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Aquatic Invasions Records

The North American toxic fungal pathogen G3 *Claviceps purpurea* (Fries) Tulasne is established in the German Wadden Sea

Stefan Nehring[1]*, Christian Boestfleisch[2], Anne Buhmann[2] and Jutta Papenbrock[2]

1 *Federal Agency for Nature Conservation, Konstantinstraße 110, 53179 Bonn, Germany*
2 *Leibniz University Hannover, Institute of Botany, Herrenhäuser Straße 2, 30419 Hannover, Germany*

E-mail: Stefan.Nehring@bfn.de (SN), C.Boestfleisch@botanik.uni-hannover.de (CB), Anne.Buhmann@botanik.uni-hannover.de (AB), Jutta.Papenbrock@botanik.uni-hannover.de (JP)

**Corresponding author*

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Abstract

Three lineages (G1, G2 and G3) within the toxic invasive ergot fungus *Claviceps purpurea* (Fries) Tulasne are known; these should be recognized as unique species, or at least as varieties. On 2 November 2011, a very dense, well established population of G3 *C. purpurea* was found on the common cord-grass *Spartina anglica* C.E. Hubbard at two localities on the German North Sea coast in the Wadden Sea (Cäciliengroden and Hooksiel). It is most likely that G3 *C. purpurea* has a North American origin and entered German coastal waters by floating sclerotia from Irish, British, or Benelux waters, where it was previously found. However, introduction via ships' ballast water coming from their native or introduced ranges is also plausible. Furthermore imports of G3 sclerotia via seed mussels collected from wild subtidal banks in Irish, British and Dutch coastal waters and released into the German Wadden Sea can currently not be excluded. Risks from this highly toxic fungus for human, grazing animals and the marine environment have been identified but not yet quantified in terms of impact. A suitable monitoring programme should be implemented to detect any unwanted impacts caused by G3 *C. purpurea* in German salt marshes at an early phase.

Key words: ergot, invasive, *Spartina anglica*, ballast water, Germany, North Sea, salt marsh

Introduction

Claviceps purpurea is an ascomycetous toxic fungus that infects the ovaries of susceptible pooid hosts. Each infected ovary is replaced by a single sclerotium. The sclerotium and the disease are commonly referred to as ergot and ergotism, respectively.

Distribution of *C. purpurea* is basically Holarctic, but it has been recorded in Arctic regions and also occurs in southern temperate and subtropical regions (Pažoutová 2002).

Three lineages (G1, G2 and G3) within this invasive species have been recognized, based on habitat association, sclerotia and conidia morphology, as well as mycotoxin (alkaloid) production (Pažoutová et al. 2000). These lineages have been further supported by Random Amplification of Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) markers as well as by the use of three DNA loci (Pažoutová et al. 2000, 2002b; Douhan et al. 2008), suggesting that this species may be more accurately described as a species complex, or at least as a variety complex (Douhan et al. 2008; Andrei 2010).

G1 isolates originate from fields, from open meadows, and from grasses growing along roads. Typical hosts of G1 are *Secale cereale, Lolium*, *Elytrigia repens, Festuca pratensis, Helictotrichon pubescens* and *Bromus* (Pažoutová 2002; Alderman et al. 2004). G1 is characterized by production of ergotamines and ergotoxines (Pažoutová et al. 2000).

Isolates of G2 are more commonly recovered from *Calamagrostis, Holcus, Molinia, Phalaroides* and *Phragmites* growing at pondsides and river banks, ditches, and forests including mountain forests (Pažoutová 2002; Alderman et al. 2004). Sclerotia of G2 contain ergocristine and ergosine (Pažoutová et al. 2000).

The third group, G3 is found in coastal salt marshes on *Spartina* and *Distichlis spicata* (Eleuterius and Meyers 1974; Pažoutová et al. 2002b; Alderman et al. 2004; Andrei 2010; for

the life cycle of *Claviceps* on *Spartina* see Gray et al. 1990). Historically G3 was defined based on its main host range also as *C. purpurea* var. *spartinae* (Duncan et al. 2002). G3 is also a chemotype producing high amounts of the mycotoxins ergocristine and ergocryptine (Pažoutová et al. 2002b).

In Germany, G1 and G2 *C. purpurea* are a long known pathogen in farmed and wild plants (e.g. Kirchoff 1929; Pažoutová et al. 2000), however, the occurrence there of G3 *C. purpurea* was unknown until now.

