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STUDIES ON FLOWER INDUCTION BY S-TRIAZINE AND CARBAMATE IN SEEDLINGS OF *ASPARAGUS OFFICINALIS* L.

1. Effects of day length, compound concentration, temperature, seedling age and cultivar on flower induction of seedlings.

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Introduction

In asparagus cultivation, a male plant is found to be more profitable than a female plant, especially because of its higher productivity and of no useless matter consumption by fruit bearing. Sex of asparagus (*Asparagus officinalis* L.), which is a dioecious plant, is clarified only by flowers two or three years after sowing. Therefore, the artificial enhancement of earlier flower induction before transplanting has been studied to enable the seedling sex distinction at earlier growth stage for obtaining only male seedlings.

Thevenin¹⁾ first observed flowering on the shoot apex of the asparagus seedlings. Hsung²⁾ tried to bring about the early flowering of seedlings by controlling temperature and day length. Abe et al^{3,4)} found that the herbicides of atrazin type or urea type significantly initiated flower induction of seedlings, and then tested various synthetic atrazin analogues to keep the seedlings alive following chemical compound treatment, sequentially found some compounds more effective in flowering induction. Inducing flowers on the apex of the shoot of asparagus seedlings by the chemical compound treatment will enable earlier distinction of the sex.

In this report, we examined the conditions of inducing flowers with the anticytokinin, s-triazine and carbamate compounds, and observed the morphogenesis of the induced flowers.

Materials and Methods

Plant materials. Seeds of *Asparagus officinalis* L. cv. Mary Washington 500W, Pole Tom, UC157, New Jersey Green (NJ Green), Limbras No. 10, Frank-

lim, Accel, Marathon, Carib Green, Jersey Giant, Welcome and Hokkai 100 were prepared. 'Mary Washington 500W' or 'UC157' were mainly used in the present experiment except for that on varietal cultivars. All seeds were washed with tap water, and immature seeds were excluded before the compound treatment.

Anticytokinin treatment. 2-chloro-4-cyclohexylamino-6-ethylamino-s-triazine and n-propyl-N-(3, 4-dichlorophenyl) carbamate (Fig. 1) were used at 50 to 400 μ M as chemicals affecting flower induction. The compounds were dissolved in a small volume of dimethyl sulfoxide (DMSO), and the solution was diluted with distilled water at prescribed concentrations. The final concentration of DMSO was adjusted to less than 0.6%.

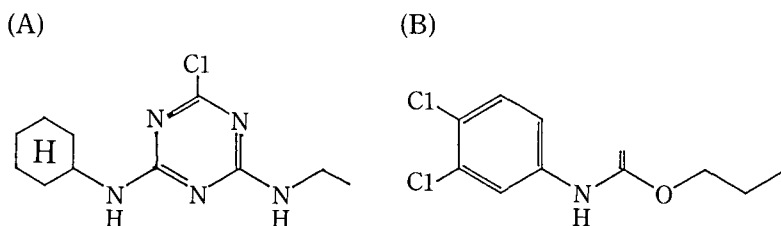


Fig. 1. Structures of compounds used for flower induction of asparagus seedlings in the present experiment.

(A) 2-chloro-4-cyclohexylamino-6-ethylamino-s-triazine.

(B) n-propyl-N-(3, 4-dichlorophenyl)-carbamate.

The developmental stage of seedlings used was synchronized by selecting seeds which had a radicle 1 or 5 mm in length after 5-7 days of water incubation.

In all treatments, 25 seeds were placed in a Petri dish (60 mm in diameter) which contained three pieces of filter papers and 5 ml of the test solution. The seeds were treated with the compounds at eight different temperatures from 5 to 40°C under darkness for 1, 2, 4, 8 and 12 days. After the compound treatment, all seeds were washed with tap water and transferred to a Petri dish which contained water alone until completion of the 12-day incubation. The seeds determined were transplanted to soil, and placed in a greenhouse.

Observation of flower induction. Thirteen days after transplanting, the number and structures of induced flowers on the apex of the first shoot of seedlings were investigated. For histological observations, flowers induced by the compounds were fixed with formalin-acetate alcohol (FAA), dehydrated, and embedded in paraplast. Sections were stained with hematoxylin.

