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Necrosis in grapevine buds (Vitis vinifera cv. Queen of Vineyard)

II. Effect of gibberellic acid (GAs) application1)

by

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Knospennekrosen bei der Rebsorte Queen of Vineyard (Vitis vinifera) II. Einfluß der Gibberellinsäure- (GA₃)Behandlung¹)

Zusammenfassung. — Bei der Sorte Queen of Vineyard wurde der Einfluß der GA_3 -Behandlung auf die Entwicklung von Knospennekrosen untersucht. GA_3 bewirkte die Ausbildung einer nekrotischen Gewebeschicht an der Basis der Mittelknospe und förderte die Entwicklung der Axillarknospen. Durch GA_3 -Behandlung der Blätter ließen sich leichter Knospennekrosen auslösen als durch direkte Behandlung der Knospen. Wurde GA_3 über den Blattstiel zugeführt, so war seine Nekrosen induzierende Wirkung 100mal stärker als bei Behandlung der Blattspreite. Nur sich entwickelnde und relativ junge Knospen waren gegen GA_3 sensibel. Um eine Knospennekrose auszulösen, mußten in den Knospen mindestens 6×10^{-6} mg GA_3 vorliegen. Zwischen 1 Woche vor und 3 Wochen nach der Blüte erfolgte die Bewegung des GA_3 in den Rebentrieben akropetal. Die mögliche Beteiligung von Gibberellinen am natürlichen Auftreten von Knospennekrosen bei wüchsigen Reben wird diskutiert.

Introduction

The use of gibberellins (GA) in horticulture has often been found to be associated with delayed bud sprouting (11), a decrease in the number of developing buds, bud necrosis, and a decrease in productivity (13, 15, 16, 17). GA application to several fruit species was found to affect the number of flowering buds (2, 6, 7, 9) in many stone fruits (almond, plum, peaches) and bud necrosis and death have also been observed (4, 5).

In the grapevine, GA_3 application has become common practice for fruit improvement. However, adverse effects, such as bud necrosis, delayed sprouting and a decrease in inflorescence number in the seeded varieties have limited its use to a few cultivars not sensitive to GA_3 (12, 13, 15, 18). In order to prevent bud necrosis, it was recommended that, particularly in seeded cultivars, only inflorescences should be treated. In varieties such as Queen of Vineyard, Alphonse Lavallee and Early Panse, GA_3 application often caused necrosis of the central bud and elongation of the axillary ones, resulting in many cases in a "split bud" (13, 14). When GA_3 was

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applied before flowering, bud necrosis was found in the lower part of the cane, while spraying after flowering caused necrosis in higher buds. In vigorous plants of Queen of Vineyard, naturally occurring necrotic buds were noted (1). Bud necrosis was found to be related to the position on the cane and stage of development of the buds and to shoot vigor (10).

The possibility that the applied GA_3 is involved in bud necrosis and its relation to natural bud necrosis and drying were the subjects of the present study.

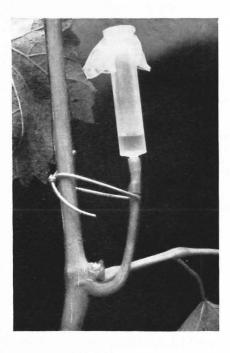


Fig. 1: Leaf petiole feeding from a 2 ml plastic syringe cylinder with a latex tube bridge to petiole.

GA_J-Zufuhr über den Blattstiel aus dem Zylinder einer 2-ml-Plastikinjektionsspritze, der durch einen Latexschlauch mit dem Blattstiel verbunden ist.

Materials and methods

Plant material, growth and pruning practices were as described in the first part of this study (10).

Gibberellin solutions (ICI Berlex, 10 % active GA_3) including Tween 20 (0.03 %) as a surfactant were either sprayed on the leaves or fed to the petioles through a rubber tube. The leaf lamina was removed just prior to attachment of the rubber tube and an open syringe cylinder of 2 ml was connected to the rubber tubing. 1 ml of GA_3 solution was introduced, taking care to remove all air bubbles from the tube (Fig. 1). An amount of 1 ml was also left on the leaf blade after spray application.

Direct application to buds was done by painting a measured volume of ${\rm GA}_3$ on the bud, using a camel hair brush.