Origin of G3 *Claviceps purpurea*

Pažoutová (2002) and other scientists located the origin of the genus *Claviceps* in the South American part of former Gondwana, and most species in the genus are tropical or subtropical. It has been postulated that species close to *C. purpurea* migrated from South America to North America after the formation of the Panama land bridge, and that they later spread to Europe (Pažoutová 2002; S. Pažoutová, pers. comm.). Although reported on the North American smooth cord-grass *Spartina alterniflora* in the USA as early as 1895 (Gray et al. 1990), G3 *C. purpurea* did not occur in Europe until 1960 (Boyle 1976). It was suggested that in the mid-20th century G3 sclerotia have been introduced into European waters from the USA via ships' ballast water (Pažoutová et al. 2002b). This hypothesis was confirmed by RAPD profiles of American and British G3 isolates from *Spartina* spp. which were uniform (Pažoutová et al. 2002b).

Germination of introduced G3 sclerotia is probably initiated by warm temperatures and occurs simultaneously with flowering in *Spartina*. Infection of *Spartina* is initiated by ascospores, with subsequent local infections resulting from the spread of conidia by insects, by wind and rainsplash, and by physical contact of inflorescences in dense swards (for more details see Gray et al. 1990).

Invasion history in Europe

Spartina has been checked annually for seed reproduction since 1958 in the vicinity of Dublin, Ireland (Boyle 1976). In the winter of 1960-61, G3 *C. purpurea* was detected in Townsend's cord-grass *Spartina* x *townsendii* at Dublin sites (Boyle 1976). It was very rare and in most cases fungal growth was arrested at early ergot formation (Boyle 1976). In the following years no records of ergot were reported from Irish coastal waters, however, in 1975 and 1976 heavy infection was apparent on *S.* x *townsendii* and common cord-grass *S. anglica* at locations in Counties Dublin, Louth, Wexford and Cork (Boyle 1976).

In 1962 and 1963 a light infection with the invasive fungus was recorded in *Spartina* marshes in Poole Harbour on the British Channel coast (Gray et al. 1990). At the same site a heavy infection occurred in November, 1971 (Gray et al. 1990). By the 1980s, massive infections emerged in several areas including Poole Harbour, the Dee and the Ribble estuaries (Gray et al. 1990). By the end of the 1990s, G3 *C. purpurea* had established populations on many but not on all *Spartina* marshes in South, East, and West Britain (Pažoutová et al. 2002b).

Although *Spartina* occurs at high stocking densities in most European countries bordering the Atlantic or the North Sea (Gray et al. 1990; Nehring and Adsersen 2009), only two published records of European G3 *C. purpurea* on *Spartina* outside Irish and British waters could be identified after extensive search in literature databases (e.g. BIOSIS, CAB, Google Scholar, SciSearch): Gray et al. (1990) reported up to 100% inflorescence infection on some Dutch *Spartina* marshes cut off by tidal barriers from sea water flooding, though no dates or specific localities were given. In autumn 2008, during examination of seed production of salt marsh species on the Belgian North Sea coast, most spikes of *Spartina* in the Yzer estuary were contaminated and/or empty (Erfanzadeh et al. 2010).

G3 *C. purpurea* is established in Germany

The ergot fungus G3 *C. purpurea* has been detected during sampling of *S. anglica* specimen for laboratory experiments at two sites in the Wadden Sea of Lower Saxony near Wilhelmshaven on 2 November 2011 (Figures 1 and 2, Appendix 1). Its sclerotia were straight or slightly curved, had ridges parallel to the long axis and were purple to black in color. They were 8 to 53 mm long and up to 5 mm wide (Figure 2).

In the two sites visited, almost all spikes of *S. anglica* were contaminated with the fungus, so the population observed seemed well established.

