In addition, damage by the harmful homogenizing treatments and damage by sonication are not additive; chloroplasts prepared at pH 7.3 are inactivated further only a very small amount by sonication. This makes it appear that the harmful homogenizing treatments, and sonic oscillation, attack at primarily the same site.

These facts lead us to suggest that there may be two pathways for dye reduction in the Hill reaction of red kidney bean chloroplasts. One of these pathways is susceptible to damage by sonic oscillation, or by a pH below 8 during homogenization; the second pathway is resistant to these treatments. The very high rates of dye reduction which can be observed under optimal conditions represent the sum of reduction along both pathways.

A similar conclusion as to the complex nature of the reducing pathway in the Hill reaction has been arrived at independently by T. Punnett on the basis of the pH optimum of different kinds of chloroplasts.

Summary

The Hill reaction rates of red kidney bean chloroplasts prepared by usual procedures were found to be low compared to those of spinach. Active chloroplasts could be prepared only if Versene was added to the homogenizing buffer. Raising the pH of the grinding buffer to 8 or over could substitute in part for the presence of Versene.

Once the leaves were ground, no further changes in chloroplast activity could be induced by reciprocation of the above treatment. Chloroplasts with low activity have apparently been damaged in a dark step, not in the light dependent part of the Hill reaction.

Sonic oscillation has only a slight effect on chloroplasts already damaged by a harmful homogenizing medium. On the other hand, it causes a rapid loss of 80 % of the activity of highly active chloroplasts. After brief sonication both types of chloroplasts are reduced to the same activity which is then resistant to extended additional sonication.

Consideration of these phenomena leads to a suggestion that there may be two pathways for dye reduction in the Hill reaction of red kidney bean chloroplasts, one sensitive, and one resistant to these inactivating conditions.

Literature Cited

1. Arnon, D. I. Copper enzyemes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris.* Plant Physiol. 24: 1–15. 1949.
2. Greenfield, Robert E. and Price, Vincent E. Liver catalase III. Isolation of catalase from mitochondrial fractions of polyvinylpyrrolidone-sucrose homogenates. Jour. Biol. Chem. 220: 607–618. 1956.
3. Hill, R. Oxygen evolved by isolated chloroplasts. Nature 139: 881. 1937.
4. Holt, A. S. and French, C. S. Oxygen production from chloroplasts. Arch. Boichem. 19: 368. 1948.
5. Lumry, Rufus, Spikes, John D. and Eyring, Henry Photosynthesis. Ann. Rev. Plant. Physiol. 5: 271–340. 1954.
6. Rabinowitch, E. I. Photosynthesis. Vol. II, pp. 1594–1595. Interscience Publishers, New York 1956.
7. Stocking, C. P. Precipitation of enzymes during isolation of chloroplasts in carbowax. Science 123: 1032. 1956.
8. Thomas, J. B., Blaauw, O. H. and Duysens, L. N. M. On the relation between size and photochemical activity of fragments of spinach grana. Biochim. Biophys. Acta 10: 230–240. 1953.

EFFECT OF GIBBERELLIN ON GROWTH, FLOWERING AND FRUITING OF THE EARLYPAK TOMATO, LYCOPERSICUM ESCULENTUM [1,2]

LAWRENCE RAPPAPORT

Department of Vegetable Crops, University of California, Davis, California

Gibberellin applied to tomato plants has been shown to induce marked stem elongation (4), to increase fresh weight (4, 15), to accelerate flowering and produce greater numbers of flowers per plant (17), and to increase fruit set (15, 17). This paper describes more definitively, the influence of gibberellins on growth, flowering, and fruit set of the Earlypak tomato.

General Procedure

Tomato seed (var. Earlypak, Ferry-Morse Seed Company, Salinas, California) was germinated in flats of vermiculite. Before the first true leaf expanded, uniform plants were transferred to flats or cans and grown in a greenhouse held at 18° to 21° C or in an outdoor lath house. Plants were grown to fruit maturity in soil benches or in large cans of soil.

A mixture of gibberellic acid ($[\alpha]D + 92°$) and gibberellin A (Ga) ($[\alpha]D + 36°$) (9) was dissolved in 0.5 % ethyl alcohol or in water. In the experiment dealing with growth response 0.05 ml of the mixture of gibberellins was applied with a micropipette to the youngest developing leaves (when about 2 cm in length) and to the adjacent vegetative apex. Similar applications were made during later growth. An atomizer was used to spray flowers and developing fruits.

