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A note on the development of a practical procedure for promoting the germination of dormant seed of grape (*Vitis* spp.)

by

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Entwicklung eines brauchbaren Verfahrens zur Förderung der Keimung dormanter Samen der Rebe (*Vitis* spp.)

Zusammenfassung. — Die Grenzen und Möglichkeiten der vorhandenen Verfahren zur Samenkeimung bei Reben werden diskutiert. Folgendes Verfahren zur Prüfung der Keimfähigkeit von Traubenkernen wurde ausgearbeitet: 24 h lang Einweichen in 0,5 M H_2O_2, weitere 24 h lang in 1000 ppm GA_3, danach 21 d lang Stratifikation bei 3—5 °C, anschließend Prüfung der Keimfähigkeit unter diurnalen Wechseltemperaturen von 20/30 °C (16/8 h). Dieses Verfahren, das an den Kernen von sieben Traubensorten erprobt wurde, erwies sich als wirkungsvoll bei der Keimungsförderung dormanter Samen und schonend gegenüber den anderen Kernen.

Introduction

To be practical a seed dormancy-breaking treatment must be able to remove dormancy from almost all dormant seeds within all seed populations (different provenances and/or cultivars) of a species, be non-injurious to non-dormant or weakly dormant seed, be easy to apply and relatively quick to achieve full germination. Freshly extracted grape seed show considerable dormancy. For those concerned with the breeding or genetic resources conservation of *Vitis* spp. this represents a considerable problem. In plant breeding it is advisable to remove dormancy physiologically when growing plants from seed in order to avoid any direct selection pressure against dormancy (to avoid vivipary) and to avoid any possible indirect selection pressure against other characters linked with dormancy. Moreover the more rapid the method of removing dormancy the better. For genetic resources conservation it is necessary to be able to remove dormancy to ensure that the genetic heterogeneity of an accession is maintained for use by breeders and to avoid confounding dormancy with non-viability in germination tests designed to monitor the viability of accessions in long-term storage.

Exposure of imbibed seed to low temperature for long periods (variously described in the literature as prechill, stratification or after-ripening) is moderately successful in breaking grape seed dormancy. Various temperatures and treatment periods have been used. Those reported as successful include 12 weeks at 5 °C (Flemion 1937, Scott and Ink 1950, Harmon and Weinberger 1959, Singh 1961), 9 weeks at 0°C (Yeou-Der *et al.* 1968), and 8 weeks at 5 °C (Chohan and Dhillon 1976). However, despite the considerable treatment period — in itself a drawback — such treatments alone are rarely totally effective in breaking seed dormancy. For example in the work of Singh (1961) treatment at 5 °C for 12 weeks with seed of 14 hybrid crosses resulted in a mean germination of only 40 % and ranged from only 8 % in one cross to a maximum 62 % germination in the least dormant cross. Thus prechill treatments are not practical in the sense discussed here.

Various other dormancy-breaking treatments which have been successful with other species have been tested for their efficacy in removing dormancy in grape (e.g. SCOTT and INK 1950, HARMON and WEINBERGER 1959, YEOU-DER *et al.* 1968, MANIVEL and WEAVER 1974). Of these, apart from the prechill treatments described above, only treatment with gibberellic acid (GA_3) has been found to be at least partly effective. GA_3 appears to be more suitable than other gibberellins for breaking grape seed dormancy (PAL *et al.* 1976). Very high concentrations of GA_3 (e.g. 8,000 ppm) are required if GA_3 is the sole method of breaking dormancy and this can result in very tall seedlings (YEOU-DER *et al.* 1968). Such deformities may result in reduced plant establishment. GA_3 concentrations can be reduced however, if treatment in GA_3 is combined with a prechill treatment. Applying GA_3 before the prechill treatment appears to be more effective in promoting germination than applying it after the prechill treatment (KACHRU *et al.* 1972, SELIM *et al.* 1981) although contradictory results have also been reported (RANDHAWA and NEGI 1964, RANDHAWA and PAL 1968). Although combining prechill and GA_3 treatments can result in much shorter overall germination periods compared to prechill alone, the GA_3 concentrations used appear to result in very high proportions of abnormal seedlings (e.g. SELIM *et al.* 1981). Such seedlings are often incapable of developing into plants. Moreover there is little evidence to suggest that the combination of treatment in GA_3 and a short prechill promotes grape seed germination sufficiently. The best combined treatment of KACHRU *et al.* (1972) was treatment in 2,000 ppm GA_3 for 48 h followed by prechill at 5 °C for 30 d. The period and environment of the subsequent germination test was not given but 81 % germination was recorded after this treatment for seed of the variety Black Muscat compared to no germination for a control treatment.

