Relation of Seededness and Ringing to Gibberellin-like Activity in Berries of Vitis vinifera¹,²

Robert J. Weaver and Robert M. Pool

Department of Viticulture and Enology, University of California, Davis, California

Introduction

Gibberellin-like substances occur in many higher plants (12, 17, 20), and several have been identified (12). The presence of gibberellin-like activity in seedless fruit² of Vitis vinifera L. was demonstrated in 1960 by Coombe (1). Although it seems probable that such a widely spread hormone also would be involved in the growth of seeded fruit, Coombe was unable to demonstrate the presence of gibberellin in crude extract from such fruit.

One objective of the present research was to study chromatographed extracts of both seeded and seedless varieties of grapes to determine the presence of gibberellin-like activity. In the past, much extraction for gibberellins was done at low pH. In our work we used 3 acid fractions. In addition a neutral extract was obtained, as several investigators have demonstrated the presence of gibberellin-like activity in neutral portions of extracts (2, 3, 4, 5, 6, 21). To broaden the spectrum a basic fraction was also examined.

A second objective was to determine the effect of ringing (girdling) on gibberellin-like activity in the berries. It is well known that a temporary ringing of certain varieties of fruit trees and grapes increases the size of developing fruits. Ringing long has been practiced to increase the size of Black Corinth and Thompson Seedless berries (7). The classical theory is the enlargement of the fruit results from the increased supply of elaborated food material trapped above the ring. Another possibility, however, is that plant hormones trapped above the ring cause fruit enlargement. One method for greatly enlarging fruits of these varieties is to treat unringed vines with exogenous gibberellins (19). Since both ringing and treatment with gibberellins result in enlarged fruit, it is likely that ringing may affect the endogenous gibberellin content of the berries.

Materials and Methods

Mature vines of Vitis vinifera L. in the University of California vineyard at Davis were utilized. Unless otherwise stated, berries were collected about 10 days after the normal shatter of berries following bloom. Ringing was done with a girdling knife that removed a 1-cm wide strip of bark from the circumference of the trunk (7).

Samples ranging from 100 to 5000 g on a fresh weight basis were taken. Varying amounts of berries could be used in the present work because no quantitative comparison of gibberellin-like activity among various experiments was planned.

After collection, the berries were frozen immediately by shaking with dry ice, lyophilized, and then stored for a period of 1 to 8 months. The material was extracted with 70% methanol at the rate of 3 liters per kg material for 48 hours at 0° (fig 1). The methanol was changed after 24 hours. The combined extracts were filtered through a Buchner funnel, and the pH adjusted to 7.5 with NaHCO₃.

The solution was concentrated to the water phase in a Rinco evaporator at 40° and, unless otherwise stated, separated into 5 fractions (fig 1). Each fraction was extracted 3 times with equal volumes of methanol. For aqueous phase, each portion was concentrated in a Rinco evaporator, and streaked on Whatman No. 3 MM paper (18 × 22 inches). Development was by the descending technique; the solvent was a mixture of isopropanol, ammonium hydroxide (28 %), and water (10: 1: 1, v v v). The chromatograms were removed when the solvent reached a point about 45 cm from the origin. After developing, the chromatograms were air-dried, cut into 10 equal sections, and compounds eluted by grinding with 100 ml methanol for 1 to 2 minutes in a Servall omni-mixer. The methanol eluates were evaporated at 40° to dryness in a Rinco evaporator and then dissolved in 0.05 % Tween-20 (polyoxyethylene sorbitan monolaurate) in 50% aqueous ethanol solutions for bioassay at a rate of 2 ml per kg of berries. In the experiment with Thompson Seedless in 1963 and with Carignane 1 ml of Tween-20 solution were used. The resulting rates were 1 ml per 100 g and 1 ml per 42 g, respectively. For the control, this wetting agent was utilized in water.

The dwarf pea bioassay adapted from the method of Köhler and Lang (8) was used to measure the gibberellin-like activity. This test is based on the

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³ In this paper seeded varieties refer to those varieties that form completely developed hard seeds. Seedless varieties are those which set fruit parthenocarpically (Black Corinth), or varieties whose embryos abort soon after fertilization so that no hard seeds are formed (Thompson Seedless, Seedless Tokay, Seedless Emperor).
observation of Lockhart (10) that gibberellins reverse the red light inhibition of elongation of dwarf peas. The peas (*Pisum sativum* L. var. Morse's Progress No. 9) were soaked 8 hours in running water. They were planted in vermiculite in the dark at 27°. Four days later seedlings ranging from 2.5 to 2.7 cm long were transferred to water culture under red light. The radiation source, which was about 70 cm above the plants, consisted of 4 red fluorescent tubes above a sheet of red cellophane. The plants were treated 24 hours after transfer with a 5-μl droplet of extract or of known gibberellin. Six days later the plants were measured from the seed to the second node. There were 10 replicate plants per treatment. Activity greater than that produced by 0.001 μg of gibberellic acid (GA₃) per plant was significant at the 5% level as shown by Duncan's multiple range test. It must be emphasized that the various gibberellins have varying activity on dwarf peas, so that our results show only relative levels of gibberellin-like activity.

