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Absorption and Translocation of Gibberellic Acid in the Grapevine

by

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Introduction

It is well known that sprays of gibberellin greatly enhance shoot elongation in many species, including grapes (Weaver and McCune 1959, Alleweldt 1959, Rives and Pouget 1959). Indications are that the hormone is rapidly absorbed and translocated to the young growing meristems and internodes, where it stimulates cell elongation. Studies with Pinto bean (Wanatabe and Scully 1957) and with Red Kidney bean and corn (Zweig et al. 1961) have revealed that foliar applications of gibberellin are very poorly absorbed into the plant. This would indicate that only a small percentage of the gibberellin applied enters the shoot, even though growth is tremendously stimulated. Thus far, experiments with young potted grape vines indicate a ready up- and downward and even a transitional movement of gibberellin in the stem (Alleweldt 1961, 1962). The direction of translocation is determined by the source-to-sink relationship. In some grape varieties the translocation seems to be inhibited by the presence of full grown leaves (Alleweldt 1962). The purposes of our experiments were to determine which portions of the shoot provide the best entry for gibberellins, and to ascertain the above mentioned translocation patterns. The latter were studied by autoradiography, using C-14-gibberellic acid.

Materials, Methods and Results

Work with nonradioactive gibberellin was done at the University of California vineyard at Davis, utilizing young shoots of Zinfandel and Tokay. Sprays were applied with a DeVilbiss No. 15 hand atomizer. In some instances gibberellin was applied to the upper surface of the leaves with a 0.1 ml pipette. One hundred μl of aqueous solution was applied per leaf in these experiments. In treating an internode, a tinfoil well was made around the stem by pressing the foil together at the bottom, using lanolin to make a good seal. To measure growth, a pin was pushed through the shoot 2 inches below the growing point at the time of treatment, for a point of reference (Weaver and McCune 1959). Potassium gibberellate was used in all treatments; Tween-20 at 0.05% (w/w) was used as the wetting agent. Ten to fourteen days after treatment, shoots were cut off 2 inches above the pin. The length, and sometimes fresh weight, of the new growth was determined.

The experiments with carbon-14-labeled gibberellic acid were done at the Forschungs-Institut für Rebenzüchtung Geilweilerhof, Germany. Potted plants of Riesling and *V. riparia* were used. The radioactive acid was produced according to the method of Petersen, DeVay, and Houston (1963), using the fungus *Gibberella fujikuroi*. The acid had an activity of 3.114 mc/mM and 0.07 or 0.16 μc was used for each treat-

ment. The compound was dissolved in 30% ethanol in water. The drops ranging in size from 5 to 40 μl were applied with a hypodermic syringe. Lanolin rings were made in an attempt to confine the drops to the intended area. Autoradiographs were made according to the method of YAMAGUCHI and CRAFTS (1953).

The experiments were done in a greenhouse at about 32° C and 90 to 95% relative humidity. Two plants were used for each treatment. Also one control plant that received no C-14-gibberellin was always autoradiographed to determine if any pseudoautoradiographs occurred. The length of time required for autoradiography was about 6 weeks. Longer exposures resulted in pseudoautoradiographs. For stem treatments the drop was applied when the shoots were in a horizontal position. As soon as the drop dried the plants were again placed erect.

Experimentation with nonradioactive Gibberellin

Experiment 1. The objective of this experiment was to compare the mature leaves with the stem as avenues of entry for gibberellin into the plants. Zinfandel shoots 14 to 18 inches long were utilized. The clusters 2½ to 4½ inches long were at a prebloom stage. With one series of shoots the upper and lower surfaces of the basal five or six mature leaves were sprayed with gibberellin at 10 ppm. Care was taken that the spray was applied only to the leaves. In a second treatment the stem, and the bases of the lateral shoots, corresponding to the portion of the stem from which sprayed leaves arose, were sprayed. In this instance care was taken not to spray the leaves. However, the dormant buds in the axils of the leaves were sprayed. A third series of shoots constituted the unsprayed control. There were 10 shoots per treatment. Treatments were made on May 13, 1963.

