Longevity of *Verticillium albo-atrum* within alfalfa stems buried in soil or maintained without soil at various temperatures.

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A large number of infected alfalfa *(Medicago sativa)* stems were produced in the greenhouse by the root-dip method of inoculation with an isolate of *Verticillium albo-atrum.* Infected stem segments (2 cm long) were buried in sterilized and non-sterilized soil or held without soil in petri plates which were incubated at -5". 5". 15". 25°, 30". and 35°C temperatures for three years. At monthly intervals stem segments were removed and plated on V-8 juice agar medium for the recovery of the pathogen. In sterile or non-sterile soil the pathogen remained viable and pathogenic throughout the test period (3 yr) only at low (-5° or 5°C) temperatures but its longevity declined with increase of temperatures. At 15°C it survived 18 months in sterile and 8 months in non-sterile soil. At 25". 30". and 35°C the longevity was reduced to 8, 7, and 6 months, respectively, in either kind of soil. However, the pathogen in stem segments placed in plates without soil survived 3 yrs at all temperatures tested, indicating its potential for surviving in alfalfa stems exposed to a wide range of temperatures (-5° to 35°C).

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Un grand nombre de tiges de luzerne *(Medicago sativa)* ont été infecteesen serre par un isolat de *Verticillium albo-atrum* selon la methode d'inoculation par trempage des racines. Des segments de tige infectes (2 cm de longueur) ont été enterres dans du sol stérilisé et non-stérilisé, ou déposés hors sol dans des boites de Pétri et mis a incuber à des temperatures de -5°, 5". 25". 30" et 35°C durant trois ans. Tous les mois, on retirait des segments de tige que l'on mettait en culture sur un milieu d'agar et de V-8 pour récupérer le pathogbne. Tant en sol stérilisé qu'en sol non-stérilisé, ce n'est qu'aux basses temperatures (-5° ou 5°C) que le pathogene a conserve sa viabilite et sa pathogénicité durant la période de test (3 ans) mais sa pérennité a diminué avec l'élévation de la tempbrature. A 15°C. il a survecu 18 mois en sol stérilisé et 8 mois en sol non-stérilisé. A 25". 30" et 35°C. sa pérennité tombait, respectivement à 8, 7 et 6 mois, dans les deux types de sol. Toutefois, le pathogbne renfermé dans les segments de tige deposes dans les boîtes de Petri sans sol, a survbcu 3 ans a toutes les temperatures d'essai, ce qui démontre son aptitude a survivre dans les tiges de luzerne exposées à un vaste ecart de temperature (-5° à 35°C).

Introduction

A recent description of Verticillium wilt of alfalfa *(Medicago sativa* L.) caused by *Verticillium albo-atrum* Reinke & Berthold and its preventive strategies have been published (1, 2). The disease can be introduced into wilt-free areas through infected or contaminated alfalfa seeds or other plant parts (3, 4, 5, 8). In England, Heale and Issac (5) noted that *V. albo-atrum,* as resting mycelium in infected alfalfa plants, can remain viable for 5 months (mo.) at the soil surface, 7 mo. at 15 cm and 9 mo. at 30 cm below ground level. They explained that the viability of the resting mycelium decreased rapidly on the soil surface because of constantly changing conditions of moisture and temperature. Recently, Keinath and Millar (6), using two soil temperatures (6° and 21°C) and three soil matrix potentials (-0.01, -0.3 and -3.0 bars), indicated that among these factors only high soil moisture (-3.0 bars) had adversely affected the persistence of the pathogen in stems buried in soil during a 16-wk test. They (6) as well as Sewell (9) found that saprophytic growth of *V. albo-atrum* in soil was extremely limited. McKeen and Thorpe (7) noted that *V. albo-atrum* from potato (*Solanum tuberosum* L.) rarely overwintered in field soil. Experimental evidence for a long-term effect of a wide range of temperatures on the survival of the alfalfa wilt pathogen in soil is scanty.

The present work was conducted for three years under controlled conditions to determine the effect of temperature on the longevity of *V. albo-atrum* within alfalfa stems in the presence and absence of soil.

