

## Biosecurity and Emerging Plant Health Problems in Turf Production and Maintenance

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### Summary

Recent research has supported the view that the distributions of many pests and diseases have extended towards higher latitudes over the last 50 years. Probably driven by a combination of climate change and trade, this extension to the ranges of hundreds of plant pathogens may have serious implications not only for agriculture, horticulture and forestry, but also for turf production & maintenance. Here we review our data relating to the current status of three emerging pest and disease problems across North West Europe (rapid blight, *Labyrinthula sp.*, the root knot nematode, *Meloidogyne minor*

and the pacific stem gall nematode, *Anguina pacificae*) and discuss the factors which may be involved in their spread and increasing impact on the turf industry. With turf production and maintenance becoming an increasingly international business, we ask if biosecurity and the promotion of plant health in turf production fields and associated sport facilities should be a greater priority for the industry. We also examine if a lack of effective biosecurity measures in the materials supply chain has led to increased plant health problems.

**Key words.** *Anguina* – *Labyrinthula* – *Meloidogyne* – nematodes – pathogens – plant disease – quarantine

### Introduction

The spread of pests and pathogens is largely mediated by anthropogenic factors such as trade and tourism, with over half of all emerging plant diseases resulting from such accidental introductions (ANDERSON et al. 2004). While human activity is the major driver for most pest and disease introductions, climatic factors appear to be the next most significant driver in the global movement of plant pathogens. Using data from the 1960 s onwards, BEBBER et al. (2013), showed that the ranges of hundreds of plant pests and disease causing organisms have moved significantly towards the poles, supporting the hypothesis of global warming driven pest and disease movement. Most recently in North West Europe we have seen the environmental impact of a succession of alien pests and pathogens such as *Chalara fraxinea* (ash dieback), *Phytophthora ramorum* (ramorum dieback/sudden oak death) and *Cameraria ohridella* (the horse chestnut leaf miner) which has highlighted the growing importance of biosecurity and plant quarantine (CHANDELIER et al. 2011; ANONYMOUS 2013). With this in mind it is timely to question if similar factors may also be affecting the spread of pests and diseases throughout the

amenity turf sector. Here we review data generated from the examination of turf samples and rootzone materials submitted to the United Kingdom Agri-Food and Biosciences Institute and the Turf Disease Centre for disease analysis over the last decade and discuss the factors promoting the spread of three emerging turf disease causing organisms in Europe, rapid blight, *Labyrinthula terrestris*, the pacific stem gall nematode, *Anguina pacificae* and the root knot nematode, *Meloidogyne minor*.

### *Labyrinthula terrestris*

Rapid Blight is a relatively new disease of cool-season turf-grasses that was initially recorded in California in 1995, but that has been increasingly confirmed in turf across Europe since 2004. The disease is caused by a unique kind of eukaryotic organism that belongs in the kingdom Chromista. In 2003, the causal organism was identified as a *Labyrinthula* species (OLSEN et al. 2005) and subsequently, a new species (*Labyrinthula terrestris*) was recorded as the cause of Rapid Blight in amenity turf (BIGELOW et al. 2005). Prior to the identification of *L. terrestris*, all known members of the genus *Labyrinthula* were restricted to

marine environments. Some of these early *Labyrinthula* species were associated with important wasting diseases of marine plants, but this apparent move into a terrestrial environment appears to be a relatively recent evolutionary development. *L. terrestris* can cause rapid and devastating turf losses in all cool-season grasses but only in turf that has developed increased levels of salinity, either from salt accumulation in the rootzone or through the use of irrigation water with a high level of salinity. This is believed to be the only turf disease whose development has been induced by human activity through the mandated use of elevated salinity irrigation water (DOUHAN et al. 2009).

