

Sex Expression in Cucumber Plants as Affected by 2-Chloroethylphosphonic Acid, Ethylene, and Growth Regulators

Received for publication January 13, 1970

SHUICHI IWAHORI,¹ JAMES M. LYONS, AND ORRIN E. SMITH

Department of Vegetable Crops, University of California, Riverside, California 92502

ABSTRACT

The effects of 2-chloroethylphosphonic acid (Ethrel), ethylene, and some growth retardants on sex expression of cucumber plants (*Cucumis sativus* L.) were investigated, with the use of a monoecious cultivar (Improved Long Green) which has a strong tendency toward maleness.

Ethrel caused increased femaleness when applied at 50 milligrams per liter at the first to the third leaf stage, but when applied at the cotyledon stage it was ineffective. The later the time of application, the higher the node at which the first female flower appeared. The total number of female flowers was about the same regardless of application time. A mixture of gibberellins A₄ and A₇ caused maleness, and Ethrel caused femaleness. However, when applied in combination at the first leaf stage the interaction was not significant. It seems, therefore, that Ethrel and gibberellins are not antagonistic but rather have different sites of action, although they have opposing effects on sex expression.

Ethylene caused femaleness but was far less effective than Ethrel, Alar (*N*-dimethylaminosuccinamic acid), CCC((2-chloroethyl)trimethylammonium chloride), Phosphon D(2,4-dichlorobenzyl-tributylphosphonium chloride), and abscisic acid did not affect sex expression of cucumber.

Cucumber flower buds, which differentiate in leaf axils of main shoots, are bisexual in their early developmental stages. Later they develop into either staminate (male) or pistillate (female) flowers (1, 7, 12). In monoecious varieties male flowers differentiate at the lower nodes, followed by female flowers at the higher nodes. The number of nodes to the first female flower and total number of female flowers are both reliable indices of sex expression.

Although sex expression in cucumber plants is determined genetically, it is modified by several environmental factors. High nitrogen, short days, low light intensity, and low night temperature are among the factors which favor femaleness. Reverse conditions tend to cause maleness (11).

The concept that sex expression of cucumber plants may be regulated by a balance between native auxins and gibberellins (2) is supported by two types of evidence. Applied auxins, especially α -naphthaleneacetic acid, induce femaleness (8, 13, 15), whereas gibberellins induce maleness (8, 23, 27). Secondly, the main shoots of andromonoecious plants bearing the male flowers contain more

extractable auxin than hermaphrodite plants with bisexual flowers (9). Furthermore, monoecious plants contain more endogenous gibberellin-like substances than gynoecious plants (2).

Recently it was demonstrated that in some plant responses auxin may exert its effect through ethylene evolution (29) and that gibberellins have generally an opposing effect to ethylene (28). Since it has long been known that unsaturated hydrocarbons (17, 18, 19) induce femaleness in cucumber, it appears that ethylene, the most potent unsaturated hydrocarbon affecting plants (4), may be the active factor for inducing femaleness.

Recently it was found that Ethrel², which releases ethylene in the presence of plant tissues (31), is remarkably effective in increasing femaleness in cucumber (14, 16, 24, 25, 30). The experiments reported here were initiated to study the effects of ethylene, Ethrel, gibberellins, growth retardants, and inhibitors on sex expression in cucumber.

MATERIALS AND METHODS

A monoecious cucumber, *Cucumis sativus* L. Improved Long Green, was used, since it shows a strong male tendency and bears only 1 or 2 female flowers in leaf axils of the main shoot through the 20th node. Seeds were sown in 5- × 5-cm peat pots filled with vermiculite and irrigated with half-strength Hoagland's solution. About 20 days after planting (first leaf stage), the peat pots were transplanted into 30-cm pots containing vermiculite. Experiments were conducted in the greenhouse during winter and spring without supplemental lighting. Although this variety is reported to be relatively insensitive to day length and temperature (7), the flowering habit of control plants was not the same in all experiments; therefore, no comparisons between different experiments were attempted.