Radioactice $^3\text{H-GA}_3$ (Amersham, England; non-specifically labelled, specific activity 6.12 mCi/mmole) was either applied on the upper and lower epidermis of the 6th leaf lamina or fed through the petiole. Radioactivity was measured by a

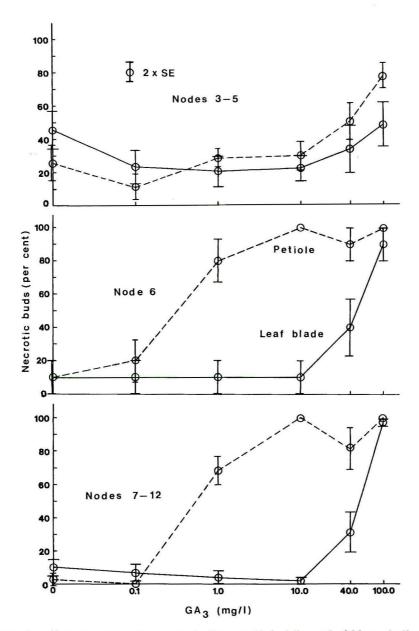


Fig. 2: The effect of various GA_3 concentrations applied at the end of bloom to the leaf blade or petiole at node no. 6 on the occurrence of necrosis in buds above and below the application site.

Der Einfluß verschiedener GA_3 -Konzentrationen auf das Auftreten von Knospennekrosen oberhalb und unterhalb des Applikationsortes. Die Substanz wurde am Ende der Blühperiode entweder über die Blattspreite oder über den Blattstiel des 6. Knotens zugeführt.

liquid scintillation counter (Packard model 3255) after buds oxidation in a Tri Carb sample oxidizer (Packard model 306). ³H-GA₃ was absorbed as tritiated water by 10 ml monophase. 7 buds were used for each ³H-GA₃ treatment; results were analyzed statistically by the Multiple Range Test.

Results

Exogenous GA_3 application (20 mg/l) to leaves and buds showed that the degree of bud necrosis was determined by the time and mode of GA_3 application (Table 1). The highest rate of bud necrosis occurred when GA_3 was applied to the leaves as a spray at flowering time. 3 weeks later, even 5 times the concentration (100 mg/l) was ineffective in causing bud necrosis. GA_3 given directly to the buds had a considerably smaller and insignificant effect.

Table 1 The effect of time and concentration of GA_3 applications on bud necrosis of grape shoots of cv. Queen of Vineyard

Der Einfluß des Behandlungszeitpunktes und der GA_3 -Konzentration auf die Knospennekrosen an den Trieben der Rebsorte Queen of Vineyard

Time of application	${ m GA}_3$ concentration (mg/l)	Organ treated¹)	Necrotic buds²) (º/₀)	
(Control)	_	_	7.0 bc ³)	
Full bloom	20	leaf	43.0 a	
**	20	bud	20 b	
21 d after full bloom	20	leaf	2.2 bc	
	20	bud	3.0 bc	
	100	leaf	3.7 bc	
	100	bud	1.5 c	

¹⁾ Leaves sprayed or buds painted at nodes no. 5-10.

Increasing the GA_3 concentration given at flowering time resulted in a higher level of necrosis when applied by petiole feeding than by laminar spray. 1 ml of a 1 mg/l GA_3 solution fed to the petiole caused a similar degree of bud necrosis as the same amount of a 40 mg/l GA_3 solution applied as a laminar spray (Table 2).

The distribution of necrosis in the buds below (nodes 3—5) and above (nodes 7—12) the point of GA_3 application (node 6) is shown in Fig. 2. GA_3 sprays to leaf blades had only a slight effect in inducing bud necrosis below the 6th node while the buds above it showed necrosis similar to that in the axil of the 6th leaf itself. This was noticeable at a GA_3 concentration of 40 mg/l and higher. The same amounts of GA_3 applied by petiole feeding affected also the buds below the 6th node when given at the high concentration of 100 mg/l, while buds at nodes 6—12 were necrotic even when 1 mg/l GA_3 was applied.

The effect of time of GA_3 application (40 mg/l) on bud necrosis is shown in Table 3. From full bloom and up to 2 weeks thereafter, GA_3 sprays caused 80—90% of the

²⁾ Results are pooled values for buds at nodes no. 5-10.

 $^{^{3}}$) Numbers followed by different letters differ significantly at P = 0.05.

