Vitis 20, 302—310 (1981)

CSIRO, Division of Horticultural Research, Adelaide, Australia

Advancing the time of ripeness of grapes by the application of methyl 2-(ureidooxy) propionate (a growth retardant)

by

J. S. Hawker, C. R. Hale and G. H. Kerridge

Die Beschleunigung des Reifezeitpunktes von Trauben durch die Anwendung von Methyl-2-(ureidooxy)-Propionat, einen Wachstumsdämpfer

Zusammenfassung. — Methyl-2-(ureidooxy)-Propionat (MUP), welches das Pflanzenwachstum verlangsamt, beschleunigte bei den *Vitis-vinifera*-Sorten Mataro und Sultana die Beerenreife um etwa 2 Wochen bzw. 1 Woche. Die Reben wurden etwa in der Mitte der ersten raschen Wachstumsphase der Beeren und ein weiteres Mal 2 Wochen später mit einer 0,1%igen MUP-Lösung besprüht. Das terminale und laterale Triebwachstum war gehemmt. Die Beerenreife war beschleunigt, wie die Entwicklung der Beerenfarbe und die Veränderungen im Gehalt an titrierbarer Säure und reduzierenden Zuckern zeigten. Die frühzeitigere Beerenreife könnte auf hormonale Veränderungen zurückgehen; allerdings war die Reife viel stärker beschleunigt als im Fall früherer Versuche, in denen während der langen Wachstumsphase der Beerenentwicklung Abscisinsäure oder Äthylen angewandt worden waren.

Introduction

Vineyard management requires some control over the time of ripening of grapes. The use of appropriate varieties and viticultural manipulations are the traditional methods of achieving this aim, but the advent of growth regulating chemicals has presented new possibilities for controlling grape ripening.

The growth of berries (fresh weight or volume) is described by a double sigmoid curve, the two periods of rapid growth (stages I and III) being separated by a period (stage II) when growth is much reduced (4). Ripening or senescence starts with the onset of stage III (onset of ripening or "veraison") and is marked as well by an increase in the growth rate, by anthocyanin development, an increased rate of sugar accumulation and by the loss of acid.

The development of fruits is generally considered to be controlled by endogenous plant hormones and exogenous hormones and growth regulators have been shown to advance or retard the onset of ripening of grapes (4, 5, 6, 7, 16). Abscisic acid (ABA) advanced ripening by 2 or 3 d when applied 11 d before normal veraison. Earlier and later applications had no effect (6). Ethylene and 2-chloroethylphosphonic acid (CEPA) hastened ripening by 4—6 d when applied during stage II (4, 7). CEPA applied during stage I delayed ripening by up to 3 weeks (4, 7). Auxins (e.g. benzothiazole-2-oxyacetic acid) delay ripening by several weeks when applied during stage I (4, 5, 7, 16).

The present paper describes the hastening by up to 2 weeks of the onset of ripening of a seedless and a seeded variety of grape by the growth retardant, methyl 2-(ureidooxy) propionate (MUP)[1]. MUP (A, below) is one of a series of chemicals

[1]) Methyl 2-(ureidooxy) propionate was kindly supplied by E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898, U.S.A. It is an experimental chemical whose potential toxicological and environmental hazards are not known.

whose use as ripeners has been patented in U.S.A. Patent 67/4829 and Australian Patent 41382/68. The simplest of the compounds listed in the patents is 2-(aminooxy) acetic acid (B).

A. $H_2NC(=O)NHOCH(CH_3)COOCH_3$ B. NH_2OCH_2COOH

Materials and methods

Glasshouse experiments

Preliminary experiments were carried out using bunches of two varieties of grapes (*Vitis vinifera* L. cultivars Cabernet Sauvignon and Riesling) on small cuttings in the glasshouse (12). The foliage and fruit were sprayed 4—5 weeks after flowering with aqueous solutions of MUP containing 0.05 % detergent (Agral 60). Shoot length was measured weekly and sugar and acid content of berries were measured as described below.