Shoots were harvested 14 days after treatment. The length of the new growth was 10.8, 16.0, and 16.3 inches, respectively, for the control, leaf treatment, and stem treatment. The $d_{.05}$ was 3.2. The same general relationship held for the fresh weight (data not presented).

Experiment 2. One objective was to study the effect of foliar development on response of the plant to gibberellin. A comparison was also made between the response obtained when the internode below the first mature leaf was treated and the response when the first mature leaf was treated. The first mature leaf, as used in this paper, refers to the first fully expanded leaf basal to the shoot tip. Uniformly growing shoots of Tokay, 17 to 20 inches long, were selected; the clusters, which were about 6 inches long, were removed. In one series the upper surface of the first mature leaf, approximately 5 inches wide, was treated with 1,000 μg of gibberellin in 100 μl of water. Much of the upper leaf surface was covered, including the major veins. In a second series the leaf apical to the first mature leaf was similarly treated. The treated leaf was about 3¾ inches wide. Treatment in a third series was made to the second leaf above the first mature leaf. In this instance the treated leaf was about 2¼ inches wide. In another series the internode just below the first mature leaf was treated with gibberellin. Untreated shoots comprised the control. Treatments were made on May 14, 1963.

Thirteen days after treatment the shoots were harvested (Table 1). All treatments resulted in increased length and fresh weight relative to the control. Treatment of the first leaf apical to the first mature leaf produced greater increases than did treatment of the first mature leaf.

Experiment 3. This experiment was designed to determine the effect of stage of development of internodes on response of the shoot to gibberellin. A comparison also was made between application to an internode and application to the leaf axil, including the bud, above the corresponding position of the nodal treatment. Shoots

Table 1

Length and fresh weight of new growth of Tokay shoots 13 days after treatment with gibberellin

Treatment	Tokay	
	Length new growth (inches)	Fresh weight new growth (grams)
Control	11.7	9.1
Stem	18.4	30.9
1st Mature leaf below tip	16.7	20.1
1st Leaf above 1st mature leaf	20.4	28.7
2nd Leaf above 1st mature leaf	18.9	26.5
$d_{.05}$	2.9	8.0

of Tokay 21 to 26 inches long were used. All clusters were removed from the shoots. In one treatment gibberellic acid at 100 μg in 100 μl water was applied to the youngest internode around which a well to hold the gibberellin could be made. This usually was the internode above the third expanding leaf below the apex. In another treatment gibberellin was applied to the internode above the first mature leaf. This internode was about 3 inches long and usually was the fifth expanding internode below the shoot apex. A third treatment was made on an older internode near the base of the plant. The stem at this location was about 3/8 inch thick. In a fourth treatment the axil of the leaf, including the bud, of the first mature leaf from the apex was treated. A final series constituted the untreated control.

Thirteen days after treatment the shoots were harvested and measurements made (Table 2). There were no significant differences among treatments when length of new growth was used as a criterion. Probably too little gibberellin was absorbed and translocated to cause measurable differences. When increase in fresh weight was used as a criterion, the greatest growth response resulted when the youngest internode was treated with gibberellin.

Experiment 4. The objective of this experiment was to compare the effect of different amounts of gibberellin applied to internodes of the same age. Tokay shoots about 24 inches long were used.

The internode above the first mature leaf from the apex was treated with 0.1, 10, 100 or 1,000 μg gibberellic acid in 100 μl aqueous solution containing 0.05% Tween-

Table 2

Response of shoots of Tokay 13 days after gibberellin was applied to internodes of various ages, or to a bud in the leaf axil

Treatment	Length of new growth (inches)	Fresh weight of new growth (grams)
Control	13.4	16.3
Youngest internode	15.2	22.5
Internode above 1st mature leaf	13.9	17.0
Oldest internode	13.4	17.9
Bud in axil of 1st mature leaf	14.3	19.3
$d_{.05}$	2.0	5.5

Table 3

Effect of varying amounts of gibberellic acid applied to the internode above the first mature leaf from the apex of Tokay grapevines

Gibberellin applied (micrograms)	Length of new growth (inches)	Fresh weight of new growth (grams)
0	12.5	13.2
1	10.7	10.7
10	11.6	11.3
100	15.7	19.8
1000	16.8	27.3
$d_{.05}$	2.3	5.2

20. The treated internodes were about 1/8 inch thick. The plants were harvested 14 days after treatment, and measurement made. Gibberellin at 100 and 1000 μg increased both shoot length and fresh weight (Table 3).