In this paper we report the results of three simple experiments in which we have attempted to improve the above procedure by the action of an additional dormancy-breaking agent — hydrogen peroxide — which we have found to be particularly effective when combined with other agents for removing seed dormancy in rice (*Oryza* spp.) (ELLIS *et al.* 1983).

Materials and methods

Seeds were extracted by hand from table grapes purchased locally, cleaned by washing in running tap water with a final wash in distilled water, blotted and then dried at 20 °C with 10—15 % relative humidity for 10 d. The drying treatment reduced seed moisture content from 30 % to 7 % (fresh weight basis) — moisture content being determined from ground seed dried at 130—133 °C for 2 h. The dried seeds were hermetically stored at 0 °C in laminated aluminium foil packets until the experiments were begun.

Seeds were extracted from both red (R) and white (W) table grapes. Those used in Experiments 1 and 2 were of three unknown cultivars of unknown origin (R_A, W_A and W_B). Seed of seven cultivars were used in Experiment 3:

R_B — cultivar Barlinka from South Africa;
R_C — cultivar Red Emperor from Chile;
R_D — cultivar Alphonse La Vallée from France;
R_E — cultivar Cardinal from Spain;
W_C — cultivar Uva Regina Puglia from Italy;
W_D — cultivar Almeria from Spain;
W_E — unknown cultivar from Italy.

Prechill treatments of 21 d at 3—5 °C and the subsequent germination tests were carried out in moist rolled paper towels. The number of seeds tested varied substantially according to the total numbers available for each seed lot. Seedlings were evaluated according to the criterion of normal germination, i.e. only those seeds which produced morphologically normal seedlings were scored as germinated.

Pretreatments — where carried out — were at 20 °C. Treatment in hydrogen peroxide (H_2O_2) preceded treatment in GA_3 solutions. Both pretreatments preceded the prechill treatment. For the H_2O_2 treatment 200 seeds were soaked in 160 ml of solution. For the GA_3 treatment seeds were soaked in twice their volume of solution. Each of these treatments was applied for 24 h and between the two treatments seeds were rinsed in distilled water and blotted.

Experiment 1 investigated the effect of 1 M H_2O_2, 2,000 ppm GA_3 and the 21 d prechill treatment on the germination of lots R_A, W_A and W_B in a factorial design. 25 seeds of W_A, 200 seeds of W_B and 120 seeds of R_A were tested at each treatment. The subsequent germination tests were at a constant temperature of 20 °C and were concluded after 63 d.

Experiment 2 investigated the effect of a limited number of different temperature regimes on the germination of seed of lots R_A and W_B which had been previously pretreated in 1 M H_2O_2 and 2,000 ppm GA_3 and prechilled for 21 d. The temperature regimes tested were constant temperatures of 20, 25, 30 and 35 °C and alternating temperatures of 20/30 °C and 20/35 °C. In the latter two regimes the higher temperatures were applied for 8 h in each 24 h cycle. 120 seeds of lot R_A and 200 seeds of lot W_B were tested in each temperature regime. Germination tests were concluded after 63 d.

Experiment 3 compared germination of seed subjected to the combined treatments of pretreatment in 0.5 M H_2O_2, 1,000 ppm GA_3 and a 21 d prechill for lots $R_{B\text{-}E}$ and $W_{C\text{-}E}$. Germination tests were carried out at an alternating temperature of 20/30 °C (16/8 h) and were concluded after 42 d. Germination test size was 200 seeds for the combined treatment but only 50 seeds for the untreated (control) germination test in which few seeds were expected to germinate.

In a number of treatments the topographical tetrazolium test for viability was carried out on seeds which had failed to germinate during the test. This was done by bisecting the imbibed seeds with a sharp knife and, provided an embryo was present, incubating one half of the bisected seed in 0.5 % tetrazolium chloride solution for 2 h at 40 °C, washing the embryos in distilled water and evaluating the staining patterns. In addition in the final experiment a tetrazolium test was carried out on a sample of the dried seed from each lot with the exception of lot R_B where insufficient seeds were available. These seeds were moistened on filter paper at 30 °C for 16 h before bisection, but the staining period had to be extended to 3 h. The number of seeds available for this test was severely limited for certain seeds lots (Table).

