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Control of vegetative growth of grapevine shoots by ethylene-releasing substances Conditions and sites of action[1])

by

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Die Kontrolle des vegetativen Wachstums von Rebtrieben durch Äthylen-liefernde Substanzen Einwirkungsbedingungen und -orte

Zusammenfassung. — Triebe der Rebsorten Muskat Hamburg und Perlette wurden mit zwei Äthylen-freisetzenden Handelspräparaten behandelt: Ethrel (2-Chloräthylphosphonsäure) und Alsol (2-Chloräthyl-tris-(2-methoxyäthoxy)silan) bei saurem und neutralem pH. Ethrel verursachte eine signifikante Hemmung des Triebwachstums, Alsol hatte keinen Einfluß auf die Wachstumsgeschwindigkeit. Ethrel war bei pH 6,9 wirksamer als bei pH 2,2; im niedrigen pH-Bereich war seine Aufnahme signifikant erhöht. In den behandelten Sprossen war vielfach das apikale Meristem geschädigt. Die Beziehung zwischen Substanzaufnahme und Wachstumsreaktion wurde bestimmt. Bei alleiniger Blattbehandlung war das Wachstum der Triebspitze nicht gehemmt.

Introduction

In a previous study of the persistence, uptake and translocation of ^{14}C-ethephon in grapevine shoots (13), we found that a considerable amount of ethephon penetrates the leaf tissue and remains there for several days. Only a slight, mainly basipetal, translocation from application sites was found.

Ethephon in aqueous solution is acidic (pH = 2.2) and releases very little ethylene. Raising its pH to 7.0 results in a marked increase in ethylene evolution (5, 8, 16, 24). Experiments to induce olive fruit abscission indicated that application of Ethrel, a commercial preparation of ethephon, at pH = 7.0 was more effective than at lower pH (5,6). Alsol, another commercial ethylene-releasing compound based on etacetasil, was found to be more effective than Ethrel in reducing the fruit-removal force in olives. Its most stable form is at pH = 6.0 (at 20 °C) and its breakdown rate is increased by either raising or lowering the pH of the solution (5; Dubach and Dupois, personal communication). In most field experiments, Alsol was more effective than Ethrel even when applied at the elevated pH of 7.0 (5, 7). However, etacetasil only slightly increased fruit coloration in grapes and prunes as compared with ethephon, and in preliminary experiments failed to inhibit grapevine shoot growth.

In the present study, the commercial ethylene-releasing compounds Ethrel and Alsol were applied at two pH levels to various organs in the upper part of growing grapevine shoots, in an attempt to follow their pathway from the site of application to the sites of action.

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Materials and methods

Mature grapevines of cvs. Perlette and Muscat Hamburg, growing in the coastal plain of Israel in experimental and commercial vineyards, were used. The ethylene-releasing compounds used for our experiments were Ethrel[1]) with ethephon (2-chloroethylphosphonic acid) as active ingredient and Alsol[2]) containing etacetasil (2-chloroethyl-tris-(2-methoxyethoxy)silane. Aqueous solution of Alsol were made up at pH = 6.9, and those of Ethrel at pH = 2.2. Buffers used for maintaining the pH of the solutions were 0.2 M potassium phosphate at pH = 7.0, and potassium citrate at pH = 2.2.

The effect of Ethrel and Alsol at different pH levels on the growth of grapevine shoots was determined after spraying the upper 20 cm of the shoots with a 0.5-l hand sprayer, until run-off. 10 shoots were treated on each of 4 vines per treatment. In addition, 10 other shoots on the same vines were sprayed with water. Shoots on separate, untreated vines were sprayed with water or buffers as additional controls.

To all the solutions, 0.03 % Triton-X 100 was added. Elongation was measured 30, 100 and 160 d after treatment. An analysis of the variance of mean growth after the treatments of control shoots on treated and untreated vines showed no interaction between treated and untreated shoots on the same vines. Thus, the control data are presented as one value.

The effect of application of Ethrel to different sites of the shoot was investigated by dipping the apex alone in Ethrel or by applying it with a brush to 2 or 3 young leaves, 3 or 4 nodes below the apex. Each treatment consisted of 30 shoots.

The time required for Ethrel to be in contact with the tissues in order to cause shoot growth inhibition was determined by dipping the upper 7 cm of growing shoots in Ethrel for 3 s, and then washing them thoroughly at different times after dipping. Elongation was determined 34 d after treatments. Each treatment consisted of 8 replicates of 5—7 shoots.

