Effects of heat stress and plant hormone treatments on growth of young Napa Camay (Vitis vinifera L.) berries

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Effects of heat stress and plant hormone treatments on growth of young Napa Gamay (*Vitis vinifera* L.) berries

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SUMMARY

Greenhouse-grown Napa Gamay grapevines, when transferred to phytotron maintained at 43℃ (day) and 22℃ (night) for 4 days (heat-stressed) during the first growth stage, yielded berries which were smaller in size and contained less total soluble solids than those on non-stressed vines but equal titratable acid contents. Heat-stressed berries sprayed with gibberellic acid (GA) grew to near normal size, but those treated with benzyladenine (BA) did not overcome heat stress. Bioassays of stressed and non-stressed, untreated berries revealed that the former contained less gibberellin-like substances than the latter.

Introduction

It is reported that the California 'hot spell', a period with high of temperatures over 38℃, lasts for 4 to 6 days. In Japan, however, the pervasion of forcing production of grapes under the structure contributes to increases in farmers' incomes but it involves the possibility of vines encountering high temperatures by unexpected accidents.

When grapevines are exposed to high temperatures during the growing season, berry growth[1)2)3)], anthocyanin formation[4)], and soluble solid accumulation[1)3)5)] are retarded. Consequently, the harvest is delayed and grapes do not reach the desirable dessert flavor or the sugar : acid ratio for attaining optimum wine quality[6)]. Berry growth inhibition may be attributed to 1) lack of import of photosynthates from wilted leaves resulting from stomatal closure as a consequence of increased abscisic acid[7)] or decrease in cytokinin content of leaves[8)]. Applications of hormones, especially gibberellins, are known to enhance berry size[9)]. In this study the hypothesis that heat stress upsets hormonal balance was tested by 1) analyzing stressed and non-stressed berries for gibberellin-like substances and 2) spraying heat stressed clusters with gibberellin (GA) or benzyladenine (BA) to see if growth inhibition can be overcome.

Materials and Methods

Heat stress and hormone treatments.

Twelve 4-year-old potted Napa Gamay vines of uniform size were grown under glass at 28℃/23°C day/night temperature. They were pruned to 3 shoots ; each shoot had an inflorescence which bloomed in mid-April.

Six vines were transferred to a phytotron kept at 43℃/22℃ day/night temperatures for 4 days from 13 May to 16 when the berries were in stage I of development. The pots were wrapped with insulation material and a coil of vinyl tubing with cold running water. A comparable number of vines was kept in the greenhouse (non-stressed). Two days after stress treatment one cluster on each vine was sprayed with 20 ppm of gibberellic acid (GA), 20 ppm benzyladenine (BA), or water (control). Three clusters on non-stressed vines were given the same treatment.

Size, soluble solid content, titratable acidity, sugar content, malate content and gibberellin determinations.

Diameters on individually marked and tagged berries on each vine were measured at 7- to 10-day intervals shortly after full bloom and until harvest. Berries were sampled at random from each vine on 4 sampling dates: 17 May, 30 May, 13 June and 19 July. The sampled berries were sorted into seeded and seedless lots, and each lot was weighed and analyzed for soluble solid content with a refractometer and for titratable acidity by neutralizing an aliquot of juice with a standard NaOH solution. The soluble solid content and titratable acidity are expressed as degree Brix and as percent acid. Individual sugars in these samples were determined by gas chromatography (Varian Aerograph, Model 1400, equipped with a 150 cm × 3.1 mm ss column packed with 3.8 % SE 30) after silylation according to Sweeley et al[10]. Malate content was determined enzymatically according to Kliewer[11].

Ethanolic extracts of stressed and non-stressed berries were developed by paper chromatography and the chromatograms analyzed for alpha-amylase enhancement using the modified barley endosperm method according to Ryugo et al.[12]

Results

Berry growth

In the phytotron the air temperature rose after 8: 00 in the morning when the stress treatment started and reached the maximum at 14: 00, followed by a rapid decrease from 21: 00. The soil temperature reached 32°C during the day even though the pots were cooled with running water, while

