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Induction of sex conversion in male Vitis[1])

by

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Introduction

Literature on natural sex conversion from functionally male to functionally hermaphroditic flowers in *Vitis* has been reviewed in previous papers (Negi and Olmo 1970, 1971). Natural sex conversion in the male *V. vinifera (sylvestris)* clone 030-44 was not due to somatic mutation or germinal mutation. Environmental conditions have been reported to influence sex conversion in male vines of *V. vinifera* L. *(sylvestris)* (Negi and Olmo 1970, 1971) and other *Vitis* species (Negrul 1936, Breider and Schieu 1938, Bethmann 1939, Levadoux 1946, Kozma 1955, Barrett 1966). Systematic attempts were started in 1965 to induce sex conversion in male vines of *Vitis* at the University of California vineyard, Davis. A preliminary report on sex conversion in a male *V. vinifera* L. *(sylvestris)* by a kinin has already been published (Negi and Olmo 1966). The present paper reports detailed studies on induction of sex conversion in the male *V. vinifera (sylvestris)* clone 030-44, several other *Vitis* species, and the hybrid 'Ganzin 1'.

Materials and Methods

Vegetatively propagated vines of the following species and a species hybrid of *Vitis* in the University of California vineyard collection at Davis were used.

Species or species hybrid	Clone	Sex
1. *V. vinifera* L. *(sylvestris)*	030-37	male
Shirwandah, Iran	030-39	male
	030-44	male
	033-64	male
	034-54	male
2. *V. cinerea* Engelm.	5009	male
3. *V. girdiana* Munson	3816	male
4. *V. longii* Prince	5427	male
	5429	male
5. *V. riparia* Michx.	5421	male
	49175	male
6. *V. rotundifolia* Michx.	'Male'	male
7. *V. rupestris* Scheele	'Constantia'	male
	'du Lot' ('St. George')	male
8. *V. vinifera* × *V. rupestris*	'Ganzin 1'	male

[1]) This paper is adapted from a portion of a thesis submitted by the senior author in partial fulfillment for the Ph. D. degree in Genetics, University of California, Davis, (February), 1969.

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Two homozygous male seedling vines numbered a26-30 and a26-66 and 2 heterozygous male seedling vines numbered a26-35 and a 26-63 from the 030-44 selfed progeny were also used.

1. Environmental factors:

a) Girdling:

Two shoots on each of 6 vines of 030-44 were girdled about 3 weeks before anthesis and the clusters were bagged. Control clusters were also bagged.

b) Nitrogen level:

Three lots of 12 dormant cuttings of 030-44 were made. Each lot was then planted in a separate glazed pot containing sterilized quartz sand. One lot was irrigated with HOAGLAND and ARNON's (1938) solution 1, the second with 1.8 × normal nitrogen and the third with no nitrogen (prepared by replacing KNO_3 and $Ca(NO_3)_2$ with KCl and $CaSO_4$ respectively).

c) Photoperiod:

Each lot of 12 cuttings of 030-44 was planted in a separate pot containing a mixture of sterilized soil, sand and peat moss in the ratio of 3 : 1 : 1. One pot was kept in the open or normal day length of approximately 12 hours, the second and third were placed in a growth chamber under 9 and 16 hours photoperiod respectively.

2. Chemical treatment:

In one series of experiments, flower clusters of the clone 030-44 at the megaspore mother cell stage were dipped for 1 minute in an aqueous solution of β-indoleacetic acid (IAA, 100 or 500 ppm), indolebutyric acid (IBA, 100 or 500 ppm), α-naphthaleneacetic acid (NAA, 25, 100 or 500 ppm), β-naphthoxyacetic acid (NOA, 100 or 500 ppm), (2-chloroethyl)-trimethyl ammonium chloride (CCC or 'Cycocel', 100, 500 or 1000 ppm), 2,3,5-triiodobenzoic acid (TIBA, 100 or 500 ppm), gibberellic acid (GA_3, 100, 200 or 500 ppm), 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (cytokinin SD 8339 or BTP) dissolved in 5% isopropyl alcohol (100, 500 or 1000 ppm), SD 8339 sonicated in distilled water (1000 ppm), 6-(4-hydroxy-3-methylbut-trans-2-enyl)-aminopurine (zeatin, 1000 ppm), 6-benzyladenine (BA, 1000 ppm), 6-furfurylaminopurine (kinetin, 1000 ppm), adenine (1000 ppm), guanine (1000 ppm), isopropyl alcohol (5%), Tween-20 (0.1%), or Tween-20 (0.1%) plus isopropyl alcohol (5%). Tween-20 at 0.1% was added as a wetting agent to the solutions, except when SD 8339 was sonicated in distilled water, or when isopropyl alcohol at 5% was used alone. Fifteen flower clusters, each on a different shoot, were used for each treatment. One hundred fifty-five control (untreated) clusters, each on a different shoot, were tagged at random. All treated and control clusters were enclosed in paper bags.

In another experiment with 030-44, inflorescences at 6 different stages of development were dipped for 1 minute in a 5-percent isopropyl alcohol solution of SD 8339 at 1000 ppm and then bagged.

In a separate experiment, 15 flower clusters of 030-44 at megaspore mother cell stage were dipped for 1 minute in a 5-percent isopropyl alcohol solution of SD 8339 at 1000 ppm and were then bagged. A few flower buds in each cluster were emasculated and the rest were removed just before anthesis. The clusters were rebagged to determine if parthenocarpic or parthenogenetic berries would be produced.

Young flower clusters of 2 homozygous and 2 heterozygous males in the 030-44 selfed progeny, those of other male clones of *V. vinifera* L. *(sylvestris)*, other *Vitis*

species listed above, and the species hybrid 'Ganzin 1' at megaspore mother cell stage were similarly dipped in a 5-percent isopropyl alcohol solution of SD 8339 at 1000 ppm. In addition, flower clusters of male clones of other *Vitis* species and 'Ganzin 1' at megaspore mother cell stage were dipped for 1 minute in an aqueous solution of gibberellic acid (100, 200 or 500 ppm) or Tween-20 (0.1%).