Results and discussion

Fig. 1 shows that seed of lots R_A, W_A and W_B were dormant, that all three dormancy-breaking agents tested had some effect in removing dormancy either singly or when combined with other agents, and that 2,000 ppm GA_3 was by far the most promotory single agent. Within lots R_A and W_B germination following the three-factor treatment, all three two-factor treatments and the GA_3 single factor treatment did not differ significantly ($P > 0.05$), but all of these results were significantly greater ($P < 0.005$)

Viability of grape seed lots assessed by the topographical tetrazolium test, normal germination after 42 d in the test at 20/30 °C for seed previously untreated or treated in 0.5 M H_2O_2 for 24 h, 1,000 ppm GA_3 for 24 h and prechilled at 3—5 °C for 21 d, and viability of seeds remaining ungerminated at the end of these tests as assessed by the topographical tetrazolium test

Vitalität von Traubenkernen aufgrund des topographischen Tetrazoliumtests, Anteil normal gekeimter Kerne nach 42tägiger Keimfähigkeitsprüfung bei 20/30 °C sowie Vitalität der nicht gekeimten Kerne am Ende dieser Prüfung · Die auf ihre Keimfähigkeit geprüften Kerne waren entweder unbehandelt oder mit 0,5 M H_2O_2 (24 h), 1000 ppm GA_3 (24 h) und Temperaturen von 3—5 °C (21 d) vorbehandelt; die abschließende Vitalitätsprüfung erfolgte ebenfalls mit Hilfe des topographischen Tetrazoliumtests

Cultivar	Tz test		Germination test			
			Untreated		Treated (H_2O_2 + GA_3 + prechill)	
	No. of seeds tested	Viability	Normal germination	Viability of seeds failing to germinate[1])	Normal germination	Viability of seeds failing to germinate[1])
		(%)	(%)	(%)	(%)	(%)
R_B	—[2])	—[2])	5	90	94 ***[3])	0 NS[4])
R_C	27	74	24	44	84 ***	0 **
R_D	32	69	22	52	70 ***	0 NS
R_E	9	100	0	90	94 ***	0 NS
W_C	79	74	0	60	62 ***	1 NS
W_D	100	97	2	88	90 ***	0 NS
W_E	19	95	6	88	91 ***	0 NS

[1]) Expressed as the percentage of the original number of seeds tested for germination. (Thus this value can be added to that for normal germination to obtain an estimate of viability for the treated and untreated seeds at the end of the germination test).
[2]) No seeds available for the tetrazolium test.
[3]) Probability of error for significant difference in normal germination between treated and untreated seeds (*** = $P < 0.005$).
[4]) Probability of error for significant difference in total viability between treated and untreated seeds (NS [not significant] = $P > 0.2$, ** = $P < 0.01$).

than either the single factor H_2O_2 treatment, the single-factor prechill treatment or the control treatment. For lot W_A, however, the germination of the three-factor treatment was significantly greater ($P < 0.05$) than the other three treatments which included treatment with GA_3; and all four treatments which included treatment with GA_3 resulted in significantly greater ($P < 0.005$) germination than the remaining four treatments.

Consequently the three-factor treatment combining treatment in 1 M H_2O_2, 2,000 ppm GA_3 with a 21 d prechill prior to the germination test was the only treatment to provide a result which was not significantly less than any other treatment in all three seed lots, treatment in 2,000 ppm GA_3 being the most effective dormancy breaking agent of the three when applied alone. However, subsequent tetrazolium tests on seeds of lots R_A and W_B which failed to germinate in test provided overwhelming evidence that the 2,000 ppm GA_3 treatment resulted in the death of a small proportion of the dormant seeds. For both lots none of the seeds that remained ungerminated following the combined treatment with all three agents were viable according to a tetrazolium test, whereas 18 % and 14 % of the original number of seeds tested were viable at the end of the germination test following the combined treatment of 1 M H_2O_2 and prechill, but only 2 % and 3 % were viable following treatment in 2,000 ppm GA_3 alone for

lots W_B and R_A respectively. Thus (by adding these values to those given in Fig. 1) we estimate that lots W_B and R_A were at least 86 % and 95 % viable respectively, and that treatment in 2,000 ppm GA_3 either alone or in combination with 1 M H_2O_2 and prechill resulted in the death of between 9—17 % ($0.03 > P > 0.00002$) and 9—12 % ($0.008 > P > 0.0022$) of seeds respectively.

