J. Amer. Soc. Hort. Sci. 107(5):860–862. 1982.

Autotoxicity of *Asparagus officinalis* L.[1]

Hsu-Jen Yang[2]
Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA 99350

Additional index words. autotoxicity, allelopathy

Abstract. **Crude aqueous extracts from dead stems, crowns, and roots from both field-grown and tissue-cultured asparagus plants delayed, but did not prevent, germination of asparagus seed. Root extract inhibited root and shoot development of asparagus seedlings grown in growth pouches. Stem and crown extracts reduced root growth but not shoot growth. The extracts of all 3 tissues caused more secondary root formation and root branching. The highest concentration of extract from crown-plus-root tissues, 5 g of tissue/100 ml water, inhibited radicle growth and killed seedlings. Toxicity of the crown-root extract was not reduced by adding activated charcoal to the extract or by autoclaving the extract. These results suggest that toxic substances in dead asparagus tissue are water-soluble and stable and may persist in old asparagus fields.**

Failures in reestablishing commercially profitable asparagus stands in old asparagus fields in Europe, Japan and North America have been frequently reported (1, 2, 3, 9, 12). The major reason for this problem is usually diagnosed as an accumulation of pathogenic organisms causing Fusarium wilt *(Fusarium oxysporum* f. *asparagi)* (1, 4), crown rot *(F. moniliforme)* (4), and root rot *(Rhizoctonia violacea)* (12) in old asparagus beds. Chemical fumigation of old asparagus fields and fungicide treatment of seeds effectively control these pathogens and improve the establishment of young asparagus plants, but stunting and wilting of seedlings still occurs (1, 5, 9, 18). Furthermore, productivity of reestablished asparagus in fumigated fields is variable (9). These observations suggest that factors other than fungal pathogens may be partially responsible for the asparagus replant problem.

Recent evidence supports the hypothesis that an accumulation of toxic substances produced by asparagus may be autotoxic and allelopathic. Asparagusic acid and related compounds which are present in asparagus shoot tissues had inhibitory effects on the growth of lettuce, rice, and radish (14, 16, 17). Dialyzed asparagus root extract reduced radicle length of lettuce (11). Asparagus root tissue mixed with soil delayed asparagus seed germination for 11 days, and water extracts of asparagus tops reduced the number of shoots in asparagus seedlings (6). These results suggest that biologically active and probably toxic substances produced by asparagus may be operative in the replant problem in old asparagus fields.

While it is certain that the microorganisms which accumulate in old asparagus beds play a role in the replant problem, there has been no substantial evidence presented that toxins produced by the asparagus plant also contribute significantly to the replant problem under field conditions. In order to determine whether asparagus toxins might affect reestablishment, it is necessary to compare the effects of plant extracts from both field-grown plants and microorganism-free plants on the germination of asparagus seeds and growth of seedlings. To this end, plants cultured in the field and plants produced in aseptic tissue culture were used as sources of extracts.

Materials and Methods

Dead asparagus plants were obtained both from established asparagus fields and from tissue cultures. Plants that had been dead for at least one growing season were collected from 4- to 5-year-old experimental plots at the Irrigated Agriculture Research and Extension Center, Washington State University, Prosser. Asparagus plants of the same lines as the field-grown plants that had been aseptically tissue-cultured in a modified Murashige and Skoog's medium (19) for 2 years were allowed to die from lack of nutrient maintenance and were then sampled. The plants were thoroughly washed and separated into stem, crown, root, and crown + root categories. They were then dried in an oven at 55°C for 5 to 7 days. The tissues were pulverized in a Wiley mill with a 40-mesh screen, extracted with distilled water by stirring for 1 hr at room temperature, and filtered through Whatman No. 1 filter paper. Crude aqueous extracts of progressively increasing concentration were prepared using 0.1, 0.5, 1.0, 3.0, and 5.0 g of dried tissue per 100 ml of distilled water.

In order to test the stability of plant-produced toxic substances, a crown + root extract of field-grown plants was prepared using 1 g tissue/100 ml water and autoclaved at 121°C and 1 kg/cm^2 pressure for 15 min. In another test, activated charcoal was added at 50, 100, 300, and 500 mg/liter of crown-root extract to determine whether the toxicity of the extract could be decreased with an adsorbent.