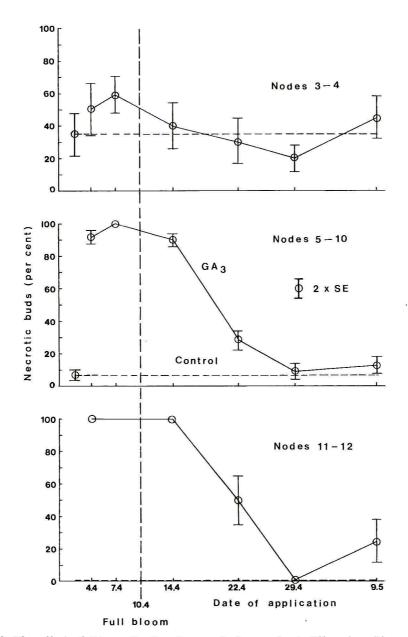


Fig. 3: The effect of GA_3 application time on bud necrosis at different positions along the shoots of Queen of Vineyard grapevines. GA_3 , 40 mg/l, was sprayed on leaves at nodes 5—10, buds were analyzed in September.

Der Einfluß des Zeitpunktes der GA_3 -Behandlung auf das Auftreten von Knospennekrosen an verschiedenen Knoten der Triebe von Queen of Vineyard. Die Blätter der Knoten 5—10 wurden mit 40 mg $GA_3/1$ besprüht; die Knospen wurden im September untersucht.

buds to become necrotic. At later applications the sensitivity of the buds to GA₃ dropped considerably, reaching the same levels as the control.

The data presented in Table 3 were analyzed further, to establish the effect of GA_3 application on bud necrosis in relation to bud position on the shoot. The buds in the axil of the treated leaves (5—10th nodes) and those above them (1—12th node) showed a high degree of necrosis when sprayed before or 2 weeks after full bloom (Fig. 3). Buds at the 3—4th nodes showed a slightly higher but insignificant level of necrosis than the equivalent control buds.

The topmost 6 leaves of growing shoots were sprayed with 40 mg/l GA $_3$ 1 week before full bloom or 1 month thereafter, when the shoots already had 25 leaves. Fig. 4 shows the relation between vine vigor and bud sensitivity to GA 3. In young shoots, a high percentage of bud necrosis was found in the treated area and above it (nodes I—VI and +1 to +6), as compared with those below the treated zone. When GA $_3$ was given after flowering to older but still growing shoots, buds above the zone of application were found to be more sensitive than those at or below the GA $_3$ treated area.

The time needed for the development of necrotic areas in the bud after GA_3 treatments was determined after a spray application of 40 mg/l GA_3 to the 5—10th

Table 2

The effect of leaf blade spraying or petiole feeding with GA₃ during flowering time on the amount of necrotic buds on grapevine shoots of cv. Queen of vineyard (results expressed as per cent necrotic buds pooled from nodes 3—12 on each shoot)

Der Einfluß der GA₃-Applikation über die Blattspreite oder den Blattstiel während der Blühperiode auf den Anteil nekrotischer Knospen an den Trieben der Rebsorte Queen of Vineyard (Ergebnisse in % nekrotischer Knospen an den Knoten 3—12 der Triebe)

Organ	GA ₃ concentration (mg/l)					
treated	0	0.1	1.0	10	40	100
Leaf blade (spray)	23 bc¹)	10 с	11 c	10 с	37 b	82 a
Petiole (feeding)	14 c	6 c	65 b	78 ab	73 ab	94 a

¹⁾ Numbers followed by different letters differ significantly at P = 0.05.

Table 3

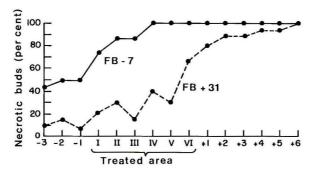
The effect of date of GA_3 application on the percentage necrosis in buds of cv. Queen of Vineyard grapevine shoots (GA_3 concentration 40 mg/l; spray application starting at full bloom on leaf blades at nodes 5—10; necrosis recorded in buds at nodes 3—12; numbers followed by different letters differ significantly at P = 0.05)

Der Einfluß des Zeitpunktes der GA_3 -Behandlung auf die Knospennekrose (%) an den Trieben der Rebsorte Queen of Vineyard (40 mg GA_3 /1; Sprühbehandlung der Blattspreiten an den Knoten 5—10; Behandlungsbeginn zur Zeit der Vollblüte; Nachweis der Nekrosen an den Knospen der Knoten 3—12; Zahlen, hinter denen unterschiedliche Buchstaben stehen, unterscheiden sich signifikant bei P=0,05)

Control	April					
	4	7	14	22	29	
10 с	88 a	91 a	82 a	33 b	9 c	

leaves at the end of the bloom period. 10 d after the application, no necrotic buds could be seen; 10 d later, 21.7 % of the buds had a necrotic layer, and 37 d after the GA_3 treatment, 70 % of the buds had a necrotic center. In a later analysis, in September, necrosis was found in 90 % of the GA_3 -treated buds.