The influence of the pH of the Ethrel solution on uptake was determined by dipping the upper parts of growing cv. Perlette shoots in Ethrel at pH = 2.2, pH = 6.9, phosphate buffer at pH = 7.0, or distilled water. The shoot tips were excised from the shoots 15 min after treatment and brought immediately to the laboratory for ethylene evolution determination.

The explants were washed thoroughly, dried with a paper towel, weighed and placed in 35-ml vials. The vials were stoppered with a rubber stopper through which samples were extracted with a hypodermic syringe for gas chromatography. Another group of shoot tips was first excised from shoots, and then dipped in the same solutions, keeping the stem's cut suface out of the solution. The explants were then treated as the former ones. The sealed tubes were put in an oven at 50 °C for 2 h. 1-ml samples of air from each tube were injected into a Packard-419 gas chromatograph equipped with an alumina column at 90 °C and a flame ionization detector. The amount of ethylene evolved by each explant was determined.

Anatomical sections were prepared 7 d after the Ethrel treatments. Sections 5 mm long, containing the apex and a subapical region, were sectioned longitudinally to 15—25 μm with a freezing microtome. Sections were stained with aceto-carmine and viewed with a light microscope.

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[1]) Amchem Products, Inc., donated by Agan Ltd.

[2]) Ciba-Geigy, donated by CTS Israel.

Results

A spray of 750 mg Ethrel/l at pH = 2.2, applied to the upper parts of actively growing cv. Muscat Hamburg shoots, caused an 80 % inhibition of shoot growth (Table 1). It also caused a 60 % decrease in the number of nodes added after treatment and a 40 % reduction in internode length. Buffered Ethrel solutions at pH = 6.9 inhibited shoot growth entirely and usually caused die-back and abscission of the apex and 2 or 3 internodes next to it. Both Ethrel treatments halted lateral shoot break almost completely.

Table 1

The effects of Ethrel and Alsol at acid and neutral pH on the growth of cv. Muscat Hamburg shoots · Vines at full bloom; shoots 70—100 cm long; buffer concentration 0.1 M; growth measured 30 d after application of chemicals

Der Einfluß von Ethrel und Alsol bei saurem und neutralem pH auf das Triebwachstum von Muskat Hamburg · Reben in Vollblüte; Triebe 70—100 cm lang; Pufferkonzentration 0,1 M; Wachstum 30 d nach Applikation der Substanzen gemessen

Treatment			Additional growth		Number of internodes added	Avg. length of internodes cm
Material	mg/l	pH	cm	% of control		
None, control	—	—	88.2 ab[1])	100	11.6	7.6
Phosphate buffer	—	7.0	83.2 b	95	11.7	7.2
Citrate buffer	—	2.2	83.2 b	94	11.1	7.5
Alsol	1000	6.9	100.3 b	114	12.4	8.2
Alsol	2000	6.9	93.9 ab	107	11.8	8.0
Alsol	1000	2.3	79.2 b	90	11.1	7.1
Ethrel	750	2.2	19.6 c	22	4.3	4.6
Ethrel	750	6.9	3.5 d	4	0	0

[1]) Figures followed by different letters differ significantly at P = 0.05.

Table 2

Effects of Ethrel on growth of cv. Perlette shoots treated on different sites of the apical part of the shoots · Ethrel concentration = 750 mg/l; apices were dipped and leaves were treated with brushes; measurements were taken 40 d after treatment

Einfluß von Ethrel auf das Triebwachstum von Perlette bei Behandlung verschiedener Bezirke des apikalen Triebteils · Ethrelkonzentration: 750 mg/l; Triebspitzen eingetaucht, Blätter eingepinselt; Messung 40 d nach der Behandlung

Treated organ	Additional growth cm ± S.E.	Number of internodes added	Internode length cm
Shoot apex	63.0 ± 3.2	11.2	5.7
Young leaves	77.2 ± 8.9	11.2	7.4
Mature leaves	76.9 ± 6.1	11.8	6.5
Untreated control	84.3 ± 4.6	11.5	7.1