The percentages of clusters producing hermaphroditic flowers at anthesis and mature berries at harvest were determined in each treatment. However, in all cases, the percentages of seeded berry and seed set were used as final measures of sex conversion. At harvest time, the fallen flowers were collected in the bags; the berries, both seeded and seedless, and the seeds were counted in each treatment. With few exceptions, each functionally male flower bud has 4 ovules-most of which abort at various stages of development. By adding the total number of fallen flowers to the total number of berries, the total number of original flower buds and ovules were determined for each treatment. The % seeded berry set per treatment was calculated by: $\frac{\text{total mature seeded berries}}{\text{total flower buds}} \times 100$, and the % seed set was calculated by $\frac{\text{total seeds}}{\text{total ovules}} \times 100$.

The viability of pollen grains from treated and control flowers was determined by the acetocarmine staining method and by germination in hanging drop cultures of a 20% sucrose solution.

The viability of seeds was tested by germinating them in flats containing sterilized soil, sand and peat moss (3 : 1 : 1) in the greenhouse at 80° F after 3 months of stratification accomplished by keeping the flats with seeds outside during winter.

Chi-square analysis was used as the statistical test of significance.

Results and Discussion

I. Environmental factors:

The different levels of nitrogen fertilization, and widely different lengths of photoperiod did not induce sex conversion in the clone 030-44. Contrary to EWART (1929), neither did girdling cause sex conversion in male vines.

II. Chemical treatment:

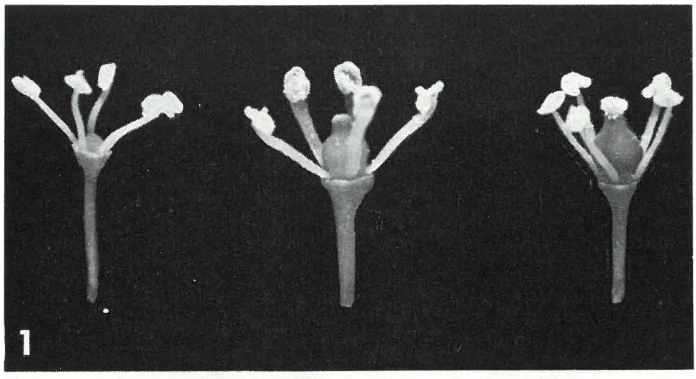
It is clear, from the data in Table 1, that of the various chemicals tried, only the cytokinin SD 8339 brought about sex conversion in 030-44. In fact, all the clusters treated with this compound produced typical hermaphroditic flowers at anthesis and normal fruit setting ensued (Fig. 1—3). Percentages of seeded berries and seed set resulting from cytokinin treatments were higher than those in the control or other treatments. This cytokinin dissolved in isopropyl alcohol (plus Tween-20) was more effective than when sonicated in distilled water without Tween-20. This may be because of its better dissolution in isopropyl alcohol and its better penetration of the flower buds with the wetting agent Tween-20. The sex converting effect of the sonicated cytokinin alone, proves that it is responsible for bringing about the change and not the isopropyl alcohol or Tween-20. IAA, IBA, NAA, NOA, CCC, TIBA, adenine, guanine and isopropyl alcohol did not increase the percentages of clusters producing hermaphroditic flowers at anthesis and mature berries at harvest, or percentages of seeded berry and seed set. Although GA_3 at 100, 200 and 500 ppm, Tween-20 at 0.1% and Twen-20 at 0.1% plus isopropyl alcohol at 5% increased the percentages of clusters producing hermaphroditic flowers at anthesis

Table 1

Effect of dipping 030-44 flower clusters at megaspore mother cell stage in various chemical solutions on sex conversion (1965—68)

Treatment	Clusters treated	Flower buds treated	Clusters with hermaphroditic flowers at anthesis	Clusters with mature berries at harvest	Seeded berry set	Seed set
	n	n	%	%	%	%
IAA, 100 ppm	15	12524	13.3	13.3	0.27	0.11
IAA, 500 ppm	15	14954	20.0	20.0	0.55	0.22
IBA, 100 ppm	15	12432	20.0	20.0	0.56	0.18
IBA, 500 ppm	15	13464	6.7	6.7	0.16	0.08
NAA, 25 ppm	15	12428	13.3	13.3	0.21	0.09
NAA, 100 ppm	15	13354	6.7	6.7	0.15	0.07
NAA, 500 ppm	15	13298	6.7	6.7	0.17	0.08
NOA, 100 ppm	15	14113	6.7	6.7	0.16	0.06
NOA, 500 ppm	15	13025	20.0	20.0	0.26	0.09
CCC, 100 ppm	15	12523	6.7	6.7	0.15	0.09
CCC, 500 ppm	15	11793	13.3	13.3	0.17	0.07
CCC, 1000 ppm	15	11984	6.7	6.7	0.18	0.06
TIBA, 100 ppm	15	13190	6.7	6.7	0.16	0.08
TIBA, 500 ppm	15	12439	6.7	6.7	0.18	0.09
GA_3, 100 ppm	15	11214	33.3	33.3	0.52	0.12
GA_3, 200 ppm	15	14916	40.0	40.0	0.48	0.18
GA_3, 500 ppm	15	12413	33.3	33.3	0.58	0.20
SD 8339 dissolved in 5% isopropyl alcohol, 1000 ppm	15	16198	100.0	100.0	15.38	5.04
SD 8339 sonicated in distilled water, 1000 ppm,	15	20477	100.0	100.0	6.56	1.66
Adenine, 1000 ppm	15	11912	20.0	20.0	0.58	0.17
Guanine, 1000 ppm	15	16195	13.3	13.3	0.18	0.08
Tween-20, 0.1%	15	13297	33.3	33.3	0.60	0.19
Isopropyl alcohol, 5%	15	17508	20.0	20.0	0.45	0.20
Tween-20, 0.1% + isopropyl alcohol, 5%	15	12193	33.3	33.3	0.58	0.16
Control, untreated	155	175504	20.0	20.0	0.65	0.25

and mature berries at harvest, they did not increase the percentages of seeded berry and seed set. These treatments, therefore, cannot be considered effective in sex conversion.