Ethrel was applied either as a droplet on the growing point or as a spray to the whole plant. Solutions of ABA and GA₄₊₇ were made by dissolving in 50% (v/v) ethanol and applied to the growing point in 10- μ l droplets with a microsyringe. Phosphon D and CCC were applied as a soil drench, and Alar was sprayed on the whole plant. For treatment with ethylene gas, plants in peat pots were enclosed in polyethylene bags into which known amounts of ethylene were injected with a syringe. Plants were exposed to ethylene for a total of 68 hr but were ventilated twice for 3 hr each at 24 and 48 hr. After each ventilation the ethylene concentration was re-established. Two sets of controls were used, one with no treatment and another enclosed in plastic bags. All

² Abbreviations: Ethrel (Amchem 68-240, Amchem Products Inc., Amber, Pa.): 2-chloroethylphosphonic acid; GA₄₊₇: a mixture of gibberellins A₄ and A₇; ABA: 3-methyl-5-(hydroxy-4-oxo-2,6,6-trimethyl-2-cyclohexene-yl)-cis-2-trans-4-pentadienoic acid, or abscisic acid; Alar: *N*-dimethylaminosuccinamic acid; CCC: (2-chloroethyl)trimethylammonium chloride; Phosphon D: 2,4-dichlorobenzyl-tributylphosphonium chloride.

¹ Present address: Experiment Farm, Faculty of Agriculture, University of Tokyo, 1-1-1 Midori-cho, Tanashi, Tokyo 188, Japan.

Table I. *Effect of Ethylene on Sex Expression of Cucumber*

Treatments as indicated begun at 19 days after planting (first leaf stage). Means with different superscripts differ significantly at $P = 0.05$.

| Treatments | Male Flower | | Female Flower | |
|------------------------------|----------------------|---------------------------------------|----------------------|--------------------------------------|
| | Node of first flower | No. of days for first flower anthesis | Node of first flower | No. of days to first flower anthesis |
| Control | 1.0 ^a | 47.0 ^a | 14.4 ^b | 61.9 ^b |
| Control in bag | 1.1 ^a | 47.0 ^a | 14.5 ^b | 60.7 ^b |
| Ethylene, 100 μ l/liter | 1.6 ^{ab} | 48.0 ^a | 9.4 ^a | 53.7 ^a |
| Ethylene, 1000 μ l/liter | 1.9 ^b | 48.2 ^a | 10.4 ^a | 55.5 ^a |
| Ethylene, 2000 μ l/liter | 1.9 ^b | 47.7 ^a | 12.4 ^{ab} | 58.2 ^{ab} |

treatments consisted of 8 plants each, unless otherwise mentioned. The node number at which the first male or female flower appeared, the total number of flowers, and days required for flowering were recorded.

RESULTS AND DISCUSSION

Effects of Ethylene. Plants were treated with 100, 1000, or 2000 μ l/liter ethylene for 68 hr starting 19 days (first leaf stages) after planting. Ethylene lowered the node number at which the first female flower developed and also gave a small but significant increase in the number of nodes to the first male flower (Table I). Ethylene had no effect on the number of days to first flower anthesis except for female flowers at 100 and 1000 μ l/liter. Of a total of 24 treated plants, 15 differentiated female flower buds between the second and fourth nodes. Ten of these buds developed into flowers. Neither the nontreated controls nor the controls enclosed in plastic bags developed female flowers below the 12th node. It is apparent from these data that ethylene causes a tendency toward femaleness when applied at the first leaf stage. The ethylene-treated plants, however, showed a general yellowing and epinasty of the cotyledons. This effect was not observed in plants treated with Ethrel unless the plants were enclosed in plastic bags from which the excess ethylene could not escape. Thus, the general effect of ethylene on plant growth probably masked the more subtle effects on sex expression. For example, at the lowest concentration used (100 μ l/liter) the influence of ethylene on femaleness, as evidenced by the node of the first flower, approached that obtained with Ethrel (Table II). However, as the concentration of ethylene was increased, the effect on femaleness was reduced. Minina and Tylkina (19), using cucumber, and Nitsch *et al.* (21), using squash, failed to induce femaleness when ethylene was applied in a closed system. Again, these results could be explained as the more general effects of high concentrations of ethylene on plant growth rather than sex expression *per se*.

Effects of Ethrel. Experiments were conducted twice with essentially the same results, and the results of only one trial are described (Table II). Ethrel at 50 mg/liter was sprayed on plants once at 10 days (cotyledon stage), 17 days (first leaf stage), 24 days (second leaf stage), or 31 days (third leaf stage) after planting; or twice at 10 and 17 days, or 24 and 31 days; or 3 times at 10, 17, and 24 days after planting. Plants not sprayed served as controls.