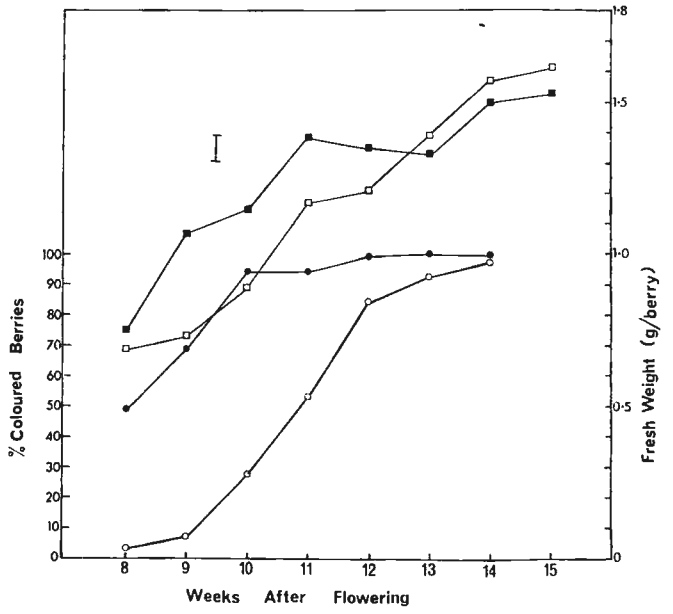

Fig. 1: Colour development in control (○) and MUP treated (●) Mataro grape berries and fresh weight of control (□) and MUP treated (■) berries. The bar represents the L.S.D. at P = 0.05 between any two means of fresh weight.

Die Farbentwicklung von Kontrollbeeren (○) und MUP-behandelten Beeren (●) der Sorte Mataro sowie das Frischgewicht von Kontrollbeeren (□) und MUP-behandelten Beeren (■). Die senkrechte Linie gibt die Standardabweichung zwischen den Mittelwerten des Frischgewichtes bei P = 0,05 % an.

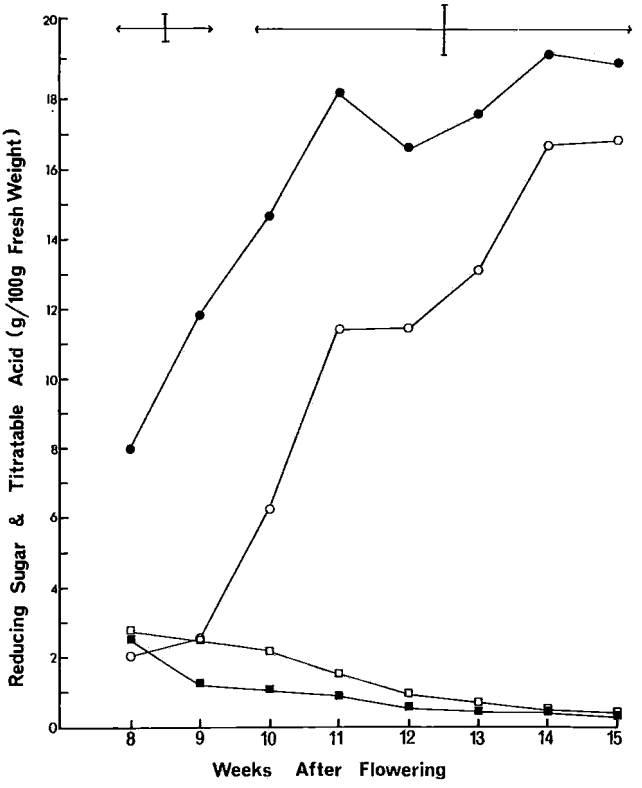

Fig. 2: Reducing sugar in control (○) and MUP treated (●) Mataro grape berries and titratable acid in control (□) and MUP treated (■) berries. The bars represent the L.S. D. at P = 0.05 between any two means of reducing sugar between the arrows. The L. S. D. for the acid values was half the size of the symbols.

Reduzierende Zucker von Kontrollbeeren (○) und MUP-behandelten Beeren (●) der Sorte Mataro sowie titrierbare Säure von Kontrollbeeren (□) und MUP-behandelten Beeren (■). Die senkrechten Linien geben die Standardabweichung zwischen den Mittelwerten der reduzierenden Zucker im Bereich der Pfeile bei P = 0,05 % an. Standardabweichung der Säurewerte halb so groß wie für die Zucker angegeben.

