

## A study of distribution of *Acremonium typhinum* in populations of red fescue in southwest England and *in vitro* growth comparisons to isolates from North American collections

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Distribution of *Acremonium typhinum* has been evaluated in several populations of red fescue in diverse habitats of southwest England. Endophytes were found most commonly in populations growing on rock surfaces, including cliff walls, stone walls, and hedge rows. They were infrequently encountered in meadows. Stromata were not observed to occur on plants in infected populations. Endophytes were isolated and *in vitro* growth rates were compared to those of several isolates from North American populations where stromata have been observed. The nonstroma-forming endophytes were found to have a reduced growth rate on fructose when compared to the stroma-forming isolates. It is proposed that low moisture conditions select for red fescue individuals containing nonstroma-forming endophytes.

Keywords: *Acremonium*, endophyte, red fescue, grasses.

Endophytic fungi bearing relationship to species of the ascomycete genus *Epichloë* (Fr.) Tul. (Clavicipitaceae) are commonly encountered in cool-season grasses (Bacon & al., 1977; Clay & Leuchtman, 1989; Latch & al., 1984; White, 1987). Many of these endophytes rarely or never produce the telomorph or any external propagules on hosts. However, when isolated they produce conidia and conidiogenous cells referable to the deuteromycete genus *Acremonium* Link sect. *Albolanosa* Morgan-Jones & W. Gams (Morgan-Jones & Gams, 1982). In some grasses *Acremonium* endophytes have been shown to give enhanced insect resistance and increased drought tolerance over endophyte-free plants (Funk & al., 1983; West & al., 1990). Because of possible beneficial effects, endophytes are being employed to increase hardiness of red fescue (*Festuca rubra* L.) turfgrass cultivars. However, en-

dophytes presently available in North American red fescue cultivars are stroma-formers. These endophytes proliferate in inflorescence primordia, forming a mycelium that traps the undeveloped inflorescence in the stroma, preventing seed formation on affected culms (Kirby, 1961; Sun & al., 1991; White, 1992). Once the stroma is formed, sexual reproduction may occur on its surface (White & Bultman, 1987). From the perspective of turfgrass seed production, stromal development reduces seed yield and limits the usefulness of endophytes in turfgrass breeding programs. In red fescue as in many other grasses, failure to form stromata results in transmission of endophytic mycelium to the next generation of the host through infection of embryos in seeds; an external mycelium on grasses is not observed (Sampson, 1933; White & Cole, 1986). Because of the importance of stromata in the life cycle of endophytes and economic implications of stromal formation to the turfgrass industry, studies were conducted on endophytes in some red fescue populations in several habitats in England to assess relative levels of endophyte infection. Red fescue populations consisted of several different subspecies depending on the particular habitat sampled. Studies on growth rates of endophytes were made to evaluate the possible relationship of growth rate to stromal development on grasses.

### Materials and methods

To evaluate endophyte infection levels in red fescue populations, 3 to 37 flower culm samples at anthesis were obtained from several habitats in southwest England. Habitats included hedge rows, stone walls, seaside sandstone cliff walls, sand dunes, and meadow areas, including pastures and non-grazed fields (Tab. 1). Populations of grasses were examined for evidence of stromata formation. Endophytes from representative plants were isolated and their *in vitro* growth rates compared to those of endophytes isolated from North American collections of chewings fescue (*Festuca rubra* L. subsp. *commutata* Gaud.) on which stromata have been found (Sun & al., 1991). Nonstroma-forming isolates collected in England are designated Belstone, Bow#1, Bow#2, Ilfracombe, and Mendips; and potential stroma-formers from collections in the United States designated Emsylva, Rose city, and RUGC (Tab. 2).

To compare growth rates of endophytes on sugars, media containing Murashige and Skoog's Salt Base, 1% agar, and 1.5% sugar (one of the following in each medium: arabinose, D-fructose, D-xylose, or glucose) were prepared and adjusted to pH 7 prior to autoclaving. Growth capacity on starch was determined using starch-milk agar. This was prepared with Murashige and Skoog's Salt Base, to which

Tab. 1. – Endophyte infection levels in red fescue populations in several different habitats.