Generally, Ethrel treatment decreased the plant height, and this effect was more pronounced as the number of applications increased. When applied at the cotyledon stage, Ethrel (as with ethylene) was completely inactive in altering sex expression in the first experiment (Table II) whereas in the second experiment a

Table II. *Effect of Time of Application of Ethrel on Sex Expression of Cucumber*

Ethrel at 50 mg/liter was applied at the various ages shown. Means with different superscripts differ significantly at $P = 0.05$.

| | Male | | | Female | | |
|---------------------------|----------------------|--------------------------------------|---------------------------|----------------------|--------------------------------------|---------------------------|
| | Node of first flower | No. of days to first flower anthesis | No. of flowers to node 20 | Node of first flower | No. of days to first flower anthesis | No. of flowers to node 20 |
| Control | 2.6 ^a | 42.7 ^a | 73.5 ^c | 12.7 ^d | 56.2 ^d | 1.6 ^a |
| Ethrel treatment at: | | | | | | |
| 10 days (cotyledon stage) | 2.4 ^a | 43.1 ^a | 71.3 ^c | 15.0 ^e | 60.5 ^e | 1.3 ^a |
| 17 days (1st leaf stage) | 13.3 ^c | 62.3 ^d | 8.0 ^{ab} | 4.2 ^a | 46.0 ^a | 11.7 ^b |
| 24 days (2nd leaf stage) | 4.7 ^{ab} | 48.2 ^b | 8.1 ^{ab} | 7.0 ^b | 49.7 ^b | 12.5 ^b |
| 31 days (3rd leaf stage) | 6.7 ^b | 53.5 ^c | 12.7 ^b | 9.7 ^c | 55.5 ^d | 10.6 ^b |
| 10 and 17 days | 13.6 ^c | 62.2 ^d | 8.6 ^{ab} | 5.7 ^{ab} | 46.7 ^a | 11.0 ^b |
| 24 and 31 days | 14.1 ^c | 63.2 ^d | 2.1 ^a | 5.5 ^{ab} | 52.2 ^c | 12.0 ^b |
| 10, 17, and 24 days | 15.1 ^c | 67.0 ^d | 3.5 ^a | 5.0 ^a | 46.2 ^a | 12.9 ^b |

few female flowers were induced at the first and second nodes on some plants. Single applications at the first through third leaf stages, however, were all very effective in causing femaleness. These plants showed a decrease in the number of male flowers and a marked increase in the number of female flowers. While there was essentially no difference between treatments in the total number of male or female flowers when applied after the cotyledon stage (17, 24, and 31 days), the number of nodes to the first flower differed between these treatments. The number of days to anthesis of female flowers was increased when the application was made to the later stages of growth. The double and triple applications showed essentially the same results as the single applications. In the multiple treatments, the first application (after the cotyledon stage) appeared to induce a maximal response in the total number of female flowers (Table II) although additional applications increased the number of nodes with a female dominant ratio. Plants treated at the first leaf stage had flowers with a female dominant ratio at the 5th through 12th nodes; those treated at the second and third leaf stages had them at the 8th through 18th, and 13th through 20th nodes, respectively (Fig. 1). Thus, the later the time of application the higher the node number with a dominant female ratio. According to Fujieda (7), plants at the first, second, and third leaf stages have already differentiated flower primordia up to the 9th, 12th, and 15th nodes, respectively. He also showed that in the second and third leaf stages, sex of flowers has already been determined up to the 4th and 7th nodes, respectively. This, together with the lack of effect of Ethrel on cotyledon stage plants, suggests that not only the size and age of the flower buds but also the age of the plants is critical for the induction of femaleness.

Effects of Ethrel and Gibberellins. Plants were treated with Ethrel (0, 10, 30, or 100 μ g) and GA₄₊₇ (0, 1, 5, or 25 μ g) in a 4 × 4 factorial combination, totaling 16 treatments. Treatments were applied separately to the growing point as 10- μ l droplets 18 days (first leaf stage) after planting.

Ethrel decreased and GA₄₊₇ increased plant height. However, development of the plants, as expressed by leaf number, was not affected by either growth regulator. The 100- μ g Ethrel treatment initially caused severe stunting of the plants, but later they recovered and initiated new growth.