In the germination tests, asparagus seed of 1 of our breeding lines, T 6, was used. The seed was surface-sterilized with 10% commercial bleach (0.5% sodium hypochlorite) for 20 min followed by 5 rinses with distilled water. This seed was then imbibed by soaking in distilled water overnight at room temperature. One hundred randomly selected seeds were placed between filter paper discs in a Petri dish containing 15 ml of either the aqueous extract or a distilled water control. The Petri dishes were placed in an incubator and maintained at 25°C. Germination counts were made every day for 12 days. Each treatment was replicated 3 times.

In the tests for seedling growth, 15 germinated seeds with radicles 0.8 to 1.0 mm in length were randomly collected and placed in a growth pouch containing 30 ml of aqueous extract or distilled water. Another 30 ml of extract or water was added to each growth pouch after 6 or 7 days to provide moisture. The growth pouches were placed in a growth chamber and maintained

[1]Received for publication Nov. 9, 1981. Scientific Paper No. 6063 Project No. 0431, College of Agriculture Research Center, Washington State University, Pullman, WA 99164.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[2]Acknowledgement is given to the Washington Asparagus Growers Association for grant funds supporting these studies.

at 22 to 28°C with a 16-hr photoperiod under 1.5 klx illumination from 20W Plant-Gro fluorescent lamps. Root and shoot lengths of the seedlings were measured after 12 to 14 days. Each experiment was repeated 3 times, and each treatment contained 3 growth pouches.

Results and Discussion

Effects of extracts from field-grown and tissue-cultured plants on seed germination and seedling development. Extracts from dead tissue of both field-grown and tissue-cultured plants delayed seed germination and reduced seedling growth (Table 1). Germination of the seeds watered with distilled water began after 3 days of incubation and exceeded 90% in 7 days. Germination of the seeds watered with the stem and crown extracts was slightly delayed and exceeded 90% in 8 days. However, germination of the seeds watered with the root extracts was significantly delayed and did not reach 90% germination until 9 days after planting. The final germination percentages were not significantly different for any of the treatments.

All of the extracts inhibited either root or shoot growth or both (Table 1, Fig. 1). An especially high degree of inhibition was produced by the root extracts. The root-extract-treated root systems produced retarded fleshy roots and poorly developed fibrous roots. Secondary roots frequently formed, and root branching occurred. Most roots at this stage had brownish tips and eventually died. The shoots of plants treated with root extract were not well-developed; they were short and weak and developed few cladophylls. In general, the root extract from tissue-cultured plants produced a greater inhibitory effect on root and shoot growth and caused more secondary root formation and root branching than the extract from field-grown plants.

The stem and crown extracts from both plant sources also reduced root growth. Treated roots were shorter than those grown with distilled water. The extracts increased secondary root formation and root branching but retarded their growth. Shoots of plants watered with stem or crown extracts of field-grown plants and the stem extract of tissue-cultured plants exhibited vigorous growth and well-developed cladophylls. There were no statistical differences in length of shoots between these treatments and the control. However, the crown extract of tissue-cultured plants significantly reduced shoot growth.

Effects of various concentrations of crown-root extract from field-grown plants on seed germination and seedling development. the effects of various concentrations of crown-root extract on seed germination and seedling growth are shown in Table 2. The 2 lowest concentrations of the extract did not affect seed germination and shoot growth. The 2 higher concentrations of the extract significantly delayed germination but did not prevent it. The extract from 0.5 g of tissue/100 ml water significantly reduced root growth but not shoot growth. Increasing the amount of tissue from 0.5 to 3 g/100 ml water retarded root and shoot growth. The highest amount, 5 g of tissue/100 ml water, completely inhibited radicle and shoot growth and killed the seedling.

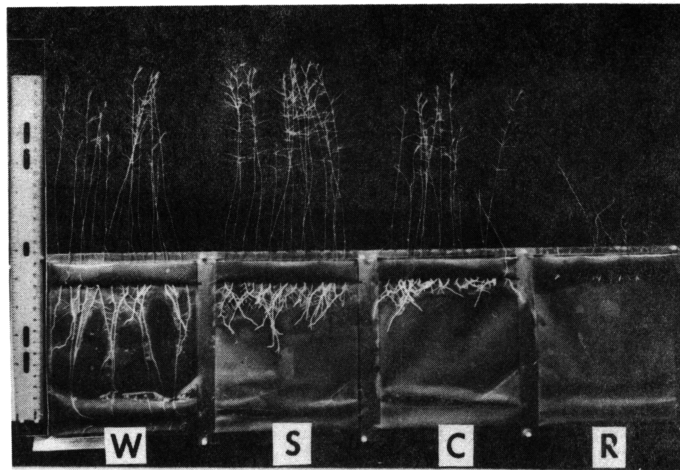

Fig. 1. Effects of the extracts of dead stems, crowns, and roots from tissue-cultured asparagus after 14 days (W = water; S = stem extract; C = crown extract; R = root extract).