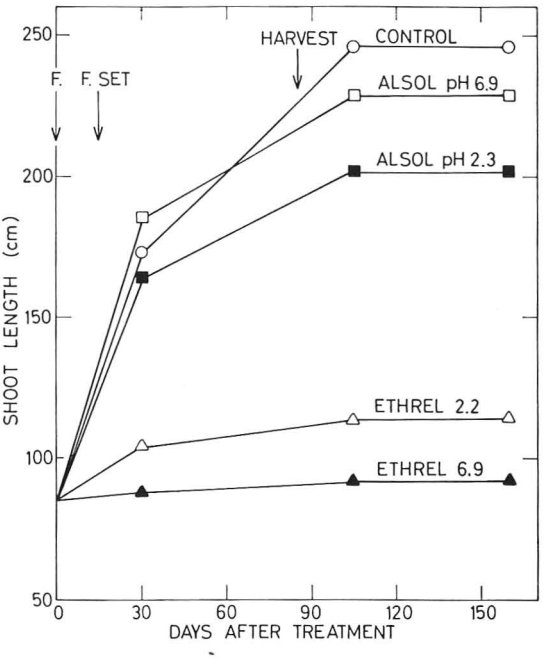

Fig. 1 (left): Split growth of a cv. Muscat Hamburg shoot following an Alsol spray on the growing region. 2000 mg/l at pH = 6.9, applied at full bloom; photograph taken 30 d after treatment.

Fig. 2 (right): Effects of Ethrel and Alsol at acid or neutral pH on the growth of cv. Muscat Hamburg grape shoots. Ethrel at 750 mg/l, Alsol at 1000 mg/l, sprayed at bloom on the upper parts of the growing shoots.

Abb. 1 (links): Gabelwuchs eines Triebes von Muskat Hamburg nach Besprühen der Wachstumszone mit Alsol. 2000 mg/l bei pH 6,9 zur Zeit der Vollblüte appliziert; photographische Aufnahme 30 d nach der Behandlung

Abb. 2 (rechts): Einfluß von Ethrel und Alsol bei saurem oder neutralem pH auf das Triebwachstum vom Muskat Hamburg. 750 mg Ethrel/l bzw. 1000 mg Alsol/l zur Blütezeit auf den apikalen Bereich der wachsenden Triebe gesprüht.

Alsol at both concentrations and pH levels tested did not significantly inhibit shoot growth. In most cases, it caused even a slight growth enhancement, as shoots became thicker and more vigorous. Sometimes it split the apical bud, which resulted in a very vigorous growth of two shoots from that bud (Fig. 1).

Another series of shoot-length measurements, performed 160 d after the treatments, revealed the duration of Ethrel's effect on shoot growth of Muscat Hamburg (Fig. 2). Very similar results were obtained with cv. Perlette and cv. Cabernet Sauvignon shoots. 6 d after dipping shoot apices in 750 mg Ethrel/l 50 % growth inhibition was found, 40 d after treatment there was only 25 % inhibition (Table 2). When mature leaves 5 nodes below the apex, or young leaves 2 or 3 nodes below the apex, were treated the growth inhibition was not significant. Dipping the shoot apex in Ethrel caused also a thickening of the subapical region and shortening of the internodes.