Symptoms of Rapid Blight can develop in turf over a wide temperature range (10–34 °C) and where applied irrigation water has salinity of 1.5dS/m or above. Infected plants may develop an orange-brown, watersoaked appearance and infected areas of turf are often sunken relative to the surrounding sward. Affected patches of turf rapidly enlarge and coalesce to produce large areas of disease that will affect all plant tissues, leading to significant turf loss. *L. terrestris* exists as colonies of individual spindle-shaped cells (approx. 15 × 5.5 µm) that move along ectoplasmic networks, secreted by specific organelles. These vegetative cells of *L. terrestris* enter the plant through natural openings or wounds and multiply by division. In addition to causing disease in cool-season turf, *L. terrestris* has also been recovered from asymptomatic warm-season grasses (OLSEN and KOHOUT 2006).

Recent molecular studies have shown that there are significant differences between isolates of *L. terrestris* from turf in the USA and those from the UK and Spain (DOUHAN et al. 2009) and suggest that the *Labyrinthula* isolated from UK turfgrass may indeed be a distinct species. This present research will provide additional molecular comparisons between *L. terrestris* isolates from the USA and European isolates cultured from turf affected by Rapid Blight.

#### *The plant parasitic nematodes Anguina pacificae and Meloidogyne minor*

Among the plant parasitic nematodes affecting turfgrass, *Anguina pacificae* displays a number of unique features: most prominent is its pathology in attacking the stems of *Poa annua*, in direct contrast to the majority of other nematodes which feed on turfgrass roots. The distribution of the nematode is also unusual. Whereas most turfgrass nematode species are found across wide geographic ranges and even continents, until recently *A. pacificae* was only found on golf greens along a narrow coastal strip of northern California (McCLURE et al. 2008). The nematode causes severe damage to *Poa annua* greens and the limited control options available to Greenkeepers has led to some Californian golf clubs to take the expensive measure of switching from *Poa annua* greens to other turf species such as creeping bentgrass, *Agrostis stolonifera*. In late 2012, symptoms consistent with those caused by *A. pacificae* appeared on a single Irish golf course and subse-

quent DNA analysis confirmed that this Californian species had indeed appeared in Europe (FLEMING et al., in press).

Root knot nematodes (*Meloidogyne*) are perhaps the most specialized of the nematodes parasitizing plants and increasingly, reports of thinning shallow-rooted turfgrass on golf courses and in sports fields in Europe and North America have been linked to these endoparasitic species (FLEMING et al. 2008; McCLURE et al. 2012). In Europe, *Meloidogyne minor* was first described in 2004, having been found on sportsturf from the British Isles and in a potato crop from the Netherlands. Increasingly detected in grassland in the British Isles and the Netherlands, this new species now causes severe symptoms in turf, especially in sand based rootzones on both *Agrostis stolonifera* and perennial ryegrass, *Lolium perenne* (KARSEN et al. 2004).

There have been ongoing discussions within the industry centering on the source of these *M. minor* (and other nematode) infections, which often appear within 12 months of turf establishment in a new green or pitch construction. Natural movement of nematodes from adjacent natural sites onto new greens, construction sand, top dressing material, turf sod and even golf shoes have all been under investigation as possible sources (FLEMING et al. 2008).

## Materials and Methods

### *Soil sampling*

Since 2002, individual turf, soil and sand samples ranging in volume from 200 to 3000 cm<sup>3</sup> were submitted by Groundsmen from the British Isles and other parts of North West Europe to the UK AFBINI Plant Pathology diagnostic laboratory and the Turf Disease Centre for disease or nematode analysis. Although samples were received and assessed from locations worldwide, analysis for plant parasitic nematodes in samples received from outside the EU was only completed at AFBINI. During this time, samples were also collected by AFBI staff during advisory visits. Samples comprised multiple cores taken from affected areas on greens using a 1.6 cm diameter × 12 cm steel auger. Each sample consisted of at least 12 cores giving a 250–300 ml total volume of sample soil. Samples of plant material and turf were also collected on-site. Nematodes were extracted from soil using decanting and sieving (HOOPER 1986a) and roots were examined for the presence of nematode galls using a stereomicroscope.