Experiments with carbon-14-gibberellic acid.

Experiment 5. In this experiment we studied the relative absorption and translocation of gibberellin when applied to various portions of the shoot. The effect of breaking the epidermis also was studied. Potted Müller-Thurgau vines with about eight nodes were used. The drops were applied just beneath the node of the first mature leaf. 0.07 μc of gibberellic acid in 5 μl was utilized. In a second treatment the base of the upper surface of the first mature leaf was treated. Two other stems were scratched in the middle of the internode with a knife blade before treatment. The buds and the axil of the first mature leaf were treated on two other vines. All plants were enclosed in plastic bags to facilitate absorption (14).

Absorption and translocation from the leaf base was slight, but better than when the intact stem was treated (Fig. 1). In a second replicate shoot (not shown) there was some movement into buds apical to the treated position. Considerable gibberellin entered the plant and subsequently translocated when the stem was scratched (Fig. 1). There was very little downward movement. The amount of translocation resulting from treatment of the bud was high (Fig. 1).

Experiment 6. This experiment was to compare absorption and translocation of gibberellin in *V. riparia* and Riesling, and to study translocation patterns from the upper surface of the base, tip, and center of the leaf. Each treatment was 0.16 μc of C-14-gibberellin in 40 μl of 30% ethanol. Treatments were made on February 18, 1964, and the plants were mounted 4 days later. With Riesling, the base, middle, and tip of the leaf were treated, but with *V. riparia* only the middle of the leaf was treated for comparison. Translocation of gibberellin was greatest when the base of the leaf was treated and least when the tip was treated. Translocation from the center was intermediate (Fig. 2). Less gibberellin was exported from the *V. riparia* leaves than from leaves of the Riesling.

Experiment 7. This experiment was designed to determine the effect of developmental stage of leaf on absorption of gibberellic acid. Müller-Thurgau plants having six to seven nodes were utilized. Leaves at five different stages of development were treated. Twenty-lambda drops containing 0.07 μc of gibberellic acid were applied to the base of the upper surface of the leaves. The youngest leaf was about 1 cm basal to the shoot tip and about 2½ cm wide. The second leaf was one node below and about four-fifths full grown, but still had a light green color. The third

