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Susceptibility of Chinese alfalfa cultivars to Verticillium wilt

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ABSTRACT

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Twenty six alfalfa cultivars (25 *Medicago sativa* and one *M. falcata*) from China were compared to the seven standard cultivars from North America and Europe for resistance to Verticillium wilt caused by *Verticillium albo-atrum*. Except for the cultivar E Qi, all 25 Chinese cultivars were susceptible to Verticillium wilt with low numbers of resistant plants (less than 21.7% in the population of each cultivar) and high disease severity index (greater than 3.43). The cultivar E Qi was moderately resistant to Verticillium wilt with 43.4% of resistant plants, which was similar to the Swedish cultivar Vertus (43.3%) but lower than the Canadian cultivar Barrier (60.2%). Since there is no report of Verticillium wilt of alfalfa in China or Taiwan, the susceptible nature of Chinese alfalfa cultivars suggests that a regulatory measure may be required to prevent this destructive disease from entering into disease free regions via pathogen-infected alfalfa seed or hay.

Key words : Verticillium wilt, *Verticillium albo-atrum*, *Medicago sativa*, *M. falcata*, alfalfa, lucerne, disease resistance

INTRODUCTION

Verticillium wilt caused by *Verticillium albo-atrum* Reinke & Berthold is an important disease of alfalfa (*Medicago sativa* L.). The disease can cause serious reductions in hay yield (24), seed yield (2) and the life of alfalfa stand (2, 15, 28, 30, 38). Although Verticillium wilt of alfalfa is wide-spread in Europe (26, 32), Canada (2, 18, 35) and U.S.A. (9, 11), there is no report on the occurrence of this important alfalfa disease in China and Taiwan.

Previous reports indicate that *V. albo-atrum* can be transmitted by infected alfalfa tissues such as seeds (7, 8, 22, 36), stems, pedicels, pods (25) and pollen (23); by insects such as pea aphid (*Acrythosiphon pisum* Harris) (21), grasshopper (*Melanoplus sanguinipes* Fabricius and *M. bivitatus* Say), alfalfa weevil (*Hypera postica* Gyllenhal) (19) or leafcutter bee [*Megachile rotundata* (Fab.)] (23); and by other means such as wind (25), water (10) or farm machinery (25). Verticillium-infected alfalfa seeds are considered an important vehicle for spread of the disease from country to country (16). Many countries have imposed strict quarantine measures to prevent spread of the disease through the seed trade. For example, Verticillium wilt of alfalfa is a disease under Class A quarantine in China (C. Li, personal communication).

The use of disease resistant cultivars is the most effective method for control of Verticillium wilt of alfalfa (24). In western Canada, the annual economic benefits of growing Verticillium wilt resistant cultivars of alfalfa was estimated at $26.6 million (Canadian dollars) (37). The objectives of this study were to assess some alfalfa cultivars from China for resistance to Verticillium wilt, and to discuss the potential risk of introducing this disease into disease-free regions or countries.

MATERIALS AND METHODS

Twenty six alfalfa cultivars including 25 *M. sativa* and

one *M. falcata* L. originating from various parts of China were compared against three Canadian cultivars, Barrier (12), AC Blue J (1) and Beaver (24); three U.S. cultivars, WL 316 (24), Sparta and Anchor (5); and one Swedish cultivar, Vertus (6, 24). Barrier (12, 24), AC Blue J (24), and Vertus alfalfa (6, 24) are resistant to Verticillium wilt, whereas Beaver (24), WL 316 (24), Sparta and Anchor alfalfa (5) are susceptible.

