

Tissue specificity by fungi endophytic in *Ulex europaeus*

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Summary. – Twenty-seven species of endophytes were isolated from whole stem and xylem segments of *Ulex europaeus*. With one exception, the frequency of endophytic fungi was greater or equal in whole stems than in xylem alone. *Phomopsis ligulata* was the most common fungus: mean values of colonization were approximately 25% for the xylem and 29% for whole stems. *Coniothyrium olivaceum*, the second most frequently isolated endophyte, colonized both tissue types equally, whilst *Fusarium lateritium* and *Coniothyrium* sp. 1 were more often isolated from whole stems. On the basis of this and previous studies it is suggested that invasion of xylem by endophytic fungi may be more prominent in shrubs than in long-lived trees.

Introduction

A preliminary study of endophytic fungi in *Ulex* spp. was undertaken by FISHER & al. (1986). They recorded endophytes isolated from *Ulex europaeus* L. and *U. gallii* PLANCK and found a positive correlation between the age of the plant material and the number of endophytic fungi colonizing the tissues. However, no attempt was made on this occasion to locate fungi within the tissues. Previous work to determine locations of endophytic fungi within the host tissue was undertaken by means of microscopical techniques (BOSE, 1947; BERNSTEIN & CARROLL, 1977; STONE, 1986; SUSKE & ACKER, 1987) but cultural work on this subject is of recent origin (FISHER & PETRINI, 1987; SUSKE & ACKER, 1987; PETRINI & FISHER, 1988). The present study aims to demonstrate the ability by endophytic fungi to colonize not only the bark but also the xylem and to obtain additional information on the endophytic character of some coprophilous fungi.

Materials and Methods

During April 1986 endophytes were isolated from two to three year old twigs taken from ten mature bushes of *Ulex europaeus* L. growing on Aylesbeare Common near Ottery St. Mary, Devon, Great Britain (grid reference SY054898). From each bush, one hundred

Table 1. Endophytic fungi of *Ulex europeus*. Taxa and number of isolations from n = 500 pieces (10 bushes).

Taxon	Intact Stem Segments	Xylem Segments
<i>Alternaria</i> sp.	1	-
<i>Aureobasidium pullulans</i> (de BARY) ARN.	-	1
<i>Chaetomium cochlioides</i> PALLISER	-	1
<i>Cladosporium tenuissimum</i> COOKE	-	3
<i>Colletotrichum</i> sp.	-	44
<i>Coniochaeta</i> cf. <i>Rosellinia xylariispora</i> COOKE & ELLIS	5	-
<i>Coniochaeta</i> spp.	90	-
<i>Coniochaeta tetraspora</i> CAIN	5	-
<i>Coniothyrium olivaceum</i> SACC.	81	97
<i>Coniothyrium</i> sp. 1	11	3
<i>Coniothyrium</i> sp. 2	-	5
<i>Cystodendron dryophilum</i> (PASS.) BUBAK	-	1
<i>Fusarium lateritium</i> NEES	26	9
<i>Gelasinospora reticulata</i> (BOOTH & EBBEN) CAILLEUX	23	-
<i>Geniculosporium</i> sp.	-	1
<i>Hypoxyylon bipapillatum</i> BERK & CURT.	-	3
<i>Orcibula parentina</i> (SCHRAD.: FR.) HUGHES	2	-
<i>Phialophora hoffmanni</i> -group	2	-
<i>Phoma</i> spp.	-	5
<i>Phomopsis ligulata</i> GROVE	296	249
<i>Phomopsis</i> sp.	-	1
<i>Podospora tetraspora</i> (WINTER) CAIN	-	1
<i>Ramichloridium schulzeri</i> (SACC.) de HOOG	-	1
<i>Sordaria fimicola</i> (ROB.) CES. & de NOT.	16	-
<i>Sporormiella australis</i> (SPEG.) AHMED & CAIN	1	-
<i>Sporormiella minima</i> (AUERSW.) AHMED & CAIN	6	1
<i>Sporormiella minimoides</i> AHMED & CAIN	4	1
STERILE MYCELIA	36	21