| Habitat      | Site or town    | County   | Infection status |        |
|--------------|-----------------|----------|------------------|--------|
|              |                 |          | Infected/tot.    | % Inf. |
| Hedge rows   | Bow             | Devon    | 12/21            | 57     |
| "            | North Tawton    | "        | 5/10             | 50     |
| "            | North Wyke      | "        | 20/25            | 80     |
| Stone walls  | Belstone        | "        | 15/20            | 75     |
| "            | Dartmoor forest | "        | 10/10            | 100    |
| "            | Mendip hills    | Avon     | 15/15            | 100    |
| Cliff walls  | Ilfracombe      | Somerset | 7/19             | 37     |
| Sand dunes   | Braunton        | Devon    | 0/37             | 0      |
| Field margin | Mendip hills    | Avon     | 0/3              | 0      |
| Old pasture  | Tadham moor     | Somerset | 0/10             | 0      |
| Woods margin | Okehampton      | Devon    | 0/30             | 0      |
| Pasture      | Bow             | "        | 0/30             | 0      |
| "            | Fern hill moor  | "        | 1/30             | 3      |

was added 0.5% nonfat dry milk, 1% fine corn starch, and 1% agar (White, 1992). To inoculate plates, pieces of mycelium approximately 1 mm in diam, cut from the margins of rapidly growing colonies on 1% glucose agar, were used. Six replicates of each isolate/medium combination were made. All plates were sealed with Parafilm and incubated at 24C in darkness for 20 days.

To evaluate growth, colony diameters were measured and are reported as mean  $\pm$  standard deviation (Tab. 2). To assess starch hydrolysis capacity, plates were flooded with 0.1% iodine solution for approximately 1 hour, after which diameters of clearing zones under or around colonies were measured (Tab. 2). Analysis of data was accomplished using statistical options available in the Statgrafics Statistical Program Package, version 2.1, made by Statistical Graphics Corp.

## Results

Red fescue populations in several habitats contained high frequencies of endophyte-infected individuals. Thirty-seven to 100% of the individuals from populations in hedge rows, and on stone walls and cliff walls, were infected with *Acremonium typhinum* (Tab. 1). Stromata were not seen in any of the seven populations sampled even though thousands of plants were present at most sites. Culm samples from sand dunes, fields, and pastures were largely endophyte-free. A single infected plant was found in 30 samples at the fern hill moor

site. This endophyte was isolated and identified as *Acremonium typhinum* Morgan-Jones & W. Gams.

In comparisons of growth rates of nonstroma-forming isolates of *A. typhinum* to stroma-forming isolates of that species, the former were seen to have significantly reduced growth capacity on fructose (Tab. 2). In addition, colonies of the nonstroma-formers on fructose were sparse, with a thin, transparent layer of appressed hyphae, while those of the stroma-formers were dense, with abundant raised, white, cottony mycelium. Significant differences between these two groups were not observed when isolates were grown on other substrates. However, isolate RUGC showed an ability to grow rapidly on xylose, while all other isolates grew very little on this sugar (Tab. 2).

Tab. 2. – Linear growth (mm) of colony and starch hydrolysis of *Acremonium typhinum* isolated from red fescue.

| Isolate                  | Glucose               | Fructose                    | Arabinose       | Xylose         | Starch        |                 |
|--------------------------|-----------------------|-----------------------------|-----------------|----------------|---------------|-----------------|
|                          |                       |                             |                 |                | Growth        | Clear           |
| <b>NONSTROMA FORMERS</b> |                       |                             |                 |                |               |                 |
| Mendips                  | 9.7±1.5a <sup>1</sup> | 1.6±0.3a                    | 12.1±1.5a       | 1.2±0.3a       | 22±0.2c       | 15±0.6b         |
| Ilfracombe               | 16±0.4b               | 2.1±0.6b                    | 17.4±0.7b       | 3.1±1.3b       | 21±0.1b       | 19±0.6d         |
| Bow #1                   | 7.2±1a                | 1.7±0.3a                    | 10.5±3.4a       | 1.2±0.2a       | 26±1d         | 19.7±1d         |
| Bow #2                   | 14.9±0.4b             | 2.5±0.5b                    | 13.6±0.7a       | 1.8±0.3b       | 18±0.9a       | 17±0.5c         |
| Belstone                 | 14±1.1b               | 2±0.2ab                     | 13.9±0.5a       | 3.5±0.4b       | 21±0.4a       | 19±1cd          |
| <b>Average</b>           | <b>12.4±3.3</b>       | <b>2±0.3</b> * <sup>2</sup> | <b>13.5±2.3</b> | <b>2.2±1</b>   | <b>18±1.5</b> | <b>21.6±2.5</b> |
| <b>STROMA FORMERS</b>    |                       |                             |                 |                |               |                 |
| Rose                     | 14±0.5b               | 4.7±0.4a                    | 14.7±0.8a       | 1.6±0.3a       | 19±0.2a       | 17±0.6b         |
| Ensylva                  | 12.3±0.7a             | 8.2±1.4b                    | 14.6±1.2a       | 2.9±0.3b       | 20±1ab        | 17.5±0b         |
| RUGC                     | 16.5±0.6c             | 12.3±0.5c                   | 17.9±0.3b       | 8.3±1.1c       | 21±1b         | 11±1a           |
| <b>Average</b>           | <b>14.3±1.7</b>       | <b>8.4±3.1*</b>             | <b>15.7±1.5</b> | <b>4.3±2.9</b> | <b>20±0.8</b> | <b>15.2±3</b>   |

<sup>1</sup>Data are reported as mean ± standard deviation; means followed by the same letter are not different according to the Duncan's multiple range test ( $P < 0.5$ ).