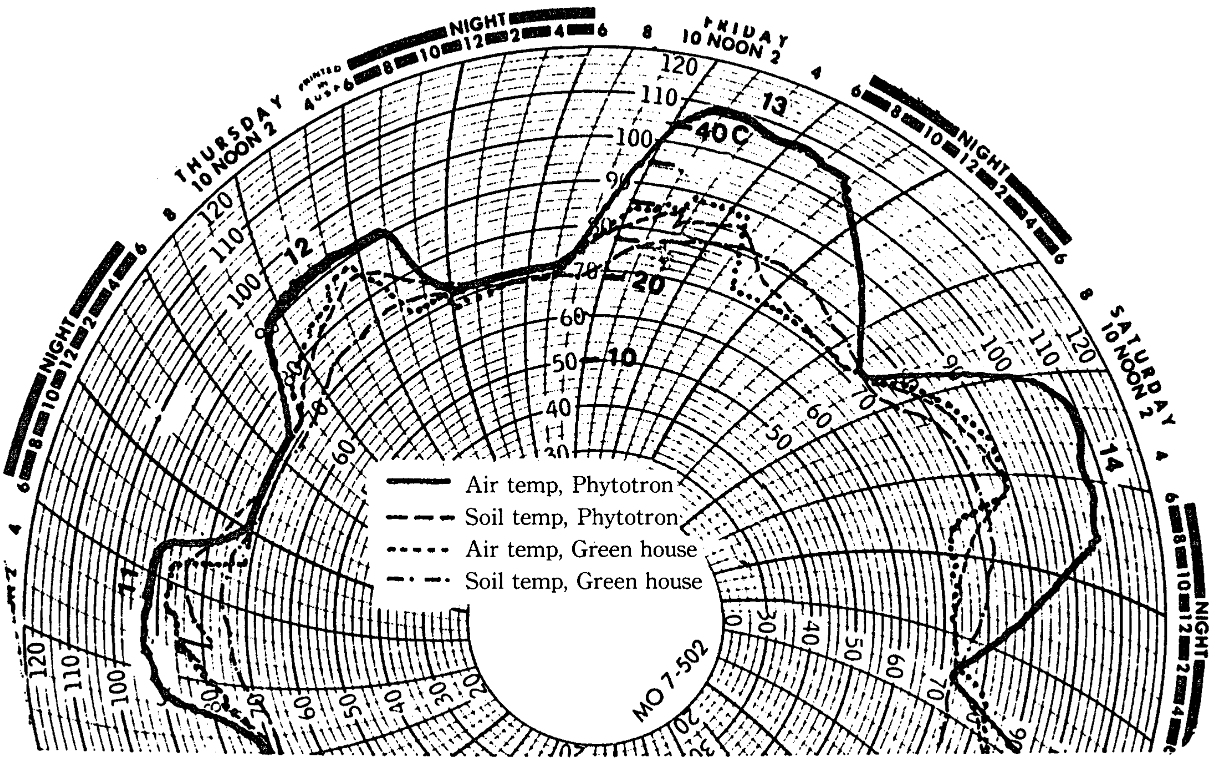

Fig. 1. Daily changes in air and soil temperature of the phytotron and the greenhouse used before and during heat stress.