pieces of about 10 mm length were taken from branches approximately 70 cm above ground level. The material was taken to the laboratory in polyethylene bags and processed within 24 hours. Before surface sterilization, half the segments were stripped of their bark, leaving the xylem only. Surface sterilization was performed by the method of FISHER & al. (1986). Segments were then placed in groups of five onto 2% Oxoid malt extract agar supplemented with 250 mg/l Terramycin (Pfizer). Plates were incubated at room temperature for up to 30 days. Isolation was by transfer of mycelia or

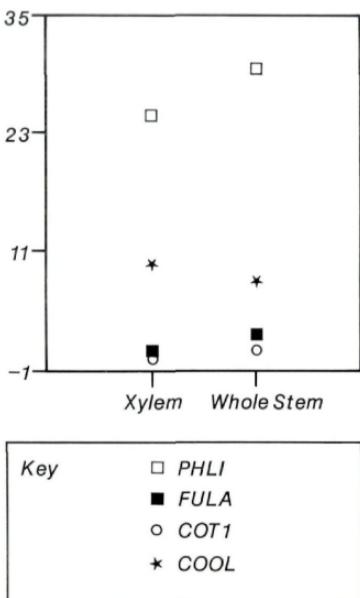


Fig. 1. Mean colonization values of xylem vs. intact stem segments for some selected fungi. The Y-axis represents the mean number of isolations for each bush for a given species. COOL: *Coniothyrium olivaceum*; COT1: *Coniothyrium* sp. 1; FULA: *Fusarium lateritium*; PHLI: *Phomopsis ligulata*.

hyphal tips to 2% malt extract agar plates. Dark UV-light was used to induce sporulation of the cultures.

Results and Discussion

The frequency of occurrence was on the whole greater or equal in whole stem segments than in xylem segments, with the notable exception of *Colletotrichum* sp., which colonized xylem exclusively (Table 1). Mean values of colonization for each bush by *Phomopsis ligulata*, a fungus described by GROVE (1935) from *Ulex* and reported by FISHER & al. (1986) as an endophyte of *Ulex europaeus* and *U. gallii*, were about 25% for the xylem and 29% for the whole stem. A t-test showed that both types of tissue are evenly colonized by this fungus with no significant difference between them. The same applies to *Coniothyrium olivaceum*, where approximately 9% of both tissue types were colonized (Fig. 1). In contrast, for *Fusarium lateritium* and *Coniothyrium* sp. 1, colonization of whole stems was significantly higher (Fig. 1, Table 1: $P<0.02$).

FISHER & al. (1986) reported *Sporormiella* spp. as frequent endophytes of *Ulex* spp. These and other coprophilous fungi were also reported by SIEBER (1985) in *Triticum aestivum* L. and by PETRINI & MÜLLER (1979) in *Juniperus communis* L. The occurrence of coprophilous fungi as endophytes of higher plants has been discussed by PETRINI (1986), who suggested that these fungi can adapt to an endophytic life style, but, as pointed out by PETRINI & FISHER (1988), their presence could partly be due to their thick-walled ascospores which on occasion may survive surface sterilization. However, the occurrence of *Sporormiella* spp. in the xylem (Table 1) indicates that they may be also true endophytes.

Recent work on endophytic fungi demonstrates that only a few endophytes are common to hosts of different families or grow in a range of different habitats (FISHER & PETRINI, 1987); these observations are confirmed by this study, because the most commonly isolated fungi have not previously been recorded from other hosts.

The investigation on *Suaeda fruticosa* (FISHER & PETRINI, 1987) and the present work suggest that invasion by endophytic fungi of the xylem in twigs of comparable diameter is more prominent in shrubs than in long-lived trees such as *Fagus sylvatica* L. and *Pinus sylvestris* L. (PETRINI & FISHER, 1988). More species of either types would have to be studied before definite conclusions can be reached.

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