Field experiments

Mature irrigated vines of *V. vinifera* L. cultivars Mataro (Monastrell, Morastrell, Balzac, Mourvèdre, a seeded red wine variety) and Sultana (Sultanina, Thompson Seedless; seedless, white), growing in the vineyard of the CSIRO Division of Horticultural Research at Merbein, were sprayed twice in December, 1968, with a 0.1 % (w/v) aqueous solution of MUP containing 0.05 % Agral 60 or detergent alone as control. Foliage and fruit were sprayed to the "run off" stage. Mataro vines were treated on the 4th and 6th weeks after flowering and Sultana vines on the 5th and 7th weeks after flowering. Single-vine plots were separated by guard vines and each treatment was replicated 10 times. Samples of fruit were taken at weekly intervals starting 3 weeks after the second application of MUP. From each vine 5 berries were taken from each of 20 bunches chosen at random making a total of 100 berries for each of the 10 vines in each treatment. Fresh weight and number of

coloured berries (Mataro) were determined for each sample. Berries were considered coloured as soon as pink pigment was visible. The berries were covered with ethanol, boiled, thoroughly homogenised and made up to 1000 ml with distilled water. After filtration, reducing sugar content was determined (1) and titratable acid was determined by titrating to the phenolphthalein end point with 0.05 N NaOH. Titratable acid is expressed as g of tartaric acid.

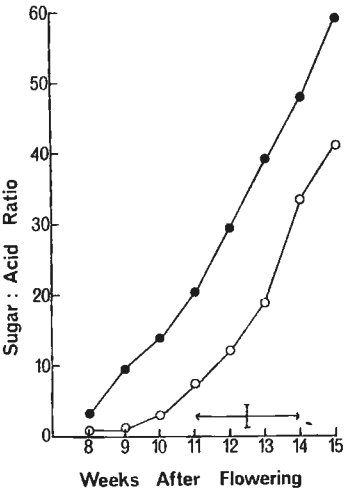

Fig. 3: Sugar : acid ratios of control (○) and MUP treated (●) Mataro grape berries. The bar represents the L.S.D. at P = 0.05 between any two means between the arrows. This covers the ratios of interest.

Zucker : Säure-Verhältnis bei Kontrollbeeren (○) und MUP-behandelten Beeren (●) der Sorte Mataro. Die senkrechte Linie gibt die Standardabweichung zwischen den Mittelwerten im Bereich der Pfeile, der in erster Linie von Interesse ist, bei P = 0,05 % an.

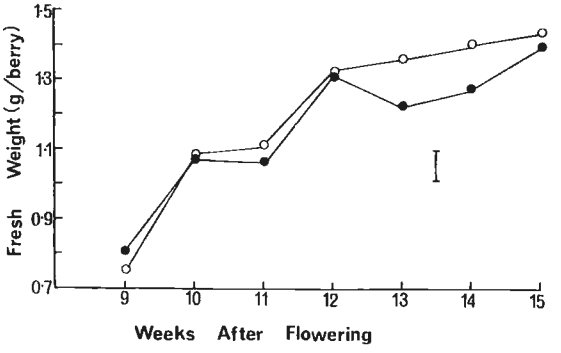

Fig. 4: Fresh weight of control (○) and MUP treated (●) Sultana grape berries. The bar represents the L.S.D. at P = 0.05 between any two means.

Frischgewicht bei Kontrollbeeren (○) und MUP-behandelten Beeren (●) der Sorte Sultana. Die senkrechte Linie gibt die Standardabweichung zwischen den Mittelwerten bei P = 0,05 % an.

Results

Glasshouse experiments

Preliminary experiments with the varieties Cabernet Sauvignon and Riesling were done in the glasshouse with 4 concentrations of MUP. A concentration of 0.1 % (w/v) resulted in cessation of shoot extension growth and sugar and acid determinations showed that berries began the second rapid growth phase about 1 week earlier than controls. High concentrations (0.2 and 1 %) killed some plants and 0.05 % slowed the growth of shoots but did not hasten the start of the second rapid growth phase of the berries.