**Experimentation and Results**

*Carignane.* Unopened flowers from this seeded wine grape were collected on May 7, 1963, about 2 to 3 weeks before full bloom; at this time the clusters were about 8 cm long. Only an F-I (neutral) and an acidic fraction were obtained. Since the ex-

**Fig. 2.** Neutral (F-I) and acidic ethyl acetate (F-IV) fractions of gibberellin-like substances extracted from flowers of Carignane collected at a prebloom stage. Activity measured on Morse's Progress No. 9 peas grown under red light. Values are averages of 10 single-plant replications.

**Fig. 1.** Flow diagram showing procedures for extraction and separation of gibberellin-like substances from grape berries. A solution of isopropyl alcohol, ammonium hydroxide, and water (10:1:1, v/v) was used as the developing solvent.
traction method differed from that for the other experiments, they are given here. Nonlyophilized flowers were ground with an omni-mixer and extracted with absolute methanol. The residue was extracted for 24 hours with more absolute methanol. The extracts were combined and reduced to the water phase under vacuum. This phase was extracted with ethyl acetate at pH 7.5. The ethyl acetate phase was washed with 0.1 N H₂SO₄ to remove basic substances. The phase containing the basic substances was adjusted to pH 2.5 with 0.1 N H₂SO₄ and then extracted with ethyl acetate. The fractions were concentrated and chromatographed.

In the F-I (neutral) fraction there was activity at Rₛ's 0 to 0.1, 0.2 to 0.3, 0.7 to 0.8, and 0.9 to 1.0 (fig 2). In the acidic fraction there was high activity from Rₛ 0.5 to 1.0, and total activity in this fraction was much greater than that in the neutral fraction.

Tokay. Berries were collected on June 27, 1963 from both seeded and seedless varieties (the seedless is a mutant arising from the seeded). Berries were extracted from each type. The water phase from the methanolic extracts was cleared with saturated lead acetate.

There was little activity in either the seeded or seedless variety in the neutral (F-I), basic (F-II), or chloroform (F-III) fractions (fig 3). There was considerable activity in the acidic ethyl acetate fraction (F-IV); this activity was more pronounced in the seeded than in the seedless variety. In both varieties there were peaks at Rₛ 0.3 to 0.4; in the seeded there was also a peak at Rₛ 0.8 to 0.9. In the acidic n-butanol fraction (F-V) in the seeded variety there was a peak at Rₛ 0.8 to 0.9.

Thompson Seedless. Berries were collected immediately after shatter on June 8, 1962, when they were 8 to 10 mm in diameter. There was much gibberellin-like activity in the neutral fraction at Rₛ 0.1 to 0.2 (fig 4). Also, there was considerable activity in the chloroform fraction, with peaks at Rₛ's
0.1 to 0.2, 0.4 to 0.5, and 0.7 to 0.8. Activity was relatively low in the acidic ethyl acetate and in the acidic n-butanol fractions. Fraction II was lost in this experiment. In the acidic ethyl acetate fraction, there was extensive damage to the growing points of the bioassay plants. Despite this, activity appeared at R\textsubscript{f}’s 0.7 to 0.8 and 0.9 to 1.0.

**Emperor.** Berries were collected in 1962. Marked gibberellin-like activity was evident only in the F-I fraction in both the seeded and seedless varieties. Activity in the seeded (Emperor) was at R\textsubscript{f} 0.9 to 1.0 and in the seedless at R\textsubscript{f} 0.8 to 1.0. These gibberellins remain to be identified. In the seeded Emperor there was slight but significant activity in the basic and acidic n-butanol fractions. In the seedless variety there was slight activity in the acidic n-butanol fraction. No activity was found in the other fractions studied. Fractions II and III of the Seedless Emperor were lost. At most of the R\textsubscript{f}’s in all fractions bioassayed, except the neutral fraction of the seedless variety, the tips of the pea plants were killed. In spite of this there was often some increase in elongation over the controls. This may indicate that if the toxic compounds are removed, many of the eluates which give negative results may prove to have considerable gibberellin-like activity.

