# Host Specialization of Different Populations of Ergot Fungus (*Claviceps purpurea*)

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**Abstract**: In our previous study of *Claviceps purpurea* three populations were found: G1 on open localities, G2 from shady or wet habitats and G3 on *Spartina* stands of coastal salt marshes. The latter two are also chemoraces. In the Czech Republic, isolates of G1 and G2 were found. The ability of four isolates representing these populations to infect and develop sclerotia on different host species (*Holcus lanatus, Helictotrichon pubescens, Phalaris arundinacea, Dactylis glomerata, Arrhenatherum elatius, Bromus inermis, Bromus erectus, Elytrigia repens, Avenella flexuosa, Lolium perenne, Poa nemoralis, Poa annua,* and different cultivars of *Poa pratensis*) was studied along with their alkaloid production. *P. pratensis* and *D. glomerata* were infected by all the isolates and sclerotia were formed by isolates 207 (G1) and 434 (G2), and on two *P. pratensis* cultivars even by 481 (G3). Infection ability (formation of sphacelial stage and honeydew) was less host-restricted than formation of mature sclerotia. G2 and G3 strains infected *A. flexuosa* without sclerotia formation. *L. perenne* was infected only once by strain 207 (G1) without sclerotia formation. *P. annua* (natural host of G2), was infected by all isolates, but no sclerotia were formed even with G2 strains. From the two G2 isolates, strain 434 from *Dactylis* formed sclerotia on five host species, whereas isolate 475 originating from *Phragmites* stand formed only sphacelia. Composition of alkaloid mixture produced in sclerotia of the same strain from various hosts confirmed that host plant does not influence the type of alkaloids produced, only their ratio.

**Keywords**: ergot; host specificity; alkaloids

It has been noted, that isolates of *C. purpurea* from certain hosts infect other grass species under artificial conditions with varying results. During the 20th century, there were many attempts at finding host-specific races of ergot. STÄGER (1905, 1908, 1923) found three races of European *C. purpurea*. In BARGER (1931), seven races (including those of Stäger) were summarized: P1 (rye, wheat, barley, *Antoxanthum odoratum*, *Hierochloe borealis*, *Arrhenatherum elatius*, *Dactylis glomerata*, *Hordeum murinum*, *Festuca pratensis*, *Phalaris arundinacea*, *Holcus mollis*, *Briza media*, *Calamagrostis arundinacea*, *Bromus sterilis*, *Poa* spp.), P2 (*Brachypodium sylvaticum, Poa annua, Millium effusum*), P3 (*Bromus erectus*, *Lolium* spp.), P4 (*Bromus erectus*, *Festuca arundinacea*, *Lolium perenne*) (MASTENBROEK & OORT 1941), P5 (*Lolium* spp., *Secale*) (BALDACCI & FORLANI 1948), P6 (*Aira, Molinia, Nardus, Phragmites*), P7 (*Poa annua* only) that was also considered *C. microcephala*. STÄGER (1922) found another race of *C. purpurea* with floating sclerotia referred as forma specialis *Phalaridis arundinaceae natans*.

Other authors, however, came to different conclusions. KYBAL and BREJCHA (1955) succeeded in infecting rye with ergot from *Phragmites* and *Molinia*, whereas CAMPBELL (1957) found no biologic barriers against cross infection at all in Canadian isolates from over 30 grass species.

Successful infection is highly dependent on the length of the period of anthesis and the inoculation technique used. Stäger's studies used three methods: spraying the heads with a conidial suspension, spraying the glumes apart and spraying the florets and dipping grass heads in conidial suspension. These methods succeeded unless the florets were closed or waxy or haired glumes protected them. CAMPBELL (1957) removed the glumes tips at anthesis and sprayed the heads with conidial sus-

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pension but when infecting *Hordeum*, it was necessary to inoculate the heads just emerging from the leaf sheath. Inoculation at anthesis was not successful.