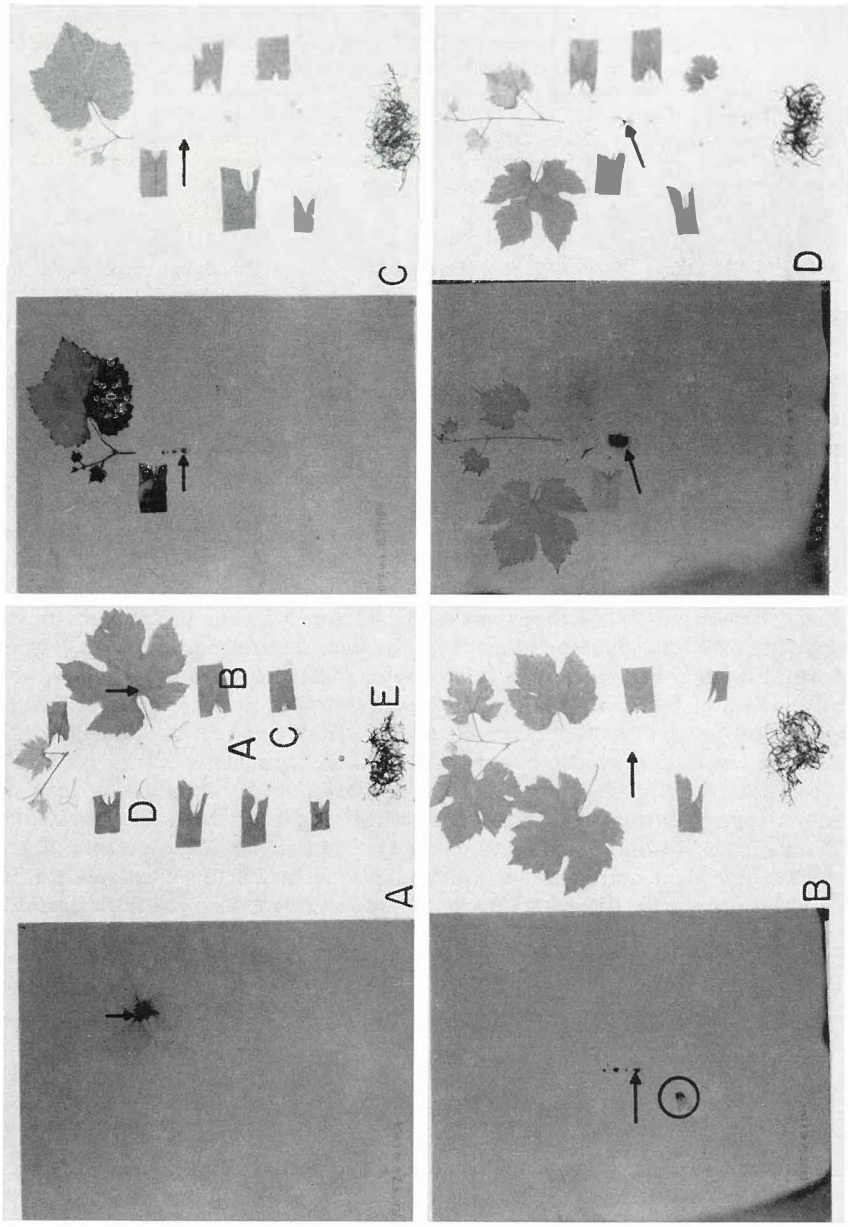

Fig. 1: Treatment of leaf (A), internode (B), internode in which epidermis was scratched before treatment (C), and of bud in leaf axil (D) with carbon-14-gibberellic acid.

Left, autoradiograph; right, mounted specimen. Arrow denotes site of treatment. A. Section of internode. B. Portion of leaf and petiole. C. Axillary bud. D. Tendril. E. Roots. The presence of carbon-14 is shown by the dark areas of the autoradiograph, the darker the area the higher the concentration of carbon-14. Note very little compound was absorbed and translocated from the leaf (A) or the node (B) treatment, but that much compound was translocated when the epidermis was scratched (C) and by the bud treatment (D). Activity in circle (B) is due to contamination and should be disregarded.