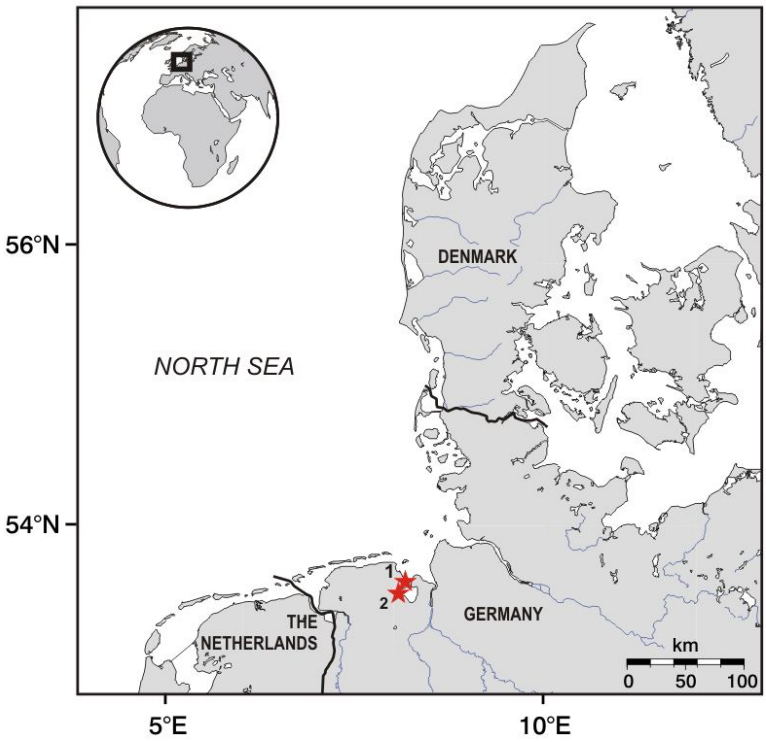

Figure 1. Record locations of ergot G3 *Claviceps purpurea* on *Spartina anglica* at Wilhelmshaven, Wadden Sea of Lower Saxony, Germany (see Appendix 1 for localities).

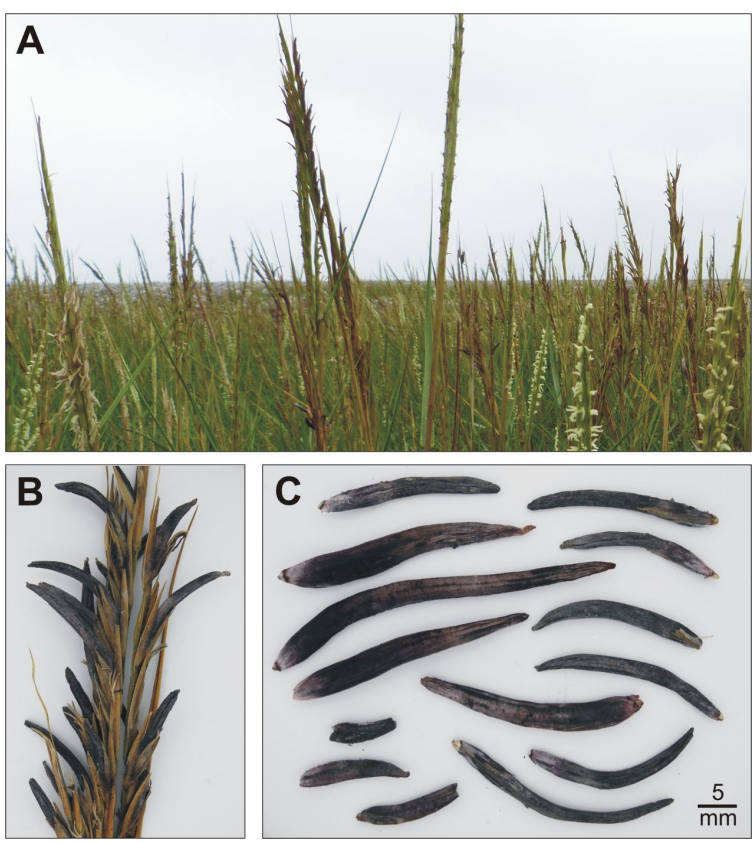

Figure 2. The ergot G3 *Claviceps purpurea* on *Spartina anglica* in a salt marsh on the German Wadden Sea coast of Lower Saxony, Cäciliengroden, 2 November 2011. **A**) Monospecific belt with massive ergot infection. **B**) Contamination of spikes with sclerotia. **C**) Isolated sclerotia. (Photographs: **A** by Anne Buhmann, **B** and **C** by Stefan Nehring).

Only flowering plants and plants with empty seed cover were free of the fungus.

During a pilot visit at the same sites on 4 October 2011 no sclerotia were detected but some *Spartina* plants were covered by orange honeydew which could have been a first sign of infection during the vegetation period (see Gray et al. 1990). However, the natural color of *C. purpurea* honeydew is whitish to beige, when a dry, orange color signalizes contamination of ergot honeydew by micro-hyperparasite *Fusarium heterosporum* Nees ex Fries, another toxic alien fungus which maybe has a slight negative effect on ergot size (Preece et al. 1994; Raybould et al. 1998; S. Pažoutová, pers. comm.). Unfortunately no samples were taken for fungal analyses at that time.

S. anglica, a fertile hybrid of *S. maritima* and *S. alterniflora*, was imported from Britain and planted in the German Wadden Sea extensively during the late 1920s and 1930s to promote sediment accretion (Nehring and Hesse 2008). By the end of the 1960s, *S. anglica* has established a continuous distribution in the German Wadden Sea (Nehring and Hesse 2008). Although many studies have been conducted on this invasive alien grass and its ecological role and impacts in German coastal waters between the first plantations and the late 2000s, no infections with ergot were recorded (Nehring and Hesse 2008). It seems very likely that G3 *C. purpurea* is a new invasive alien species to German fungi when considering its introduction to Ireland and Britain was only 50 years ago and its known invasion pattern in Europe. However, after establishment, differences in the length of lag phases and of expansion as well as in level of infection in *Spartina* marshes were noted from several areas (e.g. Eleuterius and Meyers 1974; Boyle 1976; Gray et al. 1990; Fisher et al. 2007). Furthermore, the low level ergot infection of *Spartina* could have been overlooked for some time in Germany and elsewhere because of the comparatively small size of sclerotia.