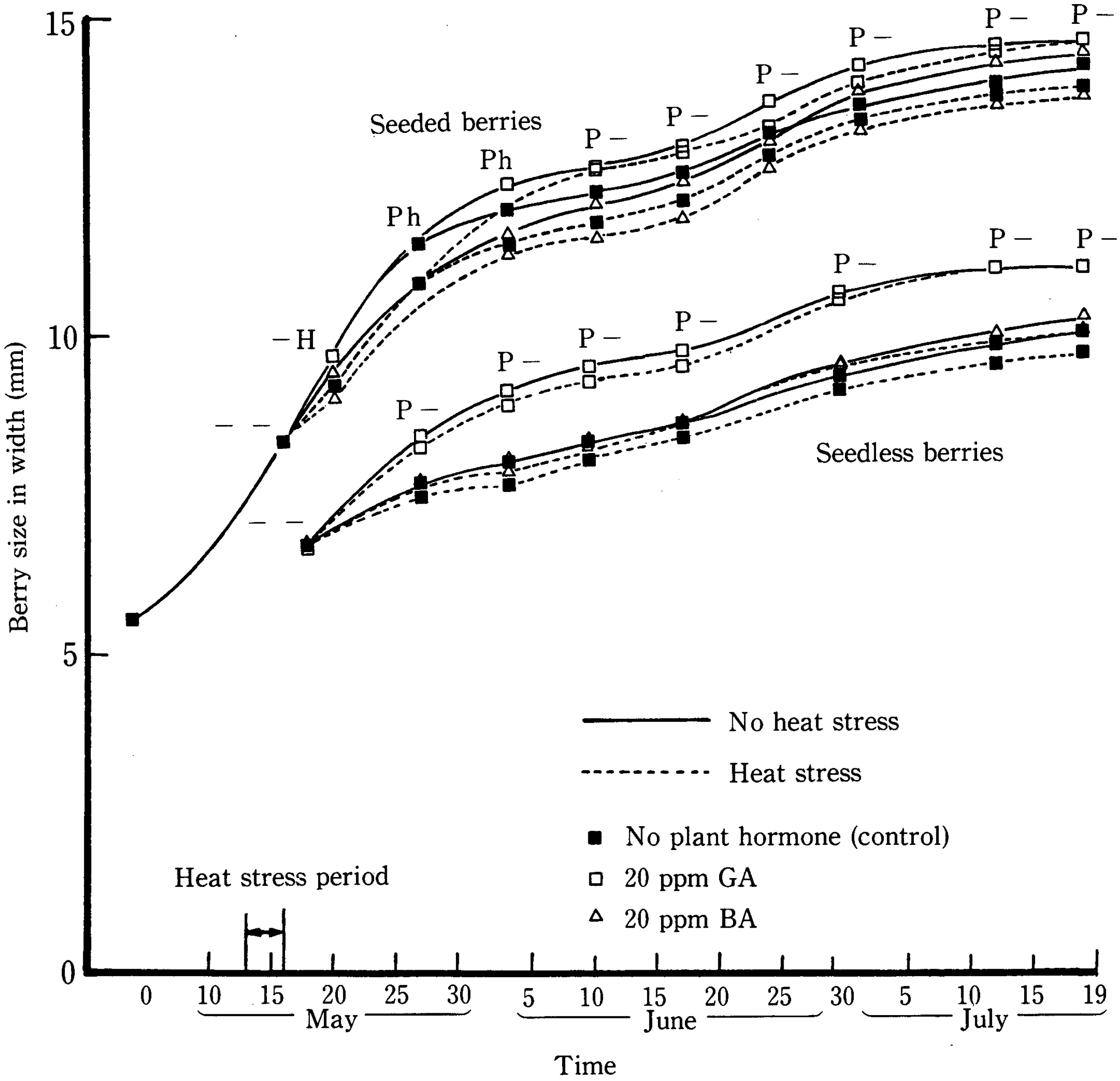

Fig. 2. Seeded and parthenocarpic berry growth after heat stress and the recovery of their growth with applications of BA and GA.
—, no significant; P, significantly different at the 1 % level with plant hormone treatments; p, at the 5 % level; H, at the 1 % level with heat stress; h, at the 5 % level.

during the night it fell below 25°C (Fig. 1).

Heat stress during stage I of berry development caused no visible injury but resulted in growth inhibition of seeded berries during the further growth of stage I after the treatment (Fig. 2 and Table 1). At harvest time significant differences were found between heat-stressed and non-stressed berries (Table 2). GA spray resulted in increased size of not only seedless but also seeded berries. Thus, it compensates for the retarded growth of the stressed berries. On the other hand, BA had no influence on reversing growth inhibition of stressed or non-stressed seeded or seedless berries. (Fig. 2, Table 1 and Table 2).

GA treatment resulted in stressed berries having a smaller length : width ratio than unstressed

Table 1. Changes in berry weight, total soluble solids and titratable acids in berry juice after treatment with and without heat stress and plant hormones.

	Berry weight (mg)			Total soluble solids (%)			Titratable acids (%)		
	Control	GA	BA	Control	GA	BA	Control	GA	BA
17 May Seeded			37.4						
No heat stress	64.1±3.6[z]			4.12±0.10			3.42		
Heart stress	56.9±2.0			4.51±0.08			3.24		
Seedless									
No heat stress	14.5±0.5			4.46±0.09			3.17		
Heat stress	14.7±0.5			5.10±0.05			3.37		
30 May Seedless									
No heat stress	36.9±1.6	47.8±2.0	37.4±1.7	5.14±0.09	4.66±0.09	5.01±0.03	3.78	3.59	3.73
Heat stress	31.3±1.3	43.0±1.6	29.1±1.4	5.48±0.04	5.48±0.04	5.31±0.12	3.98	3.57	3.94
13 June Seeded									
No heat stress	136.8±4.6	128.6±4.5	109.2±4.4	6.16±0.21	6.49±0.30	5.86±0.31	4.04	4.28	4.30
Heat stress	105.7±2.9	123.1±2.7	104.5±2.9	5.64±0.22	5.61±0.15	5.63±0.30	4.03	4.25	4.30

z ±S.D.