This phenomenon of the contradictory effects of certain dormancy breaking agents (i.e. promoting the germination of certain dormant seeds whilst causing the death of other seeds) is one which we have investigated quite extensively in rice (ELLIS *et al.* 1983). In rice our solution was to use many dormancy-breaking agents in combination, but with each agent at low concentrations in order to avoid toxic effects. Here we adopted a similar tactic, reducing the concentrations of both H_2O_2 and GA_3 from 1 M to 0.5 M and from 2,000 ppm to 1,000 ppm respectively in Experiment 3.

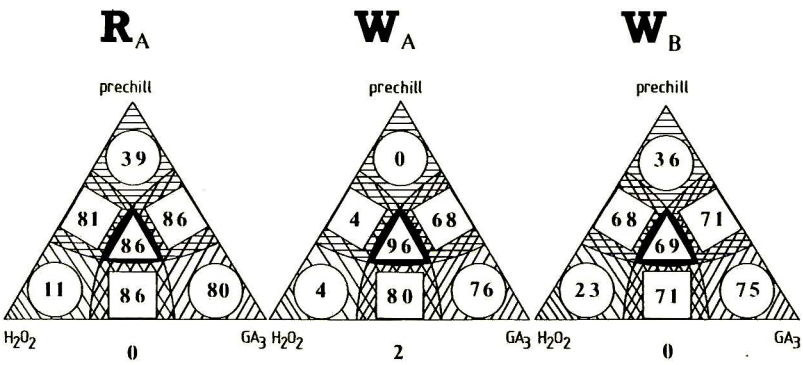

Fig. 1: The influence of soaking in 1 M H_2O_2 for 24 h, soaking in 2,000 ppm GA_3 for 24 h and 21 d prechill at 3—5 °C either singly or in combination on subsequent germination of three grape seed lots in test at 20 °C over 63 d. For each seed lot the investigation was based on a simple 2^3 factorial design. The value at the base of each triangle is the result of a 63 d germination test at 20 °C on untreated seed. Values contained within a circle at the apices of the triangles are for single-factor treatments; values contained within squares where arcs of two circles overlap are for two-factor treatments; values in the central triangles where the arcs of all three circles overlap are for the three-factor treatments. All results are presented as percentage normal germination.

Der Einfluß des Einweichens in 1 M H_2O_2 (24 h), in 2000 ppm GA_3 (24 h) sowie der Stratifikation bei 3—5 °C (21 d) — einzeln oder kombiniert — auf die nachfolgende Keimung von drei verschiedenen Traubenherkünften. Die Prüfung erstreckte sich bei 20 °C über 63 d. Werte an der Basis der Dreiecke: Keimung unbehandelter Kerne bei 20 °C nach 63 d; Werte in den Kreisen an den Dreiecksspitzen: Ergebnisse nach monofaktorieller Behandlung; Werte in den Quadraten: Ergebnisse nach bifaktorieller Behandlung; Werte in den zentralen Dreiecken: Ergebnisse nach trifaktorieller Behandlung. Alle Zahlenwerte stellen den Prozentsatz normal gekeimter Samen dar.

Much of the work on overcoming seed dormancy in *Vitis* spp. has, apparently, used uncontrolled environments for the subsequent germination tests. This is surprising since the temperature of the germination regime influences the expression of seed dormancy quite considerably in a wide range of species. One exception is the work of YEOU-DER *et al.* (1968). They tested grape seed for germination in a diurnal alternating temperature regime of 18/30 °C (the period spent at each temperature was not reported). In this investigation (Fig. 2) a similar diurnal alternating temperature regime of 20/30 °C was consistently superior to the other, admittedly few in number, constant and alternating temperature regimes in which seed were tested for germina-