Actually it cannot be excluded that G3 *C. purpurea* is even much more widely present in Europe today than is presently known. Therefore it would be useful to examine semi historic herbarium samples of *Spartina* as well as known *Spartina* swards along the German Wadden Sea and other European wetlands for infections with G3 *C. purpurea*. In order to trace the origin of *S. anglica* ergot infestation in Germany it would be useful to characterize the different G3 *C. purpurea* populations in Europe in terms of genetic diversity and to establish relationships to geographically distinct populations of *Spartina*.

It is well known and has been verified in the present case by our own observation that G3 sclerotia have the ability to float for some time in fresh, brackish and salt water. They thus comply with the prerequisite for long distance dispersal by ocean currents to German coastal waters, e.g. from the known sites of infection in Irish, British or Benelux salt marshes. However, introduction via ships' ballast water coming from North America or from introduced range of occurrence is also plausible. All first national records in Europe as well as most subsequent records in new areas were from sites located in the direct vicinity of harbors. Furthermore it should be taken into account that for many years seed mussels were collected from wild subtidal banks in Irish, British and Dutch coastal waters and deliberately introduced into the German Wadden Sea for economic mussel fishery interests (Nehls et al. 2011). In these imports unknown amounts of natural sediments, which could have been contaminated with G3 sclerotia, were also transferred and released into the environment without any treatment.

Impacts and need for monitoring

Heavy ergot infections in *Spartina* are known from many areas (e.g. USA, Eleuterius and Meyers 1974, Fisher et al. 2007; Ireland, Boyle 1976; England, Gray et al. 1990) and most obviously related to the fact that the host species tends to occur as dense, monospecific swards, and thus resembles an agricultural crop in its vulnerability to epidemics. However, it is hitherto more or less unknown which risks and impacts arise from the infection for *Spartina* and its ecosystem.

Apart from the significant reduction in seed production of *Spartina* due ergot infections, only limited evidence is available that the demand of the fungus on the host's resources may lead to host damage (Eleuterius and Meyers 1974; Gray et al. 1990; Fisher et al. 2007). However, sclerotia of American and British G3 *C. purpurea* contain high amounts of mycotoxins ergocristine and ergocryptine (Pažoutová et al. 2000; Pažoutová et al. 2002b), which are toxic even at very low concentrations. The environmental fate of these mycotoxins and their

specific impacts and risks for the natural community, for Wadden Sea visitors and for grazing animals (e.g. sheep, cattle) are completely unknown. A need for more research into these issues is evident. To this end, the first sediment and biotic samples for toxin analyses from the Wadden Sea were taken by the authors. Authors' unpublished research indicates that G3 sclerotia from the Wadden Sea coast are highly toxic, with concentration levels similar to American and British specimens.

In particular, for European coastal waters there is also a danger of G3 spreading from *S. anglica* to native grass species like *S. maritima* (Curtis) Fernald because G3 isolates have been observed on several species of *Spartina* and *Distichlis* in North-America (Gessner 1978; Fisher et al. 2007; Douhan et al. 2008). In addition, host-range studies showed that G3 isolates can also infect non-chloridoid grasses following intensive artificial inoculation but without complete sclerotia formation (Pažoutová et al. 2002a).

In conclusion, immediate and targeted monitoring of infected and non-infected *Spartina* populations is imperative in order to detect any unwanted ecological impacts by G3 *C. purpurea* in salt marshes at the early invasive phase. This is even more important when considering that salt marshes perform a variety of natural functions and provide ecosystem services that are important to the overall health and welfare of coastal zones. This management approach is in agreement with Article 40 paragraph 2 of the Federal Nature Conservation Act (BNatSchG 2009, entered into force 1 March 2010), which prescribes that in Germany the competent Federal and Länder authorities should implement suitable monitoring programmes for alien species which could be responsible for significant loss of biodiversity.

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Appendix 1. Records of ergot G3 *Claviceps purpurea* from Germany. Numbers refer to sites shown in Figure 1.

Record No. (map ref.)	Location	Record coordinates		Record date	Reference
		Latitude	Longitude		
1	North Sea, Wilhelmshaven, Hooksiel	53°38′34″N	8°03′34″E	2 November 2011	Present study
2	North Sea, Wilhelmshaven, Cäciliengroden	53°29′01″N	8°03′16″E	2 November 2011	Present study