Table 2. Effect of 4 day heat stress and plant hormone application at the first growth stage on berry quality at harvest.

	Berry weight (g)				Total soluble solids (%)				Titratable acids (%)			
	No heat stress		Heat stress		No heat stress		Heat stress		No heat stress		Heat stress	
	Seed-ed	Seed-less	Seed-ed	Seed-less	Seed-ed	Seed-less	Seed-ed	Seed-less	Seed-	Seed-less	Seed-ed	Seed-less
Control	1.96	0.82	1.85	0.81	23.4	22.9	22.0	21.6	0.910	1.046	0.911	1.035
GA	2.37	1.18	2.06	1.10	22.5	21.8	21.2	20.7	0.907	1.036	0.988	1.073
BA	2.01	0.98	1.73	0.81	22.8	22.0	21.4	21.1	0.908	1.002	0.896	0.981

Analysis of variance

	d.f.	m.s.	F	D[z]	d.f.	m.s.	F	D[z]	d.f.	m.s.	F	D[z]
Heat stress	1	0.464	7.87**	0.12	1	28.00	54.47**	0.33	1	2.73×10^{-3}	0.44	
Plant hormone	2	0.744	12.63**	0.19	2	5.48	10.66**	0.50	2	17.651	2.86	
Seeded seedless	1	19.803	366.2**	0.12	1	4.96	9.64**	0.33	1	213.2	34.55**	0.037
Interaction									HxP2	9.897	1.60	
Error	67	0.0589			67	0.51			65	6.170		

z, Significant difference at the 5 % level.
**, Significantly different at the 1 % level.

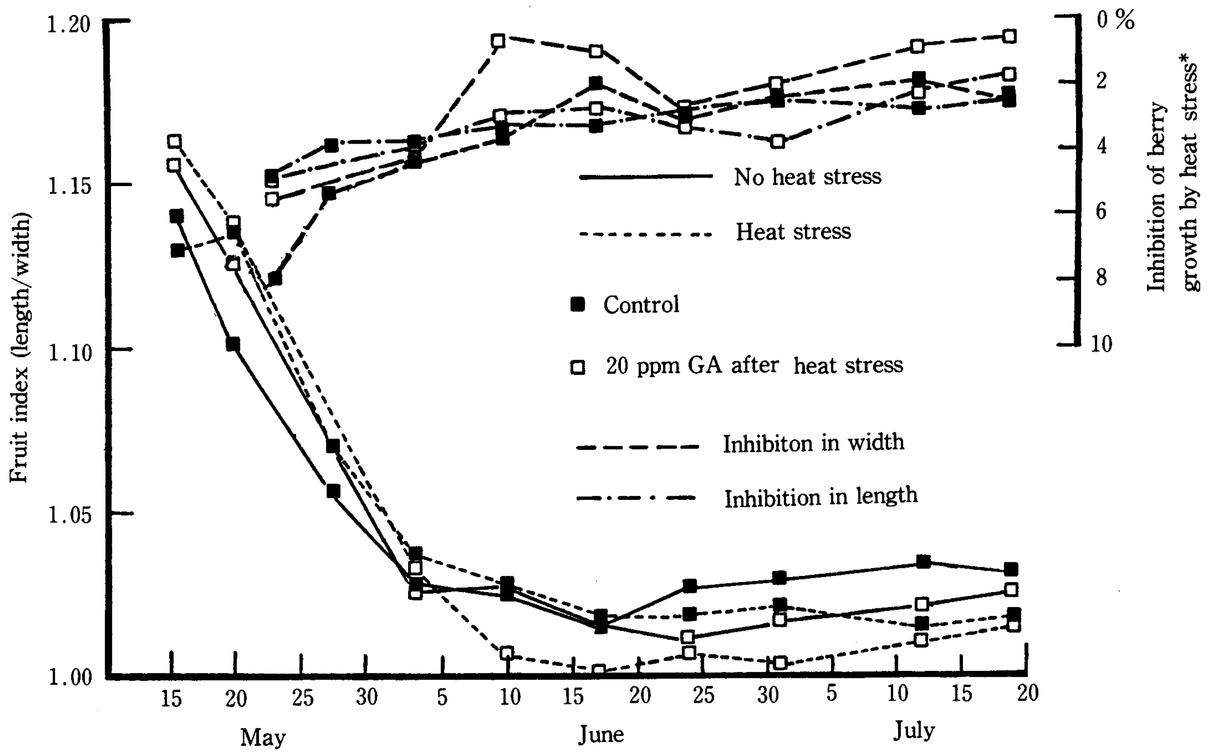

Fig. 3. Changes in berry shape and inhibition of vertical and longitudinal growth of berries after heat stress and plant hormone applications.

$$* \frac{\text{Growth of stressed berry at Ti} - \text{That of 16 May}}{\text{Growth of control berry at Ti} - \text{That of 16 May}}$$

control berries, and this ratio could be attributed to decreased inhibition in growth of berry width (Fig. 3).

