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Matching source and sink: An environmentally tailored fungal endophyte consortium increases yield in three field-grown barley cultivars

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Abstract: Environmental stresses are limiting factors in optimal agricultural crop yield, and these stresses, especially drought, are likely to become more acute due to future climate change. Crop wild relatives contain environmentally selected endophytes that can help to increase stress resistance. Our previous work with barley cultivars has shown a positive correlation between endophyte-induced yield increase and increasingly dry conditions. For this study, we hypothesised that a consortium of fungal endophytes recovered from a crop wild relative of barley growing in drought-stressed sites would enhance barley yield in similarly low moisture agricultural sites. We grew three barley cultivars on two environmentally distinct sites under three nitrogen (N) input regimes. We found that the endophyte inoculant induced an increase in grain dry weight at both sites, which experienced abnormally low local rainfall in the early growing season. The yield increase was 1.2 t/ha for standard N input, 1.1 t/ha for half N input and 0.6 t/ha with no N input. Additionally, on both sites, endophyte treatment with half N input recovered yield to that associated with untreated crops with standard N input for all three cultivars. Furthermore, the endophytes still retained their efficacy with regular foliar fungicidal crop treatments. These results show that endophytes recovered from sites with low and similar water status to the targeted barley growing sites can produce large

ABOUT THE AUTHOR

Brian R. Murphy

Brian R. Murphy is a Research Fellow in the Botany Department at Trinity College Dublin. He is recognised as a leading expert in endophyte discovery from wild relatives of crops and their application to increasing stress resistance in cereal crops. He has published 25 peer-reviewed original research and review papers as first author on endophyte/crop relationships and has appeared on national and international media on camera and in print, highlighting his research achievements. He is currently engaged in a series of projects examining the commercial potential of naturally occurring micro-organisms to improve the growth and yield of field-grown cereal crops. He is also extending the scope of his research to other important crop species. This work aims to further validate the use of micro-organisms that can reduce economically and environmentally expensive chemical fertilisers in agriculture.

PUBLIC INTEREST STATEMENT

The crop plants that feed the world are under increasing threats from global climate change, with damaging events such as severe drought likely to become more frequent. For this study, we have taken a group of micro-organisms from inside the roots of wild crop relatives and used them to improve the performance of barley crops in relatively dry growing conditions. We also found that using these micro-organism allowed us to apply less chemical fertiliser with no negative effect on the barley crop yield. This is particularly significant, as use of these chemicals is expensive for both the farmer and damaging for the environment. The use of this innovative biological crop treatment in water-stressed crop growing sites will be of great benefit both now and in the future. This type of alternative crop treatment is natural and environmentally friendly and has tremendous potential for reducing farmer's costs and reducing environmental damage.

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and significant increases in yield regardless of nitrogen input, and hold promise for application in drought-stressed sites with limited access to expensive nitrogen fertilisers.

Subjects: Environment & Agriculture; Bioscience; Food Science & Technology

Keywords: barley; biotechnology; crop wild relatives; fungal endophytes; Hordeum; yield

1. Introduction

Agricultural crops are subject to many and varied stresses that can reduce yield below the optimum. The Food and Agriculture Organization (FAO) of the United Nations estimates that between 20 and 40% of global crop yields are reduced each year due to the damage caused by plant pests and diseases (FAO, 2015a), and other studies calculate that direct yield losses caused by pathogens, animals and weeds, are altogether responsible for losses ranging between 20 and 40% of global agricultural productivity (Oerke, 2006; Oerke, Dehne, Schonbeck, & Weber, 1994; Teng, 1987; Teng & Krupa, 1980). Stresses associated with drought may become particularly acute in certain areas, and Kang, Khan, and Ma (2009) suggest that with increasing temperature and precipitation fluctuations, water availability and crop production are likely to decrease in the future. The Intergovernmental Panel on Climate Change (IPCC) predict drier summers with increased drought risk in Europe (Field, Barros, Mach, & Mastrandrea, 2014; IPCC, 2001) and farmers will have to find effective means of mitigating drought risk. Farmers in poorer regions may be hardest hit, but they do not have the resources to buy and use the expensive chemical and cultural inputs that may help to support yields under water-stressed conditions (Pandey & Bhandari, 2008). Irrigation systems can be expensive, with relatively higher economic impacts in poorer countries; two studies in Tanzania and Zambia found that diesel-powered irrigation for maize crops cost over $300/ha, and in Malawi this cost was over $1,500/ha (FAO Natural Resources Management and Environment Department, 2017).