LOVELESS (1971) found that the host-specific differences in conidial size and shape were retained even in laboratory cultures so they represent genetically fixed character. Groups of host grasses harbouring British *C. purpurea* isolates with similar size of conidia corresponded to some extent to the three races proposed by Stäger. The shortest conidia were found on hosts of P1 group; the longest conidia corresponded to f.sp. *Phalaridis arundinaceae natans*. MÜHLE (1971) observed longer conidia (7.8–9.33 µm) on *Agrostis*, *P. pratensis*, *Phleum pratense*, *F. rubra* and *D. glomerata.*

In our previous study of *Claviceps purpurea* intraspecific variation based on DNA typing, alkaloid analysis and conidial measurements, we found three different populations, G1, G2 and G3 (PAŽOUTOVÁ *et al*. 2000). These populations inhabited different habitats. Population G1 was found on fields, sunny meadows and along the roads. G2 isolates occurred in shady or wet habitats, river or pond banks and also in mountain woods (Šumava and Krkonoše Mountain in the Czech Republic). Although we collected over 100 isolates, only on three locations G1 and G2 were found together.

G3 isolates were specialized on Spartina stands (American *S. alterniflora*, British *S. maritima* and their hybrid *S. anglica*) in salt marshes with brackish water along Atlantic coast. This population was most probably introduced to Europe in 60's from North America (RAYBOULD *et al*. 1998; PAŽOUTOVÁ *et al*. 2002).

Analysis of alkaloid content revealed that G2 and G3 were also chemoraces. All G2 isolates produced mixture of ergosine and ergocristine, whereas in sclerotia of G3 ergocristine and ergocryptine was found. In the sclerotia of group G1, all other combinations of ergopeptines were found except the two encountered in G2 and G3.

G2 and G3 isolates were also characterized by elongated cylindrical conidia (6–8 µm and 10–12 µm, respectively), whereas conidia of G1 were usually oval, 5–6.5 µm long. Sclerotia of G2 and G3 floated on water as an environmental adaptation.

We suppose that groups P1, P3, P4 and P5 most probably corresponded to variable majority group G1, whereas P2, P6, P7 and Stäger's f.sp. *Phalaridis arundinaceae natans* belonged to specialized group G2.

Each of the groups was also characterized by DNA typing using RAPD and AFLP patterns and differences in the rDNA (ITS-5.8S-ITS2) sequence (PAŽOUTOVÁ *et al*. 2000, 2002). Among European isolates, two groups (confirmed as identical to G1 and G2) differing in their RAPD were observed also by JUNGEHÜLSING and TUDZYNSKI (1997).

In the present study, the ability of representative isolates of the three populations to infect and develop sclerotia on different host species was studied along with their alkaloid production.

## MATERIAL AND METHODS

Four *Claviceps purpurea* (Fr.) Tul. isolates representing populations G1, G2 and G3 (Table 1) were maintained on sucrose-asparagine agar slants T2 (PAŽOUTOVÁ *et al*. 1998). Conidia producing cultures for plant inoculation were grown on malt agar consisting of brewery malt 5 Beaum (400 ml), casein acid hydrolysate 1% (8 g), $MgSO_4 \times 7\ H_2O$ 0.1% (0.8 g), agar 3% (16 g) and cultured at room temperature. Host plants were potted and transferred from field to greenhouse ca. 30 days before inoculation. At the time of anthesis, the heads were sprayed by conidial suspension in water ($3.7–6.2 \times 10^6$ spores/ml) during three following days between 8–10 a.m. No floret manipulations were used. Honeydew formation was considered as sign of successful infection. After honeydew appeared, the infected heads were covered with open paper bags and plants transferred outside greenhouse. This step was necessary for developing mature sclerotia.

Sclerotia and/or sphacelia were collected from each plant and cultures were made from each host/strain combination. Sclerotia were washed in 2% sodium hypochlorite (twice diluted SAVO, Bochemie, CZ) for three minutes, then washed twice with distilled water and plated on sucrose–asparagine agar medium. Outgrowing mycelium was transferred on the slants after 10 days.