![Fig. 5](image-url)  
**Fig. 5.** Effect of ringing on neutral (F-I), chloroform (F-III), acidic ethyl acetate (F-IV), and acidic n-butanol (F-V) fractions of gibberellin-like substances extracted from berries of ringed (cross-hatch) and unringed (clear) Black Corinth vines. Basic (F-II) fraction not shown because of lack of activity. Activity measured on Morse’s Progress No. 9 peas grown under red light. Values are averages of 10 single-plant replications. In determining values the bases of all bars must be assumed to start at 0 line.

![Fig. 6](image-url)  
**Fig. 6.** Neutral (F-I), chloroform (F-III), acidic ethyl acetate (F-IV), and acidic n-butanol (F-V) fractions of gibberellin-like substances extracted from berries of unringed (clear) and ringed (cross-hatch) berries of Thompson Seedless at the shatter stage. Basic (F-II) fraction not shown because of lack of activity. Activity measured on Morse’s Progress No. 9 peas grown under red light. Values are averages of 10 single-plant replications. R\textsubscript{f} 0.1 to 0.7 of unringed fruit is not reported due to injury to plants from unknown toxic substances. In determining values the bases of all bars must be assumed to start from 0 line.
Effect of Ringing of Black Corinth. The trunks of 2 Black Corinth vines were ringed on June 12, 1963, about 10 days after full bloom. Forty-eight hours later the fruit from these vines and from 2 unringed vines was collected for analysis. Ringing greatly decreased the amount of gibberellin-like compounds in the neutral fraction (fig 5). In the unringed control fruit there was high neutral activity from Rf 0.7 to 1.0. There was little activity in the basic fraction either from the ringed or unringed fruit. In the chloroform fraction there were less gibberellin-like substances in girdled than in ungirdled fruit at Rf 0 to 0.5. In both the ethyl acetate and n-butanol fractions gibberellin activity was greatly increased in the ringed fruit. In the ethyl acetate fraction there were peaks at Rf’s 0.2 to 0.7 and 0.9 to 1.0. In the n-butanol fractions peaks were at Rf’s 0.2 to 0.3, 0.5 to 0.6, and 0.9 to 1.0.

Effect of Ringing of Thompson Seedless. Two vines were trunk-girdled on June 19, 1963, when the berries were in the late shatter stage. Forty-eight hours later the fruit was picked. Fruit from 2 unringed vines adjacent to those girdled were used as a control. The methanolic extract was evaporated to the water phase and then cleared with saturated lead acetate. There was no significant activity, either in ringed or unringed fruit, in the neutral, chloroform or basic fractions (fig 6). In the ethyl acetate fraction of the control fruit there was appreciable activity at Rf 0.6 to 0.9. The n-butanol fraction, especially that of the girdled berries, was very active. Toxic materials in the n-butanol extracts of unringed berries prevented growth of pea plants at Rf 0.1 to 0.7, and so these results are not present in figure 6.

Discussion

Our data show the presence of gibberellin-like activity in all 5 varieties of grapes studied. The wide diversity of active compounds in the berries is shown by the presence of gibberellin-like activity, at least in certain instances, in all fractions tested. In the 2 varieties having both seeded and seedless forms activity was present in both types. In Tokay more gibberellin was present in the seeded than in the seedless berries, indicating that seeds may be a source of the gibberellins in this variety. Gibberellins also have been demonstrated in the seeds of many other plants (17). This fits in with the observation by Müller-Thurgau (13) that there is a positive correlation between the number of seeds per berry and berry size. Our results with Tokay may find support by the results of Nitsch et al. (16), who showed that the peak of hormone production in Concord and Concord Seedless varieties corresponded with the development of the testa. However, the bioassay used by these authors tested for auxins as well as for gibberellins. Coombe (11) failed to find gibberellin-like activity in seeded grape varieties, but this may be explained by the fact that the nonpurified extracts he used probably contained considerable amounts of inhibitors. In the present study evidently these were removed at least in part by solvent fractionation and chromatography.

In the first experiment with Thompson Seedless (fig 4), and with control and ringed Black Corinth, there was much activity in the chloroform fraction. Hayashi et al. (6), working with potato peelings, ascribed gibberellin activity in chloroform extracts to GA3 and GA20, which are soluble at pH 6. However, this does not explain our results with grapes, because we found at least 3 peaks of activity. Also, GA3 and GA20 have similar Rf’s in the isopropanol solvent system and so should account for only 1 of the peaks.

In the second experiment with Thompson Seedless (fig 6) greatest activity was found in the acidic ethyl acetate and acidic n-butanol fractions, while in the first experiment (fig 4) most occurred in the neutral and chloroform fractions. The berries collected in 1963 for the second experiment were collected at a later stage than those in 1962. Also, the season was cooler in 1963. These results indicate that stage of development and climatic conditions may affect the form of the gibberellins present.