The extent and type of damage caused by GA_3 was found to depend on the stage of bud development. Mature, fully differentiated buds are insensitive to GA_3 . In mature buds which continue to differentiate, GA_3 damage was manifested by central bud necrosis without any external malformation. In young buds, GA_3 caused elongation of the lower internodes, development of a necrotic layer below the apex, and eventual death of the central bud (Fig. 5).



Nodal position on the shoot

Fig. 4: The effect on bud necrosis of GA₃ application before and after full bloom to the top 6 leaves of growing shoots. Queen of Vineyard shoots were sprayed with 40 mg/1 GA₃ 1 week before or 1 month after full bloom (FB), which was on April 12.

Einfluß der GA₃-Behandlung der oberen 6 Blätter wachsender Triebe von Queen of Vineyard vor und nach der Vollblüte. Die Triebe wurden 1 Woche vor oder 1 Monat nach der Vollblüte (FB), die auf den 12. April fiel, mit 40 mg GA₃/l besprüht.

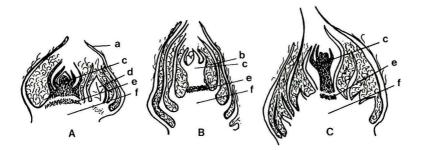


Fig. 5: Necrosis development in control and GA_3 treated Queen of Vineyard buds. — A: Control with necrotic central bud. GA_3 -treated with elongating active central bud. C: GA_3 -treated with elongated necrotic central bud. — a = scales, b = hairs, c = central bud, d = axillary bud, e = necrotic layer, f = bud base.

Entwicklung der Nekrosen in Kontrollknospen und mit GA_3 behandelten Knospen von Queen of Vineyard. — A: Kontrolle mit nekrotischer Mittelknospe. B: Sich streckende aktive Mittelknospe nach GA_3 -Behandlung. C: Gestreckte nekrotische Mittelknospe nach GA_3 -Behandlung. — A_3 -Beh

The axillary buds elongated rapidly after the necrotic decline of the central bud. In a longitudinal cut of such buds, the actively developing axillary buds near the hollow necrotic center are seen (Fig. 6). This rapid development of the axillary buds pushes the scale leaves covering the whole bud to open, resulting in a "split bud" formation (Figs. 7 and 8).

Translocation of GA_3 into the different buds along the vine was followed by use of tritiated GA_3 , which was introduced by petiole feeding (10 mg/l) or smeared

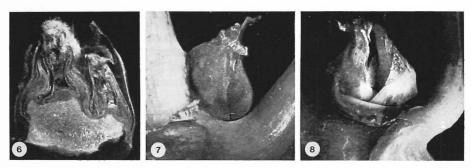


Fig. 6: Longitudinal cut through a GA₃-treated bud with necrotic center and developing axillary bud.

Figs. 7 and 8: General view of a normal bud (7) and a "split bud" (8) induced by ${\rm Ga_3}$ treatments to cv. Queen of Vineyard grapevine.

Abb. 6: Längsschnitt durch eine GA_3 -behandelte Knospe mit nekrotischem Zentrum und sich entwickelnder Axillarknospe.

Abb. 7 und 8: Gesamtansicht einer normalen Knospe (7) und einer "gespaltenen" Knospe (8), ausgelöst durch GA₃-Behandlung von Queen of Vineyard.

Table 4

Translocation of ${}^3\mathrm{H-GA}_3$ in cv. Queen of Vineyard grapevine shoots and per cent bud necrosis along the shoot after petiole feeding (10 mg/l) or leaf blade application (40 mg/l) to the leaf at the 6th node (radioactivity in buds measured after 9 h; necrosis determined on parallel shoots; 1 ml of solution was applied in both methods)

Translokation von ³H-GA₃ in den Trieben der Rebsorte Queen of Vineyard und Knospennekrosen (%) längs der Triebe nach Aufnahme über den Stiel (10 mg/l) oder über die Spreite (40 mg/l) des Blattes am 6. Knoten (Radioaktivität in den Knospen nach 9 h gemessen; Nekrosen an parallelen Trieben bestimmt; bei beiden Methoden wurde 1 ml Lösung appliziert)