Ethrel increased the node number at which the first male flower developed (Fig. 2A) and concomitantly delayed anthesis of the first male flower. Applied alone, GA₄₊₇ did not significantly affect sex expression. When GA₄₊₇ was applied in combination

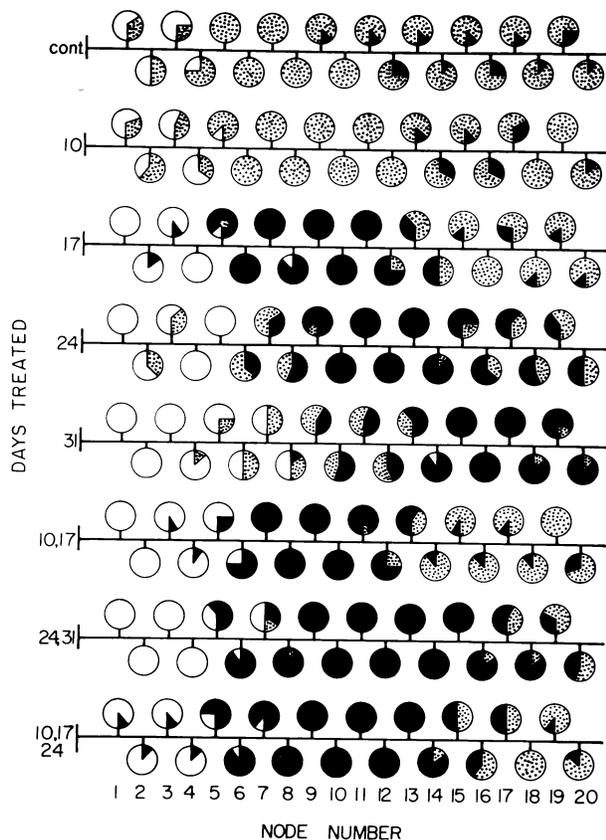


FIG. 1. Effects of time of application of Ethrel on sex expression of cucumber plants. Ethrel (50 mg/liter) was sprayed on certain days after seeding as indicated at left side of the figure. The circle represents the ratio of sex of each node. Clear areas represent percentage of aborted buds; dotted and black areas represent male and female flower-bearing nodes, respectively.

with Ethrel (10 or 30 μg), it did not change the node number or anthesis of the first male flower. However, in combination with 100 μg of Ethrel, GA_{4+7} considerably decreased the number of nodes to the first male flower compared to Ethrel alone. Figure 2B shows that Ethrel alone or in combination with GA_{4+7} markedly decreased the number of male flowers. As the amount of applied Ethrel increased, fewer male flowers opened. GA_{4+7} had no effect on the number of male flowers, and no statistically significant interaction was found.

On the other hand, Ethrel lowered the number of nodes to the first female flower and accordingly shortened the days required to anthesis (Fig. 2C). Ethrel also increased the number of female flowers (Fig. 2D), but the effect was decreased by the addition of GA_{4+7} . When analyzed statistically, the effects of GA_{4+7} alone in increasing the node number (Fig. 2C) and decreasing the number of female flowers (Fig. 2D) were highly significant. However, the interactions of GA_{4+7} and Ethrel were not statistically significant.

Scott and Leopold (28) showed that ethylene and gibberellins had opposing effects on the growth of lettuce hypocotyls, invertase activity of beets, and α -amylase activity of barley. Also, Fuchs and Lieberman (6) concluded that the effects of gibberellin and ethylene on growth of etiolated pea seedlings were antagonistic, and similarly Robinson *et al.* (24) reported antagonistic effects of gibberellin and Ethrel on cucumber sex expression. In the results presented here (Fig. 2) Ethrel induced a strong female tendency, while in contrast gibberellin treatment resulted in fewer female flowers, higher female node, and delay in the first female flower. These results are in general agreement with those reported