Results

About 92% of the seeds treated with the compounds germinated and about 82% of the germinated seeds grew. The following seed germination percentages were obtained: more than 85% in 'Welcome', 'Jersey Giant', 'Franklin', 'Accel', 'Mary Washington 500W', 'Pole Tom', 'Marathon', 'Carib Green' and 'UC157', and 71 to 73% in 'Limbras No. 10', 'Hokkai 100' and 'NJ Green'. In addition, about

84% of the germinated seeds grew into seedlings. Even one-day treatment with carbamate or s-triazine enhanced the flower induction of seedlings, and 38 to 57% seedlings flowered through 4- to 8-day treatments with 200 μM solution of carbamate or s-triazine (Fig. 2). Percentage of flower induction increased with the increase of the concentrations of both the compounds (Fig. 3). Differences in

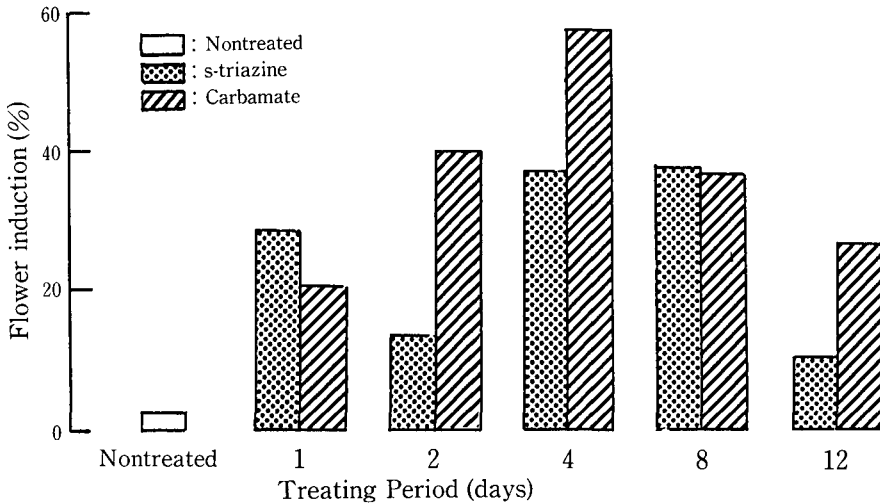


Fig. 2. Effects of periods of carbamate or s-triazine treatment on flower induction of asparagus seedlings. Seeds (cv. Mary Washington 500W) were treated with each compound at 200 μM .
 Flower induction rate (%) = (Number of flower-inducing seedlings/Number of developed seedlings) \times 100

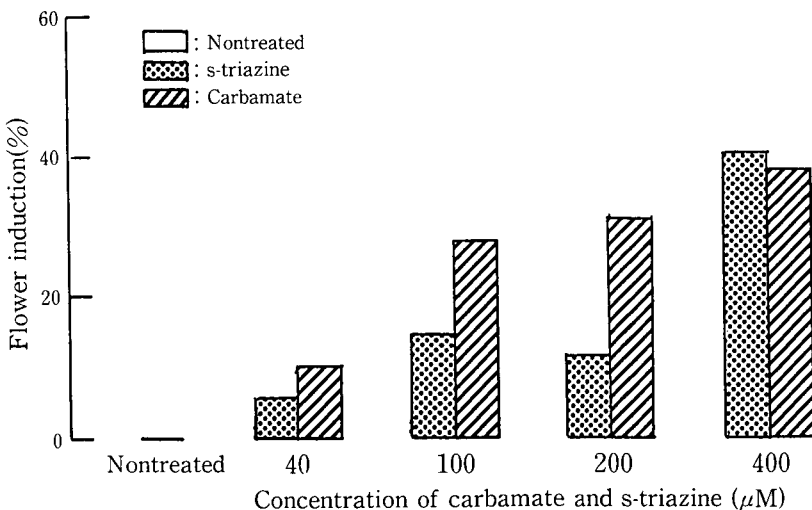


Fig. 3. Effects of concentrations of carbamate and s-triazine compounds on flower induction of asparagus seedlings. Seeds (cv. Mary Washington 500W) were treated with each compound for eight days.
 Flower induction rate (%) = (Number of flower-inducing seedlings/Number of developed seedlings) \times 100

the rates of flower induction depended on the cultivars. In all cultivars, the treatment with carbamate brought about higher percentages of flower induction than that with s-triazine. The cultivars used showed the following flower induction rates: less than 30% in 'Welcome', 'Jersey Giant' and 'Limbras No. 10', while higher percentage (75 to 88%) in 'Marathon', 'Carib Green' and 'UC157' (Fig. 4).