### *Labyrinthula isolation and DNA extraction*

Ribosomal DNA (rDNA) sequences of 4 European isolates of *L. terrestris* (2 from Portugal, 1 from Co Cork, Ireland, 1 from Spain) were compared with published sequences of 3 isolates from the USA and Scotland previously characterised by DOUHAN et al. (2009). *Labyrinthula* was isolated, cultured and the DNA extracted and sequenced using

the methods detailed by DOUHAN et al. (2009). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (TAMURA et al. 2011).

#### Nematode Morphometric analysis

For measuring morphological characters, nematodes were mounted live in water on microscope slides covered with a thin layer of 2 % water agar and a cover-slip placed over the nematode on the slide. On occasions, nematodes were killed in hot water (65 °C) and fixed in TAF (HOOPER 1986b). Nematodes were photographed on a Nikon Eclipse 50i compound scope and measurements determined using NIS-Elements version 4.10, Nikon imaging software. Specimens were then compared to the published measurements for *A. pacifica* (CID EL PRADA and MAGGENTI 1984) and *Meloidogyne* (BRZESKI 1998; KARSSSEN et al. 2004)

#### Nematode DNA extractions and DNA sequencing

Individual nematodes were cut into pieces on a microscope slide in 20 µl Worm Lysis Buffer (WLB) [WLB; 50 mM KCl, 10 mM Tris pH 8.2, 2.5 mM MgCl<sub>2</sub>, 60 µg mL<sup>-1</sup> proteinase K (Roche), 0.45 % NP40 (Fisher Scientific), 0.45 % Tween 20 (Sigma), 0.01 % gelatine]. The solution was then transferred into a PCR tube and incubated in an Applied Biosystems 2720 Thermal Cycler [program: 60 °C for 60 min, 96 °C for 10 min] for DNA isolation. Amplification of the ITS1, 5.8 s and ITS2 regions of the rDNA cistron was performed using PCR primers, rDNA1 and rDNA2 (VRAIN et al. 1992). The PCR assay contained 1 µl DNA, 1 µl rDNA1 primer, 1 µl rDNA2 primer (each at 10 µM), 12.5 µl GoTaq® Green Master Mix 2X Promega, 9.5 µl H<sub>2</sub>O performed with the thermocycler program [program: 94 °C for 5 min, 35 cycles; 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and a single 72 °C for 5 min]. *PCR purification*: PCR products were purified using a ChargeSwitch PCR Clean-Up Kit (Life Technologies), following the manufacturer's protocol. DNA sequences were generated for the forward and reverse strands by Queens University Belfast Genomics Core Technology Unit and the consensus

sequences constructed with Geneious version 5.5.6 (Bio-matters).

## Results

### Labyrinthula terrestris

The comparison of rDNA sequences showed low genetic distances (0.002–0.004) among European isolates but larger differences between North American and European *L. terrestris* (Table 1). This was confirmed in the phylogenetic analysis of the rDNA sequences which showed the European isolates clustering separately from the North American isolates (Fig. 1).

### Anguina pacifica

*Anguina pacifica* was initially detected on samples from a *Poa annua* putting green from a single golf course in County Dublin, Ireland. Inspection of the site in December 2012 showed that the infection was present on a turf nursery and 3 competition greens (greens 1, 5 and 16). Damage was most extensive on the turf nursery, where symptoms had first appeared. Within 12 months nema-

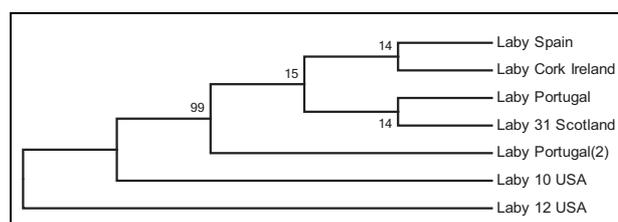


Fig. 1. *Labyrinthula terrestris*: Phylogenetic analysis of 7 *Labyrinthula* (Laby) isolates using Maximum Parsimony, based on the LSU/ITS-rDNA sequence which comprises ITS1, 5.8S, ITS2 and a portion of the LSU including the variable D1 and D2 regions (DOUHAN et al. 2009). Bootstrap support is indicated above nodes and is based on 500 replicates

Table 1. *Labyrinthula terrestris*: Pairwise genetic distance comparisons among 7 *Labyrinthula* isolates from Europe and the USA computed using the maximum composite likelihood model within MEGA 5 (TAMURA et al. 2011). Isolates Laby 31, 10 and 12 from DOUHAN et al. 2009.