DNA was prepared from cultures grown on cellophane covered sucrose-asparagine plates (PAŽOUTOVÁ *et al*. 2000). Mycelium was powdered in liquid nitrogen and extracted by phenol–chloroform and extraction buffer as described in PAŽOUTOVÁ *et al*. (1998) with an additional purification step (PAŽOUTOVÁ *et al*. 2000).

Table 1. Origin of *C. purpurea* isolates used in the study

| Isolate | Host | Location | Origin | Population |
|---|---|---|---|---|
| 207 | *Triticum aestivum* | field | Kansas, USA | G1 |
| 434 | *Dactylis glomerata* | mountain wood | Phillipsreut, DE | G2 |
| 475 | *Phragmites* sp. | pond bank | Sobotka, CZ | G2 |
| 481 | *Spartina anglica* | saltmarsh | Lincegrove, UK | G3 |

RAPD analysis with primer 257 (CGTGATGTCAGTGATGC) (PAŽOUTOVÁ *et al*. 2000) confirmed that patterns of reisolated strains were identical with that of the inoculation culture.

Alkaloid content and composition were assayed by HPLC (PAŽOUTOVÁ *et al*. 2000).

Inoculation experiments and their evaluation were performed at Grassland Station Zubří, DNA typing and alkaloid analysis was done at Institute of Microbiology Czech Academy of Sciences, Prague.

## RESULTS

Natural hosts for isolates from our collections are summarized in Table 2. Although there were preferred

Table 2. List of natural host species for continental *C. purpurea* populations

| G1 | G1 + G2 intermediary | G2 |
|---|---|---|
| *Bromus* spp. | *Alopecurus pratensis* | *Agrostis* sp. |
| *Carex* sp. | *Ammophila arenaria* | *Calamagrostis* spp. |
| *Echinopogon* sp. | *Arrhenatherum elatius* | *Festuca rubra* |
| *Elymus caninus* | *Dactylis* spp. | *Holcus* sp. |
| *Elytrigia repens* | *Festuca ovina* | *Milium effusum* |
| *Festuca arundinacea* | *Glyceria fluitans* | *Molinia coerulea* |
| *F. gigantea* | *Phleum* spp. | *Phalaroides arundinacea* |
| *Lolium* spp. | *Poa pratensis* | *Phragmites communis* |
| *Helictotrichon pubescens* | | *Poa annua* |
| *Leymus arenarius* | | |
| *Phalaris tuberosa* | | |
| *Secale cereale* | | |
| *Sesleria tatrae* | | |
| *Spartina fusiformis* | | |
| *Triticum aestivum* | | |

Table 3. Infection and sclerotium forming ability of two *C. purpurea* isolates (1999)

| | Isolate | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 207 | | | | 475 | | | |
| Host | C | HD | St. No. | Stroma | C | HD | St. No. | Stroma |
| *H. lanatus* | 29 | * | 0 | – | 19 | * | 0 | – |
| *H. pubescens* | 17 | *** | 1.65 | SC | 26 | ** | 1.58 | SP |
| *P. pratensis* (cv. Bohemia) | 26 | *** | 3.35 | SC | 18 | ** | 0 | – |
| *P. pratensis* (cv. Midnight) | 14 | *** | 3.14 | SC | 15 | * | 0 | – |
| *P. arundinacea* | 27 | * | 0.15 | SP | 17 | * | 0 | – |
| *D. glomerata* | 24 | ** | 1 | SC | 26 | ** | 1.19 | SC |
| *A. elatius* | 21 | * | 0.05 | SP | 28 | * | 0 | – |
| *B. inermis* | 19 | *** | 1.00 | SC | 23 | 0 | 0 | – |
| *E. repens* | 12 | * | 0.33 | SC | 8 | 0 | 0 | – |
| *P. annua* | 17 | * | 0 | – | 32 | ** | 0 | – |
| *L. perenne* | 34 | * | 0 | – | 20 | 0 | 0 | – |

C – number of flowering culms; HD – intensity of honeydew formation; St. No. – number of stromata formed per culm; SP – sphacelial stroma; SC – sclerotial stroma; *, **, *** – weak, medium or intensive honeydew formation