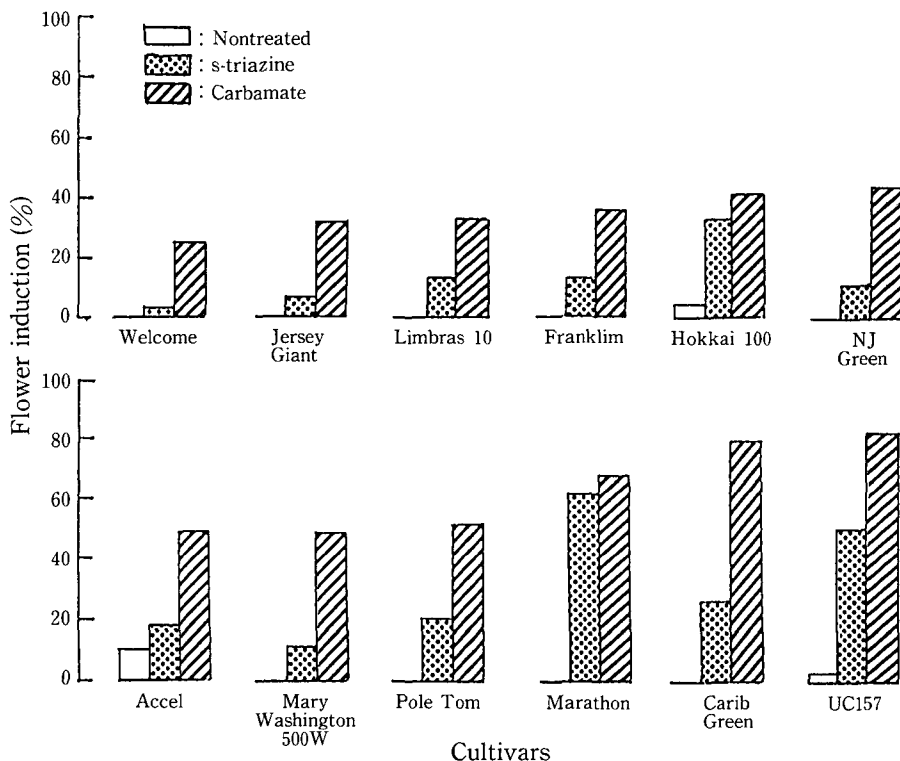


Fig. 4. Effects of carbamate and s-triazine compounds on flower induction at an early developmental stage of seedling of various asparagus cultivars. Seeds were treated with carbamate or s-triazine at $200 \mu\text{M}$ for eight days. Flower induction rate (%) = (Number of flower-inducing seedlings/Number of developed seedlings) \times 100

Low temperature (5°C) or high temperature (35° to 40°C) in treating period delayed the beginning of germination, whereas these temperatures did not decrease germination percentages, and scarcely inhibited the growth subsequent to germination. Flower induction rates gradually increased according as the treating temperature rose (Table 1).

The seeds before radicle emergence or the seedlings with a less than 1 mm-long radicle responded more actively to the flower induction enhancement by the compounds than the seedlings with a more than 5 mm-long radicle (Table 2).

Three kinds of flower setting modes were recognized in the present experiment. Most of the seedlings developed from treated seeds bore a flower on the apex of the first shoot with cladophylls elongated (Fig. 5-A). The flower setting

Table 1. Influence of temperatures in carbamate compound treatment on flower induction of asparagus seedlings (cv. UC157).

Temperature of treatment (°C)	No. of treated seeds	No. of germinated seeds	No. of developed seedlings	Flower induction of seedlings	
				Number	%
5	50	50	48	15	31.3
10	50	49	49	14	28.6
15	50	50	47	17	36.2
20	50	47	47	29	61.7
25	50	49	47	37	78.7
30	50	47	46	31	67.4
35	50	48	41	35	85.4
40	50	50	49	45	91.8

Seeds were treated with carbamate compound at 200 μ M for eight days.

Flower induction rate(%)=(Number of flower-inducing seedlings/Number of developed seedlings) \times 100.

Table 2. Influence of the developmental stage of asparagus seedling (cv.UC157) at a time of carbamate compound treatment on flower induction.

Developmental stages according to length of radicle (mm)	No. of tested seeds	No. of developed seedlings	No. of flower-inducing seedlings	%
0	50	50	31	62.0
1	50	50	36	72.0
5	50	50	10	20.0

Seeds with a radicle 1 to 5 mm in length were selected after 5 or 7 days of water incubation, before the 4-day compound solution treatment started from seeding on a filter paper in Petri dish.

Flower induction rate(%)=(Number of flower-inducing seedlings/Number of developed seedlings) \times 100.

was rarely observed on the apex of the first shoot which has a lateral shoot with cladophylls (Fig. 5-B), and on the apices of both the first shoot and 1 or 2 lateral shoots that bore no cladophyll (Fig. 5-C).