Neutral activity (refers to activity found in the neutral fraction) was present in the first experiment with Thompson Seedless (fig 4). Black Corinth, and both seeded and seedless Emperor. Hayashi and Rappaport (3). Hayashi et al. (6) and Hashimoto and Rappaport (2) have demonstrated the presence of neutral gibberellin activity in potato peelings and bean seeds, respectively. Neutral gibberellin-like compounds also have been found by Wierzchowski and Wierzchowska (21) in culture filtrates of Gibberella fujikuroi. The method of extraction, however, would indicate that these substances are basic in nature. It is of interest that we found slight but significant activity in Emperor berries in the basic fractions since, to our knowledge, this previously has not been reported in higher plants.

The possibility that bound gibberellin may be of importance is supported by work with other plant materials. Hayashi and Rappaport (4) found that when they chromatographed aged neutral extracts from potato peelings, the neutral activity disappeared; however, new activity showed up at an Rf associated with acid gibberellins. Lazer et al. (9) showed in residue experiments with malt that the longer the period of extraction, the greater was the amount of labeled GA3 that could be recovered. They postulated the degradation of a hypothetical bound gibberellin to explain this phenomenon. Hashimoto and Rappaport (2) reported an increase in activity in the neutral fraction when bean seeds (Phaseolus vulgaris L.) were incubated with GA3, and postulated an autoregulatory mechanism for the control of endogenous gibberellin levels. When grapes were treated with GA3, (Weaver and Pool,
unpublished data), an increase in gibberellin activity was observed in the neutral and chloroform fractions. McComb (11) demonstrated bound gibberellin in mature runner bean (Phaseolus multiflorus) seeds. Yamaki (22) recently reported an increase in the acid gibberellin content of acid-hydrolyzed extracts of Mung bean (Phaseolus mungo) seeds.

Hayashi et al. (6) found gibberellin-like activity in the acidic n-butanol fraction of potato peelings but ascribed it to incomplete extraction of the acid gibberellins with ethyl acetate. In our experiment with Thompson Seedless in 1963, activity in the n-butanol fraction was much greater than that in the ethyl acetate fraction. Sembner et al. (18) demonstrated the presence of gibberellins in butanol fractions from Phaseolus coccineus L. They reported part of this activity was due to GA₆ and part to Phaseolus e, a gibberellin bound to carbohydrates and ninhydrin-positive compounds. Murakami (14, 15) demonstrated in several plants the presence of gibberellin A₃ β-glucoside. The Rp of this compound (0.4) in ammoniacal isopropanol is similar to the peak we obtained in the n-butanol fraction of ringed Thompson Seedless in 1963. The presence of a gibberellin glycoside in ringed fruit would not be surprising since it is well known that much accumulation of carbohydrates occurs above the ring.

Our data show that ringing is associated with great differences in content of gibberellin-like substances in berries. Previous work with crude extracts by Coombe (1) failed to reveal an increase in gibberellin-like activity as a result of ringing. It is now necessary to discover which gibberellins are involved in fruit development and hopefully the mechanisms involved.

In the fruit from unringed Black Corinth there was high activity in the neutral fraction, some in the chloroform fraction, but little or none in the acidic ethyl acetate or acidic n-butanol fractions. Ringing removed the activity in the neutral fraction, but greatly increased the activity in the acidic ethyl acetate and acidic n-butanol fractions. One can postulate that ringing triggers a transformation of the neutral activity (possibly a bound or precursor form) to forms that are found in the acidic ethyl acetate or acidic n-butanol fractions. One can further hypothesize that it is the activity in the latter 2 fractions that is important in development of Black Corinth berries. In Thompson Seedless (ringing experiment) there was no significant activity in the neutral fraction. However, these berries were collected late (June 19), and an earlier collection might have shown considerable activity in the neutral and in the chloroform fractions.

**Summary**

Extracts of flowers, berries, or both, of 5 varieties of grapes (Vitis vinifera L.) were analyzed for gibberellin-like activity. Extracts using 3 organic solvents, chloroform, ethyl acetate, and n-butanol, were made at low (2.5), neutral, and basic (8.0) pH values. For the chromatographic separation of gibberellin-like activity a developing solvent consisting of a mixture of isopropanol, ammonium hydroxide and water (10:1:1, v/v) was used. Dwarf peas grown under red light were used for the bioassay.

Gibberellin-like activity was found in all varieties of grapes tested, and at least in certain instances, in all fractions tested. In the 2 varieties having both seedless and seeded forms activity was present in both types. There was more gibberellin-like activity in seeded than seedless Tokay.

Ringing had a tremendous influence on gibberellin content of the berries of seedless varieties. In Black Corinth ringing greatly decreased the content of neutral gibberellin-like activity, but increased the activity in the acidic ethyl acetate and acidic n-butanol fractions.

**Literature Cited**


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