<sup>2</sup>An asterisk indicates that the stroma-former average is significantly different from the nonstroma-former average for a particular medium according to the students t-test ( $P < 0.05$ ).

## Discussion

Differences in endophyte-infection levels may in part be explained by the occurrence of distinct subspecies of red fescue in specific habitats. On sand dunes, *F. rubra* subsp. *arenaria* (Osbeck) Syme. predominates (Hubbard, 1984). Of 37 culms of this subspecies examined, all were free of endophyte infection (Tab. 1). This subspecies was not observed, and to our knowledge has never been reported, to be infected by *Epichloë*, perhaps an indication that it is an unsuitable host. In hedge rows and stone walls, strong creeping red fescue (*F. rubra* L. subsp. *rubra*) is most common, forming large dense patches. This grass was found to harbor *A. typhinum* in 50 to 100% of the individuals at these sites. Turfgrass cultivars of this subspecies in use in the United States are commonly infected by a stroma-forming endophyte that has been identified as *Epichloë typhina* (Pers.: Fr.) Tul. (Sun & al., 1991). On sandstone cliff walls, *F. rubra* subsp. *pruinosa* (Hack) Piper is common. The infection level in a population of this subspecies was 37%, however, these culm samples had been degraded by other fungi to the extent that *Acremonium* mycelium could have been obscured in some cases and the actual level of infection may be higher. In meadows, subsp. *rubra* and other red fescue subspecies, such as subsp. *megastachys* Gaud. and *multiflora* (Hoffn.) Wallr., were abundant. These grasses were sampled at some sites, but were free of endophyte infection. The predominance of endophyte-infected individuals in populations on rock surfaces may have an ecological rather than a taxonomic explanation. These habitats are difficult to colonize due to limitations of moisture and nutrients, however, once colonized they are rarely disturbed. Populations may persist for centuries, forming large slowly spreading mats, underneath which soil layers are frequently very thin and moisture in particular may be periodically scarce. The long lived individuals in these populations are likely bombarded by infective propagules originating on plants bearing stromata of *Epichloë* that are common in the vicinity on species of *Agrostis* L., *Holcus* L., and *Dactylis* L. (White & Baldwin, 1992). It is expected that some of the endophytes successfully infect hosts and may produce stromata.

In a previous study it was shown that stromal development increases relative transpiration in grasses (White & al., 1993). An increased water loss in habitats where water is limiting may compromise survival of hosts. Thus it is expected that low moisture conditions provide selection against stromal development. It has been demonstrated in tall fescue (*Festuca arundinacea* Schreb.) that a nonstroma-forming endophyte, *Acremonium coenophialum* Morgan-Jones & W. Gams, gives increased drought tolerance to hosts through an increase in osmotic adjustment potential of meristems (West & al.,



1990). Perhaps in red fescue similar enhancements in drought tolerance are derived from endophytes once stromata are no longer formed. Under low moisture conditions red fescue plants associated with nonstroma-forming endophytes may have a selective advantage over endophyte-free individuals. In contrast, in meadows moisture is more abundant, and populations are much less stable, composed of shorter lived individuals. In these habitats, any given host individual has a reduced opportunity for infection since it may be exposed to fewer infective propagules over its shorter life span and once infected, strong selection against stroma-formation may not occur due to abundant soil moisture.

One feature responsible for failure of endophytes to develop stromata may be related to growth rate of the endophyte on host available sugars or other energy compounds. The reduced growth of the nonstroma-forming endophytes on fructose is significant since this monomer is abundant in grass meristematic tissues (Volenec & Nelson, 1984). Glucose and fructose, both component monomers of sucrose, are released into the apoplast through cleavage of the disaccharide by fungal and plant invertases (Volenec & Nelson, 1984). It seems likely that these sugars provide most of the energy for construction of stromata on culms. The observed reduced ability to gain energy for growth from fructose likely results in a reduced ability to grow rapidly enough on inflorescence primordia to trap that organ in a mycelium and complete stroma development (Kirby, 1961; White & al., 1991). Selection for low moisture tolerance apparently is correlated with reduced growth capacity on fructose, at least in the populations studied. It is probable that other features of endophytes account for increases in drought tolerance over endophyte-free plants.

Optimizing moisture relations with the environment may have been an important factor in the evolution of nonstroma-forming endophytes in cool-season grasses. Many species, such as tall fescue and perennial ryegrass (*Lolium perenne* L.) were perhaps initially infected by *Epichloë* in moist regions where stroma-formers are abundant. In drier habitats or seasons, stroma formation may have been selected against. Enhanced drought tolerance and pest resistance due to the nonstromal forms might have been secondarily selected for, increasing these endophytes in populations of grasses.

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