In the observations of flower organ structures, it was clarified that the male flower induced by the compound treatment had some matured anthers containing pollens and an immatured ovary (Fig. 6-A, D, F), and the female flower had a matured ovary with ovules (Fig. 6-C, F, G). The observational method made it possible to distinguish the sexuality of flowers of plants of *Asparagus officinalis* L. In these cases, however, certain cautions were required especially because a malformed flower and some male flowers often had an immature ovary (Fig. 6-B).

Discussion

In the present study, it was shown that without inhibiting the germination, the seed treatment with solutions containing two anticytokinins, carbamate and s-

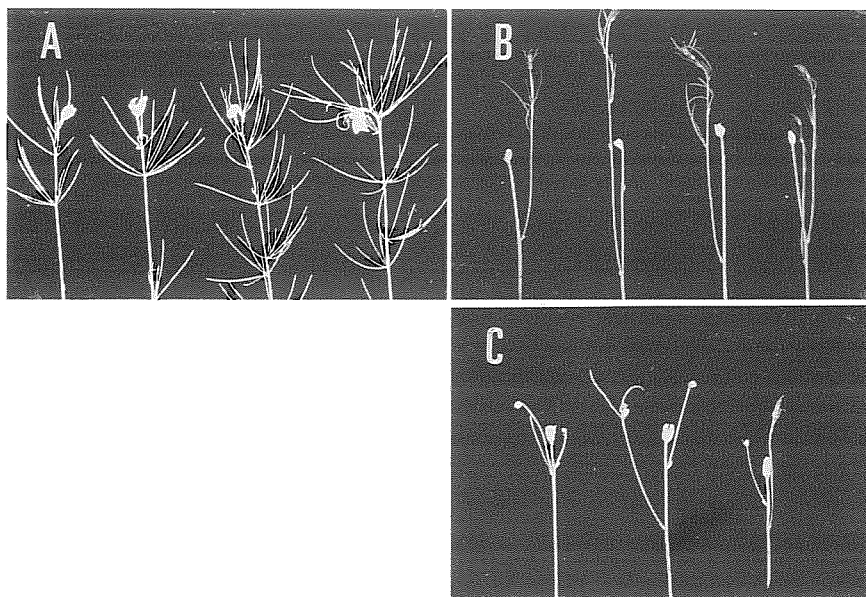


Fig. 5. Modes of terminal flower setting in seedlings treated with compounds.
(A) On the apex of the first shoot with cladophylls elongated.
(B) On the apex of the first shoot with a lateral shoot bearing cladophylls.
(C) On the apices of both the first shoot and 1 or 2 lateral shoots that bear no cladophylls.

triazine, significantly enhanced flower induction at the top of the first shoot of seedlings. The 4- to 8-day treatments at 200 to 400 μM of the compounds were optimal for flower induction. From the higher flower induction percentage in carbamate treatment, it was found that carbamate had higher activities in inducing flowers than s-triazine.

Ungerminated seeds and seedlings with a radicle 1 mm in length showed the highest response to flower induction enhancement by the synthetic compound treatments. The flower-inducing effect of the compounds decreased considerably in the seedlings which had grown at the developmental stages with a 5-mm radicle or a primordium of the first shoot. This may show that the compounds effectively work at the early stage of germination. This consideration agrees with the report of Yanosaka et al⁵⁾, and was explained from the fact that the phenomenon was related closely to an activity of anticytokinins. In the future, the synthetic compound-caused flower induction will be one of the interesting phenomena in plant physiology.

The results from several replicates in the present experiments clarified that some differences in the percentage of flower induction were recognized among the used varieties. These facts also agree approximately with the results reported by Yanosaka et al⁵⁾. Further studies will be required to obtain a higher rate of flower induction.