Bud position	Petiole feeding			Leaf blade application			
	³ H (cpm/bud)	GA ₃ (mg/bud)	Necrosis	³ H (epm/bud)	GA ₃ (mg/bud)	Necrosis	
9	3972	6.7×10^{-6}	100	407	0.9×10^{-6}	20	
8	3746	6.3×10^{-6}	90	139	$0.3 imes 10^{-6}$	50	
7	3576	6.0×10^{-6}	80	212	0.5×10^{-6}	30	
6	5561	9.4×10^{-6}	100	277	0.6×10^{-6}	40	
5	186	0.3×10^{-6}	40	283	0.6×10^{-6}	30	
4	679	1.2×10^{-6}	40	415	0.9×10^{-6}	40	
3	101	0.2×10^{-6}	22	940	2.1×10^{-6}	30	

on the leaf blade (40 mg/l) of the leaf at the 6th node. Buds from the 3—9th nodes were sampled 9 h after treament. On shoots with petiole feeding of $^3\text{H-GA}_3$, the axillary bud of the fed petiole (6th bud) contained as much as 9.4×10^{-6} mg $^3\text{H-GA}_3$. A slightly lower level of $^3\text{H-GA}_3$ was found in each of the three buds above it (Table 4). The buds below the application site contained lower levels of $^3\text{H-GA}_3$. Laminar application of $^3\text{H-GA}_3$ was found to be ineffective and the amounts detected were insignificant.

Discussion

The application of gibberellic acid to the leaves of cv. Queen of Vineyard grapevine caused more bud necrosis than when applied directly to the buds. As shown previously with cv. Barlinka grapevines (15), the leaves having a larger exposed area and a smaller penetration barrier apparently allow a better penetration and translocation of GA3 to the buds. Petiole feeding of GA3 caused a higher degree of bud necrosis at lower GA3 concentration than either spraying or smearing the GA3 on the lamina. The degree of bud necrosis depended to a large extent on the stage of development of the buds. Upper, young still differentiating buds on mature canes were affected by GA3 to the same extent as those on young, still growing shoots. It is obvious, then, that bud damage was determined by the bud's stage of development and to a lesser extent by the shoot's age. Further support for this idea comes from the observation that the number of necrotic buds found at or above the GA₃ treated zone was greater than that found below the zone of GA₃ application. GA3 has been found to cause bud internode elongation, and in young buds to affect dormancy (3). Since the GAs applied to the leaves must be translocated to the buds, the direction of GA₃ translocation will affect the position of necrotic buds on the shoot. Hale and Weaver (8) found that GA3 moved downward, along with leaf metabolites. Our results show that the small degree of bud necrosis below the zone of application correlated with low levels of labelled 3H-GA3 in the lower buds (below the 3H-GA3-treated area). This may indicate that the lower bud did not constitute a strong metabolic sink for GA3 at the time the experiment was conducted. However, it is also possible that the lower buds were not affected by GA₃, since they were already fully differentiated and had ceased to grow.

Generally, the naturally occurring necrosis in the central bud on a Queen of Vineyard grapevine was similar to that induced by exogenous treatments of GA_3 . Exogenous application of GA_3 (18) also enhances the rate of shoot growth in most grapevine cultivars. As the natural necrosis is induced only during active shoot growth, and mainly in vigorous vineyards, it is suggested that high levels of endogenous gibberellins are involved in the development of necrosis in the central buds of vigorously growing shoots. This would need verification by direct analysis for endogenous gibberellins in buds at various stages of development and different degrees of shoot vigor.

Summary

The effect of gibberellic acid application on the development of necrosis in cv. Queen of Vineyard grapevine buds was studied. GA_3 caused the development of a necrotic layer at the base of the central bud and promoted the development of the

axillary buds. GA_3 application to leaves caused necrosis in the buds more readily than direct application to the buds. GA_3 fed to the petiole was 100 times more active in inducing necrosis than leaf application. Only developing and relatively young buds were sensitive to GA_3 . A minimum of 6×10^{-6} mg GA_3 in the bud was needed to cause bud necrosis. During the period from 1 week before to 3 weeks after bloom, the movement of GA_3 in the grape shoot was acropetal. The possible involvement of gibberellins in the natural necrosis of buds on vigorous grapevines is discussed.

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