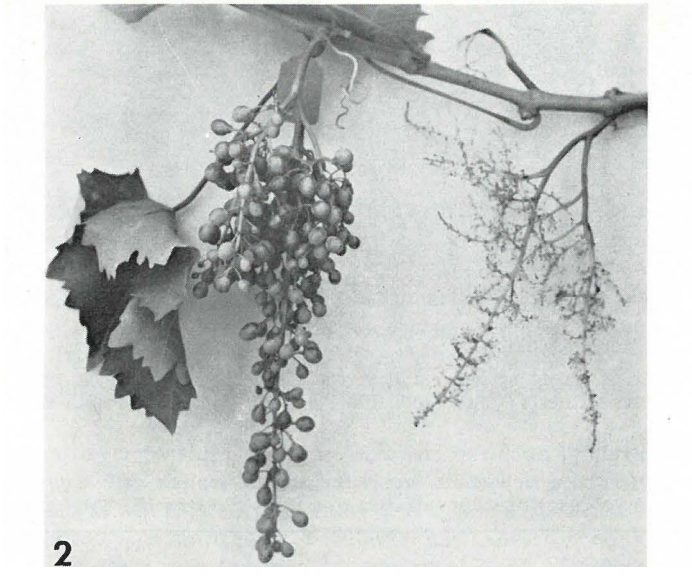
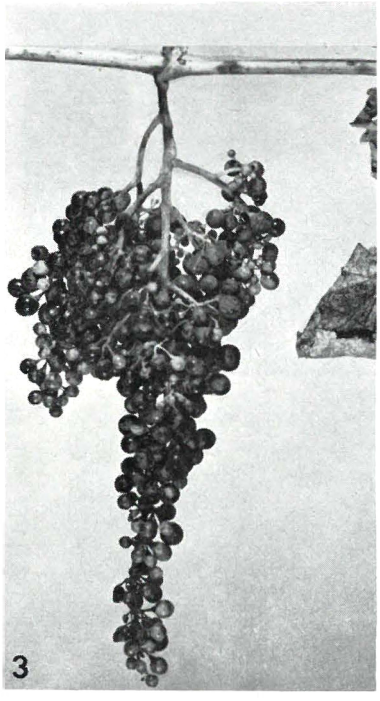


Fig. 1: Individual grape flowers: Left, functional male of 030-44; middle, hermaphroditic-induced by dipping a male flower bud of 030-44 at megaspore mother cell stage in the cytokinin SD 8339 at 1000 ppm; right, normal hermaphroditic of variety Muscat of Alexandria, × ca. 5.

Fig. 2: Effect of dipping 030-44 flower cluster at megaspore mother cell stage in SD 8339 at 1000 ppm on fruit set: Left. 11 weeks after dipping; right, control, × ca. 1/4.

The effects of 3 different concentrations of SD 8339 on sex conversion in 030-44 are presented in Table 2. A concentration of 1000 ppm was the most effective, followed in decreasing order by 500 and 100 ppm.

Of the various cytokinins tested (each at 1000 ppm) on sex conversion in 030-44, SD 8339 and zeatin were the most effective, followed by benzyladenine and kinetin (Table 3). SD 8339 and zeatin did not have significantly different effects. On the basis of percentages of seeded berry and seed set, all the cytokinin treatments, all of the treated clusters had hermaphroditic flowers at anthesis and mature berries at harvest, except in the kinetin treatment where only 46.7% of the treated clusters had hermaphroditic flowers at anthesis and mature berries at harvest. SD 8339 has



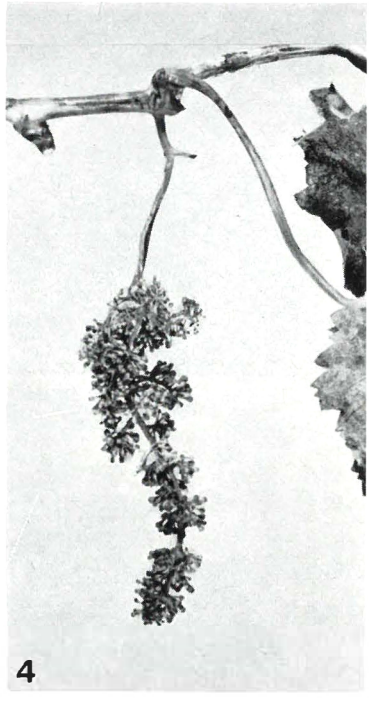


Fig. 3: A cluster of 030-44 with mature berries at harvest produced by dipping it in SD 8339 at 1000 ppm at megaspore mother cell stage, × 2/5.

Fig.4: A cluster of 030-44 at harvest that was dipped in SD 8339 at 1000 ppm one day before anthesis (stage 6 in Table 4), × ca. 2/3. Compare with Fig. 3.

Table 2

Effect of dipping 030-44 flower clusters at megaspore mother cell stage in 5% isopropyl alcohol solutions of varying concentrations of the cytokinin SD 8339 on sex conversion

Concentration of SD 8339	Clusters treated	Flower buds treated	Clusters with hermaphroditic flowers at anthesis	Clusters with mature berries at harvest	Seeded berry set	Seed set
ppm	n	n	%	%	%[1]	%[2]
1000	15	16198	100.0	100.0	15.38	5.04
500	15	4811	100.0	100.0	9.04	2.96
100	15	5592	100.0	100.0	6.31	1.85
0 (Control)	155	175504	20.0	20.0	0.65	0.25

[1]) Difference of more than 1.3% of seeded berry set between any two treatments is significant at 5% level.