In the experiments concerned with stem heights,

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[2] This paper is based on Project 1175 D.

measurements were taken at the appearance of the first flower clusters, and the distance between the cotyledonary node and the node below the first flower cluster is reported.

Experimental

Stem Elongation: The influence of repeated applications of Ga at specific intervals during growth was investigated. Using tomato plants grown in cans of soil, 25 μgm of Ga was applied to developing leaves at each of certain nodes as they appeared (table I). The final application coincided with anthesis of the first flower cluster. In plants treated at the first node increases in stem elongation were observed within a week. However, treatment of the succeeding nodes produced no observable stem height increases, and by anthesis of the first flower cluster no significant differences in stem elongation had resulted.

Table I

Stem Elongation and Flowering of Earlypak Tomato Plants as Influenced by Single or Repeated Applications of Gibberellin at the Indicated Nodes

Nodes treated	Times treated	Total gibberellin applied	Total ht	No. of nodes preceding 1st flower cluster	Time from seeding to flowering
no.	*no.*	*μgm/plant*	*cm*		*days*
...	0	0	45	7	53
1	1	25	45	8	49
1,4	2	50	48	9	48
1,4,8	3	75	46	8	48
1,4,8,12	4	100	51	8	48
L.S.D. at 1 %			N.S.*	N.S.*	0.7

* N.S. = Not significant.

The effect of higher concentrations and repeated dosages was determined by treating tomato plants twice weekly with 0, 25, 50, 100 or 200 μgm per plant until the first flower cluster appeared. Repeated treatment with 25 μgm and 50 μgm per plant did not significantly affect stem elongation; with 100 and 200 μgm per plant, however, slight and marked differences, respectively, were obtained (table II).

The surprising lack of response by anthesis to single or repeated applications of 25 and 50 μgm per plant (tables I and II) and the longer stems resulting from repeated applications of 100 and 200 μgm per plant of Ga indicated a further study of the growth responses of young Earlypak tomato plants. Single applications of 2.5, 25, and 50 μgm of Ga per plant to the first expanding leaves stimulated stem elongation markedly. In contrast to elongation (measured at anthesis of the first flower cluster) of plants treated repeatedly until anthesis (tables I and II), significant effects resulted from single applications with 2.5 or 25 μgm per plant when plants were measured during early growth (table III). Stem height increased no more with 50 μgm per plant, however, than with 25 μgm per plant.

Table II

Effect of Gibberellin * on Elongation and Flowering of Earlypak Tomato Plants

Gibberellin, μgm/plant	Ht, cm	No. of nodes preceding 1st flower cluster	Days to flowering
0	47.0	8	58
25	49.0	8	54
50	54.6	8	53
100	58.7	8	52
200	70.5	8	59
L.S.D. at 1 %	10.4	N.S.**	3.7

* Application made twice weekly from the appearance of the first expanding true leaves until the appearance of the first flower cluster.
** N.S. = Not significant.

In a more detailed study, concentrations of 0, 15, 150, 300, and 450 μgm per plant were applied to the first exapnded leaf. Six days after treatment plants receiving 150 to 450 μgm, as compared with untreated plants, elongated significantly. The greatest stem height increase resulted from applications of 450 μgm per plant (fig 1).

Fresh and Dry Weights: Reports regarding the influence of gibberellins on fresh and dry weight are inconsistent (1, 4). Since such increases in response to an applied chemical would be extremely significant, fresh and dry weights were determined. Ga at 50 μgm per plant was applied to the first expanded leaves of Earlypak tomato plants. Ten days later, these plants, together with some which received no Ga, were cut at the cotyledonary node and weighed. Fresh weight was 61 % more in the treated plants.