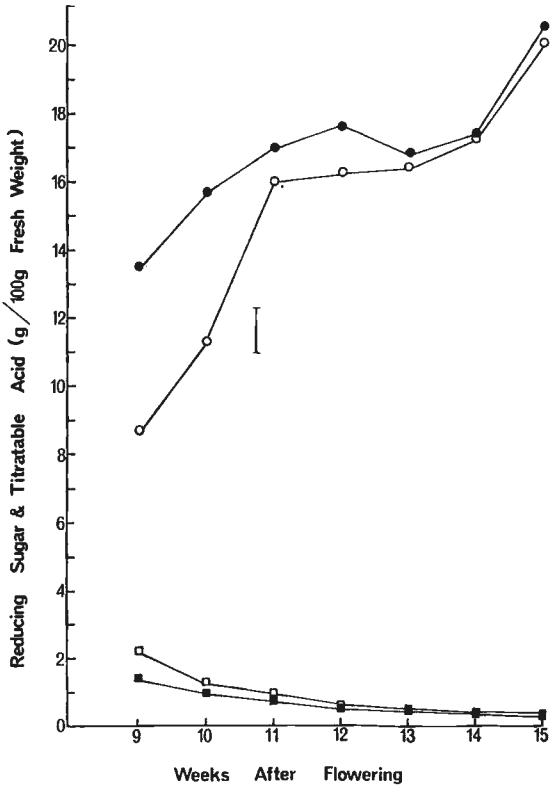

Fig. 5: Reducing sugar in control (○) and MUP treated (●) Sultana grape berries and titratable acid in control (□) and MUP treated (■) berries. The bar represents the L. S. D. at P = 0.05 between any two means of reducing sugar. The L.S.D. for acid values was half the size of the symbols.

Reduzierende Zucker von Kontrollbeeren (○) und MUP-behandelten Beeren (●) der Sorte Sultana sowie titrierbare Säure von Kontrollbeeren (□) und MUP-behandelten Beeren (■). Die senkrechte Linie gibt die Standardabweichung zwischen den Mittelwerten der reduzierenden Zucker bei P = 0,05 % an. Standardabweichung der Säurewerte halb so groß wie für die Zucker angegeben.

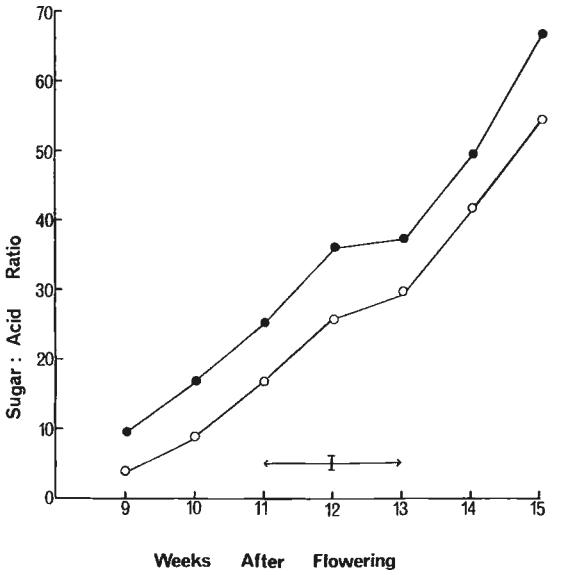

Fig. 6: Sugar : acid ratios of control (○) and MUP treated (●) Sultana grape berries. The bar represents the L.S.D. at P = 0.05 between any two means between the arrows. This covers the ratios of interest.

Zucker : Säure-Verhältnis bei Kontrollbeeren (○) und MUP-behandelten Beeren (●) der Sorte Sultana. Die senkrechte Linie gibt die Standardabweichung zwischen den Mittelwerten im Bereich der Pfeile, der in erster Linie von Interesse ist, bei P = 0,05 % an.