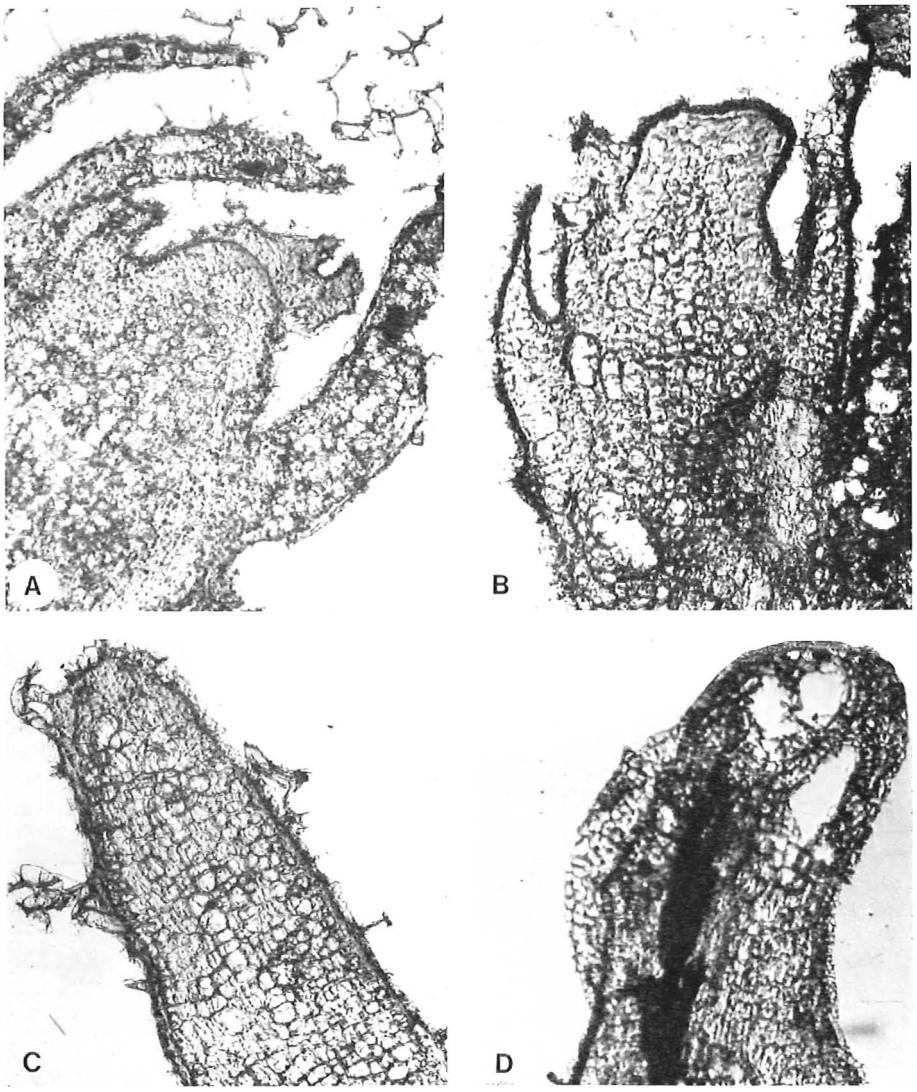

Fig. 3: Longitudinal sections of apices of cv. Muscat Hamburg shoots, 7 d after Ethrel treatment. — A: Untreated apex. × 75. B: Treated apex. C: Primordium of an untreated apex. × 188. D: Primordium of leaf or scale of treated apex. — Ethrel at 500 mg/l, pH = 2.2; sections 15-20 μm prepared with a freeze microtome and stained with aceto-carmine.

Längsschnitte durch Triebspitzen von Muskat Hamburg 7 d nach Ethrelbehandlung. — A: Unbehandelte Triebspitze. 75 ×. B: Behandelte Triebspitze. C: Primordium von unbehandelter Triebspitze. 188 ×. D: Primordium eines Blattes oder einer Schuppe von behandelter Triebspitze. — 500 mg Ethrel/l, pH 2,2; 15-20 μm dicke Gefrierschnitte mit Carmin-Essigsäure gefärbt.

Table 3

Ethylene evolution from apical parts of cv. Perlette shoots following dipping in Ethrel · The apical parts of shoots were dipped for 3 s and washed 15 min thereafter · Ethrel concentration = 750 mg/l; phosphate buffer at pH 7.0, 0.1 M

Freisetzung von Äthylen aus denapikalen Triebteilen von Perlette nach Eintauchen in Ethrel · Die apikalen Triebteile wurden 3 s eingetaucht und 15 min danach abgewaschen · Ethrelkonzentration: 750 mg/l; 0,1 M Phosphatpuffer von pH 7,0

Treatment	pH	nl C_2H_4 · g fr.wt.$^{-1}$ · h^{-1} ± S.E.	
		Intact shoots	Detached explants
Ethrel + phosphate buffer	6.9	260.8 ± 29.0	183.6 ± 14.3
Ethrel	2.2	363.7 ± 23.6	242.1 ± 28.7
Water	5.6	19.7 ± 3.6	21.4 ± 7.0
Phosphate buffer	7.0	15.5 ± 2.1	54.3 ± 7.3

Table 4

The effect of application duration of Ethrel to cv. Perlette shoot apices on their growth inhibition. Apex dipped in 750 mg Ethrel at pH = 6.9; apices were thoroughly washed at various time intervals after ethrel application; elongation was determined 34 d after treatment.

Einfluß der Einwirkungsdauer von Ethrel auf die Hemmung des Triebwachstums bei Perlette · Triebspitzen in 750 mg Ethrel/l von pH 6,9 getaucht und zu verschiedenen Zeitpunkten nach der Applikation gründlich abgewaschen; Trieblängenzuwachs 34 d nach der Behandlung ermittelt

Time from dipping until wash min	Additional growth cm ± S.E.
Without Ethrel	25.3 ± 3.8
0	17.5 ± 4.9
1	24.6 ± 3.8
15	16.4 ± 3.5
60	10.6 ± 1.7
1440	2.9 ± 0.5
Without wash	3.0 ± 1.6

Ethrel applied at pH = 2.2 was absorbed better by the upper parts of intact or detached cv. Perlette shoots than when applied at pH = 6.9 (Table 3), although at the higher pH Ethrel caused considerably stronger shoot growth inhibition.