[2]) Difference of more than 0.2% of seed set between any two treatments is significant at 5% level.

been reported to be more effective than benzyladenine in increasing fruit set and fruit enlargement in *V. vinifera* grapes, while kinetin was mentioned to have no such effects (Weaver, van Overbeek and Pool 1966). These authors attributed its (SD 8339) effectiveness to its greater solubility and mobility. Zeatin has been found to

Table 3

Effect of dipping 030-44 flower clusters at megaspore mother cell stage in various cytokinins on sex conversion

Cytokinin	Concentration	Clusters treated	Flower buds treated	Clusters with hermaphroditic flowers at anthesis	Clusters with mature berries at harvest	Seeded berry set	Seed set
	ppm	n	n	%	%	%	%
SD 8339	1000	15	16198	100.0	100.0	15.38	5.04
Zeatin	1000	15	3750	100.0	100.0	15.01	5.08
Benzyladenine	1000	15	13520	100.0	100.0	5.36	1.77
Kinetin	1000	15	16212	46.7	46.7	2.03	0.70
Control	0	155	175504	20.0	20.0	0.65	0.25

[1]) Difference of more than 1.3% of seeded berry set between any two treatments is significant at 5% level.

[2]) Difference of more than 0.2% of seed set between any two treatments is significant at 5% level.

be considerably more active than other known cytokinins in the carrot root tissue and soybean callus assays (Miller 1965, Letham 1966). According to Letham (1967), the high activity of zeatin is evidently associated with the presence in the side chain of an allylic hydroxyl group, which would be very reactive.

The negative effect of adenine on sex conversion (Table 1), suggests that is has to be substituted at the sixth position to bring about the change, as all the cytokinins are N^6- substituted adenines (Strong 1958, Skoog and Strong 1965, Helgeson 1968).

Inflorescence development at the time of treatment is an important factor. The results of applying SD 8339 (1000 ppm) at 6 different development stages of the flower buds of 030-44 are presented in Table 4. It is apparent that treatment at the megaspore mother cell or third stage produced the highest percentages of seeded berry and seed set. This was followed by treatment 3—4 days before megaspore mother cell differentiation (second stage). Treatments at the first 4 stages resulted in 100.0% of the clusters forming hermaphroditic flowers at anthesis that later produced berries, whereas, at the fifth and sixth stages, only 46.7 and 26.7% respectively did so. On the basis of percentages of seeded berry and seed set, the effects of the treatments at different developmental stages were also significantly different from one another. These differences may partly be dependent upon the number of male flower buds with normal ovules at the time of dipping. With the exception of treatment at the sixth stage, treatments at all other stages were effective in sex conversion. Treatment at the sixth stage caused only the ovaries to swell in almost all flowers and prevented them from abscissing (Fig. 4).

It was found that the SD 8339 treatment, at megaspore mother cell stage, did not induce parthenocarpic or parthenogenetic berry development in 030-44. However, treating the emasculated flowers of some hermaphroditic *V. vinifera* grape varieties with this cytokinin has resulted in a set of berries (Weaver, van Overbeek and Pool 1966). Crane and van Overbeek (1965) reported that this cytokinin also induced parthenocarpy in the Calimyrna fig.

Table 4

Sex conversion in 030-44 as determined by stage of flower bud development when dipped in the cytokinin SD 8339 at 1000 ppm

Treatment	Developmental stages of flower buds		Clusters treated	Flower buds treated	Clusters with hermaphroditic flowers at anthesis	Clusters with mature berries at harvest	Seeded berry set	Seed set
	Stages in the ovules	Stages in the anthers	n	n	%	%	%[1])	%[2])
SD 8339, 1000 ppm	1. Nucellus in the form of protuberance	Microspore mother cells	15	19397	100.0	100.0	6.16	1.93
	2. 3—4 days before megaspore mother cell differentiation; hypodermal archesporial cell observed in many ovules	Microspore mother cells undergoing meiotic divisions	15	14201	100.0	100.0	11.37	3.50
	3. Megaspore mother cells	Young microspores	15	16198	100.0	100.0	15.38	5.04
	4. Tetrad of megaspores with chalazal cell functioning	Microspore nucleus dividing	15	13385	100.0	100.0	9.80	3.08
	5. Some ovules have 2-nucleate embryo sacs while others have degenerated embryo sacs	2-celled pollen grains	15	17310	46.7	46.7	1.97	0.54
	6. Most ovules have degenerated embryo sacs, while a few have embryo sacs with normal egg apparatus and secondary nucleus	Mature 2-celled pollen grains with 3 germ-pores	15	15708	26.7	26.7	0.66	0.24
Control, untreated			155	175504	20.0	20.0	0.65	0.25

[2]) Difference of more than 0.2% of seed set between any two treatments is significant at 5% level.

[1]) Difference of more than 1.3% of seeded berry set between any two treatments is significant at 5% level.

Table 5

Effect of dipping 030-44 flower clusters at megaspore mother cell stage in various cytokinins on pollen viability

Cytokinin	Concentration	Stainability Test			Germination Test		
		Total pollen grains examined	Pollen grains stained	Stained normal pollen grains	Total pollen grains counted	Pollen grains germinated	Germination
	ppm	n	n	%	n	n	%
SD 8339	1000	600	553	92.2	600	337	56.1
Benzyladenine	1000	600	550	91.7	600	330	55.0
Kinetin	1000	600	551	91.8	600	340	56.7
Control	0	600	553	92.2	600	345	57.5

Table 6

Germination of seeds from hermaphroditic flowers and subsequent fruits of 030-44 produced by dipping flower clusters at megaspore mother cell stage in various cytokinins

Cytokinin	Concentration	Total seeds planted	Seeds germinated	Germination
	ppm	n	n	%
SD 8339	1000	300	226	75.3
Benzyladenine	1000	300	210	70.0
Kinetin	1000	300	221	73.7
Control	0	300[1])	214	71.3

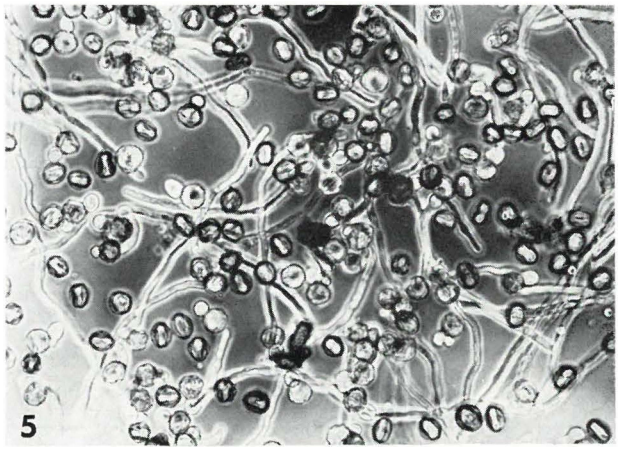
[1]) Seeds from fruits produced by natural hermaphroditic flowers.