	1	2	3	4	5	6	
Spain 1	Laby Spain						
Portugal 2	Laby Port 2	0.002					
Portugal 3	Laby Port 1	0.000	0.002				
Ireland 4	Laby Cork	0.002	0.004	0.002			
Scotland 5	Laby 31 Scot	0.000	0.002	0.000	0.002		
USA 6	Laby 10 USA	0.044	0.047	0.044	0.045	0.044	
USA 7	Laby 12 USA	0.044	0.047	0.044	0.045	0.044	0.000

tode symptoms had spread to an additional 4 greens and a practice chipping green. Nematode levels were highest in greens with a longer history of symptoms and infection (Fig. 2). Coincident with the presence of galls in the plant stems, infective juvenile *A. pacificae* were also present in rootzone samples from all affected greens. The infection risk from plant material was confirmed with the discovery of a single infected *Poa annua* plant in a sample of 5000 cm<sup>3</sup> plant debris collected from the golf club shoe cleaning station (an air jet cleaning system) in September 2013. Microscopic analysis of turf cores removed during aeration of infected greens showed that large numbers of stem galls containing infective juveniles and eggs were also present in this material.

Meloidogyne minor

The construction of a new golf course in Ireland offered the opportunity to examine the dynamics of *M. minor* establishment in new golf greens. The development comprised

USGA design creeping bentgrass greens built over a 3 year period resulting in completed greens at different stages of development. All greens used the same creeping bentgrass variety and source of sand, which was from a local sand extraction site. Tees were constructed using similar grade sand but sourced from a freshwater lake site. Fig. 3 shows that *M. minor* was first detected in greens 16 months after seeding and that the numbers of *M. minor* juveniles increased with age of green. Yellow-patch symptoms (typical of *M. minor* infection) first appeared in the new turf after 14 months. No *M. minor* or other plant parasitic nematodes were detected in 3 year old tees from the course. Similarly, no *M. minor* were found in 20 samples taken from various natural locations around the golf course.

To assess the potential of turf sod, construction sand and top dressing sand as a source of plant parasitic nematode infection, 18 samples of commercial material from various locations were examined for the presence of nematodes.

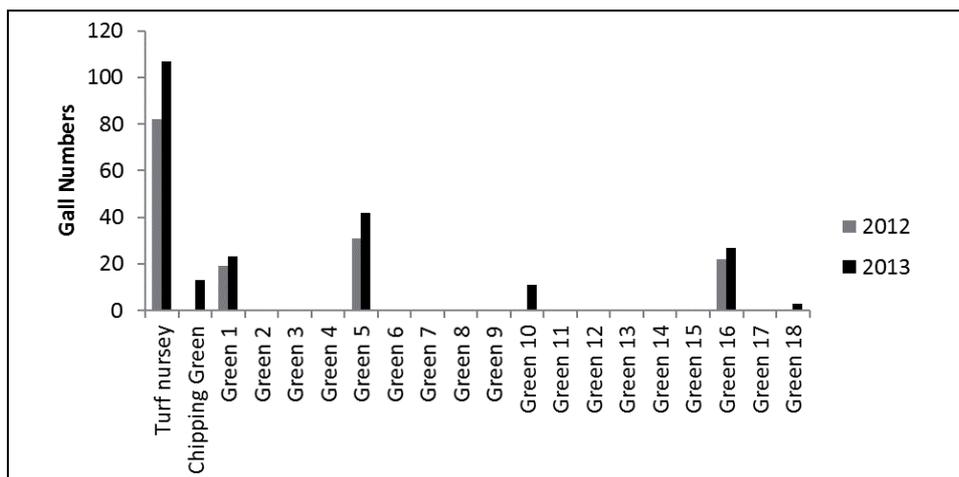


Fig. 2. *Anguina pacificae*: Numbers of galls in 100 cm<sup>3</sup> samples taken in November 2012 and 2013 from 20 *Poa annua* golf greens from an Irish golf club.