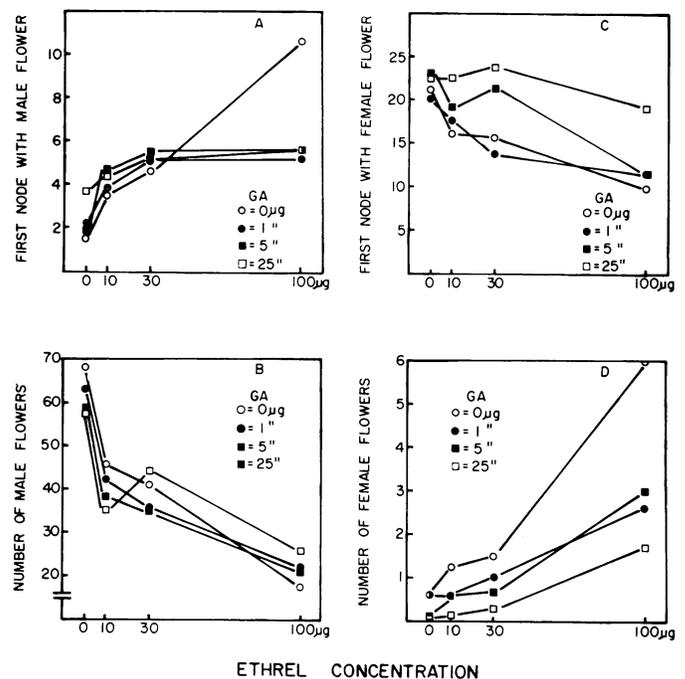


FIG. 2. Effects of Ethrel and GA_{4+7} on sex expression of cucumber plants as expressed by first node with male flower (A), number of male flowers (B), first node with female flower (C), and number of female flowers (D). Counting of flowering discontinued at 59 days after seeding. Total number of flowers through 20th node.

previously (6, 24, 28) on the opposing nature of Ethrel and gibberellin. However, since the interaction here was not significant, it would suggest that these growth regulators may act independently, rather than as antagonists. The sites of action are still unknown, but the opposing effects on sex expression may be explained through an effect on hormone balance affecting general plant rather than on the flower bud itself.

Effects of Growth Retardants and Inhibitors. Bromocholine bromide (22) and allyltrimethylammonium bromide (20) have been reported to cause femaleness in some cucumbers, and Alar in muskmelon (10). In addition, ABA is known to inhibit gibberellin action in α -amylase synthesis in barley endosperm (5). To compare the activity of these kinds of compounds with Ethrel on sex expression in cucumber, ABA, Alar, Phosphon D, and CCC were applied at the first leaf stage. ABA (1.2 or 20 μg) was applied to the growing point as 10- μl droplets, and Alar (5000 mg/liter) was applied as a spray. Phosphon D and CCC (2 or 10 mg in 2 ml of solution) were applied as soil drenches. Each treatment consisted of five plants. Phosphon D (10 mg) killed the plants within 1 day of treatment. All the other treatments decreased the plant height, but none of them affected sex expression. The absence of any effect on sex expression, in this study of these compounds known to affect gibberellin levels and/or action in higher plants, strongly supports the view that Ethrel and gibberellins must act independently in this response.

LITERATURE CITED

1. ATSMON, D. AND E. GALUN. 1960. A morphological study of staminate, pistillate and hermaphrodite flowers in *Cucumis sativus* L. *Phytomorphology* 10: 110-115.
2. ATSMON, D., A. LANG, AND E. N. LIGHT. 1968. Contents and recovery of gibberellins in monoecious and gynoecious cucumber plants. *Plant Physiol.* 43: 806-810.
3. BEN-YEHOSHUA, S., S. IWAHORI, AND J. M. LYONS. 1969. Ethylene and Ethrel: Role in development of fig fruit. *J. Amer. Soc. Hort. Sci.* Submitted for publication.

