Folia Cryptog. Estonica, Fasc. 49: 73–76 (2012)

First records of two ascomycetes on *Phleum pratense* in Estonia

Triin Varvas & Bellis Kullman

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences,
Kreutzwaldi 5, 51014 Tartu, Estonia.
E-mails: triin.varvas@emu.ee, bellis.kullman@emu.ee

Abstract: Within a study on endophytic fungi of Timothygrass (*Phleum pratense*), two ascomycetes were recorded for the first time in Estonia. Based on morphological and molecular methods, the species were determined as *Alternaria arbusti* and *A. viburni* (teleomorph *Lewia viburni*), new to *P. pratense*.

Kokkuvõte: Kahe uue põldtimuti kottseene esmaleiud Eestis

Uurides põldtimuti (*Phleum pratense*) endofüütseid seeni leiti Eestile kaks uut kottseene liiki. Kasutades puhaskultuuri ja molekulaarseid tunnuseid määrati liigid *Alternaria arbusti* ja *A. viburni* (teleomorf *Lewia viburni*), mis on uued mikroseened põldtimutile.

INTRODUCTION

The important and widespread fungal genus *Alternaria* was originally described by Nees in 1816 (Simmons, 2002); it comprises ca 300 species of fungi commonly isolated from plants, soil, food, and air (Kirk et al., 2008). Prior to our study, only 14 species of this genus had been recorded in Estonia, according to eElurikkus database (http://elurikkus.ut.ee/). The genus includes many species that produce mycotoxins harmful to their plant hosts as well as to humans and animals (Simmons, 2007; Agrios, 2005; Christensen, 2005). The total losses caused by the species of this genus are estimated to be among the highest brought about by any plant pathogen (Agrios, 2005). The species of this anamorphic genus cause serious problems in agriculture by reducing crop yield and causing spoilage in storage (Andersen et al., 2001), but many species also produce secondary metabolites that are used as biocontrol agents (Yandco-Ables et al., 2006; Mohan Babu et al., 2002).

The genus *Alternaria* comprises many species-groups differing in several morphological characters. *A. arbusti* and *A.viburni* belong to the *A. infectoria* species-group, which is characterized by the production of long secondary conidiophores between conidia (Simmons, 2002). *A. arbusti* is known as the causal agent of leaf lesions on Asian pear (*Pyrus pyrifolia*) (Simmons & Roberts, 1993). The degree of the pathogenicity of *A. viburnum* is unclear. The above species have previously been identified only during plant pathogen studies. We found *A. arbusti* and *A.viburni* during a study on endophytic fungi of Timothygrass. It has been reported that microenvironmental conditions can change the lifestyle of fungi from mutualistic to parasitic (Kniskern & Rausher 2006, Johnson & Oelmüller 2009). Considering the above circumstances, the genus *Alternaria* deserves in-depth research of both its ecology and toxicology. The purpose of this paper was to give a brief overview of the two fungal species, *A. arbusti* and *A.viburni,* new to Estonia.

MATERIALS AND METHODS

Samples of Timothygrass (*Phleum pratense* L.) were collected for endophyte studies in autumn 2008 from the FAHM (Free Air Humidity Manipulation) experimental plots, located in Rõka, Tartu County, Estonia (58°14'44"N, 27°17'58"E).

Plants were processed for endophytes by surface sterilization of the tissues and planting the material on agar media. From plants, 5 × 5 mm fragments were cut and the surfaces were disinfected by rinsing in 96% ethanol for 1 min, followed by repeated rinsing in sterile water. Fragments were cut into smaller sections and placed in Petri dishes (9 cm diam.) on potato dextrose agar (PDA, Merck KGaA Germany). The subsequent pure fungal cultures were studied

macroscopically and microscopically using a Zeiss Axioskop 40 FL microscope, AxioCam MRc camera and the Axio Vison 1.6 program. Species were determined using taxonomic monographs and manuals by Domsch et al. (1980), Simmons (2007) and Ellis & Ellis (1997).

For molecular identification, 7–10 days after inoculation of subcultures, a small piece of mycelium with agar was cut and preserved in sodium dodecyl sulphate (SDS). Genomic DNA was extracted according to Gardes & Bruns (1993). ITS regions of rDNA were amplified by PCR using primers ITS1F (5´ to 3´: CTT GGT CAT TTA GAG GAA GTA A) and ITS4 (5´ to 3´: TCC TCC GCT TAT TGA TAT GC). PCR product was purified with Exo-Sap (Fermentas, Lithuania) following the manufacturer's instructions. The samples were sequenced in Macrogen (Korea) with primers ITS1 (5` to 3`: TCC GTA GGT GAA CCT GCG G) and ITS5 (5` to 3`: GGA AGT AAA AGT CGT AAC AAG G). The sequences were edited using the program Sequence Scanner 1.0 (Applied Biosystems, Foster City, California, USA) and compared with the sequences in the GenBank using the BLASTn algorithm. Sequences with <97% ITS rDNA sequence similarity were considered homospecific. The voucher strain of *A. arbusti* culture was deposited in the collection of fungal living cultures [TFC 2010-011] at the Estonian University of Life Sciences and the sequences of both taxa were deposited in the GenBank (accession no JN688919 *A. arbusti*, JN688921 *A. viburni*).