tion for both lots. Note also that the ranking of germination test temperatures with respect to final percentage germination was identical for seed of both lots (Fig. 2). Although the differences in final percentage germination between the alternating temperature regime of 20/30 °C and the constant temperature regime of 25 °C were not significant ($P > 0.2$) for either seed lot when expressed in terms of the total number of seeds tested, this is not surprising since very few of the viable seeds in each lot remained dormant at 25 °C (viability of R_A and W_B was estimated to be 95 % and 86 % respectively). Expressing germination as the proportion of viable seeds which germinated showed germination at 20/30 °C to be significantly greater than germination at 25 °C ($P < 0.005$ for seed of both lots). That is dormancy was broken in a significantly greater proportion of seeds at 20/30 °C than at 25 °C. Consequently an alternating temperature regime of 20/30 °C was used to test seed for germination in the final experiment. In addition since no further germination occurred after 21 d in any germination test (Fig. 2) the test period was shortened from 63 to 42 d. Of the four constant temperature regimes used 25 °C consistently gave the most rapid germination and the highest proportion of germinating seeds (Fig. 2). It is therefore suggested that anyone wishing to germinate grape seeds but unable to provide an alternating temperature regime of 20/30 °C should use 25 °C.

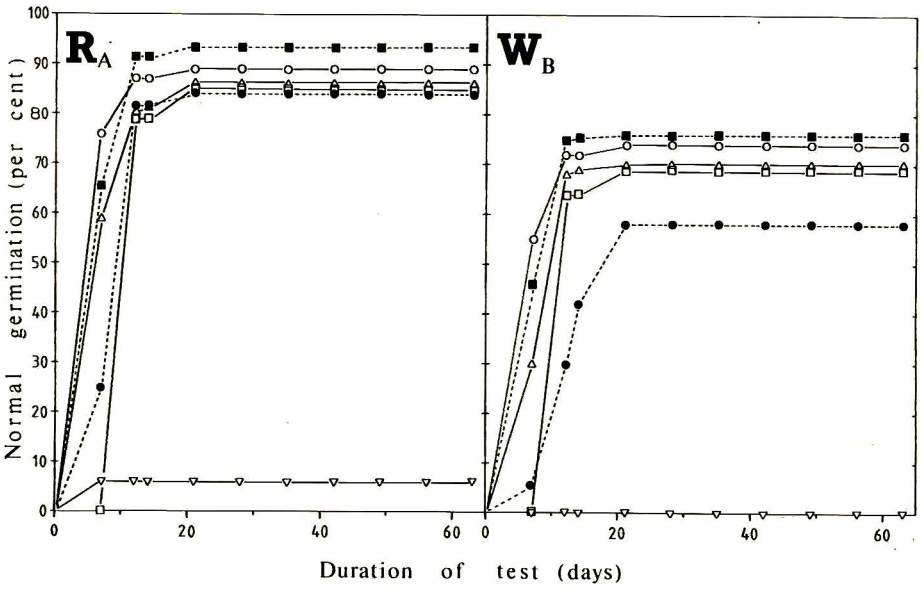

Fig. 2: Germination progress curves of two grape seed lots treated in 1 M H_2O_2 for 24 h, in 2,000 ppm GA_3 for a further 24 h, prechilled at 3—5 °C for 21 d and then tested at 20 °C (□—□), 25 °C (○—○), 30 °C (△—△), 35 °C (▽—▽), 20/30 °C (■----■), or 20/35 °C (●----●) for 63 d. In the alternating-temperature treatments the first temperature quoted was applied for 16 h and the second temperature for 8 h in each 24-h cycle.

Zeitlicher Verlauf der Keimung bei zwei Traubenkernherkünften nach Behandlung mit 1 M H_2O_2 (24 h), 2000 ppm GA_3 (weitere 24 h) und Stratifikation bei 3—5 °C (21 d). Die Keimprüfung wurde 63 d lang bei 20 °C (□—□), 25 °C (○—○), 30 °C (△—△), 35 °C (▽—▽), 20/30 °C (■----■) oder 20/35 °C (●----●) durchgeführt. Bei der Anwendung der Wechseltemperaturen wirkte die an erster Stelle aufgeführte Temperatur täglich 16 h, die zweite 8 h lang ein.