Alfalfa seeds were planted in Cornell Peat-Lite Mix™ (4) in Root-trainer books™ (Spencer-Lemaire Industries Ltd., Edmonton, Alberta, Canada) and kept for 10 wk in a growth room at 20/15 C temperature regime with 16-h photoperiod. A conidial suspension containing 7 x 10^6 to 1 x 10^7 spores ml^{-1} was prepared from an alfalfa strain of *V. albo-atrum* (LRC 112) grown on V8-juice agar in Roux flasks at room temperature (20±2°C) for 12 days. Prior to inoculation, plant shoots were trimmed to 3-4 cm height and plant roots were cut. Plants were inoculated by soaking the injured roots in the spore suspension for 10 min. These plants were then kept in the growth room for 4 wk and rated for disease severity and incidence using the method described by Huang and Hanna (20). Disease severity was scored on a scale of 1 to 5 (31): 1 = no symptoms; 2= one or two leaflets showing slight mottling, but no distinct symptoms; 3= distinct symptoms on leaves of one stem; 4= severe symptoms on leaves of multiple stems; and 5= dead. Plants rated 1 and 2 were classified as resistant (31). Two experiments were conducted during 1992-94. For the first experiment, 10 alfalfa cultivars including six Chinese, two Canadian, one U.S. and one Swedish cultivar were used. There were two replicates per cultivar (treatment) and 51 plants per replicate. For the second experiment, 26 alfalfa cultivars including 20 Chinese, one Canadian, four U.S. and one Swedish cultivar were used. There were nine replicates per cultivar (treatment) and 12 plants per replicate. Each experiment was repeated once. A disease severity index (DI) was calculated for each cultivar using the formula: DI = Σ[nW]/T where n= number of plants; W= wilt rating, 1 to 5; T= total number of plants.

Data on percentage of resistant plants and disease severity index of the tested cultivars in each experiment were analysed for statistical significance using analysis of variance (ANOVA) for a randomized block design. For each experiment, the two runs were analysed separately. Since similar results were obtained for both runs, data was presented in combined form using two runs as replicates. Separation of means for each experiment was determined by Duncan's multiple range test. All statistical analyses were conducted using SAS/STAT® software (34).

RESULTS AND DISCUSSION

For the first experiment, the level of resistance to *V. albo-atrum* differed significantly ($P<0.05$) among the alfalfa cultivars used (Table 1). Of the four cultivars tested as controls, the two Canadian cultivars, Barrier (Fig. 1) and AC Blue J, were resistant to Verticillium wilt with more than 59% of resistant plants in the tested populations and a disease severity rating of less than 2.20. The Swedish cultivar, Vertus, was also resistant to the disease but the percentage of resistant plants in this cultivar was significantly ($P<0.05$) lower than that of Barrier and AC Blue J. The North American cultivar, Anchor, was susceptible with only 7% of resistant plants in the population and a disease severity rating of 3.64 (Table 1). These results confirm previous reports that Barrier (20), AC Blue J (24) and Vertus (6, 11, 20, 24) are resistant and Anchor (20) is susceptible to Verticillium wilt.

In the first experiment, all of the six Chinese cultivars including five *M. sativa* (cv. Tsau Yuan No. 1, Tsau Yuan No. 2, Ho Ten, Tsau Don and Gong Nong No. 1) and one *M. falcata* were susceptible to Verticillium wilt, with less than 22% of resistant plants in the tested populations and a disease severity rating of more than 3.43 (Table 1). In the second experiment, the level of resistance to Verticillium wilt was significantly different ($P<0.05$) among the 26 alfalfa cultivars including 20 Chinese cultivars (Table 2). Of the six control cultivars tested, the Canadian cultivar, Barrier was the most

Table 1. Resistance of 10 alfalfa cultivars (six from China) to Verticillium wilt caused by *Verticillium albo-atrum* (1992)[1]

Cultivar	Source	Resistant Plants (%)[2]	Disease Severity Index[3]
Barrier	Canada	60.1 a [4]	2.20 a [4]
AC Blue J	Canada	59.1 a	2.14 a
Vertus	Sweden	35.4 b	2.57 a
Tsau-Yuan #1	China	21.7 bc	3.43 b
Medicago falcata	China	19.2 bc	3.58 b
Tsau Yuan #2	China	18.5 bc	3.45 b
Ho Ten	China	11.5 bc	3.91 b
Tsau Don	China	9.5 c	3.81 b
Gong Nong #1	China	7.3 c	4.05 b
Anchor	U.S.A.	7.0 c	3.64 b