Table 4. Infection and sclerotium forming ability of four *C. purpurea* isolates (2000)

| | Isolate | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 207 (G1) | | | | 475 (G2) | | | | 434(G2) | | | | 481 (G3) | | | |
| Host | C | HD | St. No. | Stroma | C | HD | St. No. | Stroma | C | HD | St. No. | Stroma | C | HD | St. No. | Stroma |
| *H. lanatus* cv. Hola | 20 | ** | 0 | – | 20 | ** | 2.2 | SP | 28 | ** | 4.28 | SC | 17 | * | 0.71 | SP |
| *P. pratensis* (PI 235489) | 34 | *** | 1.50 | SC | 44 | *** | 0 | – | 36 | *** | 2.83 | SP+SC | 22 | *** | 0 | – |
| *P. pratensis* cv. Slezanka | 37 | ** | 0.19 | SC | 38 | ** | 0 | – | 44 | ** | 5.93 | SC | 34 | * | 0 | – |
| *P. pratensis* (PI 286210) | 34 | *** | 3.70 | SC | 30 | *** | 0 | – | 22 | *** | 2.05 | SC | 24 | *** | 0.63 | SP |
| *P. pratensis* cv. Krasa | 31 | *** | 5.52 | SC | 34 | *** | 0 | – | 43 | *** | 0.44 | SC | 40 | * | 0.58 | SC |
| *H. pubescens* | 16 | *** | 3.38 | SP+SC | 16 | ** | 1.13 | SP | 19 | ** | 0.89 | SP | 20 | * | 0.40 | SP |
| *P. nemoralis* cv. Dekora | 36 | *** | 0.03 | SC | 30 | *** | 0.40 | SC | 56 | *** | 0.57 | SC | 43 | * | 0 | – |
| *D. glomerata* cv. Niva | 16 | ** | 2.19 | SC | 20 | ** | ND | YSP | 19 | ** | 13.58 | SC | 16 | ** | 0 | – |
| *P. arundinacea* | 13 | * | 0 | – | 11 | * | ND | YSP | 20 | *** | 11.45 | SC | 12 | * | 0.25 | SC |
| *P. annua* | 13 | * | 0 | – | 33 | *** | 0.06 | SP | 10 | *** | 0 | – | 20 | *** | 0.60 | SP |
| *A. elatius* cv. Baca | 13 | *** | 0.62 | SC | 19 | * | 0.84 | SP | 24 | * | 0 | – | 23 | * | 0 | – |
| *B. erectus* | 21 | *** | 1.57 | SC | 12 | 0 | 0 | – | 14 | 0 | 0 | – | 13 | 0 | 0 | – |
| *E. repens* | 14 | * | 0.07 | SC | 18 | 0 | 0 | – | 13 | 0 | 0 | – | 17 | 0 | 0 | – |
| *L. perenne* | 22 | 0 | 0 | – | 21 | 0 | 0 | – | 32 | 0 | 0 | – | 34 | 0 | 0 | – |
| *A. flexuosa* | 30 | 0 | 0 | – | 30 | *** | 0 | – | 33 | * | 0 | – | 29 | * | 0 | – |

C – number of flowering culms; HD – intensity of honeydew formation; St. No. – number of stromata formed per culm; SP – sphacelial stroma; SC – sclerotial stroma; YSP – young sphacelial stage hidden in floret; ND – not determined; *, **, *** – weak, medium or intensive honeydew formation

grass species found for each group, some hosts were shared by G1 and G2. Population G3 occurred only on *Spartina alterniflora* and interspecific hybrid *S. anglica* (*S. alterniflora* × *S. maritima*).

Isolate 207 (G1) originated from wheat field in Kansas with extremely continental climate. The two G2 isolates differ in their original location. 475 was collected on *Phragmites* stand growing in water and 434 originates from *Dactylis glomerata* from the woods on the Bavarian side of Šumava Mountains. Isolate 481 was included to test the degree of specialization of G3.