In another study the shoots of plants given 2.5, 25 or 50 μgm of Ga were cut and weighed 17 days after treatment. Fresh-weight (grams) increases over the control were obtained with all treatments; however, there was no significant difference between treatments with 2.5 and 25 μgm of Ga (table III). The shoots

Table III

The Influence of a Single Application of Gibberellin Applied to the First Expanding True Leaves on Stem Heights, Fresh Weights, and Dry Weights of Earlypak Tomato Plants

Gibberellin	Days after treatment			Fresh wt per plant	Increase in fresh wt over control	Increase in dry wt over control
	9	13	15			
μgm/plant	*Height, cm*			*gm*	*%*	*%*
0 (control)	2.5	4.8	7.5	3.8	...	...
2.5	4.9	8.0	11.3	4.8	26.1	18.5
25	8.3	12.0	15.4	4.5	18.1	14.8
50	9.2	13.3	16.8	5.3	40.0	40.7
L.S.D. at 1 %	2.7	5.9	5.2			

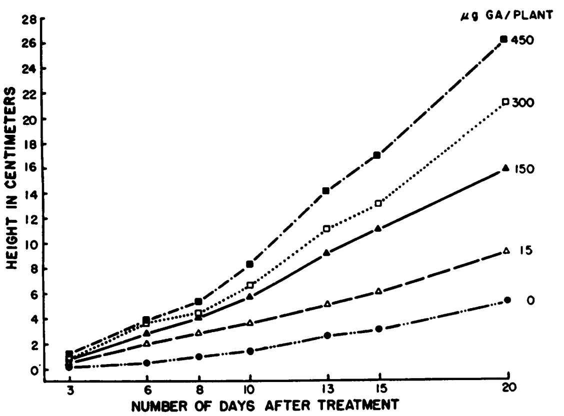

FIG. 1. The effects of 0, 15, 150, 300 and 450 μgm of gibberellin per plant applied to the vegetative apex on stem elongation of Earlypak tomato plants.

were placed in a 70° F forced-draft oven and dried for 24 hours. The percentage of dry matter of treated plants did not vary significantly from that of the controls. However, increases in total dry matter (% dry wt × fresh wt per plant) were obtained, especially with the 50 μgm application. The differences in fresh and dry weights between treatments at 2.5 and 25 μgm were not significant.

MORPHOLOGIC EFFECTS: Repeated foliar applications at concentrations above 25 μgm per plant frequently resulted in abnormal extension of the leaf rachis and leaflet petioles and in chlorosis of the leaves (1). In addition, the normally incised tomato leaves frequently developed entire margins (fig 2). Leaflets frequently rolled inward along the central vein after spraying at high concentrations. Axillary growth (tillers) decreased as concentration increased from 50 to 200 μgm per plant. At higher concentrations of Ga, spindly, weak plants developed. When

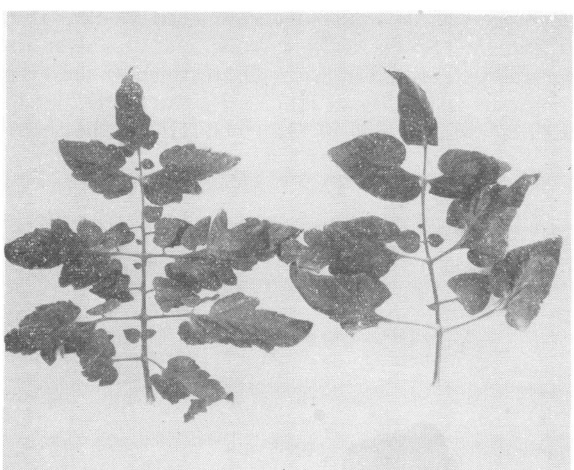

FIG. 2. Morphologic effects of gibberellin at concentrations above 500 μgm/ml. Notice the continuous margins, extension of leaflet petioles, and fewer leaflets per blade of treated leaf on the right.

early developing flowers of the first cluster were sprayed before anthesis with concentrations above 50 μgm, pedicels, sepals, petals, and pistils became enlarged. Fruits from such flowers usually developed abnormally.

FLOWERING: Wittwer and Bukovac (17) showed that while flowering in certain determinate tomato varieties may be hastened by early application of gibberellins, flowering of indeterminate varieties is relatively unaffected. Earlypak, like Pearson from which it is derived, is a determinate variety.