Since the cytokinins had such a marked effect on stimulating the gynoecium, their effects on pollen development and viability were determined. Data on viability of pollen collected from flowers previously dipped in SD 8339, benzyladenine, and kinetin and from control flowers of 030-44 are presented in Table 5. It is evident that the percentages of stained, normal pollen grains and pollen germination are almost equal in treated and control flowers (see Fig. 5, 6).

The data in Table 6 show that viability of seeds obtained from hermaphroditic flowers and subsequent fruits of 030-44 induced by SD 8339, benzyladenine, and kinetin was normal, and was not different from the control where the seeds collected were from fruits produced by natural hermaphroditic flowers (Fig. 7).

Effect of SD 8339 on sex conversion in some other male clones and seedling vines of *V. vinifera* L. *(sylvestris):*

It may be seen from the data in Table 7 that male siblings of 030-44, numbered 030-37, 030-39, 033-64 and 034-54, also responded to the SD 8339 treatment at megaspore mother cell stage in the same manner as 030-44. Two homozygous male seedling vines, numbered a26-30 and a26-66 and two heterozygous males, a26-35 and a26-63, obtained in the 030-44 selfed progeny also responded to SD 8339 treatment at megaspore mother cell stage (Table 8). The percentage of seeded berry and



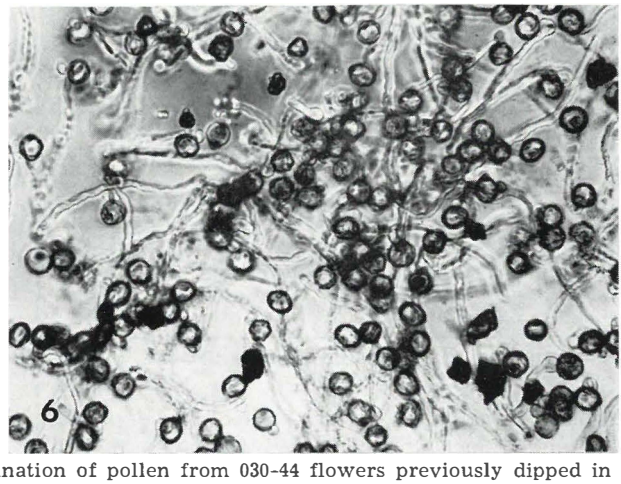


Fig. 5: Germination of pollen from 030-44 flowers previously dipped in SD 8339 at 1000 ppm, × 155.
Fig. 6: Germination of pollen from control flowers of 030-44, × 155.

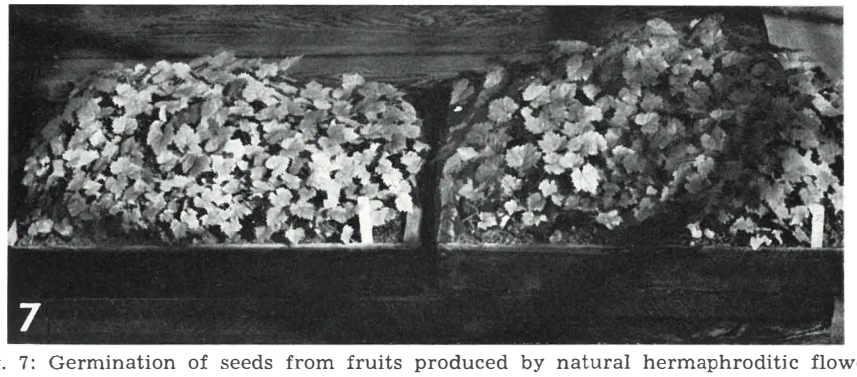


Fig. 7: Germination of seeds from fruits produced by natural hermaphroditic flowers (left) and from SD 8339 (1000 ppm)-induced hermaphroditic flowers and subsequent fruits (right) of 030-44, × 1/10.

Table 7

Effect of dipping flower clusters of some male seedling sibs of 030-44 at megaspore mother cell stage in the cytokinin SD 8339 at 1000 ppm on sex conversion

Clone	Treatment	Clusters treated	Flower buds treated	Clusters with hermaphroditic flowers at anthesis	Clusters with mature berries at harvest	Seeded berry set	Seed set
		n	n	%	%	%	%
030-37	SD 8339, 1000 ppm	15	12626	100.0	100.0	10.72	2.98
	Control, untreated	15	11422	6.7	6.7	0.10	0.05
030-39	SD 8339, 1000 ppm	15	11804	100.0	100.0	10.49	3.09
	Control, untreated	15	12826	13.3	13.3	0.17	0.09
033-64	SD 8339, 1000 ppm	15	11332	100.0	100.0	13.54	4.00
	Control, untreated	15	10942	13.0	13.3	0.46	0.16
034-54	SD 8339, 1000 ppm	15	12416	100.0	100.0	10.93	2.67
	Control, untreated	15	11315	0.0	0.0	0.00	0.00

seed set on these seedling vines, however, was much less than those of 030-44 and its sibs discussed above. The reason for the lower percentages of set in these F_1 males is obscure. However, the difference in the genotype of these F_1 males did not alter their response to cytokinin. In these male *V. vinifera* clones and seedling vines also, the viability of pollen was not affected by the cytokinin SD 8339 treatment and the seeds obtained showed normal germination.