Effects of crown-root extracts from field-grown plants with activated charcoal added or autoclaved on germination and seedling development. The germination of seeds watered with extract with or without charcoal added began after 4 days and exceeded 90% in 9 days. After 12 days, there was no significant difference in percentage of germination or in root and shoot growth between plants in the extracts with and without charcoal added.

The autoclaved extract did not affect seed germination, or improve root or shoot growth as compared to the nonautoclaved extract. However, none of the seedlings grown with autoclaved extract had reddish-brown discoloration spots on crowns and roots, whereas a few seedlings grown with nonautoclaved ex-

Table 1. Effects of the extracts of stems, crowns, and roots from field-grown and tissue-cultured asparagus plants on seed germination and seedling development of asparagus.

Treatment	Germination (%) 7 days	9 days	12 days	Root length[x] (cm)	Shoot length[x] (cm)	Seedlings with secondary and branching roots (%)
Water (Control)	92.0a[y]	97.7a	98.7a	11.4a	16.0a	11.2c
Extract[z]						
Stem FG	84.3b	97.0a	98.0a	5.8bc	16.1a	38.6b
TC	87.7ab	96.7a	97.0a	6.4b	17.0a	30.3b
Crown FG	80.7bc	95.3ab	96.3a	6.5b	16.6a	34.0b
TC	85.0b	96.3a	97.7a	4.8c	14.4b	31.1b
Root FG	70.7d	88.0c	96.0a	2.4d	12.6c	38.7b
FG	76.0cd	90.3bc	96.7a	1.1e	5.4d	55.6a

[z]The extracts were prepared by stirring 1 g of ground tissue in 100 ml distilled water for 1 hr at room temperature. FG = field-grown plants, TC = tissue-cultured plants.
[y]Mean separation in columns by Duncan's multiple range test, 5% level. The data were transformed to angles for test of significance.
[x]After 14 days.

Table 2. Effects of various concentrations of field-grown asparagus crown-root extract on seed germination and seedling development of asparagus.

LConcn (g of dried tissue/100 ml water)	Germination (%)			Root length[y] (cm)	Shoot length[y] (cm)
	7 days	9 days	12 days		
0	90.7a[z]	96.7a	97.3a	10.5a	13.6a
0.1	87.0a	96.3a	96.3a	9.9a	13.4a
0.5	88.7a	95.7a	96.3a	7.3b	13.7a
1.0	77.0a	95.7a	97.3a	5.7b	13.4a
3.0	38.0b	86.0b	91.7a	1.4c	3.9b
5.0	37.7b	86.0b	93.3a	0.0d	0.0c

[z]Mean separation in columns by Duncan's multiple range test, 5% level. Data were transformed to angles for test of significance.
[y]After 12 days.

tracts did. These spots were diagnosed as being due to infection by *Fusarium* pathogens. Autoclaving eliminated the microorganisms in the extract but did not reduce or destroy the toxic substances in the extract.

Asparagus is a perennial crop, and a planting will often be productive for over 20 years. During such a long period, pathogens and toxic substances present in asparagus tissues will accumulate in the soil. This study has shown that crude aqueous extracts from microorganism-free tissue-cultured plants are equally as toxic to asparagus seedlings as extracts from field-grown plants. This indicates that the asparagus replant problem may be due to autotoxicity as well as pathogen accumulation.

Strong evidence is presented here to support the hypothesis that asparagus is highly autotoxic and produces toxins that inhibit its reestablishment in old asparagus fields. Furthermore, asparagus has been found to be allelopathic to other plant species (6, 10, 11, 14, 16, 17).

The chemical mechanisms of autotoxicity and allelopathy in asparagus are poorly understood. Yanagawa (14) and Yanagawa et al. (16, 17) found that asparagusic acid and related compounds isolated from asparagus were inhibitory to seedlings of several crop species. However, asparagusic acid is not toxic to asparagus seedlings (15). Moreover, this compound is highly unstable under environment extremes (15) and would not be expected to persist in the soil for long periods. Further investigation will be needed in order to determine what compounds produced the autotoxic effects observed in this study.