Treatment of plants at specific nodes with 25 μgm (table I) or twice weekly with 25, 50, or 100 μgm hastened flowering significantly without affecting the number of nodes below the first flower cluster (table II). No significant differences in flower numbers in the first two clusters resulted from applications of 0.05, 0.5, 2.5, or 25 μgm/ml of Ga made at the appearance of the first expanded leaf. Applied to the vegetative apex of tomato plants at first an-

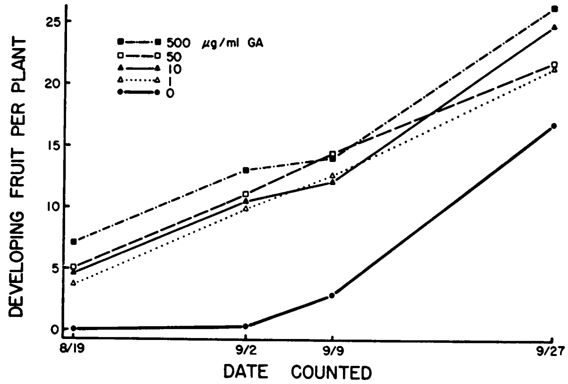

FIG. 3. The pattern of fruit set from the appearance of early developing fruit to the appearance of the first ripe fruit as affected by flower sprays of 0, 1, 10, 50 and 500 μgm/ml gibberellin.

thesis, similar treatments failed to affect subsequent flowering or fruit numbers.

FRUIT SET: Commencing August 4, 1956, the second and succeeding four flower clusters of tomato plants were sprayed twice weekly (for a period of 8 weeks) with Ga at concentrations of 0, 1, 10, 50, and 500 μgm/ml. Figure 3 shows the total number of developing fruits counted (per plant) preceding the appearance of the first ripe fruits. Figure 4 indicates the number of ripe normal and parthenocarpic fruit per plant harvested on specific dates. The fruit set of all sprayed flower clusters was rapid and almost complete, but in untreated plants fruiting was largely delayed until the appearance of the 5th or 6th cluster. Total fruit set of unsprayed plants never reached that of plants treated with gibberellin (fig 3).

Statistical analysis revealed that sprays of 1 μgm/ml were as effective as 500 μgm/ml in setting Earlypak tomatoes. The decrease in production of ripe normal fruit (fig 4) resulted from the frequency of harvesting (10/11 and again on 10/14) rather than from any apparent differences in the pattern of fruit set. The average numbers of ripe normal fruit harvested per plant are shown in table IV. Ga sprays frequently induced parthenocarpy (fig 4 and table IV) with associated underdevelopment and poor ripening characteristics. However, when seeds were present in fruits from sprayed flowers, fruits generally developed normally.

In one experiment, spraying fruits twice weekly did not appear to affect fruit size, although the vegetative portions (calyx and pedicel) were characteristically enlarged. The restricted plant-growing area provided in 5-gallon cans of soil made the recording of fruit weights valueless.

Discussion and Conclusions

Rapid stem elongation is seen in many plants after treatment with extremely minute amounts of the gibberellins (1, 2, 3, 4, 5, 6, 9, 13, 14, 15, 18). With Earlypak tomato, although minor differences are apparent soon after treatment, pronounced stem height increases are obtained primarily with very high concentrations of Ga within 10 days after application. Stem elongation is marked when the first expanded leaf is treated during early growth (4 to 6 leaves), although by anthesis neither single nor repeated applications of up to 50 μgm of Ga produced significant differences in elongation. By anthesis only with repeated applications at concentrations above 50 μgm did stem elongation differ significantly from that of untreated plants. The results demonstrate the decreasing responsiveness to Ga as plants develop. A

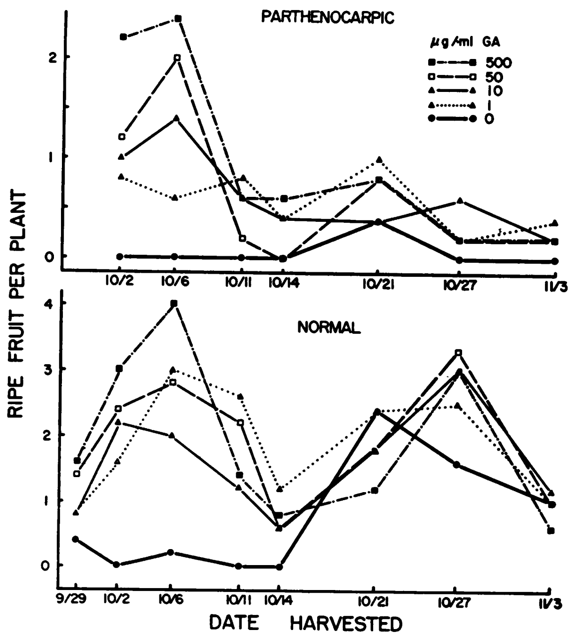

FIG. 4. The influence of flower sprays containing 1, 10, 50 and 500 μgm/ml of gibberellin applied twice weekly on the numbers of ripe normal and parthenocarpic fruit harvested at the indicated dates.