1. Data for Resistant Plants (%) and Disease Severity were analysed using two runs as replicates.
2. Resistant plants are the percentage of plants with disease severity ratings of 1 or 2.
3. Disease rating: 1 = no symptoms, 5 = plant dead.
4. Means in each column followed by same letter are not significantly different at 0.05 level (Duncan's Multiple Range Test).

Fig. 1. Three alfalfa cultivars, Barrier (left), E Qi (middle) and Gong Nai Si (right) showing differences in resistance to Verticillium wilt. Note the Canadian cultivar Barrier is resistant whereas the Chinese cultivars, E Qi and Gong Nai Si are moderately resistant and susceptible, respectively.

resistant with more than 60% of resistant plants and a disease severity rating of 2.39. The Swedish cultivar, Vertus, was also resistant to the disease, but the number of resistant plants in this cultivar was significantly ($P < 0.05$) lower than that of Barrier. The other four North American cultivars, Sparta, WL316, Beaver and Anchor, were susceptible with less than 23.1% of resistant plants in the populations and a disease severity rating higher than 3.13 (Table 2). Results of the second experiment were consistent with those of the first experiment, and confirm reports that Barrier (20) and Vertus (6, 11, 20, 24) are resistant, WL316 is moderately resistant (20) to susceptible (24), and Beaver (24), Anchor (20), and Sparta (20) are susceptible to Verticillium wilt.

Of the 20 Chinese alfalfa (*M. sativa*) cultivars tested in the second experiment, only the cultivar E Qi was moderately resistant to Verticillium wilt. This cultivar contained 43.4% of resistant plants which was similar to Vertus (43.3%), but was lower than Barrier (60.2%) (Table 2). The disease severity index in E Qi was significantly higher than that of Vertus and Barrier. The other 19 Chinese alfalfa cultivars were all susceptible to Verticillium wilt, although the pecentage of resistant plants varied among cultivars, ranging from 16.3% in cv. Yu Xian to 0.5% in cv. Ya Zhou (Table 2). The disease severity index was high in these susceptible cultivars, ranging from 3.59 in cv. Ding Xi to 4.38 in cv. Min Feng. Compared to the resistant cultivars, many diseased plants in the susceptible cultivars were dead (Fig. 1). Due to the heterogeneous nature of alfalfa cultivars and multigenic nature of resistance to *V. albo-atrum* (29), all the cultivars tested contained some resistant plants, even in the susceptible cultivars from China. This suggests the possibility of improving the level of resistance to Verticillium wilt using resistant plants in each cultivar through further breeding and cultivar development by mass selection.

Verticillium wilt of alfalfa was first reported in Sweden in 1918 (17) and it was found in other European countries including Germany (33), Denmark, Holland (13), and Britain (27). Although Verticillium wilt of alfalfa is firmly established throughout most northern European countries (32), the disease was not found in North America (26) until the early 1960s in Canada (3) and until 1976 in U.S.A (11). There is no report of occurrence of Verticillium wilt of alfalfa in China or Taiwan. However, previous studies indicate that the temperature requirement for optimum growth of alfalfa strains of *V. albo-atrum* was 25℃ (10) to 26℃ (14). Heale (14) suggests that it is possible for this pathogen to spread into relatively warmer area where *Verticillium* dahliae Kleb. is the major cause of wilt diseases in crops. The climatic conditions may be suitable for the establishment of this disease in northern China and the cool, mountainous areas of Taiwan. The absence of Verticillium wilt of alfalfa in China and Taiwan may be due to the lack of inoculum of *V. albo-*

Table 2. Resistance of 26 alfalfa cultivars (20 from China) to Verticillium wilt caused by *Verticillium albo-atrum* (1994) [1]