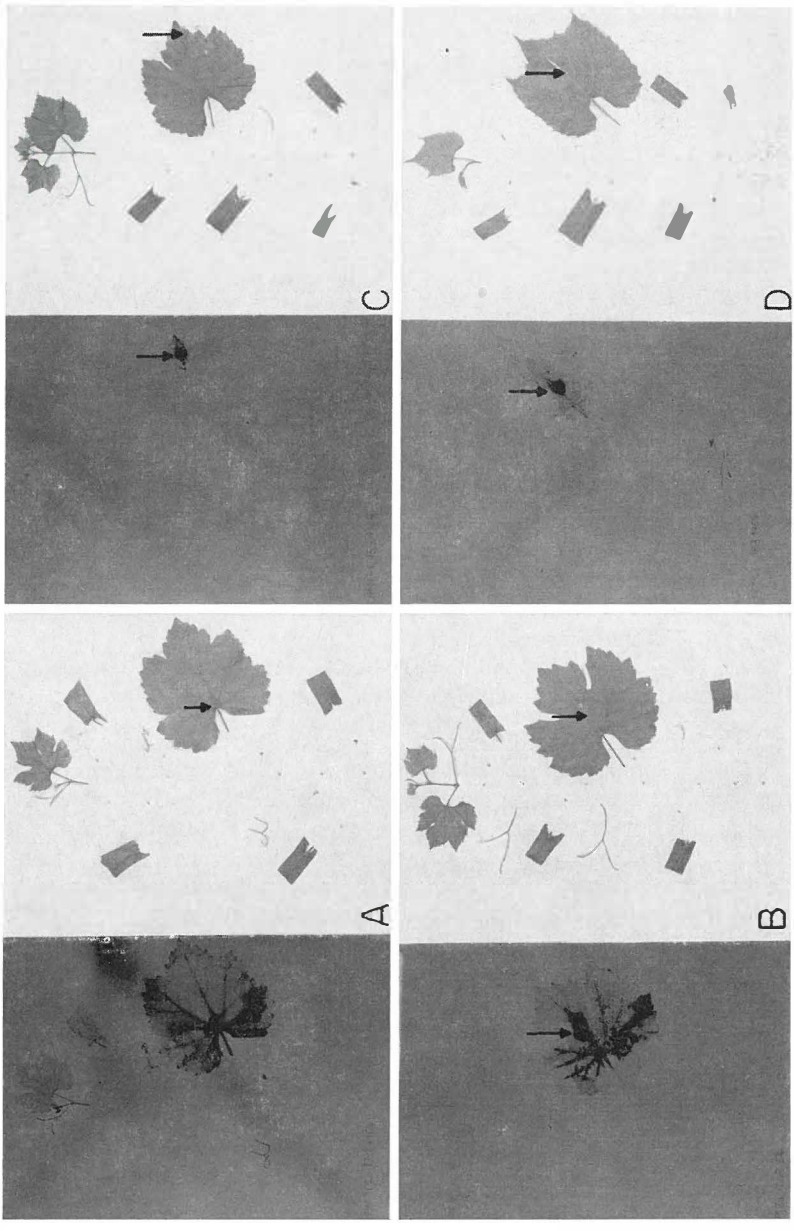

Fig. 2: Plant of Riesling treated with carbon-14-gibberellin at base of leaf (A), middle of leaf (B), or near tip of leaf (C), and plant of *V. riparia* treated at the center of leaf (D). Left, autoradiograph, right, mounted specimen. Note that most translocation of compound occurred when base of leaf was treated (A) and least when tip (C) was treated. Less movement of compound resulted when center of *V. riparia* leaf (D) was treated than when center of Riesling leaf (B) was treated. The spread of radioactive compound on the leaves was mainly a result of creeping of the drop on the surface of the leaf.

leaf was the second mature leaf from the base of the shoot and was full grown and green. The fifth leaf was the basal mature leaf, and usually was smaller than those apical to it. Five days after treatment the shoots were harvested and mounted.

Little translocation occurred from the treated leaves, indicating how difficult it is for gibberellic acid to be absorbed. The most movement occurred from the leaf that was four- fifths full size; some gibberellin moved into the young tip above the leaf. No export occurred from the oldest leaves, probably because the compound could not penetrate into these tougher leaves. There was no movement from one replicate of the youngest leaves, but in the other replicate there was some gibberellin in the large leaf below it.

Experiment 8. The objective was to determine relative rates of absorption and translocation of gibberellic acid by studying shoots harvested at various intervals after leaves were treated with the acid. On January 18, 1964, the basal portion of the upper surface of the first mature leaf on each of 12 shoots was treated, with 0.07 μc of gibberellic acid in 20 μl of 30% ethanol. Two plants were harvested at 6 hours or 1, 2, 4, 7, or 10 days post-treatment, respectively. Autoradiographs then were prepared.

No visible movement of gibberellic acid out of the leaf occurred during the first day. By 2 days a small amount of gibberellin had been exported to the growing tip. At 4 and 7 days the amount that had moved to the tip was progressively greater. Ten days after treatment there was some activity in the tip, but it was not as great as from shoots collected 7 days after treatment.