TABLE IV

AVERAGE NUMBER OF NORMAL AND PARTHENOCARPIC RIPE EARLYPAK TOMATOES HARVESTED FROM SEPT. 20 TO NOV. 3, 1956 FOLLOWING TWICE-WEEKLY SPRAYS OF GIBBERELLIN

GIBBERELLIN, μGM/ML	NO. OF FRUIT/PLANT	
	NORMAL	PARTHENOCARPIC
0	3.4	0.4
1	13.6	4.8
10	11.8	4.6
50	12.4	4.4
500	15.8	7.4
L.S.D. at 1 %	5.6	1.4

possible explanation for the disparity between these results and those reported by Bukovac and Wittwer (4) is that they collected samples earlier in growth when differences in stem elongation were also apparent in the present studies. These differences, however, were negated by the time anthesis of the first flower cluster had occurred, possibly because of the decreasing receptiveness of older plants to Ga. In addition, important varietal differences occur. In concurrent studies plants of the Potentate variety were taller by anthesis when treated during early growth with 50 μgm per plant of gibberellic acid.

Increases in fresh and dry weights of plants grown with a continuous supply of gibberellin in water culture were reported by Brian et al (1) and Marth and coworkers (13). Bukovac and Wittwer reported a 50 % increase in fresh and dry weight of celery (4). However, working with several tomato varieties, they did not obtain dry-weight increases with as much as 20 μgm per plant. In the present experiments Ga stimulated consistent stem elongation, leaf enlargement (rachis and leaflet petioles), and fresh-weight increases. Dry matter content (% dry wt × fresh wt per plant) was increased, especially when very high concentrations were used. The later harvest of plants in these experiments may account for the disparity in results.

Kato (7) reported that gibberellic acid increased respiration and water uptake in pea stem segments. Increased cell elongation has also been found following treatment with gibberellins (8). In Earlypak tomato fresh-weight increases seem to be coupled with greater water uptake as well as increases in dry matter content. These increases point to further critical studies of gibberellin effects on the mechanism of water uptake, cell enlargement, and cell division.

Gibberellin profoundly affects the reproductive development of Earlypak tomatoes. While flowering was accelerated only slightly (3 to 6 days) by treatment with gibberellins, this and other papers (5, 10, 11, 12, 13, 15, 17) suggest further studies of the influence of Ga on flowering of various plants. The value of leaf numbers preceding the appearance of flower parts as an index of accelerated flowering has received considerable emphasis (16). It is of especial interest, therefore, that although Ga hastened or did not affect flowering, it did not influence the number of nodes preceding the appearance of flowers in these experiments. This indicates the need for careful consideration of the plant material when leaf numbers are used as an index of flowering. The implication is that in some plants Ga may accelerate maturation of vegetative parts preceding flowering. This is further emphasized by the induction of flowering in biennial (10) and winter annual plants (5) without cold treatment.

The pronounced fruit-setting ability of gibberellin is of interest both physiologically and economically. The search for new growth regulators to control fruit setting of tomatoes has made fleetingly prominent a variety of chemicals which experimentation and commercial use have proved only partially satisfactory. Aside from their typical formative and other undesirable effects, these compounds are generally effective within a limited range of concentrations below which fruit set is inadequate and above which injury to both fruit and plants frequently results. While it is emphasized that the quality of fruits harvested from sprayed flowers in these experiments was not evaluated critically, similar responses to sprays of 1 to 500 μgm/ml Ga indicate the desirability of continued investigation under temperature conditions unfavorable for fruit set.