Natural sex conversion was observed in all the male *V. vinifera (sylvestris)* clones studied except in 034-54, which did not show this phenomenon in 3 consecu-

tive years (1966—68). The *V. vinifera* male seedling vines a26-30, a26-35 and a26-66 did not exhibit natural sex conversion in 4 years of observation (1964, 1966—1968), while a26-63 showed this phenomenon in all 4 years. Thus, in these male *V. vinifera (sylvestris)* clones and seedling vines, there was no correlation in their tendency for natural sex conversion and response to applied cytokinin.

Table 8

Effect of dipping flower clusters of some genetically homozygous and heterozygous male vines from 030-44 selfed progeny at megaspore mother cell stage in the cytokinin SD 8339 at 1000 ppm on sex conversion

Vine	Genetic Constitution	Treatment	Clusters treated	Flower buds treated	Clusters with hermaphroditic flowers at anthesis	Clusters with mature berries at harvest	Seeded berry set	Seed set
			n	n	%	%	%	%
a26-30	Homozygous	SD 8339, 1000 ppm	15	11619	100.0	100.0	0.94	0.34
		Control, untreated	15	12710	0.0	0.0	0.00	0.00
a26-35	Heterozygous	SD 8339, 1000 ppm	15	12418	100.0	100.0	0.86	0.34
		Control, untreated	15	13216	0.0	0.0	0.00	0.00
a26-63	Heterozygous	SD 8339, 1000 ppm	15	11912	100.0	100.0	0.93	0.36
		Control, untreated	15	12918	6.7	6.7	0.11	0.03
a26-66	Homozygous	SD 8339, 1000 ppm	15	13911	100.0	100.0	0.85	0.33
		Control, untreated	15	11218	0.0	0.0	0.00	0.00

Effect of SD 8339, GA_3 and Tween-20 applied to flower clusters at megaspore mother cell stage on sex conversion in male clones of other *Vitis* species and the species hybrid 'Ganzin 1':

It is apparent, from the data in Table 9, that GA_3 or Tween-20 had practically no effect on sex conversion in male vines of 'Ganzin 1', *V. cinerea* 5009, *V. girdiana* 3816, *V. longii* '5427' and '5429', *V. riparia* '5421' and '49175', *V. rotundifolia* 'Male', and *V. rupestris* 'Constantia' and 'du Lot' ('St. George'). Although all the clusters treated with SD 8339 developed morphologically hermaphroditic flowers at anthesis, the percentages of clusters with berries and percentages of seeded berry and seed set at harvest varied from species to species (see Fig. 8—11). In the case of *V. rupestris*, variation occurred from clone to clone. *V. longii* and *V. riparia* showed the greatest response, followed by *V. rupestris* 'Constantia', 'Ganzin', *V. girdiana*, *V. cinerea* and *V. rupestris* 'du Lot' ('St. George'). *V. rotundifolia* was, by far, the least responsive; it produced only 1 seeded and 1 parthenocarpic berry from 1188 treated flowers. The different clones of *V. longii* and *V. riparia* were almost equally responsive. The difference in physiological response to the cytokinin SD 8339 shown by these species and the species hybrid could be either due to anatomical differences in their flower buds or to slight genetic differences. Morphologically, the majority of flowers of male vines of these species and the species hybrid was of type 1 (described by Negi and Olmo 1970), though varying numbers of flower types 2 and 3 were observed in all the species and the species hybrid. Flowers of type 4 were never found in male vines of *V. cinerea* 5009, *V. girdiana* 3816, *V. rotundifolia* Male, and *V. rupestris* du Lot (St. George) during 5 consecutive years of observation (1964—1968). On rare occasions, flowers of type 4 were observed in the male vines of 'Ganzin 1', *V. longii* 5427 and 5429, *V. riparia* 5421 and 49175, and *V. rupestris* '*Constantia*'. This means that occasional natural sex conversion was observed in male vines of Ganzin 1, *V. longii* and *V. riparia* clones, and *V. rupestris* Constantia, while it was not observed in the male vines of other species and clones studied. Thus, unlike the male clones and seedling vines of *V. vinifera* L. *(sylvestris)*, the male clones of these species and the species hybrid exhibited a positive correlation between natural sex conversion and the physiological response to the cytokinin SD 8339.

SD 8339 had no effect on pollen viability of these species and the species hybrid 'Ganzin 1', as more than 90% stained (normal) pollen grains were present in treated and control flowers. Percent pollen germination varied from species to species but did not vary between treated and control flowers of the same species and the species hybrid.

Data on the germination of seeds obtained from SD 8339-induced hermaphroditic flowers showed that the germination was more than 50% in each of the species and the species hybrid. Where possible, % seed germination in treated and control were compared and the differences were not greatly different.

A negative effect of GA, IAA, spermine tetrahydrochloride and isopropyl alcohol on sex conversion in male vines of 6 *Vitis* species was reported by Iizuka and Hashizume (1968). Gargiulo (1968) reported that isopropyl alcohol and Tween-20 did not convert sex in male vines of 6 grape rootstock varieties, and amphidiploid hybrid of *V. vinifera* × *V. rotundifolia* and a backcross of this amphidiploid with *V. vinifera*. SD 8339 at 1000 ppm has been reported to convert sex in male vines of *V. thunbergii* (Iizuka 1967) and Ganzin 1 (Gargiulo 1968). Gargiulo (1968) further reported that SD 8339 (1000 ppm) treatment to flower clusters of male vines of rootstocks 3306 C, 3309 C, 110 R, 420 A and 34 E.M., an amphidiploid hybrid of *V. vini-*

Table 9

Effect of dipping flower clusters of male clones of some *Vitis* species and the species hybrid „Ganzin 1" at megaspore mother cell stage in SD 8339, GA_3 or Tween-20 on sex conversion (1966-68)