Five of the seven lots tested at 20/30 °C showed considerable dormancy (Table). The combined treatment of a 24 h soak in 0.5 M H_2O_2 followed by a 24 h soak in 1,000 ppm GA_3 with a subsequent 21 d prechill at 3—5 °C and germination testing at 20/30 °C resulted in a significant increase in germination over the control in all seven seed lots. Moreover, virtually no dormant seed remained ungerminated at the end of this procedure (Table), and comparison with the results of both the initial tetrazolium tests for viability and those after the germination test of untreated seed provide no evidence of any significant damage to seed resulting from the use of the combined dormancy breaking procedure.

There are a number of reports in the literature which demonstrate that the use of high concentrations of gibberellins can result in seedlings of increased height (e.g. YEOU-DER *et al.* 1968, PAL *et al.* 1976); in extreme cases seedlings may be too etiolated for subsequent growth and development. No such problems were encountered here; the combined treatment on the seven seed lots had no noticeable effect on subsequent seedling height. SELIM *et al.* (1981), using a number of compounds, including gibberellins, to break dormancy encountered considerable problems with high proportions of abnormal seedlings in all their treatments — including the control treatments. Again no such problems were encountered here. It is possible that the 30 °C germination test temperature of SELIM *et al.* (1981) was too high because there is some evidence here that 30 °C should be regarded as a super-optimal germination temperature, the progress of germination at 25 °C being both more rapid and complete (Fig. 2).

Although the evidence presented here is limited, nevertheless we believe that it is sufficient to demonstrate the advantage of this combined procedure for promoting the germination of grape seed compared to either of the procedures suggested previously — 12 weeks prechill at 5 °C (SCOTT and INK 1950), or treatment in 2,000 ppm GA_3 followed by a prechill treatment (KACHRU *et al.* 1972). Consequently we urge those involved in the breeding and genetic resources conservation of *Vitis* spp. to compare the efficacy of this procedure with that currently in use.

Although we are confident of the benefits of this procedure over those currently in use, this does not mean that a better procedure could not be developed given more time and a greater supply of seed. We have three suggestions for anyone attempting to develop an even better dormancy breaking procedure. First we are by no means certain that the prechill treatment need be as long as 21 d. Reduction to 14 or even 7 d may be possible. Secondly 0.01 M 2-mercaptoethanol added to the germination medium in our experience promotes the germination of dormant grape seed. It may be possible for this agent to replace or be combined with one or more of the agents used here but we have been unable to test this. Finally KACHRU *et al.* (1972) had considerable success in removing dormancy quickly and simply by allowing running water to drip through seeds in muslin bags for up to 16 d. One problem they found was that seeds began to germinate in the bags and were easily damaged such that these germinated seeds were incapable of further growth. As a practical procedure in gene banks, or elsewhere, a long washing procedure would be fraught with similar operational difficulties. Nevertheless it occurs to us that a shorter washing period of 2 d or so in combination with other dormancy breaking agents might result in a procedure as effective as that developed here.

Summary

The effect of hydrogen peroxide (H_2O_2), gibberellic acid (GA_3) and a short exposure of imbibed seed to 3—5 °C (prechill) on grape seed germination were investigated fac-

torially. All three factors had some effect, either singly or in combination with one or more of the other agents, in promoting the germination of dormant seed. 2,000 ppm GA_3 was the best single agent for breaking dormancy but tetrazolium staining revealed that this treatment caused the death of a small proportion of seed. Comparison of germination tests at 20, 25, 30 and 35 °C showed 25 °C to be the best constant temperature for rapid full germination. Seed were also tested for germination in two diurnal alternating temperature regimes — 20/30 °C and 20/35 °C, the higher temperatures being applied for 8 h in each 24 h cycle. The alternating temperature of 20/30 °C proved better for testing seed for germination than either 20/35 °C or 25 °C, the best constant temperature. The following germination test procedure was devised for grape seed: a 24 h soak in 0.5 M H_2O_2, a further 24 h soak in 1,000 ppm GA_3, followed by a 21 d prechill at 3—5 °C with subsequent testing for germination in a diurnal alternating temperature regime of 20/30 °C with the higher temperature being applied for 8 h in each daily cycle. This procedure was tested with dormant seed of seven lots of diverse origin and found to be very effective in promoting the germination of dormant seed whilst not damaging to other seeds. In the opinion of the authors the above procedure represents a suitable practical dormancy breaking procedure for seed of *Vitis* spp.

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