Two experiments were made – one in 1999 with strain 207 and 475, one in 2000 using strains 207, 434, 475, 481 and extended range of host species. Isolate 207 (G1) infected almost all host grasses except *Avenella flexuosa*. In the first experiment, infection of *Lolium perenne* manifested by honeydew formation was observed, but no visible sphacelia or mature sclerotia were found. In the next year, *L. perenne* was not infected at all. On *E. repens*, which belongs to natural G1 hosts, almost no sclerotia occurred in both experiments. On *P. arundinacea* and *P. annua*, natural hosts of G2, either sphacelia only or no stromata at all were found.

Isolate 475 failed to infect *Bromus* spp., *L. perenne* and *E. repens* (natural hosts of G1). However, the formation of mature sclerotia was erratic and occurred only on *D. glomerata* in the first experiment and on *Poa nemoralis* in the second experiment.

Isolate 434 infected all natural hosts of G2 group and also the intermediary ones. Mature sclerotia were formed on all these hosts with the exception of *Arrhenatherum elatius* and *P. annua*. The best yield of sclerotia was on *D. glomerata*.

Isolate 481 (the most specialized G3) infected the same hosts as 434, but completely mature sclerotia occurred only on *P. arundinacea* (only three pieces) and one cultivar of *P. pratensis*. However, the sphacelial stage observed on *H. pubescens* was developed enough to contain typical alkaloids.

Alkaloid analysis was done on samples with sufficient amount of sclerotia. Because sclerotia were small and some samples contained only 10–15 mg of material still firmly associated with glumes, percentage of alkaloids per sclerotium mass was not calculated. Table 5 shows that host plant indeed does not influence the type of alkaloids produced, only partially the ratio of respective components in the alkaloid mixture.

Table 5. Alkaloid content in sclerotia of *C. purpurea* from different host species

| Isolate | Host | Ergosine | Ergocornine | Ergocryptine | Ergocristine |
|---|---|---|---|---|---|
| **1999** | | | | | |
| 207 | *P. pratensis* (cv. Midnight) | 22.7 | 0.0 | 65.7 | 11.6 |
| 207 | *D. glomerata* | 17.7 | traces | 76.4 | 5.8 |
| 207 | *B. inermis* | 21.8 | 2.1 | 74.6 | 1.5 |
| 207 | *P. pratensis* (cv. Bohemia) | 23.3 | 0.0 | 65.9 | 10.8 |
| 207 | *E. repens* | 16.0 | 0.0 | 78.8 | 5.2 |
| 207 | *H. pubescens* | 18.8 | traces | 67.3 | 13.9 |
| 475 | *D. glomerata* | 22.4 | 0.0 | 0.0 | 77.6 |
| **2000** | | | | | |
| 207 | *B. erectus* | 23.5 | 2.9 | 73.2 | 0.4 |
| 207 | *D. glomerata* (cv. Niva) | 23.0 | traces | 61.7 | 15.3 |
| 207 | *P. pratensis* (cv. Krasa) | 17.9 | traces | 63.7 | 18.4 |
| 207 | *P. pratensis* (PI 286210) | 18.6 | 0.0 | 65.2 | 16.2 |
| 434 | *H. lanatus* | 49.9 | 0.0 | 1.3 | 48.8 |
| 434 | *D. glomerata* (cv. Niva) | 53.4 | 0.0 | 0.7 | 45.9 |
| 434 | *P. arundinacea* | 48.5 | 0.0 | 1.1 | 50.4 |
| 434 | *P. pratensis* (PI 286210) | 41.6 | 0.0 | 1.5 | 56.9 |
| 481 | *H. pubescens* | 0.0 | 0.0 | 22.5 | 77.5 |
| 481 | *P. pratensis* (cv. Krasa) | 0.0 | 0.0 | 28.4 | 71.6 |

Alkaloid content is given as percentage of total area of alkaloid peaks
Traces – peak visible but with area under 1%

## DISCUSSION

Host species typical for open dry locations like *E. repens* and *Bromus* spp. are not infected by the representatives of G2 and G3 populations from wet and/or shady habitats. Also, G1 isolate showed limited capacity for sclerotia formation of hosts natural for G2 group. These results suggest that certain host preferences developed in the populations adapted to different environments. However, there were differences in performance under experimental conditions of two G2 isolates as well.