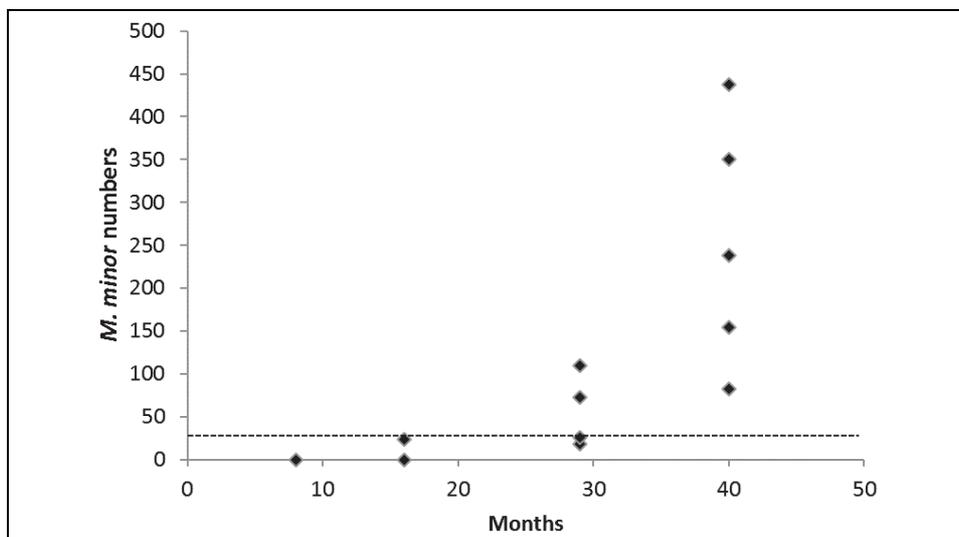


Fig. 3. *Meloidogyne minor*: Infective juvenile numbers in 100 cm<sup>3</sup> soil from 12 *Agrostis stolonifera* golf greens of varying ages. Horizontal dotted line indicates damage threshold.

Turf sod contained the highest levels of plant parasitic nematodes. Samples from the British Isles and Europe contained significant levels of ecto- and endoparasitic species, including in the case of the Irish sample, *M. minor* (Table 2).

Top dressing sand sourced from European and Asian locations generally contained very low numbers of nematodes, with non-parasitic bacterial and fungal feeding species dominating. One sample of top dressing material sampled in Ireland (but sourced originally in Great Britain) did contain high levels of the stubby root nematode, *Trichodorus* (Table 3).

Construction sand used in building golf greens and soccer pitches within the British Isles also contained low numbers of nematodes (Table 4). Again most nematodes were

non-parasitic, but low levels of plant parasites were found in some sands. One sample (C2005b), taken directly from the extraction site used to source construction sand sample C2005a, contained significantly higher levels of nematodes, including infective juvenile *M. minor* and other *Meloidogyne* species.

## Discussion

The data presented in this study indicate a real potential for the accidental spread of pests and pathogens in the turfgrass industry. In particular, the pattern of development of pest and disease outbreaks in new constructions, has led to the suspicion that pathways linked to the move-

Table 2. Numbers of nematodes in 100 cm<sup>3</sup> soil from 5 samples of turf sod from north west Europe tested between 2007 and 2013.

	Ireland TS2007	Ireland TS2008	England TS2010	The Netherlands TS2011	Germany TS2013
Bacterial/fungal	2358	355	1733	528	7138
<i>Tylenchus</i>	0	7	13	0	54
<i>Heterodera</i>	328	19	3	0	109
<i>Pratylenchus</i>	0	0	16	56	0
<i>Longidorus</i>	0	0	0	6	0
<i>Meloidogyne minor</i>	13	2	0	0	0
<i>Meloidogyne naasi</i>	0	0	2	5	0
<i>Hemicycliophora</i>	0	0	0	0	0
<i>Helicotylenchus</i>	10	63	5	63	52
<i>Rotylenchus</i>	0	0	0	0	191
<i>Tylenchorhynchus</i>	45	12	21	0	0
<i>Heterodera</i> cysts	21	0	0	0	1
<i>Meloidogyne</i> galls	0	0	2	1	3

Table 3. Numbers of nematodes in 1000 cm<sup>3</sup> sand from 6 samples of top dressing sand tested between 2009 and 2012.