Soluble solids content and titratable acidity

Heat stress increased the % Brix of both seeded and seedless immature berries after the stress but decreased it at the ripening. GA and BA treatments reduced it as well (Table 1). The constituents of sugars in ripened fruits were mainly fructose and glucose with a trace of sucrose. Heat stress reduced the levels of these sugars in seeded berries. GA-applied berries contained less fructose and more glucose than those of the control. Heat stress had no effect on titratable acidity through whole growth stage (Table 1). Heat-stressed berries contained less malate than non-stressed ones (Table 3).

Gibberellin-like substances in berries

Assays of heat-stressed and non-stressed Napa Gamay berries for gibberellin-like substances revealed that exposure to heat reduced the native GA content below that of comparable berries that were not heat-stressed (Fig. 4).

Discussion

Experiments with Napa Gamay vines revealed that a) immature grapes in stage I can tolerate high temperatures more than at onset of veraison[13], and b) vines tolerate heat stress better if the roots were cooled than if they were also stressed like the top[13]. These observations suggest that high respiratory activity in leaves, berries and roots is a factor in this phenomenon. There is also a varietal difference of heat injuries: Thompson Seedless gapevines caused shrivelling of berries with leaf chlorosis by heat stress during stage I of berry development[3]. Thus, as temperatures are raised above

Table 3. Effect of 4 day heat stress and plant hormone application at first growth stage on sugar and malate contents in berry juice.

	No heat stress		Heat stress	
	Seeded	Seedless	Seeded	Seedless
Fructose (%)				
Control	12.75	11.85	12.33	11.65
GA	12.24	10.66	11.74	11.23
BA	12.85	10.12	10.96	10.44
Glucose (%)				
Control	13.88	13.11	13.57	11.16
GA	14.80	11.30	14.07	12.29
BA	14.44	10.34	13.38	12.05
Total sugar (%)				
Control	26.15	24.69	25.90	22.81
GA	27.04	21.96	25.81	23.52
BA	27.29	20.46	24.34	22.49
Malate (%)				
Control	0.384	0.427	0.331	0.427
GA	0.431	0.474	0.469	0.484
BA	0.384	0.481	0.282	0.417

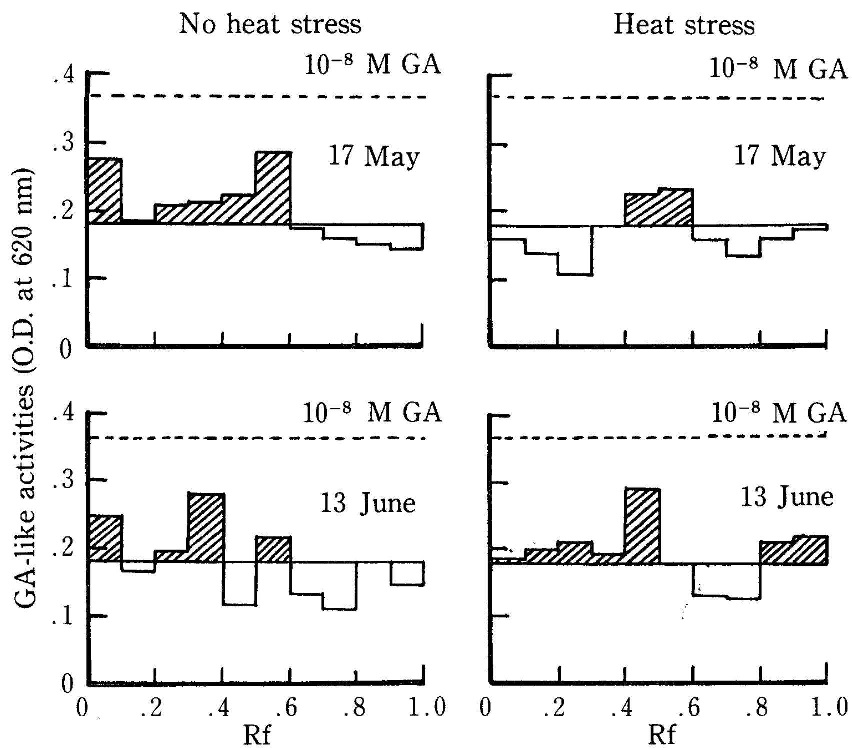

Fig. 4. Reduced GA activities in berry flesh after heat stress
Solvent : iso-propānol-ammonium water-water (10 : 1 : 1v/v)