4. BURG, S. P. AND E. A. BURG. 1965. Ethylene action and the ripening of fruits. *Science* 148: 1190-1196.
5. CHRISPPELS, M. J. AND J. E. VARNER. 1966. Inhibition of gibberellic acid induced formation of α -amylase by abscisin II. *Nature* 212: 1066-1067.
6. FUCHS, Y. AND M. LIEBERMAN. 1968. Effects of kinetin, IAA, and gibberellin on ethylene production, and their interactions in growth of seedlings. *Plant Physiol.* 43: 2029-2036.
7. FUJIEDA, K. 1966. A genecological study on the differentiation of sex expression in cucumber plants. *Bull. Hort. Res. Sta. Ser. D.*, No. 4: 43-86 (In Japanese with English summary).
8. GALUN, E. 1959. Effects of gibberellic acid and naphthaleneacetic acid on sex expression and some physiological characters in the cucumber plant. *Phyton* 13: 1-8.
9. GALUN, E., S. IZHAR, AND D. ATSMON. 1965. Determination of relative auxin content in hermaphrodite and andromonoecious *Cucumis sativus* L. *Plant Physiol.* 40: 321-326.
10. HALEVY, A. H. AND Y. RUDICH. 1967. Modification of sex expression in muskmelon by treatment with the growth retardant B-995. *Physiol. Plant.* 20: 1052-1058.
11. HESLOP-HARRISON, J. 1957. The experimental modification of sex expression in flowering plants. *Biol. Rev.* 32: 38-89.
12. ITO, H., T. KATO, K. HASHIMOTO, AND T. SAITO. 1954. Factors responsible for the sex expression of Japanese cucumber. II. Anatomical studies on the sex expression and the transformation of the cucumber flowers induced by pinching. *J. Hort. Ass. Jap.* 23: 65-70 (In Japanese with English summary).
13. ITO, H. AND T. SAITO. 1956. Factors responsible for the sex expression of Japanese cucumber. III. The role of auxin on the plant growth and sex expression (1). *J. Hort. Ass. Jap.* 25: 101-110 (In Japanese with English summary).
14. IWAHORI, S., J. M. LYONS, AND W. L. SIMS. 1969. Induced femaleness in cucumber by 2-chloroethanephosphonic acid. *Nature* 222: 171-172.
15. LAIBACH, F. AND F. J. KRIBBEN. 1950. Der Einfluss von Wuchsstoff auf die Bildung männlicher und weiblicher Blüten bei einer monözischen Pflanze. *Ber. Deut. Bot. Ges.* 62: 53-55.
16. MCMURRAY, A. L. AND C. H. MILLER. 1968. Cucumber sex expression modified by 2-chloroethanephosphonic acid. *Science* 162: 1397-1398.
17. MEHNIK, F. J. 1958. Acetylene treatment as a method of increasing the formation of fruitful female flowers in cucumber. *Doklady Vsesojuz Akad. Sel'sk. Nauk.* 21(11): 20-23 (Russian). Cited from *Hort. Abstr.* 29: 1426 (1959).
18. MININA, E. G. 1938. On the phenotypical modification of sexual characters in higher plants under the influence of the conditions of nutrition and other external factors. *C. R. Acad. Sci. U.S.S.R. (Doklady)* 21: 298-301.
19. MININA, E. G. AND L. G. TYLKINA. 1947. Physiological study of the effect of gases upon sex differentiation in plants. *C. R. Acad. Sci. U.S.S.R. (Doklady)* 55: 165-168.
20. MITCHELL, W. D. AND S. H. WITTEW. 1962. Chemical regulation of flower sex expression and vegetative growth in *Cucumis sativus* L. *Science* 136: 880-881.
21. NITSCH, J. P., E. B. KURZ, JR., J. L. LIVERMAN, AND F. W. WENT. 1952. The development of sex expression in cucurbit flowers. *Amer. J. Bot.* 39: 32-43.
22. OTA, T. 1962. Studies on BCB (Bromocholine bromide). V. Effects of BCB on the growth and flowering of cucumber plant (1). *J. Jap. Soc. Hort. Sci.* 31: 329-336 (In Japanese with English summary).
23. PETERSON, C. E. AND L. D. ANHDER. 1960. Induction of staminate flowers in gynocious cucumbers with GAs. *Science* 131: 1673-1674.
24. ROBINSON, R. W., S. SHANNON, AND M. D. DE LA GUARDIA. 1969. Regulation of sex expression in the cucumber. *Bioscience.* 19: 141-142.
25. RUDICH, J., A. H. HALEVY, AND N. KEDAR. 1969. Increase in femaleness of three cucurbits by treatment with Ethrel, an ethylene releasing compound. *Planta* 86: 69-76.
26. RUSSO, L., JR., H. C. DOSTAL, AND A. C. LEOPOLD. 1968. Chemical regulation of fruit ripening. *Bioscience.* 18: 109.
27. SAITO, T. AND H. ITO. 1963. Factors responsible for the sex expression of Japanese cucumber. XIII. Physiological factors associated with the sex expression of flowers (2). Role of gibberellin. *J. Jap. Soc. Hort. Sci.* 32: 278-290 (In Japanese with English summary).
28. SCOTT, P. C. AND A. C. LEOPOLD. 1967. Opposing effects of gibberellin and ethylene. *Plant Physiol.* 42: 1021-1022.
29. SHANNON, S. AND M. DE LA GUARDIA. 1969. Sex expression and the production of ethylene induced by auxin in the cucumber (*Cucumis sativum* L.). *Nature* 223: 186.
30. SIMS, W. L. AND B. L. GLEDHILL. 1969. Ethrel effects on sex expression, growth development in pickling cucumbers. *Calif. Agric.* 23(2): 4-6.
31. YANG, S. F. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. *Plant Physiol.* 44: 1203-1204.