After applying Ethrel to the upper parts of shoots, it has to be in contact with the tissues for at least 15 min in order to cause significant growth inhibition (Table 4). Ethrel continues to diffuse into the shoot tissues for more than 1 h after the treatment.

Anatomical sections of apices 7 d after Ethrel treatment showed a reduction in staining with aceto-carmine in comparison with sections from control apices. In the treated apices the cell walls deteriorated, cell separation occurred, and a distortion of cell layers arrangement in the apical meristem was apparent (Fig. 3). Large lesions developed in some apical structures, mainly the primordia of leaves and scales.

Discussion

Treating growing grapevine shoots with Ethrel at pH = 6.9 caused a stronger inhibition of shoot growth than when treated at pH = 2.2. This was reported also in studies of inducing olive fruit drop (6, 7). The stronger growth inhibition induced by Ethrel at the higher pH could not be the result of better uptake, since uptake of Ethrel at this pH was 30—50 % less than at the lower pH. This was to be expected, as low pH enhances uptake of acids by increasing the dissociation in the cuticule and plasmalemma (21). Although Alsol was more effective than Ethrel in reducing fruit removal force in olive, it was inactive in inhibiting grapevine shoot growth. Lowering the pH of Alsol solutions which results in increased ethylene evolution (7) did not alter its lack of effect on grapevine shoot growth. Thus, either Ethrel has a specific effect on grapevine shoot growth (22) or Alsol is not taken up by the grapevine shoots. It was also suggested that under our environmental conditions Alsol decomposes too rapidly to be effective (5). With Ethrel, the rate of ethylene release does not seem to be too rapid in this system, as the higher rate of evolution at pH 6.9 was more active in inhibiting growth than when the low pH treatments were used. It was suggested (17) that, under the specific environmental conditions, the rate and duration of ethylene evolution following an ethephon treatment were different. Thus, it is possible that Ethrel at pH = 6.9 was optimal for the specific process of grapevine shoot growth inibition under our conditions. Ethrel treatments inhibited apical growth along with the outgrowth of lateral buds (2, 3, 10). Quinn *et al.* (19) also found a reduction in lateral shoot formation in chrysanthemums following the application of Ethrel at a low concentration. However, they found a slight increase in formation of the laterals, after high Ethrel concentrations were used.

Ethrel caused damage to the apical meristems and primordia, similar to its effect on cherry (23) and apricot (9); though to a lesser extent. The new growth following Ethrel treatments was characterized by a decreased number of nodes, shorter internodes, and smaller leaves (20), which resembled the effect of gamma irradiation on grape apical meristems (18).

Ethylene was shown to inhibit DNA synthesis in the apices, probably by inducing the accumulation of a DNA polymerase inhibitor (4). However, enhanced ethylene-induced DNA synthesis was shown in the subapical region (14).

The clear cell degradation in and below the shoot apex due to Ethrel application could be explained on the basis of ethylene-induced senescence causing increased cellulase, pectinase and polygalacturonase activities (1). Furthermore, Freytag *et al.* (11) showed also ethylene-induced alteration of the orientation of the endoplasmatic reticulum and ribosome distribution and density in the cells.

Many studies indicated that ethylene reduces auxin synthesis, enhances its binding, metabolism and inhibits its polar transport (1, 12, 15), which could lead to inhibited growth, such as found with the grapevine shoots in this study. The reason for the fact that Ethrel had no effect on the older, mature tissues is still not clear.

Summary

Grapevine shoots of the cvs. Muscat Hamburg and Perlette were treated with two commercial ethylene-releasing substances, Ethrel (2-chloroethylphosphonic acid) and Alsol (2-chloroethyl-tris-(2-methoxyethoxy)silane) at acid and neutral pH. Ethrel

caused significant shoot growth inhibition, Alsol had no effect on the growth rate. Ethrel was more active at pH 6.9 than at pH 2.2, and its uptake was significantly higher at the low pH. In many cases the apical meristem of Ethrel-treated shoots was damaged. The relation of uptake to growth response was determined. Leaf blade treatments alone did not inhibit the growth of the apex.