Discussion

Results of experiments with nonradioactive gibberellin on young shoots of Zinfandel and Tokay indicated that the compound was about equally absorbed by both leaves and stems. In these experiments large amounts of gibberellin were used, and even though absorption was shown to be poor with C-14-gibberellin, sufficient entered the plant to stimulate shoot growth. About the same response was obtained whether the stem just basal to the first mature leaf, or the upper surface of the first mature leaf, was treated. The young and tender tissues apparently are most readily entered by gibberellin. For example, the young expanding leaf above the first mature leaf absorbed more gibberellin than did the first mature leaf. The youngest leaves treated were about one-half the size of the first mature leaves, so both could be considered to be exporting leaves (5).

More effective wetting agents no doubt would reduce greatly the amount of gibberellin required. This is evidenced by the report that more gibberellin was absorbed into bean plants at 100% humidity than at lower humidities (14).

Smaller amounts of gibberellin were utilized with carbon-14-gibberellic acid because of its lower availability. One striking result was the slight penetration of the carbon-14-gibberellin into intact plants, even though the plants were grown at high humidity. Penetration was very poor through leaves and nodes, but was high through buds. Perhaps the gibberellic acid reaches the active portion of the bud by penetrating between the overlapping bud scales, or perhaps it may move through cracks in the buds into the xylem, where it is carried rapidly into the upper parts of the plant. However, the exact mechanism of penetration through the buds is not known.

The route of translocation of the gibberellic acid varied with the treatment. Application of the compound to intact leaves and stems resulted in phloem movement

as revealed by the accumulation of gibberellin in the young growing shoot tip and young growing points. The movement is a source-to-sink relationship, and the actively growing shoot tip provides a sink for the compound. This pattern was already assumed by one of the authors in earl, experiments (ALLEWELDT 1961) in which the direction of translocation could be altered by removing the tip of the actively growing treated shoots or tips of shoots arising lower on the spur than the treated shoot. In the former instance the tips removed were above the point of application and in the latter below it. When gibberellin was applied to a scratched stem, movement in the xylem was indicated. This is evidenced by the presence of the acid in the older leaves apical to the point of application. The gibberellic acid is carried upwards in the transpiration stream, and very little downward movement occurs. It was identified in both old and new leaves.

The results obtained in the present experiment where different portions of a leaf were treated with gibberellin were similar to those obtained by WEAVER and DEROSE (11) using 2,4-dichlorophenoxyacetic acid on bean leaves. In both instances the greatest amount of translocation out of the leaf occurred when the basal portion of the leaf was treated and the least when the apical portion was treated.

The comparison between Riesling and *V. riparia* indicates differences in penetration into and export from the leaves, thus confirming earlier results with nonradioactive gibberellin (ALLEWELDT 1961). In these earlier experiments even higher amounts of gibberellin did not stimulate shoot growth of *V. riparia* if applied to mature leaves. The result obtained with radioactive gibberellin cannot, however, give a satisfactory answer to the question, whether gibberellin is inactivated after entering *V. riparia* leaves. The presence of antigibberellins in some plants, already predicted by BIRD and ERGLE (1961) in their experiments with Gossypium, has been revealed by ZIEGLER et al. (1966). Most export of radioactive gibberellin occurred from leaves four-fifths full size, but still light green and tender. Such leaves offer relatively easy penetration and are also fully exporting. The fact that no radioactive gibberellin moved out of the leaf during the first day, and that the most radioactivity was present after 7 days indicates that gibberellin is slowly penetrating the leaf over a long period. The duration of penetration also seems to be influenced by environmental conditions since experiments conducted during the main growing season (May through August) have shown that maximum response to gibberellin occurs if treated leaves are cut off approximately three days after application.