Field experiments

Variety Mataro. — Fruit on vines which had been treated with MUP began the second rapid growth phase about 2 weeks earlier than the grapes on control vines. Colour development, increase in fresh weight (Fig. 1), decrease in titratable acid concentration and increase in reducing sugar concentration (Fig. 2) occurred earlier in fruit from treated vines. Grapes used for dry wine production are normally harvested when their sugar : acid ratio is about 30. With treated grapes this value occurred at about the 12th week after flowering which was 2 weeks earlier than with the untreated fruit (Fig. 3).

Variety Sultana. — MUP also advanced the ripening of the seedless white variety, Sultana, although it did not cause a more rapid increase in fresh weight. Sugar and acid values showed that the ripening of treated grapes was between 1 and 1.5 weeks ahead of controls (Fig. 4, 5 and 6).

Effect of MUP on growth of shoots. — The growth of shoots of both varieties was arrested by the application of MUP. No visual damage occurred to the mature Sultana leaves which were present at the time of application. Some Mataro leaves showed small necrotic lesions but these constituted less than 1 % of the total leaf area.

Discussion

MUP inhibits the growth of sugarcane stems possibly by inhibition of cell division in the meristems. At the same time an increase in the rate of sugar storage in

the stems is observed (Robinson, Glasziou and Rochescouste, personal communication). Few cell divisions occur in the pericarp of grape berries after half-way through the first growth phase and further increase in the volume of the berries is mainly due to cell expansion (3, 9). For this reason MUP was applied to grapevines 4 or 5 weeks after flowering so as to avoid interference with cell division in the berries. In the present work it was shown that MUP inhibits shoot elongation in four varieties of *V. vinifera* and advances the start of the second rapid growth phase of the berries but has no effect on the subsequent rate of sugar accumulation (Fig. 2).

Studies on source-sink interactions in grapevines indicate that limiting the size of the source (leaves) can delay the ripening of grape berries depending on the relative sizes of sources and sinks (2, and references cited therein). Increasing the size of the sink (viz. fruit load) also delays ripening in some varieties in the field (17, 18). Decreasing the size of the sink by bunch thinning either experimentally (Hale, unpublished data) or commercially, does not result in a measurable hastening of the onset of ripening. Inhibiting growth of the shoots (thereby removing a competitive sink; 8) by the application of CEPA had no consistent effect on ripening (10, 15). The slight fluctuations in ripening were attributed to differences in vigour between vines (15). Applications of the growth retardant N-dimethylaminosuccinamic acid to grapevines resulted in a delay of the onset of ripening (13). In the present experiment the growth retardant, MUP, decreased shoot growth but unlike the other growth retardants mentioned above, caused a considerable hastening of the onset of ripening. In view of the differences in response to the different growth retardants it seems unlikely that the hastening of ripening caused by MUP can be explained by the decrease in the size of the competitive sink, i.e., the growing shoots. It seems more likely that MUP had a direct effect on the grape berries, as it is known that growth regulators affect their rate of development (see "Introduction").

Advancement and retardation of the onset of ripening by other compounds has been observed before, but the advancement previously reported has not been as great as the 2 weeks reported here with MUP. The mode of action of MUP appears to be different to that of ethylene or ABA because it is effective during stage I, a stage of development when ABA is ineffective (6) and when ethylene delays the onset of ripening (7).

Endogenous concentrations of ethylene, auxins, gibberellins and ABA change during berry development (11, 14), and it is possible that MUP changed the relative concentration or activity of, or substituted for, or antagonized one or more of these hormones which resulted in the hastening of ripening. The inhibition of 1-aminocyclopropane carboxylate synthase, a key enzyme in ethylene biosynthesis, by aminooxyacetic acid, the simplest compound described by patent specifications covering MUP, might be of relevance in this context (19).

The magnitude of the effect of MUP in the field might be increased by application of different concentrations at different times. It is also possible that other related compounds may have similar or greater effects than MUP. These questions along with potential toxicological and environmental hazards remain unanswered. However, we have demonstrated that a worthwhile hastening of ripening of grapes can be achieved by the application of a chemical in the field, a fact which may eventually be of value to commercial vignerons.