Literature cited

1. Abeles, F. B., 1973: Ethylene in plant biology. Academic Press, New York.
2. Anderson, A. S., 1976: Regulation of apical dominance by ethephon, irradiance and CO_2. Physiol. Plant. 37, 303—308.
3. Anonymous, 1967: 2-Haloethanephosphonic acids as ethylene releasing agents for the control of plant growth development Amchem Products Inc. Information sheet No. 35.
4. Apelbaum, A., 1974: Reduction in extractable DNA polymerase activity in *Pisum sativum* seedlings by ethylene. Plant. Physiol. 54, 125—128.
5. Ben-Tal, Y., Klein, I. and Lavee, S., 1979: The role of the source of ethylene on the development of an abscission layer in olive pedicels. in: Geissbuhler, H. (Ed.): Advances in pesticide science, 347—350. Pergamon Press, Oxford, New York.
6. — — and Lavee, S., 1976: Increasing the effectiveness of ethephon for olive harvesting. Hort Science 11, 489—490.
7. — — and — — , 1976: Ethylene influence on leaf and fruit detachment in Manzanillo olive trees. Sci. Hort. 4, 337—344.
8. Biddle, E., Kerfoot, D. G. S., Kho, Y. H. and Russel, K. E., 1976: Kinetic studies of the thermal decomposition of 2-chloroethylphosphonic-acid in aqueous solution. Plant Physiol. 58, 700—702.
9. Bradley, M. V., Marey, N. and Crane, J. C., 1969: Morphological and histological effects of Ethrel on the apricot *Prunus americana* L. as compared with those of 2,4,5-T. J. Amer. Soc. Hort. Sci. 94, 316—318.
10. Edgerton, L. J. and Blanpied, G. D., 1968: Regulation of growth and fruit maturation with 2-chloroethylphosphonic acid. Nature 219, 1064—1065.
11. Freytag, A. M., Berling, J. D. and Linden, J. C., 1977: Ethylene-induced fine structure alteration in cotton and sugarbeet radicle cells. Plant. Physiol. 60, 140—143.
12. Goldsmith, M. H. M., 1977: Polar transport of auxin. Ann. Rev. Plant. Physiol. 28, 439—478.
13. Hirschfeld, G. and Lavee, S.: Persistence uptake and translocation of ^{14}C-ethephon in Perlette and Cardinal grapevines. (Submitted to Austral. J. Plant Physiol.)
14. Kang, B. G. and Burg, S. P., 1973: Influence of ethylene on nucleic acid synthesis in etiolated *Pisum sativum*. Plant Cell Physiol. 14, 981—988.
15. Lieberman, M. and Knegt, E., 1977: Influence of ethylene on IAA concentration in etiolated pea epicotyl tissue. Plant Physiol. 60, 475—477.
16. Maynard, J. A. and Swan, J. M., 1963: Organo-phosphorous compounds. I. 2-chloralkylphosphonic acid as phosphorylating agents. Austral. J. Chem. 16, 596—608.
17. Olien, W. C. and Bukovac, M. J., 1978: The effect of temperature on rate of ethylene evolution from ethephon and from ethephon treated leaves of sour cherry. J. Amer. Soc. Hort. Sci. 103, 199—202.
18. Pratt, C., 1959: Radiation damage in shoot apices of "Concord" grapes. Amer. J. Bot. 46, 103—109.
19. Quinn, J., Kiplinger, D. C. and Tayama, H., 1977: The effect of Florel (ethephon) and Shell-SD-8339 (DBA) on chrysanthemum. S. Ohio Flor. Ass. Bull. 569, 6.
20. Sacks, R. M. and Hackette, W. P., 1972: Chemical inhibition of plant height. HortScience 7, 440—447.
21. Sargent, J. A., 1965: The penetration of growth regulators in leaves. Ann. Rev. Plant. Physiol. 16, 1—12.
22. Shulman, Y., Hirschfeld, G. and Lavee, S., 1980: Vegetative growth control of six grapevine cultivars by spray application of (2-chloroethyl)phosphonic acid (ethephon). Amer, J. Enol. Viticult. 31, 288—293.

23. WILDE, M. H. and EDGERTON, L. Y., 1975: Histology of ethephon injury on "Montmorency" cherry branches. HortScience 10, 79—81.
24. YANG, S. F., 1969: Ethylene evolution from 2-chloroethylphosphonic acid. Plant. Physiol. 44, 1203—1204.

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