Alternatives to traditional and expensive drought amelioration methods are urgently required, and microbial crop treatments, especially endophytes, may provide part of the solution. Endophytes are a class of plant-associated micro-organisms that have shown particular promise in agriculture (Achatz et al., 2010; Busby, Ridout, & Newcombe, 2015; Kuldau & Bacon, 2008; Murphy, Doohan, & Hodkinson, 2014; O'Hanlon, Knorr, Jørgensen, Nicolaisen, & Boelt, 2012; Rodriguez, White, Arnold, & Redman, 2009; Saikkonen, Gundel, & Helander, 2013; Schulz & Boyle, 2006). Endophytes (bacteria, fungi and unicellular eukaryotes) live at least part of their life cycle inter- or intra-cellularly inside plants, usually without inducing pathogenic symptoms. This can include competent, facultative, obligate, opportunistic and passenger endophytes (Hardoim, van Overbeek, & van Elsas, 2008). Endophytes can have several functions and/or may change function during their life-cycle (Murphy et al., 2014).

Previous studies have found that crop wild relatives contain environmentally selected endophytes that can help to increase drought stress resistance (Kannadan & Rudgers, 2008; Knapp, Pintye, & Kovács, 2012; Kuldau & Bacon, 2008; Murphy, Martin Nieto, Doohan, & Hodkinson, 2015a; Yokoya, Postel, Fang, & Sarasan, 2017). Our previous work with barley cultivars demonstrated a positive correlation between endophyte-induced yield increases and increasingly dry conditions (Murphy, Hodkinson, & Doohan, 2017). It is important to obtain further field validation of the positive effect of these endophytes on barley yield to demonstrate effectiveness over several growing seasons, so for this study we aimed to conduct a new set of field trials using a consortium of fungal endophytes. We investigated if endophtyes would influence barley yield at two widely separated sites that normally experience differing amounts of rainfall, thus supporting or rejecting our hypothesis that endophytes for drought tolerance can be selected from crop wild relatives growing in similarly low moisture conditions. Positive results from this study could provide evidence of the potential for endophytes to relieve some of the economic and social costs associated with water stress in crops.

2. Materials and methods

2.1. Endophyte inoculum

The endophyte inoculum consisted of a mix of equal volumes of four fungal endophytes (Table 1) that were isolated from a wild barley relative, *Hordeum murinum* subsp. *murinum* (L.). These strains were obtained from the endophyte collection at the University of Dublin, Trinity College Dublin, Ireland. These particular endophyte strains were chosen because they were previously shown to enhance the growth, development and yield of cultivated barley in growth cabinet experiments (Murphy, Doohan, & Hodkinson, 2015; Murphy, Martin Nieto, Doohan, & Hodkinson, 2015b). The strains had also significantly increased barley grain yield on a relatively dry field site (Murphy et al., 2017). The field sites from which the endophytes were recovered were characterised by a relatively high soil salinity (mean 1.39 bars), high soil pH (mean 7.7), southern exposure and low soil moisture content (mean 3.03%), with two sites having no measurable soil moisture (Table 2).

2.2. Crop cultivar selection

Three spring barley cultivars (the feed cultivar Mickle, the feed and malting cultivar Planet and the malting cultivar Propino) were selected as the experimental subjects (seed source Goldcrop Ltd. Cork, Ireland). Mickle (Sumitt × Yard, Breeder: Syngenta Seeds Ltd.) is an established British variety with very high yield potential. Propino (Quench × NFC Tipple, Breeder: Syngenta Seeds Ltd.) is widely planted across Europe. Planet (Tamtam × Concerto, Breeder: RAGT Seed) is a new cultivar which is progressing very well across Europe as well as in New Zealand and Argentina. These were chosen on the basis that they are modern and also internationally recognised, so the results will be relevant for a number of years and will be more easily shared for comparative purposes with other growers.