Summary

Gibberellin (Ga) stimulated growth, flowering, and fruit set of Earlypak tomato. Total stem elongation of plants, as measured from the cotyledonary node to the node preceding the first flower cluster, was significantly increased by twice-weekly applications of Ga at concentrations of 100 and 200 μgm, but not by concentrations of 25 or 50 μgm per plant. Neither single nor repeated treatments with 25 μgm applied to the expanding leaves at the first, fourth, eighth, or twelfth nodes produced significant stem height differences. Stem elongation of young plants (4 to 6 leaves) was increased, as compared with the control, at concentrations of 2,5, 15, 25, 50, 150, 300 and 450 μgm per plant. Both fresh-weight and dry-matter content of young Earlypak tomato plants were significantly increased. Leaf enlargement, the development of entire instead of incised margins, and an inward rolling of leaflets resulted from treatments with high concentrations of Ga.

Single or repeated applications of Ga at concentrations of 25, 50 and 100 (but not 200) μgm per plant hastened flowering by 3 to 6 days without affecting the number of nodes preceding the first flower cluster. Setting of normal and parthenocarpic fruit was increased by repeated floral sprays of Ga at concentrations from 1 to 500 μgm/ml. Spraying the developing fruit did not increase fruit size.

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Literature Cited

1. Brian, P. W., Elson, C. W., Hemming, H. G. and Radley, Margaret The plant growth promoting properties of gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*. Jour. Sci. Food Agr. 12: 602–612. 1954.
2. Brian, P. W. and Hemming, H. G. The effect of gibberellic acid on the shoot growth of pea seedlings. Physiol. Plantarum 8: 669–681. 1955.
3. Brian, P. W., Hemming, H. G. and Radley, Margaret A physiological comparison of gibberellic acid with some auxins. Physiol. Plantarum 8: 899–912. 1955.
4. Bukovac, M. J. and Wittwer, S. H. Gibberellic acid and higher plants: I. General growth responses. Agr. Expt. Sta., Michigan, Quart. Bull. 39: 307–320. 1956.
5. Harrington, J. F., Rappaport, Lawrence and Hood, K. J. The influence of gibberellin on stem elongation and flowering of endive. Science 125: 601–602. 1957.
6. Hayashi, T. and Murakami, Y. Biochemical studies of bakanae fungus. XXVIII. The physiological action of gibberellin. Jour. Agr. Chem. Soc. Japan 27: 672–675. 1953.
7. Kato, J. Effect of gibberellin on elongation, water uptake, and respiration of pea stem sections. Science 123: 1132. 1956.
8. Kato, Y. Responses of plant cells to gibberellin. Bot. Gaz. 117: 16–24. 1955.
9. Lang, A. Stem elongation in a rosette plant, induced by gibberellic acid. Naturwiss. 43: 257–258. 1956.
10. Lang, A. Induction of flower formation in biennial Hyoscyamus by treatment with gibberellin. Naturwiss. 43: 284–285. 1956.
11. Lang, A. Bolting and flowering in biennial *Hyoscyamus niger,* induced by gibberellin. Plant Physiol. 31 (Suppl.): 35. 1956.
12. Lang, A. Gibberellin and flower formation. Naturwiss. 43: 544–545. 1957.
13. Marth, P. C., Audia, W. V. and Mitchell, J. W. Effect of gibberellic acid on growth and development of various species of plants. Plant Physiol. 31 (Suppl.): 43. 1956.
14. Phinney, B. O. Growth response of single-gene dwarf mutants in maize to gibberellic acid. Proc. Nat. Acad. Sci., U.S. 42: 185–189. 1956.
15. Rappaport, L. Growth regulating metabolites. California Agriculture 10: 4. 1956.
16. Rappaport, L. and Wittwer, S. H. Flowering in head lettuce as influenced by seed vernalization, temperature and photoperiod. Proc. Amer. Soc. Hort. Sci. 67: 429–437. 1956.
17. Wittwer, S. H. and Bukovac, M. J. Some effects of gibberellin on flowering and fruit setting. Plant Physiol. 32: 39–41. 1957.
18. Yabuta, T. and Hayashi, T. Biochemistry of the bakanae fungus (2). Isolation of gibberellin, a metabolic product of *Gibberella fujikuroi* Wr. which promotes the growth of rice seedlings. Jour. Agr. Chem. Soc. Japan 15: 257. 1939.