Species or species hybrid	Clone	Treatment	Clusters treated	Flower buds treated	Clusters with morphologically hermaphroditic flowers at anthesis	Clusters with mature berries at harvest	Seeded berry set	Seed set
			n	n	%	%	%	%
V. vinifera × *V. rupestris*	'Ganzin 1'	SD 8339, 1000 ppm	15	7616	100.0	53.3	2.17	0.71
		GA_3, 100 ppm	15	7098	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	6561	0.0	0.0	0.00	0.00
		GA_3, 500 ppm	15	6078	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	6897	0.0	0.0	0.00	0.00
		Control, untreated	15	6916	0.0	0.0	0.00	0.00
V. cinerea	'5009'	SD 8339, 1000 ppm	15	10512	100.0	40.1	1.10	0.27
		GA_3, 100 ppm	15	9919	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	9111	0.0	0.0	0.00	0.00
		GA_3, 500 ppm	15	8898	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	11616	0.0	0.0	0.00	0.00
		Control, untreated	15	9892	0.0	0.0	0.00	0.00
V. girdiana	'3816'	SD 8339, 1000 ppm	15	6718	100.0	46.7	1.96	0.57
		GA_3, 100 ppm	15	6843	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	5612	0.0	0.0	0.00	0.00
		GA_3, 500 ppm	15	6968	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	6812	0.0	0.0	0.00	0.00
		Control, untreated	15	5817	0.0	0.0	0.00	0.00
V. longii	'5427'	SD 8339, 1000 ppm	15	4416	100.0	100.0	8.11	3.20
		GA_3, 100 ppm	15	3710	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	2912	6.7	6.7	0.10	0.05
		GA_3, 500 ppm	15	3616	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	3917	0.0	6.7	0.00	0.00
		Control, untreated	15	3896	6.7	6.7	0.07	0.04
	'5429	SD 8339, 1000 ppm	15	3684	100.0	100.0	8.95	3.34
		GA_3, 100 ppm	15	3608	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	2998	0.0	0.0	0.00	0.00
		GA_3, 500 ppm	15	3511	6.7	6.7	0.12	0.06
		Tween-20, 0.1%	15	4014	6.7	6.7	0.09	0.04
		Control, untreated	15	3819	0.0	0.0	0.00	0.00
V. riparia	'5421'	SD 8339, 1000 ppm	15	3611	100.0	100.0	8.84	3.89
		GA_3, 100 ppm	15	3618	6.7	6.7	0.08	0.03
		GA_3, 200 ppm	15	2898	0.0	0.0	0.00	0.00
		GA_3, 500 ppm	15	3475	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	3817	0.0	0.0	0.00	0.00
		Control, untreated	15	3986	6.7	6.7	0.09	0.05

Table 9

(Continued from Table 9)

Species or species hybrid	Clone	Treatment	Clusters treated	Flower buds treated	Clusters with morphologically hermaphroditic flowers at anthesis	Clusters with mature berries at harvest	Seeded berry set	Seed set
			n	n	%	%	%	%
V. riparia	'49175'	SD 8339, 1000 ppm	15	4116	100.0	100.0	8.49	3.81
		GA_3, 100 ppm	15	3691	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	3411	6.7	6.7	0.11	0.05
		GA_3, 500 ppm	15	2820	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	3916	6.7	6.7	0.06	0.02
		Control, untreated	15	3812	6.7	6.7	0.08	0.04
V. rotundifolia	Male	SD 8339, 1000 ppm	15	1188	100.0	6.7	0.08	0.02
		GA_3, 100 ppm	15	979	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	1014	0.0	0.0	0.00	0.00
		GA_3, 500 ppm	15	1147	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	1218	0.0	0.0	0.00	0.00
		Control, untreated	15	1197	0.0	0.0	0.00	0.00
V. rupestris	'Constantia'	SD 8339, 1000 ppm	15	3145	100.0	100.0	4.02	1.38
		GA_3, 100 ppm	15	3286	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	3315	0.0	0.0	0.00	0.00
		GA_3, 500 ppm	15	3293	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	3090	0.0	0.0	0.00	0.00
		Control, untreated	15	4002	0.0	0.0	0.00	0.00
V. rupestris	'du Lot' ('St. George')	SD 8339, 1000 ppm	15	6167	100.0	33.3	0.68	0.25
		GA_3, 100 ppm	15	5582	0.0	0.0	0.00	0.00
		GA_3, 200 ppm	15	5113	0.0	0.0	0.00	0.00
		GA_3, 500 ppm	15	6866	0.0	0.0	0.00	0.00
		Tween-20, 0.1%	15	5917	0.0	0.0	0.00	0.00
		Control, untreated	15	6175	0.0	0.0	0.00	0.00

fera × *V. rotundifolia* and a backcross of this amphidiploid with *V. vinifera* before anthesis resulted in the production of hermaphroditic flowers at anthesis, but later the clusters dried up and no berries developed. The reason for this, in the amphidiploid hybrid and in the backcross of this with *V. vinifera*, could be that they were genetically sterile and thus no fertilization took place. The reason for no fruit setting in the rootstocks is not clear. IIZUKA and HASHIZUME (1968) reported that the cytokinins BTP (SD 8339), 6-benzyladenine and 6-benzyladenosine (each at 1000 ppm) converted sex successfully with a normal fruit set in male vines of *V. coignetiae*, *V. thunbergii* var. *typica*, *V. linsecumii*, *V. aestivalis*, *V. vulpina*, and *V. labrusca* when their flower clusters were treated 18—35 days before anthesis, while treat-