Isolate 475, although equally capable of infection and honeydew formation as the isolate 434, mostly did not form sclerotia or even visible sphacelial stage on most of the host species (Table 4). Possible explanation might be the origin of isolate 475 from reed stand at the pond bank, which is an environment completely different from our experimental field.

As expected, isolate 481 (G3) originating from the most specialized environment of British salt marshes had also limited infection ability for land grasses. However, sclerotia formation was observed on three hosts natural for the group G2.

The most common host genus is *Poa*, which was infected by all the isolates and sclerotia were formed by 207 (G1) and 434 (G2), on two cultivars even by 481 (G3). On the other hand, isolate 475 repeatedly did not form stromata on *P. pratensis*, only on *P. nemoralis*.

*A. elatius* was weakly infected and sclerotia formation was rare. When we collected sclerotial specimens at the location in Březno near Mladá Boleslav, there were always only occasional occurrences on this grass. We observed 1–2 sclerotia per head and single sclerotia-carrying plants in healthy stands along the road, although at the same time, *L. perenne* and *D. glomerata* in the nearby locations were massively infected. Isolates from these three specimens belonged all to G1. It seems that *A. elatius* is naturally resistant to *C. purpurea* infection.

From the presented results we may conclude that resistance of different host grasses and their cultivars may vary with the *C. purpurea* isolate used for inoculation. Therefore the experiments testing the resistance of new cultivars should be made with *C. purpurea* strains of defined population or at least originating from similar locations for which the cultivar is intended.

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**Abstrakt**

PAŽOUTOVÁ S., CAGAŠ B., KOLÍNSKÁ R., HONZÁTKO A. (2002): **Hostitelská specializace různých populací námele** (*Claviceps purpurea*). Czech J. Genet. Plant Breed., **38**: 75–81.

Naše předchozí studie odhalila existenci tří populací uvnitř druhu *C. purpurea*: G1 z otevřených lokalit, G2 ze stinných nebo vlhkých lokalit a G3 na porostech trav rodu *Spartina* spp. ze slanisek atlantického pobřeží. Schopnost čtyř izolátů, zastupujících tyto populace, infikovat různé hostitele, vytvářet zralá sklerocia a produkovat alkaloidy byla sledována na druzích *Holcus lanatus*, *Helictotrichon pubescens, Phalaris arundinacea, Dactylis glomerata, Arrhenatherum elatius, Bromus inermis, Bromus erectus*, *Elytrigia repens*, *Avenella flexuosa*, *Lolium perenne, Poa nemoralis, Poa annua* a kultivarech *Poa pratensis*. *P. pratensis* a *D. glomerata* byly infikovány všemi izoláty a sklerocia vytvořily izoláty 207 (G1) a 434 (G2), na dvou kultivarech *P. pratensis* i izolátem 481 (G3). Infekční schopnost (tvorba medovice a sfaceliálního stadia) byla méně závislá na hostitelském druhu než tvorba sklerocií. Kmeny G2 a G3 infikovaly *A. flexuosa*, avšak netvořily sklerocia. *L. perenne* bylo infikováno pouze v jednom pokusu kmenem 207, a to bez tvorby sklerocií. *P. annua* (přirozený hostitel G2) byla infikována všemi izoláty, ale ani izoláty G2 nevytvořily sklerocia. Ze dvou izolátů G2 použitých ve studii kmen 434 původem z *Dactylis* tvořil sklerocia na pěti hostitelských druzích, zatímco kmen 475 pocházející z *Phragmites* dosáhl pouze sfaceliálního stadia. Složení směsi alkaloidů produkovaných týmž izolátem na různých hostitelských travách potvrdilo, že rostlina neovlivňuje složení směsi, pouze poměr jednotlivých složek.

**Klíčová slova**: námel; hostitelská specifita; alkaloidy

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