2.3. Seed treatment

A dressing was applied to the seeds as a thin film using a Hege 14 seed dresser (Wintersteiger AG, Austria), a device designed to apply treatments to small amounts of seed (100–2,500 g). The seed dressing consisted of a liquid carrier to aid seed adhesion (Incotec DISCO™) mixed with one of two treatment solutions at a ratio of 50:50. The two treatments were: (1) untreated (plain water) or (2) an endophyte spore solution (approximately 1,500 fungal spores per seed, in pure water suspension). The seed was spun at high speed in the seed dresser while being spray-dressed, resulting in the application of 4 mL of the liquid treatment per kg of seed. The seed dressing dries within minutes. The coated seed was thereafter stored in burlap bags at room temperature (~20°C) before sowing.

2.4. Experimental procedure

Two field trials were conducted on sites that experience different climatic environments (Table 2) and are on different soil types with variable characteristics of fertility (Table 3). The two field trial sites were located in the south of Ireland at Carrigtwohill, Co. Cork (51° 54′ 38.53″ N, 8° 16′ 49.00″ W, 16 m a.s.l.), and at Backweston, Co. Kildare, in the east of Ireland (51° 51′ 38.52″ N, 8° 2′ 56.54″ W, 51 m a.s.l.). Barley crops had been grown on the Carrigtwohill site for the last 3 years, whereas the Backweston site had been planted with barley only for the previous year, after a year left fallow.

The barley seeds were dressed with each of the treatments and the seeds sown by direct drilling at a density of 193 kg/ha for Mickle, 207 kg/ha for Planet and 202 kg/ha for Propino. Four replicate

Table 1. The strain code and ITS sequence similarity of endophyte strains used in this study, showing the relationship to NCBI GenBank accessions

Endophyte strain ID	GenBank accession	Nearest NCBI BLAST match	Pairwise similarity %
TCD04060502A	KM492844	*Cladosporium* sp., KX090309	83
TCD04060502C	KM492845	*Penicillium* sp., HE975035	87
TCD04090103A	KM492837	*Penicillium* sp., MF136581	95
TCD04090604A	KM492839	*Metarhizium* sp. EU307891	95

Table 2. Environmental variables for collection sites. Soil pH, moisture content %, salinity are mean values ± standard error (n = 10)

Site	Exposure	Soil measurements			
		pH	Moisture content %	Salinity*	Soil type#
1	South	7.8 ± 0.09	9.1 ± 2.6	1.18 ± 0.04	SM
2	South	7.7 ± 0.03	0 ± 2.8	1.49 ± 0.02	SM1
3	South	7.7 ± 0.04	0 ± 2.8	1.51 ± 0.02	SM1
MEANS		7.7	3.03	1.39	

Notes: *Salinity is Osmotic Pressure in bars.
#Soil type SM is Silty Sand in ASTM D-2487 soil classification.

Table 3. Field trial sites soil characteristics

Site character	Field trial site	
	Carrigtwohill	Backweston
Soil pH	6.8	6.9
Phosphorous mg/L	5.5	16.9
Potassium mg/L	100	65.8
Copper mg/L	0.396	1.8
Manganese mg/L	3.84	14.1
Zinc mg/L	0.717	1.6
Soil type	Sandy silt	Clayey silt
Years cultivated	>10	>10

trial plots of 1.8 × 12.5 m were sown for each treatment, giving a total of 72 plots per field site. A month before seed sowing, 500 kg/ha 0/10/20 (N/phosphorus (P)/potassium (K)) + 250 kg/ha Granlime was applied as a top dressing, and 250 kg/ha of Sulfacan was applied immediately before sowing. Seeds were sown on 27th March (Carrigtwohill) and 5th April (Backweston). Three nitrogen (N) input regimes were applied during the growing season: (1) standard nitrogen input for the site (100%); for Carrigtwohill the full rate was 163 kg N/ha and for Backweston 120 kg N/ha), (2) half of standard nitrogen input (50% N) and (3) no extra nitrogen (0% N). Where appropriate, nitrogenous fertiliser was applied to the growing crops during the growing season at Zadoks growth stage (GS) 32 (second node detectable; Zadoks et al., 1974); the full rate fertiliser plots received 452 kg/ha Sulfacan and half of these amounts was applied to the 50% fertiliser treatment plots. Plots not receiving N fertiliser were covered by plastic sheeting during the application process to prevent unwanted drift from fertilised plots.