TAXONOMIC DESCRIPTIONS

Alternaria arbusti E. G. Simmons, Mycotaxon 48: 103 (1993).
Teleomorph – Unknown.
Culture description – Colonies loosely woolly with sparse greyish aerial hyphae. Colony colour dark-grey to black or light-grey to dark brown with a lighter border. Reverse side has either concentric rings from light yellow to dark brown or dark hypha radiating out from the centre (Fig. 1). Only juvenile club-shaped conidia observed, forming chains or singly, divided by transverse and longitudinal septations, 15–32 × 4–12 µm. Conidia colour light to medium brown.
Substrata – *P. pratense* leaf (studied Estonian material); originally isolated from leaves of *Pyrus pyrifolia* (Simmons, 1993).
Holotype – BPI 802727.
Comments – The species was identified by molecular methods. It has earlier been recorded on cherry fruit (*Prunus sp.)* as a nontoxigenic species (Simmons, 1993). A study on the secondary metabolites of the *Alternaria* species established that *A. arbusti* does not produce a potential phytotoxic compound present in some other members of the *A. infectoria* species-group. At the same time, it produces another biologically active metabolite — infectopyrone (Christensen et al., 2005).

Alternaria viburni E. G. Simmons, Mycotaxon 83: 132 (2002).
Teleomorph – *Lewia viburni* E.G. Simmons & McKemy, in Simmons, Mycotaxon 83: 130 (2002) (see also a description of teleomorph Simmons, 2007).
Culture description – Colony cottony with sparse whitish aerial hypha. Colony colour dark-grey-brownish to light-grey. Reverse side light grey and smooth with some concentric rings. No conidia observed. Culture was contaminated upon re-culturing, pure culture or culture photo not preserved. Substrata: *P. pratense* leaf (studied Estonian material); originally isolated from leaves of *Viburnum* sp. (Simmons, 2002).
Holotype – BPI 841915.
Comments – The species was identified by molecular methods. This little known species could have a great potential in the research of secondary metabolites. A chemotaxonomic marker study conducted on the *A. infectoria* species-group by Christensen et al. (2005) showed that *A. viburni* produced novae-zelandin A and novae-zelandin B – secondary metabolites with a chemical structure similar to that of some phytotoxic compounds from other fungi.

A. arbusti and *L. viburni* are new endophytic fungi on *Phleum pratense*. The species of the genus *Alternaria* and even its strains can cause diseases in different plants by producing distinct host-specific toxins. In this case pathogenicity only occurs in the presence of a specific toxin (Markham & Hille, 2001). Considering this, the toxin production of the strains isolated from *Phleum pratense* should be determined for pathogenicity.