40°C, injuries appears sooner in many varieties[1]. The threshold temperature range for visible symptoms to appear is between 40°C and 43°C. These temperatures are about 10°C higher than that for optimum photosynthesis, so this process is impaired long before heat stress symptoms appear[14].

Comparisons between cumulative growth curves of berries on heat stressed and non-stressed vines in this study reveal that stress resulted in growth retardation. Heat-stressed berries were more spherical than unstressed ones. Stressed Napa Gamay berries also had a smaller length : width ratio than unstressed ones ; GA treatment of stressed berries resulted in nearly round berries (Fig. 3). This can be explained as follows ; stress is inhibitory more to latitudinal growth and GA application can relieve the inhibition.

Kliewer et al[2]. reported that Cardinal and Pinot Noir vines grown at 30°C had berries lower in weight, titratable acidity and malate contents, but the soluble solid content was the same as in those grown at 20°C. With Tokay grapes, soluble solid contents were decreased with high temperature[5]. High night temperature also reduced berry weight, total soluble solids and free acids in Muscat of Alexandria grapes[1]. Thus, it is obvious that heat stress shows the same detrimental effect on berry qualities as the usual high temperature in spite of its short exposure. Glucose is the predominant sugar in immature berries ; fructose level increases as the berries ripen so that at harvest the glucose : fructose ratio is nearly unity[6]. Heat-stressed seeded Napa Gamay berries had uniformly lowered glucose, fructose and malate contents ; GA-treated seeded berries had slightly higher malate content than untreated ones. Glucose : fructose ratio of Napa Gamay berries did not decrease as it did in heat-stressed Chardonnay berries[15]. This contradiction may owe to varietal difference, exposure time of heat stress and berry maturity. Besides, in stressed berries there is a lag in accumulation of soluble solids and organic acids and in decrease in acidity[3].

The growth inhibition by heat stress could be attributed to increasing abscisic acid content in leaves or reducing a) photosynthetic capacity and b) synthesis and export of growth promoting substances. The findings that a) the level of GA-like compounds in berries was reduced after the vines were exposed to heat stress and b) a single application of GA at 20 ppm would nearly reverse the effect of heat stress, indicate that fruit cells temporarily lost their ability to synthesize GA. But since the photosynthetic apparatus was virtually at a standstill during the stress period, growth regulators may have been present but were not loaded onto the phloem and exported to the berries. Since leaves and buds are known to produce growth promotors (and inhibitors) and those adjacent to the clusters were not assayed for GA or cytokinins, it cannot be stated from our data whether it was the fruit cells and/or the leaves and buds that lost their ability to synthesize these hormones.

These data indicate that when a heat spell is predicted, applications of GA or promalin to grape clusters immediately before and after a period of hot weather will overcome the detrimental effects of heat stress by restoring growth and quality potentials to the berries[3].

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熱ストレスと植物ホルモン処理の Napa Gamay (*Vitis vinifera* L.) 未熟果の生長に及ぼす影響

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要　　約

ポット植醸造用ブドウ Napa Gamay をもちい，果実発育第一期にファイトトロン中で昼間43℃，夜間22°Cの熱ストレスを4日間与え，その後温室で育てた。ストレスを受けないものに比べ，ストレスを受けた有核・無核果粒とも処理直後より発育の劣る傾向を示し，収穫時の果粒重も劣った。その果汁の固形物含量はストレス後しばらくの間高かったが成熟期には対照区より低く，フラクトーズとグルコーズ含量は低下していた。熱処理直後に20ppm ジベレリン処理すると熱ストレスで低下した発育は回復し，その効果はとくに無核果粒に対して著しかった。一方，ジベレリン処理で有核果・無核果とも固形物含量が低下したが適定酸度への影響はみられなかった。

オオムギ胚乳テストによるジベレリン活性は熱処理によって低下したが，その後回復した。