The growing crop received the following fungicidal treatments: GS 39, 750 g/L Fenpropimorph at 0.2 L/ha (Corbel®) and 250 g/L Prothioconazole at 0.4 L/ha (Proline®); GS 41, 41.6 g/L Epoxiconazole + 41.6 g/L Fluxapyroxad + 66.6 g/L Pyraclastrobin at 1.8 L/ha (Ceriaxl®) and 500 g/L Chlorothalonil at 1.0 L/ha (Bravo®). The independent growers at each field site recommended this fungicide regime for their particular conditions. At GS 39, the insecticide Sumi-Alpha (Interfarm UK Ltd.), with the active ingredient Esfenvalerate, was applied at 165 mL/ha. All crops were machine-harvested in August and dried before measuring grain yield and protein content. Grain yield comparisons at a moisture content of between 16 and 19% were based on yield per plot.

Statistical analyses were carried out using two-way ANOVA with Bonferroni correction, Pearson's Product Moment Correlation (r) and two-tailed Student's t tests (Datadesk 7.01 and Microsoft Excel 2010®).

3. Results

3.1. Yield analysis
For grain yield comparisons between endophyte-treated and untreated barley controls, a significant sample interaction was detected between field site and barley cultivar (P < 0.01). In every case for every reported comparison, the yield was greater for the endophyte treated plots (Table 4). The overall mean yield increase for all cultivars and both sites was 1.1 t/ha (14%), with an increase of 1.2 t/ha for standard N input, 1.1 t/ha for half N input and 0.6 t/ha with no N input. For standard N input, the endophyte treatment induced an increase in yield of 1.4 t/ha for Mickle, 1.5 t/ha for Planet and 0.7 t/ha for Propino. Additionally, for all three cultivars, endophyte treatment with half N input recovered yield to that associated with untreated crops with standard N input. For half N input, the endophyte treatment induced an increase in yield of 0.9 t/ha for Mickle, 1.3 t/ha for Planet and 0.7 t/ha for Propino. With no N input, the endophyte treatment induced an increase in yield of 0.4 t/ha for Mickle, 0.8 t/ha for Planet and 0.7 t/ha for Propino. With the endophyte treatment, the grain yield at Backweston was a mean of 1.2 t/ha greater than at Carrigtwohill, and was 1.7 t/ha greater without endophyte treatment.

Yield comparisons for the individual cultivars revealed that the endophyte treatment induced a mean overall increase for all N inputs of 1.3 t/ha for Planet, 0.9 t/ha for Mickle and 0.8 t/ha for Propino. For each field site, yield increase associated with endophyte treatment was significant (P < 0.05) for all N inputs (except for no N input at Backweston). For the Carrigtwohill field site, endophyte treatment induced a mean yield increase of 0.9 t/ha for Mickle, 1.4 t/ha for Planet and 0.8 t/ha for Propino; for Backweston, the increases were 0.9 t/ha for Mickle, 1.0 t/ha for Planet and 0.7 t/ha for Propino.

3.2. Grain quality
The 1,000 grain weight (TGW) was significantly greater (P < 0.05) with endophyte treatment for all cultivars and all N input regimes on both sites and in most of the individual treatment comparisons. The overall mean TGW increase was 1.8 g (3%), with an increase of 2.1 g (4%) for standard N input, 1.6 g for half N input (12%) and 1.7 g (3%) with no additional N (Table 5). For standard N input, the endophyte treatment induced an increase in TGW of 0.4 g for Mickle, 2.2 g for Planet and 3.4 g for Propino. For half N input, the endophyte treatment induced a **decrease** in TGW of 2.4 g for Mickle, and an increase of 3.7 g for Planet and 3.4 g for Propino. With no N input, the endophyte treatment induced an increase in TGW of 0.2 g for Mickle, 3 g for Planet and 2.9 g for Propino.

The percentage of nitrogen (N) in the grains did not differ significantly between most treatments, except for Mickle with half N input (P < 0.05) and Propino with no N input (P < 0.05). With standard N input, grain N% was between 1.66 and 1.81%, with half N between 1.63 and 1.69%, and with no N between 1.46 and 1.55%.

The specific weight (kph) for grain was between 65.1 and 67.0 for all treatments, with no significant differences between treatments. We detected a positive correlation between grain yield and both TGW (r < 0.01) and grain N% (r < 0.05).