Summary

Methyl 2-(ureidooxy) propionate (MUP), a plant growth retardant, hastened the ripening of grape berries of *Vitis vinifera* L. cultivars Mataro and Sultana by about 2 weeks and 1 week, respectively. The vines were sprayed with 0.1 % solution of MUP about halfway through the first rapid growth phase of the fruit and again 2 weeks later. Terminal and lateral shoot growth was inhibited. Ripening of berries was advanced as measured by development of colour, and changes in titratable acid and reducing sugar content. The earlier ripening of the berries may have been due to hormonal changes but the advancement was much greater than that found previously with abscisic acid or ethylene applied during the slow growth stage of berry development.

Acknowledgements

The authors thank B. W. J. Michael, R. J. McGuire and H. van Dijk for valuable technical assistance.

Literature cited

1. Arnold, W. N., 1965: β-Fructofuranosidase from grape berries. Biochim. Biophys. Acta 110, 134—147.
2. Buttrose, M. S., 1966: The effect of reducing leaf area on the growth of roots, stems and berries of Gordo grape-vines. Vitis 5, 455—464.
3. Coombe, B. G. 1960: Relation of growth and development to changes in sugars, auxins and gibberellins in fruits of seeded and seedless varieties of *Vitis vinifera*. Plant Physiol. 35, 241—250.
4. — — and Hale, C. R., 1973: The hormone content of ripening grape berries and the effects of growth substance treatments. Plant Physiol. 51, 629—634.
5. Hale, C. R., 1968: Growth and senescence of the grape berry. Austral. J. Agricult. Res. 19, 939—945.
6. — — and Coombe, B. G., 1974: Abscisic acid — An effect of the ripening of grapes. Bull. Roy. Soc. N. Z. No. 12, 831—836.
7. — — , — — and Hawker, J. S., 1970: Effects of ethylene and 2-chloroethylphosphonic acid on the ripening of grapes. Plant Physiol. 45, 620—623.
8. — — and Weaver, J. R., 1962: The effect of developmental stage on direction of translocation of photosynthate in *Vitis vinifera*. Hilgardia 33, 89—131.
9. Harris, J. M., Kriedemann, P. E. and Possingham, J. V., 1968: Anatomical aspects of grape berry development. Vitis 7, 106—119.
10. Hirschfield, G. and Lavee, S., 1980: Control of vegetative growth of grapevine shoots by ethylene-releasing substances. Conditions and sites of action. Vitis 19, 308—316.
11. Iwahori, S., Weaver, R. J. and Pool, R. M., 1968: Gibberellin-like activity in berries of seeded and seedless Tokay grapes. Plant Physiol. 43, 333—337.
12. Mullins, M. G., 1966: Test-plants for investigations of the physiology of fruit in *Vitis vinifera* L. Nature 209, 419—420.
13. Peterson, J. R., 1974: Effect of N-dimethylaminosuccinamic acid on Cabernet Sauvignon grapevines. Austral. J. Exp. Agricult. Animal Husb. 14, 126—128.
14. Sacher, J. A., 1973: Senescence and postharvest physiology. Ann. Rev. Plant Physiol. 24, 197—224.
15. Shulman, Y., Hirschfield, G. and Lavee, S., 1980: Vegetative growth control of six grapevine cultivars by spray application of (2-chloroethyl) phosphonic acid (ethephon). Amer. J. Enol. Viticult. 31, 288—293.
16. Weaver, R. J. 1962: The effect of benzothiazole-2-oxyacetic acid on maturation of seeded varieties of grapes. Amer. J. Enol. Viticult. 13, 141-149.
17. — — and McCune, S. A., 1960: Effects of over-cropping Alicante Bouschet grapevines in relation to carbohydrate nutrition and development of the vine. Proc. Amer. Soc. Hort. Sci. 75, 341—353.

18. Winkler, A. J., 1954: Effects of overcropping. Amer. J. Enol. Viticult. 5, 4—12.
19. Yu, Y., Ademas, D. O. and Yang, S. F., 1979: 1-Aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis. Arch. Biochem. Biophys. 198, 280—286.

Eingegangen am 11. 8. 1981

J. S. Hawker
CSIRO,
Division of Horticultural Research
G.P.O. Box 350
Adelaide
South Australia, 5001