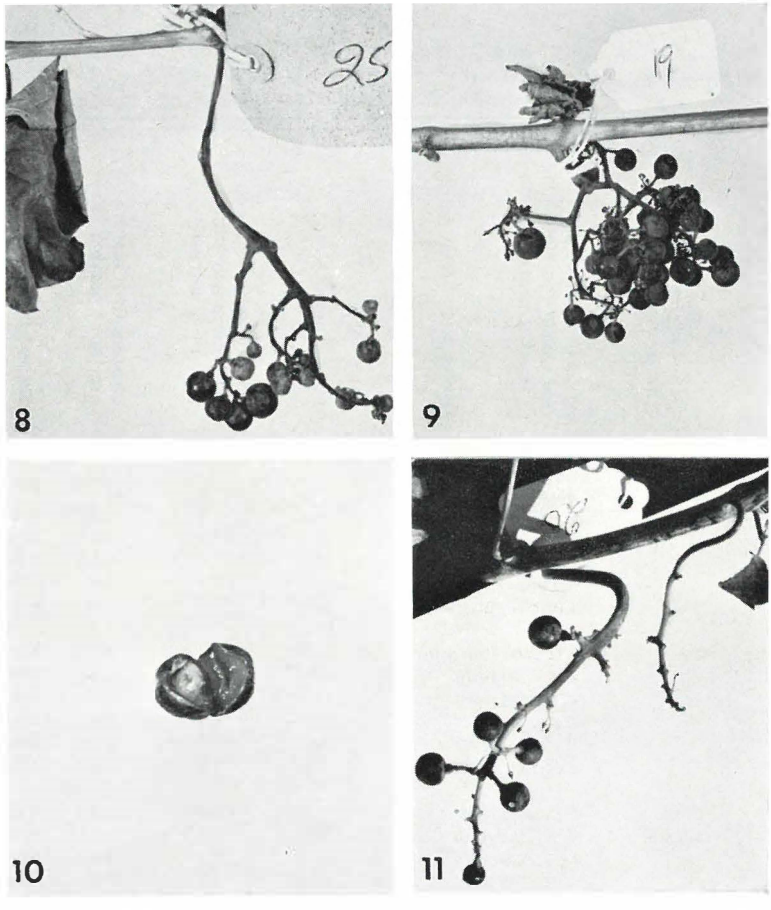


Fig. 8—11: Effect of dipping flower clusters of male clones of *Vitis* species and a species hybrid at megaspore mother cell stage in SD 8339 at 1000 ppm on fruit set: Fig. 8, Ganzin 1, × ca. ⅔; Fig. 9, *V. riparia* '5421', × ca. ²/₃; Fig. 10, *V. rotundifolia* Male, × ca. 2, Fig. 11: *V. rupestris* du Lot (St. George), × ca. ⅔.

ment of flower clusters of these species with these cytokinins 14—17 days before anthesis did not result in successful sex conversion and no berries developed. Though they did not study the stage of flower bud development anatomically at the time of treatments, their findings, however, indicate the importance of stage of flower bud development at the time of treatment.

It may be pointed out here that the treatment of flower clusters of female vines of *V. vinifera* L., at different developmental stages with SD 8339 at 1000 ppm, did not convert the sex though the pistil and other floral parts were enlarged. Similar findings were reported by Iizuka and Hashiizume (1968) on treating the flower clusters of female vines of *V. coignetiae* and *V. thunbergii* with cytokinin BTP (SD 8339), benzyladenine and benzyladenosine.

It can be deduced from these results that natural sex conversion in male vines of *Vitis* (see Negi and Olmo 1970, 1971) is associated in some manner with endogenous cytokinins. Sex conversion in male vines of *V. vinifera* L. *(sylvestris)* was attributed

to the influence of environmental, both local and seasonal, conditions (NEGI and OLMO 1970, 1971) and to a number of minor modifying genes (NEGI and OLMO 1971). Though the exact environmental factor and the number of minor modifying genes that cause sex conversion are not determined, it is possible that the influence of these factors may be regulating cytokinin production in the flower buds. Cytokinin activity has been detected in the bleeding sap of hermaphroditic *V. vinifera* grapevines (LOEFFLER and VAN OVERBEEK 1964, SKENE and KERRIDGE 1967, SKENE 1968), and it is thought to be synthesized in the roots (LOEFFLER and VAN OVERBEEK 1964, MULLINS 1967, SKENE and KERRIDGE 1967). However, it has been found by the authors (NEGI and OLMO 1970, 1971) that in the case of male vines, certain shoots and clusters have a larger number of converted flowers than others. Moreover, on a single shoot all clusters do not have converted flowers and in an individual cluster all the flowers are not converted. If cytokinin is synthesized in the roots and is translocated upward in the xylem, it is likely that all shoots of a vine, all clusters on a shoot, and all flower buds in a cluster would receive about equal quantities. As our results (NEGI and OLMO 1970, 1971) do not indicate this to be the case, it appears, on the other hand, that an individual flower bud or an individual cluster acts as a unit. Modifying genes under certain environmental conditions bring about synthesis of cytokinin in these units and the pistils develop normally. Thus, natural sex conversion results.

Practical application of this method of sex conversion by cytokinins:

1. Production of all-male populations:

With this method of sex conversion it is possible to self male vines, some of the progeny of which are homozygous for male transmission. On crossing these homozygous males with females or hermaphrodites, populations consisting of all-male vines can be obtained. This is very useful and economical in breeding for rootstocks where frequently only male vines are selected because of their unfruitfulness and greater vigor.

If this method of sex conversion can be successfully used in *Asparagus,* it will have commercial advantage, since male plants yield 25% more than female (ROBBINS and JONES 1928).

2. Use in crosses involving dioecious species:

In certain crosses involving dioecious species only the male plants survive and reach maturity. With this method, their sex may be converted and they may then be selfed or used as female parents in further breeding.

Summary

Shoot girdling, widely different levels of nitrogen and photoperiod and a number of chemical agents were ineffective in promoting sex conversion in a male *V. vinifera (sylvestris)* clone 030-44.

The cytokinins SD 8339, zeatin, benzyladenine and kinetin converted sex from male to hermaphrodite in the clone 030-44. The first two cytokinins were more effective than the others. A concentration of 1000 ppm of SD 8339 was the most effective, followed in decreasing order by 500 and 100 ppm. Treatment was most effective at the time of megaspore mother cell formation.

SD 8339 also induced sex conversion in other male clones and seedling vines of *V. vinifera* L. *(sylvestris)* and in male clones of other *Vitis* species, and a species hybrid 'Ganzin 1'.

In male clones and seedling vines of *V. vinifera* L. *(sylvestris),* there was no correlation in frequency of natural sex conversion and that induced by SD 8339, but such a correlation was found in male clones of other *Vitis* species and a species hybrid.

The cytokinins did not affect pollen viability and the seeds obtained germinated normally.

Association of natural sex conversion with endogenous cytokinins in male vines of *Vitis* is discussed.

Two possible practical applications of this method of sex conversion by cytokinins are also mentioned.

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