3.3. Environmental variables
The Backweston site has a greater background soil fertility than the Carrigtwohill site (personal communication), which was confirmed by soil tests conducted prior to site preparation (Table 3). These tests showed that the soil at Backweston had greater values for Phosphorous (×3), Copper (×4.5), Manganese (×3.7) and Zinc (×2.2).

Both sites received relatively low rainfall during the growing season when compared with historical data (Table 6). When analysed over the 3 years that we have conducted field trials (from 2015 to 2017), we found a direct negative correlation between total growing season rainfall and endophyte-induced yield increase (r < 0.05), so lower rainfall during the growing season was

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Table 4. Mean barley grain yield (t/Ha) ± standard error. Statistically significant differences (ANOVA, $P < 0.05$) between endophyte and untreated trials are highlighted in bold underline

Cultivar	Field trial location									MEAN
	Carrigtwohill			Backweston			Both locations combined			
Treatment	**Standard N**	**50% N**	**No N**	**Standard N**	**50% N**	**No N**	**Standard N**	**50% N**	**No N**	
Mickle										
Endophyte	**11.1 ± 0.1**	9.4 ± 0.5	4.6 ± 0.2	12.6 ± 0.1	11.2 ± 0.4	7.3 ± 1.3	**11.9**	10.3	6.0	9.4
Untreated	9.7 ± 0.1	8.7 ± 0.1	4.3 ± 0.2	11.4 ± 0.5	10.1 ± 0.5	7.0 ± 1.2	10.5	9.4	5.6	8.5
Planet										
Endophyte	**11.1 ± 0.3**	**9.4 ± 0.1**	4.4 ± 0.4	11.6 ± 0.6	**11.3 ± 0.6**	7.8 ± 1.2	**11.4**	10.4	6.1	9.3
Untreated	8.7 ± 0.1	8.1 ± 0.2	4.0 ± 0.3	11.1 ± 0.3	10.0 ± 0.2	6.7 ± 1.1	9.9	9.0	5.3	8.0
Propino										
Endophyte	9.8 ± 0.1	**8.8 ± 0.2**	4.4 ± 0.3	11.2 ± 0.2	10.1 ± 0.2	8.1 ± 1.0	10.5	**9.5**	6.3	8.8
Untreated	9.2 ± 0.1	7.7 ± 0.2	4.0 ± 0.3	10.4 ± 0.1	9.8 ± 0.3	7.2 ± 0.6	9.8	8.7	5.6	8.0
MEAN										
Endophyte	**10.7**	**9.2**	**4.5**	**11.8**	**10.9**	7.7	**11.3**	**10.1**	6.1	9.2
Untreated	9.2	8.2	4.1	10.9	9.9	6.9	10.1	9.0	5.5	8.1

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Table 5. Mean barley 1,000 grain weight (TGW) g ± standard error. Statistically significant differences (ANOVA, $P < 0.05$) between endophyte and untreated treatments are highlighted in bold underlined italics

	Field trial location									
Cultivar	**Carrigtwohill**			**Backweston**			**Both locations combined**			**MEAN**
Treatment	**Full N**	**50% N**	**No N**	**Full N**	**50% N**	**No N**	**Full N**	**50% N**	**No N**	
Mickle										
Endophyte	53.4 ± 1.0	52.4 ± 0.8	52.9 ± 0.9	***60.0 ± 0.1***	***55.7 ± 1.3***	***59.4 ± 0.3***	56.7	54.1	56.1	55.6
Untreated	54.1 ± 0.8	54.0 ± 0.3	53.6 ± 0.7	58.6 ± 0.4	***59.1 ± 0.6***	58.2 ± 0.3	56.3	56.5	55.9	56.2
Planet										
Endophyte	***52.4 ± 0.2***	***54.1 ± 0.3***	***53.2 ± 0.1***	***58.2 ± 0.5***	***57.8 ± 0.1***	***59.5 ± 0.4***	55.3	***56.0***	56.4	55.9
Untreated	49.2 ± 0.7	51.2 ± 0.6	50.9 ± 0.3	57.0 ± 0.2	53.5 ± 0.7	56.2 ± 0.1	53.1	52.3	53.5	52.9
Propino										
Endophyte	***52.8 ± 1.1***	***52.8 ± 0.6***	52.6 ± 1.0	***59.6 ± 0.8***	***58.5 ± 0.3***	***57.7 ± 0.1***	56.2	***55.7***	55.2	55.7
Untreated	49.1 ± 0.4	50.3 ± 0.5	51.0 ± 0.7	56.5 ± 0.2	54.3 ± 0.1	55.7 ± 0.5	52.8	52.3	53.3	52.8
MEAN										
Endophyte	***52.9***	***53.1***	***52.9***	***59.3***	***57.3***	***58.9***	56.1	55.3	55.9	55.7
Untreated	50.8	51.8	51.8	57.4	55.6	56.7	54.0	53.7	54.2	53.9

