves that heat stress neither damaged the photosynthetic apparatus nor changed the photosynthate translocation. However, it affected the distribution patterns of 14C photosynthates in grapevines, suggesting a changing sink−source relationship between plant parts. Matsui et al[2,3] found lowered levels of gibberellin-like activity in young heat-stressed berries and their partial recovery of berry growth by application of gibberellin. Thus, changing the sink—source relationship might be the most important in the factors due to heat stress.

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果実成熟初期の熱ストレスによるブドウ有核品種（*Vitis vinifera* L.）の品質低下

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（1991年7月20日受理）

要　　約

1．果実成熟初期に Napa Gamay 品種を昼間43℃夜間28℃のファイトトロンに持ち込み，4日間熱ストレスを与えた。ポットを冷却しなかった場合，すべての果房は日焼けを生じ，果粒は萎縮した。しかし，ポットを冷却すると，いくらか萎縮果粒もあったが外見上正常な果房を着けた。しかし，このような房の健全な果粒でも熱ストレスを与えないものに比べて，果粒径，果粒重，固形物含量や酸度は低下した。

2．同様に，40℃で8日間熱ストレスを与えた。この場合も，外見上の障害はなかったが，果粒径，グルコーズとフラクトーズ含量，酸度やリンゴ酸含量は減少した。その程度は熱ストレスを受けるときの果粒の熟度に影響され，緑熟果粒がもっとも感受性大であった。

3．気孔の拡散抵抗を測定し，熱ストレス前後の葉の蒸散を調査した。ストレスを与えている間は気孔の蒸散は押さえられたが，温室にもどすと処理前の状態に回復した。

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