Materials and Methods

A culture of *V. albo-atrum* isolated from alfalfa grown in Saskatchewan in 1983 was used in this study. Sub-cultures were maintained on V-8 juice agar medium on which the pathogen sporulated profusely in 2-4 days at room temperature (22° ± 2°C). To obtain sufficient numbers of infected stems, about 200 Vernal alfalfa plants (5-wk-old with stems cut back) were inoculated by soaking their trimmed roots in a water suspension of conidia (6×10^6 / ml) for 15-20 minutes. Inoculated plants were then transplanted individually into 10 cm plastic pots containing a mixture of garden loam, peat, and sand (3:1:1 by volume) in the greenhouse (i.e. by the customery root-dip method of inoculation). After 5-6 wk of regrowth when the plants started showing wilt symptoms, stems were cut into 2 cm long pieces, surface-sterilized in 2% NaOCl solution (1% available chlorine) for 5 min. and plated on 2% water agar medium with 4 pieces per plate. *V. albo-atrum* grew out from more than 75% of the pieces (about 2200). The infected pieces (with the fungus visible on the surface) were either

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buried in sterilized and non-sterilized soil adjusted to 20 ± 2% moisture content (matric potential 0.5 bar) or kept without soil in 9 cm petri plates.

These plates (each containing 4 pieces) were sealed with air-tight tapes and placed in 6 incubators set at -5°, 5°, 15°, 25°, 30°. and 35°C. In each incubator there were 30 sets of 3 plates (sterile, non-sterile and no soil). At monthly intervals, one set from each temperature was examined for the viability of the pathogen. Stem pieces were removed from the incubated plates and plated on clarified V-8 juice agar (4 pieces per plate). In 2-4 days, if viable, the pathogen grew out from the pieces and produced typical verticillate conidiophores with numerous conidia. The result was recorded as the presence or absence of the fungus in each plate. The pathogenicity of representative surviving cultures was tested on 5-wk-old Vernal alfalfa seedlings every 6 months by the root-dip method of inoculation as described above.

Results and Discussion

During the first 6 months, *V.* albo-atrum was recovered from the stem pieces in all plates incubated at -5° to 35°C. The effect of soil temperature on the longevity of the pathogen became apparent in the following months (Table 1). At -5° and 5°C. it remained viable in sterilized or non-sterilized soil throughout the experimental period (3 yr). At 15°C, it survived 18 mo. in sterilized and 8 mo. in non-sterilized soil. At 25°, 30°, and 35°C its longevity was reduced to 8, 7, and 6 mo., respectively. Only at 15°C, a possible adverse effect of antagonistic microorganisms (in non-sterile soil) on the pathogen was indicated because it survived 10 mo. longer in sterilized soil.

Table 1. Survival (+ or –) of *V.* albo-atrum in infected alfalfa stems buried in sterile (st) and non-sterile (nst) soil incubated at -5° to 35°C from 6 to 36 months.

		Months of incubation						
Temperature (C)	Soil	6	7	8	9	18	24	36
-5°	st	+	+	+	+	+	+	+
	nst	+	+	+	+	+	+	+
5°	st	+	+	+	+	+	+	+
	nst	+	+	+	+	+	+	+
15°	st	+	+	+	+	+	–	–
	nst	+	+	+	–	–	–	–
25°	st	+	+	+	–	–	–	–
	nst	+	+	+	–	–	–	–
30°	st	+	+	–	–	–	–	–
	nst	+	+	–	–	–	–	–
35°	st	+	–	–	–	–	–	–
	nst	+	–	–	–	–	–	–

In stem pieces held in plates without soil, the pathogen remained viable for the entire period (3 yr) at all temperatures from -5° to 35°C, indicating its strong survival ability in alfalfa stems that are not buried in soil.

Pathogenicity tests showed that all representative samples of the surviving cultures remained as virulent as the initial isolate used to produce the infected stems, irrespective of incubation temperatures. It would appear that the range of temperature (-5° to 35°C) used in this work would hardly affect the survival or virulence of the pathogen. It should be mentioned that the moisture content of the soil remained nearly at the initial level (15-20% or 0.3 to 0.5 bar).

Conclusions

This study provided an experimental evidence of the effect of temperature on the longevity of *V.* albo-atrum in alfalfa stems buried in soil. Low temperatures (-5° and 5°C) were most favorable for a long-term survival (3 yr or more) of the pathogen, but higher temperatures (25° to 35°C) progressively reduced its longevity. However, the pathogen within host tissues without the association of soil was remarkably tolerant to higher temperatures. This preliminary in *vitro* study indicated that infected alfalfa sterns might serve as a continual source of infection in the field and that the pathogen would not be affected by -5° to 35°C air temperatures.

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