Cultivar	Source	Resistant Plants (%) [2]	Disease Severity Index [3]
Barrier	Canada	60.2 a [4]	2.39 a [4]
E Qi	China	43.4 b	2.90 b
Vertus	Sweden	43.3 b	2.54 a
Sparta	U.S.A.	23.1 c	3.13 b
Yu Xian	China	16.3 cd	3.68 cdef
Ding Xi	China	15.3 cde	3.59 cde
Xi Feng	China	13.6 cdef	3.68 cdef
WL316	U.S.A.	13.1 cdef	3.47 c
Tu Mu #2	China	10.1 cedf	3.88 defghi
Gong Nong #2	China	8.9 defg	3.69 cdef
Tu Mu #1	China	8.1 defg	3.64 cde
Ao Han	China	7.6 defg	3.94 efghij
Beaver	Canada	6.7 defg	3.65 cde
Jia Mu Si	China	6.1 defg	3.79 cdefg
Chang Wu	China	5.9 defg	4.05 fghijk
Xin Mu #1	China	5.7 defg	3.88 defghi
Xian Yang	China	5.5 defg	3.77 cdefg
Anchor	U.S.A.	5.3 defg	3.54 cd
Bei Jiang	China	4.7 efg	4.13 ghijk
Sha Wan	China	4.4 efg	3.84 cdefgh
Yang Gao	China	4.1 efg	4.23 ijk
Min Feng	China	3.9 fg	4.38 k
Wu Zi Wan	China	3.9 fg	4.06 fghijk
Gong Nai Si	China	2.6 fg	4.17 hijk
Qing Yang	China	1.1 g	4.35 k
Ya Zhou	China	0.5 g	4.30 jk

1. Data for Resistant Plants (%) and Disease Severity were analysed using two runs as replicates.
2. Resistant plants are the percentage of plants with disease severity ratings of 1 or 2.
3. Disease rating: 1 = no symptoms, 5 = plant dead.
4. Means in each column followed by same letter are not significantly different at 0.05 level (Duncan's Multiple Range Test).

atrum. Heale et al. (16) indicated that quarantine measures could help to prevent outbreaks of Verticillium wilt in disease-free areas. The highly destructive nature of *V. albo-atrum* on Chinese alfalfa suggests that a quarantine regulation similar to that in China may also be required in Taiwan in order to reduce the risk of introducing *V. albo-atrum* contaminated or infected alfalfa seeds or hay into the disease-free areas.

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摘　要

黃鴻章[1,3]、Acharya, S. N. [1], Hou, T. J. [2], Erickson, R. S. [1], Dalton, R. E. [1], and Mueller, C. A. [1] 1999. 中國苜蓿品種對黃萎病之罹病性檢定. 植病會刊 8:67-72. ([1.] Agriculture & Agri-Food Canada, Research Centre, Lethbridge, Ablerta, Canada T1J4B1 ; [2.] Grassland Research Institute, Chinese Academy of Agricultural Sciences, Huhehot, Inner Mongolia, China ; [3.] 連絡作者：電子郵件 huangh@em.agr.ca)

用人工接種方法檢定來自中國大陸的26個苜蓿品種對黃萎病菌 (*Verticillium albo-atrum*) 的罹病性反應。結果顯示除了鄂旗 (E Qi) 品種具有中等抗病性外，其餘26個品種均呈現高度感病性，與北美洲及歐洲等對照品種相較，鄂旗品種之抗病性與Vertus (歐洲品種) 相近，但是比Barrier (加拿大品種) 為差。苜蓿黃萎病在中國與台灣尚無報導，鑑於本病害容易經由種子與有病植株莖幹傳播。在無病地區宜將本病納入檢疫對象，以杜絕此一新病害之侵入。

關鍵詞：苜蓿黃萎病，*Verticillium albo-atrum*, 抗病檢定、中國大陸