Table 6. Rainfall data for the crop growing periods and associated endophyte-induced mean yield increase for three barley cultivars, over all nitrogen inputs*

	Historical	2015		2016		2017	
	Mean rainfall mm 1991–2015	Rainfall mm	Yield increase	Rainfall mm	Yield increase	Rainfall mm	Yield increase
Ballybane	423	467	3%	–	–	–	–
Carrigtwohill	374	–	–	480	3%	307	14.5%
Backweston	331	–	–	326	6%	298	10%

Note: *2015 and 2016 yield data from Murphy et al. (2017).

related to a greater overall endophyte-induced yield increase. If growth period rainfall was greater than the seasonal norm (averaged over 25 years from 1991 to 2015) then the endophyte treatment had relatively little effect on barley grain yield, whereas with lower than average rainfall the endophyte related yield increase was greater. A near normal amount of rain gave an intermediate endophyte-induced yield increase.

Over the three growing seasons on all sites, we found a positive correlation between the endophyte-induced yield increase and both the mean growing season air temperature ($r < 0.05$) and the total potential evapotranspiration ($r < 0.05$) (Table 7).

4. Discussion

The results from this study confirm, expand upon and add robustness to the positive endophyte effect on barley grain yield when endophyte source and target environments are matched. Lower rainfall, greater potential evapotranspiration and higher air temperature during the growing season were all significantly related to a greater overall endophyte-induced yield increase. The endophytes were originally recovered from *H. murinum* plants growing on dry sites experiencing relatively low rainfall and with a southern exposure, where the genotype-specific endophyte associations in these particular soils may contribute to environment-specific host needs (Lundberg et al., 2012). The endophytes may offer an extended genetic resource to the plants enabling increased drought stress resistance (Reynolds & Tuberosa, 2008).

When compared with historical data, both experimental field sites received relatively low rainfall during the growing season. The same degree of beneficial endophyte effect was not seen with previous results from wetter growing seasons (Murphy et al., 2017), so it seems that this endophyte consortium would be most effective for barley on sites that normally experience <330 mm rainfall during the growing season. It is of particular note that both sites experienced an exceptionally dry April and May, which is the critical early growth and establishment period for barley; April plus May

Table 7. Site specific climate data for the crop growing period showing mean endophyte-associated yield increase. Air temp. figures are means*

Site, year	Air temp. °C	Potential evapo., mm	Degree days below 15.5°C	Mean yield increase %, (t/ha)
Ballybane, 2015	11.9	334.3	597	2.0, (0.2 t/ha)
Carrigtwohill, 2016	12.7	362.2	505	3.0, (0.4 t/ha)
Backweston, 2016	12.7	365.9	576	11.0, (0.4 t/ha)
Backweston, 2017	13.0	378.4	534	12.0, (0.87 t/ha)
Carrigtwohill, 2017	13.3	374.8	410	12.0, (1.03 t/ha)

Note: *2015 and 2016 yield data from Murphy et al. (2017).

rainfall at Carrigtwohill was only 63 mm (compared with a more typical 150 mm) and was only 53.5 mm at Backweston (more typically 135 mm).

Large differences in yield were apparent between field sites for both endophyte and control treatments. The yields at Backweston were greater with every treatment, and especially for the control plots with no N input where the yield at Backweston was 2.8 t/ha greater. This may be due to the more favourable background soil fertility, where amounts of crucial yield-associated elements were greater (P (×3), Cu (×4.5), Mn (×3.7), Zn (×2.2)). Phosphorous may be the key determinant here, and the endophytes may have increased the mobilisation of this key nutrient (Owen, Williams, Griffith, & Withers, 2015). While yield in general was higher at Backweston for all treatments the relative endophyte-induced yield increase was similar (0.9 t/ha at Backweston, representing a 10% increase, versus 1.0 t/ha at Carrigtwohill, representing a 14% increase) showing that this endophyte consortium can be yield beneficial on sites with widely differing background soil fertility.