	Ireland TD2009	Ireland TD2010	England TD2010	England TD2010	Singapore TD2011	Dubai TD2012
Bacterial/fungal	12	13	1	0	2	5
<i>Tylenchus</i>	0	2	0	0	0	0
<i>Heterodera</i>	0	0	0	0	0	0
<i>Pratylenchus</i>	0	1	0	0	1	0
<i>Longidorus</i>	0	0	0	0	0	0
<i>Meloidogyne minor</i>	0	0	0	0	0	0
<i>Hemicycliophora</i>	0	0	0	0	0	0
<i>Helicotylenchus</i>	0	0	0	0	0	0
<i>Rotylenchus</i>	0	0	0	0	0	0
<i>Trichodorus</i>	0	35	0	0	0	0
<i>Tylenchorhynchus</i>	0	0	0	0	0	0

Table 4. Numbers of nematodes in 1000 cm<sup>3</sup> sand from 7 samples of Irish and UK construction sand tested between 2005 and 2012.

	Ireland C2005a	Ireland C2005b*	Ireland C2008	England C2006	England C2010	England C2011	England C2012
Bacterial/fungal	16	238	8	12	0	3	2
<i>Tylenchus</i>	0	17	0	0	0	0	0
<i>Heterodera</i>	0	8	0	0	0	0	0
<i>Pratylenchus</i>	0	18	0	0	0	0	0
<i>Longidorus</i>	0	0	0	0	0	0	0
<i>Meloidogyne minor</i>	0	6	1	0	0	0	0
<i>Meloidogyne naasi</i>	0	2	0	0	0	0	0
<i>Hemicycliophora</i>	0	0	0	0	0	0	0
<i>Helicotylenchus</i>	1	24	0	0	0	0	0
<i>Rotylenchus</i>	0	0	0	0	0	0	0
<i>Tylenchorhynchus</i>	0	39	0	0	0	0	0

\* Sample taken directly from sand extraction site which was the original source of sample Ireland C2005a

ment of construction and maintenance materials may be implicated (FLEMING et al. 2008).

Rapid blight is particularly interesting as its emergence as a significant turf disease may be directly linked to human activity, specifically the use of saline irrigation water. A key question then is, has this pathogen also been spread by human activities, perhaps on the equipment and shoes of golfers? First detected on North American golf greens, rapid blight infections have started to appear in regions of Europe including Ireland, Spain and Portugal (ENTWISTLE 2012). DOUHAN et al. (2009) showed that a Scottish isolate appeared to differ genetically from North American *L. terrestris*. The apparent distinction of European and North American isolates (Fig. 1) is confirmed in this study supporting the view that it is unlikely the disease has made its way from North American greens to Europe. Rather, native European *L. terrestris* populations appear to be the source of this disease in Europe and exactly where it resides in the natural environment should be the study of further research. The emergence of native pests and diseases in response to changing environmental conditions (resulting either from human or climatic drivers) is likely to be an increasingly common phenomenon in the future and the turfgrass industry must be ready to identify and respond to such events.