Summary

1. Studies were made on the absorption and translocation patterns of nonradioactive gibberellin and carbon-14-gibberellin.
2. When the mature leaves of young shoots of Zinfandel or the stem and the leaf axils were sprayed with nonradioactive compound at 10 ppm about equal stimulation of shoot growth resulted. Much response to gibberellin was obtained when the first leaf above the first mature leaf was treated. Treatment of the internode below the first mature leaf gave results similar to that of treating the first mature leaf. Another experiment showed that the young internodes gave more response than older ones to gibberellin.
3. Studies with radioactive gibberellin showed that intact internodes and leaves were very poor avenues for entry of gibberellin. Movement was mainly to the actively growing shoot tip. Considerable movement through the buds occurred, and after

the internode was scratched, much gibberellin entered the plant and moved upwards in the xylem.

4. Most carbon-14-gibberellin was absorbed and translocated when the base of the upper surface of the leaf was treated and least when the tip was treated. Treatment of the center of the leaf gave intermediate results. Less gibberellin was exported from *V. riparia* leaves than from the Riesling. Leaves about four-fifths full size and still light green afforded some penetration and resultant translocation, but little or no penetration occurred in the older leaves. In one experiment no visible movement of gibberellic acid out of the leaf occurred during the first day, and most movement was demonstrated after seven days.

Acknowledgements

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Literature Cited

1. ALLEWELDT, G.: Die Wirkung der Gibberellinsäure auf einjährige Reben bei verschiedener Photoperiode. Vitis 2, 23—33 (1959).
2. — —: Weitere Untersuchungen über die sortenspezifische Gibberellinreaktion der Reben. Z. Pflanzenzüchtg. 45, 178—193 (1961).
3. — —: Die Bedeutung des Blattalters für die Gibberellinreaktion von Reben. Symposiumsbericht Gießen, 1.—3. Dez. 1960, In: Eigenschaften und Wirkungen der Gibberelline. Springer-Verl., Berlin, Göttingen, Heidelberg, 150—157 (1962).
4. BIRD, L. S. and D. R. ERGLE: Seedling growth differences of several cotton varieties on the influence of gibberellin. Agron. J. 53, 171—172 (1961).
5. HALE, C. R. and R. J. WEAVER: The effect of developmental stage on direction of translocation of photosynthate in *Vitis vinifera*. Hilgardia 33, 89—131 (1962).
6. PETERSEN, L. J., J. E. DE VAY and B. R. HOUSTON: Effect of gibberellic acid on development of hypocotyl lesions caused by *Rhizoctonia solani* on Red Kidney beans. Phytopathology 53, 630—633 (1963).
7. RIVES, M. and R. POUGET: Action de la gibberelline sur la compacité des grappes de deux varietés de vigne. C. R. Séances Acad. Agr. France (Paris) 45, 343—345 (1959).
8. WANATABE, R. and N. J. SCULLY: The translocation of C^{14} gibberellic acid and/or its metabolic fragments in the Pinto bean. U. S. Atomic Energy Comm., Argonne National Lab. ANL 5732, 195—197 (1957).
9. WEAVER, R. J. and S. B. MCCUNE: Response of certain varieties of *Vitis vinifera* to gibberellin. Hilgardia 28, 297—350 (1959).
10. — — and — —: Bioassay for testing activity of gibberellins on grape shoots. Amer. J. Enol. and Vitic. 10, 185—190 (1959).
11. — — and H. R. DE ROSE: Absorption and translocation of 2,4-dichlorophenoxyacetic acid. Bot. Gaz. 107, 509—521 (1946).
12. YAMAGUCHI, S. and A. S. CRAFTS: Autoradiographic method for studying absorption and translocation of herbicides using C^{14}-labeled compounds. Hilgardia 28, 161—191 (1958).
13. ZIEGLER, H., D. KÖHLER und B. STREITZ: Ist 2-Chlor-9-fluorenol-9-Carbonsäure ein Gibberellinantagonist? Z. Pflanzenphysiol. 54, 106—118 (1966).
14. ZWEIG, G., SHOGO YAMAGUCHI and G. W. MASON: Translocation of C^{14}-gibberellin in Red Kidney bean, normal corn and dwarf corn. Adv. in Chem. Series 28, 122—134 (1961).

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