Reduction or inactivation of toxic substances produced by plants in agricultural soils can be accomplished through prolonged periods of leaching, adsorption, volatilization, and microbial action (7, 8, 13). The progressive deactivation of allelopathic substances in asparagus roots in soil with the passage of time has been noted (10). In this study, extracts from asparagus tissues which had been dead for at least 8 months in the field still contained toxic substances that caused detrimental effects on seedling development. Hanna (2) reported that a 4-year crop rotation of 3 years in alfalfa and 1 year in barley, or the 1st and 3rd years in a leguminous cover crop, and the 2nd and 4th years in barley between removal of the old asparagus and replanting, is not sufficient to insure commercial yields. Sawata (9) stated that established fields of asparagus were plowed under during World War II in Hokkaido, Japan, and other crops were grown in these fields for several years. After the war, many farmers replanted asparagus in these fields. The results was poor growth and production. Perhaps longer periods of rotation are required.

Activated charcoal has been widely used to inactivate pesticides in the soil (7) and provides a potential treatment for fields with poor reestablishment. However, our laboratory results indicate that activated charcoal does not detoxify asparagus extracts and thus shows little promise as a field treatment.

Preliminary observations indicate that some selections obtained from inbreeding cv. 'Mary Washington' grow well and are productive in old asparagus fields. This suggests that breeding autotoxin-resistant lines of asparagus is possible. This may be the best way to overcome the problem of poor reestablishment in old asparagus fields.

Literature Cited

1. Gorgan, R. G. and K. A. Kimble. 1959. The association of *Fusarium* wilt with the asparagus decline and replant problem in California. Phytopathology 49:122–125.
2. Hanna, G. C. 1938. Fertilizer trials with asparagus on peat sediment soil. Proc. Amer. Soc. Hort. Sci. 36:560–561.
3. Hanna, G. C. 1947. Asparagus production in California. Calif. Agr. Ext. Serv. Cir. 91.
4. Johnston, S. A. 1977. The etiology and control of *Fusarium* root rot of asparagus. PhD Thesis, Rutgers, The State Univ. of New Jersey, New Brunswick.
5. Lacy, M. L. 1979. Effects of chemicals on stand establishment and yields of asparagus. Plant Dis. Rptr. 68:612–616.
6. Laufer, G. A. and S. A. Garrison. 1977. The effect of asparagus tissue on seed germination and asparagus seedling growth; Possible allelopathic interactions. HortScience 12:385. (Abstr.)
7. Putnam, A. R. 1978. Allelopathy in agroecosystems. Annu. Rev. Phytopath. 16:431–451.
8. Rice, E. L. 1974. Allelopathy. Academic Press, New York.
9. Sawata, E. 1967. Asparagus (in Japanese). Sheibundo-Shinkosha, Tokyo, Japan.
10. Shafer, W. E. and S. A. Garrison. 1980a. Effects of decomposing asparagus root tissue on lettuce, tomato, and asparagus seed emergence. HortScience 15:406. (Abstr.)
11. Shafer, W. E. and S. A. Garrison. 1980. Effects of asparagus root extracts on lettuce and asparagus seed germination and growth. HortScience 15:406. (Abstr.)
12. Stahl, M. 1970. Die Wurzelfaule des Spargels. Z. PflKrankh. 77:353–367.
13. Swain, T. 1977. Secondary compounds as protective agents. Annu. Rev. Plant Physiol. 28:479–501.
14. Yanagawa, H. 1978. Structure and physiological properties of asparagusic acid (in Japanese). Kagaku to Seibutsu. 16:124–129.
15. Yanagawa, H. 1979. Preparation and determination of asparagusic acid. Method in Enzymology. 62:181–184.
16. Yanagawa, H., T. Kato, and Y. Kitahara. 1973. Asparagusic acid-S-oxides, new plant growth regulators in etiolated young asparagus shoots. Tetrahedron Lett. 13:1073–1075.
17. Yanagawa, H., T. Kato, Y. Kitahara, N. Takahashi, and Y. Kato. 1972. Asparagusic acid, Dihydroasparagusic acid, and S-acetyldehydroasparagusic acid, new plant growth inhibitors in etiolated young *Asparagus officinalis*. Tetrahedron Lett. 25:2549–2552.
18. Weibe, J. 1967. Soil and seed treatment effects of *Fusarium* wilt of asparagus seedlings. Hort. Res. Inst. Ont. 1966:33–36.
19. Yang, Hsu-Jen. 1977. Tissue culture technique developed for asparagus propagation. HortScience 12:140–141.