An accidental trans-Atlantic introduction does appear to be the source of the Irish outbreak of the nematode *Anguina pacifica*. The natural range of this species is limited to the North Californian coastline and its appearance on an Irish golf course was as unexpected as it was unwelcome. The data from this study (Fig. 2) indicate that it has spread initially from one green (probably a turf nursery, where infection levels were highest) to other parts of the course, with aeration and turf repair activities the most

likely means of movement. Golf shoes are not entirely eliminated as a means of spread of this plant parasite. *Anguina pacifica* can survive many months in a desiccated state, with an ability to infect its hosts once suitable environmental conditions are re-established (MCCLURE et al. 2008). The widespread use of “soft-spikes” on golf shoes has been promoted as a means of minimizing “wear and tear” on golf putting surfaces. While physical damage may be reduced, these shoe sole designs tend to accumulate higher amounts of plant material than traditional metal spikes, and *Anguina*-infected plant material could conceivably be transferred around a golf course by players on their shoes. This is one possible route by which this Californian nematode could have travelled to Ireland, however as it was first detected on a turf nursery, which does not usually come into contact with players, it seems less likely in this case. The infected turf nursery has been used as a demonstration and testing location for maintenance equipment and contaminated machinery, originally used in the USA, seems a more likely source of the *A. pacifica*. This route of entry of a plant parasitic nematode is not unique, with USA military vehicles returning from Europe to New York State believed to be the source of a potato cyst nematode (*Globodera rostochiensis*) outbreak on Long Island in 1941 (CHITWOOD et al. 1942).

This highlights the importance of effective biosecurity in the turfgrass industry. Where appropriate, consideration should be given by Groundsmen to cleaning any used machinery brought on-site. Similarly, during maintenance procedures, areas with a significant disease or pest problem may need to be treated only after work on healthy areas has been completed and rigorous post-use cleaning should be routine. While the accidental transfer of pests and pathogens on machinery and golf equipment is a risk,

contaminated construction and maintenance materials are likely to pose a greater biosecurity risk. Our data indicate that turf sod can be a high risk material with significant and potentially damaging levels of plant parasitic nematodes in most samples tested. The industry may need to consider regular testing of turf production sites for nematode levels and if appropriate, treatment of the growing turf with nematicides in order to minimize the risk of introducing plant parasitic nematodes into new constructions and renovations. Living turfgrass is also likely to harbor a greater range of pathogens, often in a latent or non-symptomatic state (Kate ENTWISTLE, pers. comm.).

Many new golf and sports field constructions use turfgrass seed as an alternative to turf sod but even this approach has significant risks for turf health. Typically, new constructions show little sign of plant parasitic nematodes within the rootzone immediately after establishment, however nematode levels (especially those of endoparasites such as *M. minor*) often become detectable within 12 months of seeding. In sports fields using undersoil heating, nematode populations may build up significantly faster. In the case of *M. minor*, damage (small chlorotic patches of turf) usually becomes apparent between 12 and 18 months after seeding. Our data from a recent Irish golf construction project have shown that contamination of new greens from a natural *M. minor* population already existing on the site is unlikely and it seems certain that construction sand is the source of inoculum for these loci of *M. minor* infection. While the analysis of construction and top dressing sands has indicated numbers of plant parasitic nematodes to be low, the large volumes of material used on golf courses and sports fields ensure that, without the use of more stringent biosecurity measures, the introduction of plant parasitic nematodes and subsequent damage may be inevitable. The normal practice for preparing construction and top dressing materials involves extraction from a suitable site, grading and washing before delivery to the end-user. While most plant parasitic nematodes will be removed during this process we have shown that low levels of nematodes can remain in the material, posing a threat to future turf health.

While the option of heat or chemically sterilizing construction and maintenance materials may be too expensive for most of the industry, simple procedures such as storage of materials before use, may reduce the risk from pests such as *M. minor* and the wide range of other plant parasitic nematodes found in turfgrass (VANDENBOSSCHE et al. 2011). In the absence of hosts, plant parasitic nematode levels will decline in stored sand and soil and if left for a sufficient period (i.e. 9–12 months), they may eventually disappear, eliminating the risk to new constructions.

Over the past decade, the appearance of many new pests and diseases has raised the significance of biosecurity, quarantine and plant health to European agriculture, horticulture and forestry (ANONYMOUS 2013). The turfgrass industry too has witnessed new plant health problems, but the awareness of biosecurity issues has lagged behind

that in other sectors. It is the responsibility of plant pathologists and those involved in the education of turfgrass professionals to address this problem and prepare the industry for dealing with future plant health risks.

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