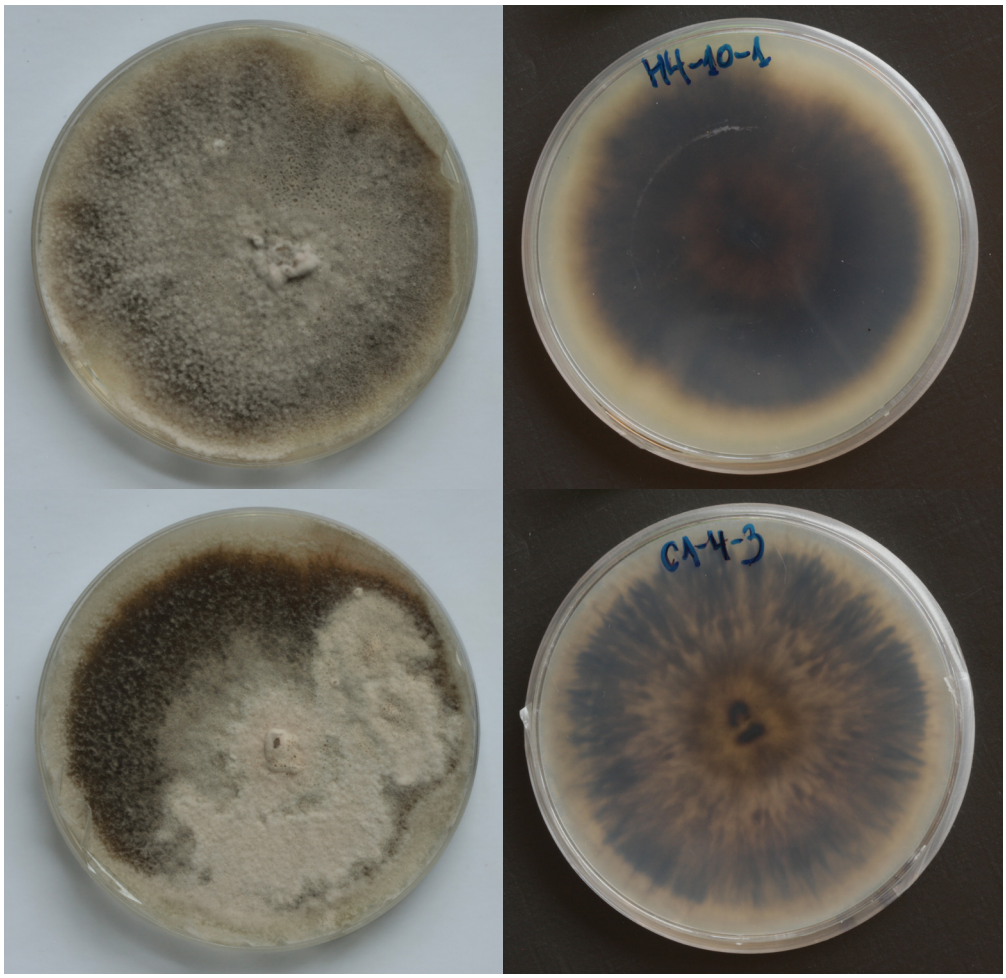

Fig. 1. Two *Alternaria arbusti* isolates (surface and reverse) on PDA at 21 days.

ACKNOWLEDGEMENTS

We wish to express our thanks to K. Kasekamp for performing molecular analyses and for cooperation. Financial support was received from the Ministry of Education and Research of Estonia (target financing SF0170057s09).

REFERENCES

Ables, C. Y., Rosskopf, E. N. & Charudattan, R. 2006. Putting plant pathogens to work: progress and possibilities in weed biocontrol part 2. Improving weed control efficacy. APS Net Plant Pathology Online. http://www.apsnet.org/online/feature/weed2. Cited 07.05.2012

Agrios, G. N. 2005. Plant Pathology (5th edition). Elsevier-Academic Press, San Diego, CA. 922 pp.

Andersen, B., Kroger, E. & Roberts, R. G. 2001. Chemical and morphological segregation of *Alternaria alternata*, *A. gaisen* and *A. longipes*. *Mycological Research* 105: 291–299.

Christensen, K. B., Van Klink, J. W., Weavers, R. T., Larsen, T. O., Andersen, B. & Phipps, R. K. 2005. Novel chemotaxonomic markers of the *Alternaria infectoria* species-group. *Journal of Agricultural and Food Chemistry* 53(24): 9431–9435.

Domsch, K. H., Gams, W. & Anderson, T. H. 1980. *Compendium of soil fungi*. IHW-Verlag, Eching, Germany. 859 pp.

eElurikkus – http://elurikkus.ut.ee/ Cited 13.05.2012
Ellis, M. B. & Ellis, J. P. 1997. *Microfungi on Land Plants. An Identification Handbook.* Richmond Publishing, Slough. 868 pp.
Gardes, M. & Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
Johnson, J. M. & Oelmüller, R. 2009. Mutualism or parasitism: life in an unstable continuum. What can we learn from the mutualistic interaction between Piriformospora indica and Arabidopsis thaliana? *Endocytobiosis Cell Research* 19: 81–111.
Kirk, P. M., Cannon, P. F., Minter, D. W. & Stalpers, J. A. 2008. *Ainsworth and Bisby's Dictionary of the Fungi.* 10th ed, CABI Bioscience, UK. 784 pp.
Kniskern, J. M. & Rausher, M. D. 2006. Environmental variation mediates the deleterious effects of *Coleosporium ipomoeae* on *Ipomoea purpurea*. *Ecology* 87: 675–685.
Markham, J. E. & Hille, J. 2001. Host-selective toxins as agents of cell death in plant-fungus interactions. *Molecular Plant Pathology* 2(4): 229–239.
Mohan Babu, R., Sajeena, A., Seetharaman, K., Vidhyasekaran, P., Rangasamy, P., Som Prakash, M., Senthil Raja, A. & Biji, K. R. 2002. Host range of *Alternaria alternate* – a potential fungal biocontrol agents for waterhyacinth in India. *Crop Protection* 21(10): 1083-1085.
Simmons, E. G. 1993. *Alternaria* themes and variations (63–72). *Mycotaxon* 48: 91–107.
Simmons, E. G. 2002. *Alternaria* themes and variations (305–309). *Mycotaxon* 83: 127–145.
Simmons, E. G. 2007. *Alternaria*: an identification manual. APS Press. 775 pp.
Simmons, E. G. & Roberts, R. G. 1993. *Alternaria* themes and variations (73). *Mycotaxon* 48: 109–140.