HortScience 32(6):1085–1086. 1997.

Evaluation of *Asparagus officinalis* Cultivars for Resistance to Stem Blight by Using a Novel Inoculation Method

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Additional index words. Phomopsis asparagi, seedling, breeding, disease resistance

***Abstract. Asparagus officinalis* L. cultivars were evaluated for resistance to asparagus stem blight caused by *Phomopsis asparagi* (Sacc.) Bubák under controlled environmental conditions. The plants were inoculated with the vinyl tube and cotton inoculation method. Disease severity assessments, based on the percentage of diseased plants and the disease index, were made 4 weeks after inoculation. Estimates of the percentage of diseased plants ranged from 33% to 80%, and the disease index ranged from 28 to 79. None of the cultivars and lines showed high resistance, but there were significant differences in disease susceptibility among the cultivars and lines.**

Stem blight caused by *Phomopsis asparagi* (Sacc.) Bubák (Bubák, 1906) is a disease that causes great damage to asparagus production throughout Japan. The causal fungus first forms small lesions or spots on the lower part of the asparagus stem. The lesions continue to expand and produce yellowish-brown spindle-shaped spots. Pycnidia usually appear at the central part of a spot and become secondary sources of infection (Sakai et al., 1992a). Infected stems often die when the lesions expand enough to surround them, which makes this disease more devastating than other asparagus diseases in Japan.

Although benomyl and tetrachloroisophthalonitrile (TPN) are used for control, these fungicides are relatively expensive and not always effective (Sakai et al., 1992a). A resistant cultivar is indispensable to lower production costs. Several authors (Sakai et al., 1992b; Tu et al., 1988) reported cultivar evaluation studies on resistance to asparagus stem blight in the field; however, they found no resistant cultivars and lines, mainly because of the inconsistency of the disease pressure. A reliable screening method is necessary for efficient selection for resistant genotypes.

Sonoda et al. (1995) developed the vinyl tube and cotton inoculation (VC inoculation) method for asparagus stem blight. It can produce a controlled number of lesions on the lower part of the stem, where the first infection often occurs. This inoculation method is applicable to seedlings and mature plants. The objective of the present investigation was to evaluate *A. officinalis* cultivars and lines for resistance to asparagus stem blight by using this recently reported inoculation method.

Materials and Methods

Twenty-four cultivars and two lines of asparagus were screened for resistance to asparagus stem blight in two experiments. The control cultivar was 'UC157'. Each seedling was grown in a pot. Shoots of 2- to 5-month-old seedlings were cut back to ground level to produce uniform new shoots. Newly emerged shoots were VC inoculated with a *P. asparagi* isolate collected from the asparagus field in the Fukushima Prefecture Agricultural Experiment Station 2 weeks after the shoOts had been cut, i.e., when the cladophylls had just fully expanded. Absorbent cotton (1 cm^2) was put around the stem, covering at least one scale leaf ≈3 cm above the soil level. A chlorinated vinyl tube (20 mm long, 8 mm in diameter) slit lengthwise was put on the absorbent cotton to hold it (Fig. 1). Then 0.6 mL of *P. asparagi* inoculum (10^6 spores/mL) was infiltrated gently into the cotton with a pasteur pipette. After inoculation, the plants were transferred to a controlled-environment chamber at 25 °C and 95% to 100% relative humidity under low-light conditions. The absorbent cotton and vinyl tube were removed after 3 d, then the plants were transferred to a glasshouse at 25 ± 3 °C under natural daylight. The assessments were made 4 weeks after inoculation.

Disease severity assessments were based on the percentage of diseased plants and the disease index (DI) was calculated by using disease severity grades: 0 = none; 1 = small lesions (<0.2 cm) ; 2 = spreading lesions; 3 = large lesions (>1 cm); 4 = pycnidia formation. Inoculated stems that developed pycnidia before the assessments were evaluated and discarded to prevent secondary infection by the pycniospores. The percentage of diseased plants was calculated from standards where disease severity grades 0 and 1 represented resistant plants, and grades 2 to 4 represented susceptible plants. Twelve plants each were used for three replications. These data were

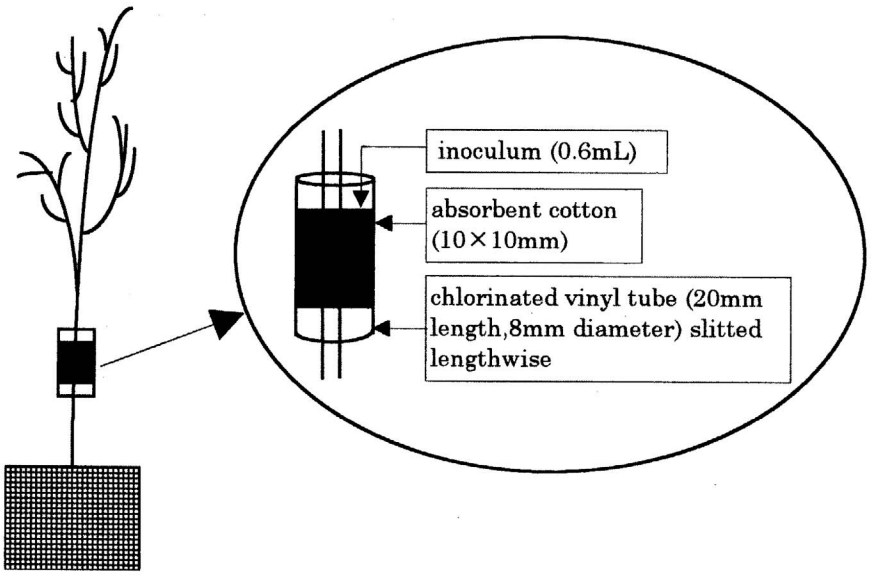

Fig. 1. Schematic of the vinyl tube and cotton inoculation method for testing the resistance of asparagus to stem blight.