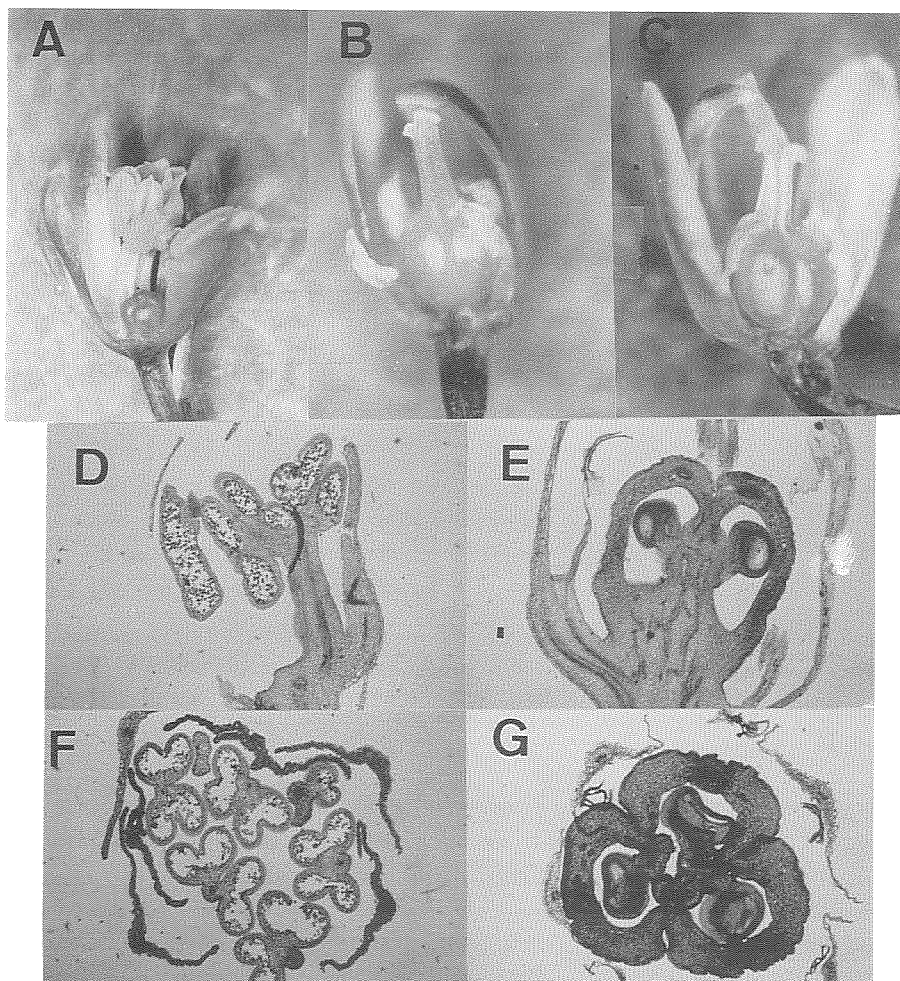


Fig. 6. Appearances and internal structures of flowers formed on shoot apices of seedling treated with anticytokinin, carbamate. (A) Male flower. (B) Bisexual flower. (C) Female flower. (D) Longitudinal section of male flower. (E) Longitudinal section of female flower. (F) Cross section of male flower. (G) Cross section of female flower.

Thevenin¹⁾ and Hsung²⁾ controlled flowering under various conditions during raising seedlings, and pointed out that the high temperature was effective in flowering. The higher temperature (30° to 40°C) delayed the seed germination, but promoted flowering. These results suggest that higher temperatures (30° to 40°C) show a promotive effect on flowering and may be associated with promoting the flowering.

It was possible to distinguish the sex by the observation of stamen and pistil formed inside the flower. Yanosaka et al.⁵⁾ obtained asparagus fruits by crossing female with male, of which all flowers were induced by the chemical compound

treatment. The fact suggests that both the pollen and ovary functioned normally. This will enable the phenomenon to be applied not only in the distinction of the sex of asparagus seedlings but also in the reduction of cross breeding duration. Further researches on the fertility of pollens and ovaries in the flowers artificially induced will be required to establish a practical method available for excellent cultivar breeding.

Summary

Defined conditions of anticytokinin (s-triazine and carbamate) treatment to induce flower bud formation at the apices of the first shoot of seedlings of *Asparagus officinalis* L. were investigated using cv. Mary Washington 500W, Pole Tom, UC157, New Jersey Green, Limbras No. 10, Franklim, Accel, Marathon, Carib Green, Jersey Giant, Welcome and Hokkai 100, and the structures of induced flowers were observed.

The synthetic compounds used in these experiments did not strongly inhibit the germination or the growth of the seedlings. The seeds treated with carbamate at 200 to 400 μM for four days or with s-triazine at 400 μM for four to eight days flowered in high percentage.

Differences in percentage of flower induction depended on cultivars. It was found that carbamates had a tendency to affect flower induction in higher percentage than s-triazines. The 8-day treatment with 200 μM carbamate at high temperatures (35°-40°C) enhanced earlier flower induction. In the carbamate treatment, a lowering of flower induction percentage of the seedlings with a radicle more than 5 mm in length made it clear that the carbamate activities to induce early flowering were influential in the short beginning of germination.

Most of the induced flowers set on the apices of the first shoot of seedlings with elongated cladophylls about two weeks after the compound treatment. By means of observing the developmental stages of anthers containing pollens or of a developed ovary with ovule, it was possible to distinguish the sex of the anticytokinin-induced flowers of the seedling.

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