Received for publication 11 Mar. 1996. Accepted for publication 11 Nov. 1996. 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.

[1]Vegetable Breeding.
[2]Vegetable Breeding.
[3]Plant Pathology.

analyzed using the new Duncan's multiple range test, $P \leq 0.05$ (Duncan, 1955; Harter, 1960).

Results and Discussion

Inoculated plants produced symptoms only from the inoculated area starting 3 d after inoculation. In the first experiment, the mean of diseased plants ranged from 33% to 76%, the DI ranged from 28 to 63 (Table 1). 'Larac' and 'Huchels Schneewittchen' different significantly from 'Greenwich', 'Gynlim', and 'Eros' in the percentage of diseased plants and ID. In the second experiment, the mean of diseased plants ranged from 42% to 80%. The DI ranged from 42 to 79 (Table 1). 'Huchels Leistungsauslese', 'Huchels Alpha' and 'Lucullus 522' different significantly from 'Grande', 'Golia', 'Thukikou 2Gou' 'Thielim', 'Apollo', 'Presto', 'Jersey Knight', and 'UC157' in the percentage of diseased plants and the DI. The disease severity in the control cultivar UC157 was moderate for both experiments.

There were differences among cultivars and lines in degree of susceptibility, although none of them showed high levels of resistance. These results were similar to the reaction of *A. officinalis* to *Stemphylium* leaf spot (Bansal, 1988). According to the mean percentage of diseased plants and DI of our two experiments, the cultivars were assigned to three groups. The resistant group consisted of 'Greenwich', 'Gynlim ', and 'Eros'. The susceptible group consisted of 'Larac', 'Huchels Schneewittchen', 'Huchels Leistungsauslese', 'Lucullus 522', and 'Huchels Alpha'. Three cultivars of the resistant group present possible breeding material for resistance to asparagus stem blight.

Our results show that the VC inoculation method can reveal differences in susceptibility to stem blight among cultivars. Under natural conditions in the field, it is difficult to control environmental factors that favor infection, such as temperature, humidity and mechanical wounds.

Sometimes no infection occurs because of dry weather (Sakai et al., 1992a). Inoculum in sprayed spore suspensions tends to drip to the bottom of the stem, because of the thin needle-like shape and smooth surface of the cladophylls, which is likely to cause a variation in the inoculation point and infection rate. When *A. officinalis* plants were inoculated by needle, no cultivar showed any disease resistance (Sonoda et al., 1995).

In our study, since the DI and mean percentage of infected plants showed the same tendency, the difference in susceptibility among cultivars seems to originate from resistance during infection. The VC inoculation method can control environmental factors concerning host–pathogen interaction during infection, such as infection court and environmental conditions.

Compared with traditional inoculation procedures, VC inoculation has proven effective for the selection of lines resistant to stem blight disease. With method, it is also possible to select stem blight-resistant individuals from clonally propagated or pollen-originated homogeneous plants as breeding material.

Table 1. Mean percentage of diseased plants and disease index (DI) of *Asparagus officinalis* cultivars and lines screened for resistance to asparagus stem blight.

Cultivar	Origin	Infected plants (%)[z]	DI
Experiment 1			
Greenwich	U.S.A.	33 a	23 a
Gynlim	Netherlands	37 ab	28 a
Eros	Italy	39 ab	36 ab
Jersey Giant	U.S.A.	43 a–c	36 ab
Marte	Italy	45 a–d	35 ab
Andreas	France	48 b–d	44 a–c
Vulkan	Germany	50 b–d	48 bc
Thukikou 3Gou	Japan	56 d	51 cd
Larac	France	74 e	63 d
Huchels Schneewittchen	Germany	76 e	69 d
UC157 (control)	U.S.A.	56 cd	41 bc
Experiment 2			
Grande	U.S.A.	42 a	42 a
Golia	Italy	46 a	45 a
Thukikou 2Gou	Japan	50 a	44 a
Thielim	Netherlands	50 a	49 a
Apollo	U.S.A.	52 a	52 a
Presto	Germany	53 a	51 a
Jersey Knight	U.S.A.	55 a	52 a
Atlas	U.S.A.	56 ab	55 ab
Steline	France	59 ab	58 ab
Jersey Gem	U.S.A.	60 ab	57 ab
Hermes	Germany	62 ab	61 ab
Argo	Italy	64 b	60 ab
Huchels Leistungsauslese	Germany	78 b	77 b
Huchels Alpha	Germany	78 b	76 b
Lucullus 522	Germany	80 b	79 b
UC157 (control)	U.S.A.	51 a	48 a

[z]Mean percentage of diseased plants was calculated from: disease severity grade 0 and 1 are resistant, and 2 to 4 are susceptible. Column means were calculated using the new Duncan's multiple range test, $P \leq 0.05$.

[y]Disease index = $\Sigma \frac{\text{(Number of plants classified into each grade} \times \text{Disease severity grade number)}}{\text{Total number of plants employed} \times 4} \times 100$

Disease severity grade: 0 = none, 1 = small lesions, 2 = spreading lesions, 3 = large lesions, 4 = pycnidia formation. Column means were calculated using the new Duncan's multiple range test, $P \leq 0.05$.

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