Endophyte-induced yield increases were recorded for all N input regimes, suggesting that the endophytes are equally effective regardless of the amount of fertiliser applied (Murphy et al., 2017). Remarkably, and probably of most interest to farmers, endophyte treatment with half N input recovered yield to that associated with untreated crops with standard N input. This would represent a tremendous economic saving for the farmer, for whom chemical fertiliser costs represent as much as 0.26 of direct costs (Connolly, Moran, & Galway, 2015), and these costs have generally risen over time (FAO, 2015b). Savings of such magnitude would be equally beneficial in reducing environmental pollution (Dobermann & Nelson, 2013).

Overall, TGW was greater with endophyte treatment for all cultivars on both sites, and in most individual treatment comparisons, indicating that the yield increase was not just due to a greater number of grains/heads. Achatz et al. (2010) also found that the basidiomycete fungal endophyte *Piriformospora indica* improved this trait in barley, but Waller et al. (2005) found no *P. indica* effect on TGW. Other yield traits that we did not record may have been contributory factors; Sanchez-Rodriguez et al. (2017) found that the endophyte-associated yield increase was associated with an increase in spike number.

While grain N content decreased along with decreasing N input, as expected (Ercoli, Lulli, Mariotti, Masoni, & Arduini, 2008), we found no endophyte associated effect on grain N%. Newsham (2011), in a meta-analysis of the effects of dark septate endophytes (DSE) on plant responses, reported DSE-associated increases in plant shoot N%, but this was a trait we did not measure. We did, however, detect a positive correlation between grain yield and both TGW and grain N%.

The endophyte associated yield increases were correlated with decreasing rainfall during the growing season, but the barley plants were not critically drought stressed in this study. However, we may look to other studies on drought stressed plants to gain some clues as to the mechanisms involved.

Our previous work with these endophyte strains indicated the preferential allocation of resources to aboveground tissue in drought stressed barley plants treated with the endophytes (Murphy, et al., 2015a), and this is supported by the endophyte-induced increases in grain yield found in the current study. The original source species from which the endophytes were recovered (*Hordeum murinum*) has been shown to preferentially use resources for seed production in drought (Myrna Johnston, Alfredo Olivares, & Carolina Calderón, 2009), and this crop wild relative association may also be a factor in the yield increases we found in the present study.

Kannadan and Rudgers (2008) found that endophytes caused *Poa alsodes* (grove bluegrass) plants to up-regulate water conservation mechanisms faster in response to drought, resulting in increased total plant biomass. Achatz et al. (2010) demonstrated that the positive effect of *P. indica* on grain yield is due to accelerated growth of barley plants early in development, and these early beneficial effects may be a factor in our study, particularly as the growing sites experienced ~60% less rainfall than normal during April–May.

Water-deficiency adversely affects crop growth by generating reactive oxygen species (ROS), and other studies have reported endophyte associated reductions in ROS by increasing the total polyphenol, reduced glutathione, catalase, peroxidase and polyphenol oxidase as compared to control plants (Khan et al., 2013; Torres, White, Zhang, Hinton, & Bacon, 2011). Drought tolerance has been reported to be influenced by endophyte-derived hormones. For example, abscisic acid and gibberellins produced by the endophyte *Azospirillum lipoferum* have been shown to be involved in alleviating drought stress symptoms in maize (Cohen, Travaglia, Bottini, & Piccoli, 2009).

Water deficit is the commonest environmental stress factor limiting plant productivity, and involves multiple modes of action (Bohnert & Jensen, 1996). However, the complexity of abiotic stress responses in plants make any precise identification of all the factors involved very difficult (Lata, Chowdhury, Gond, & White, 2018). Key processes of stress tolerance include signalling pathway components such as transcription factors, heat shock proteins, chaperones and late embryogenesis abundant proteins, ROS scavenging and synthesis of osmoprotectants (Augé & Moore, 2005). Ion and water transporters, and a range of related processes appear to be common across plant species (Langridge, Paltridge, & Fincher, 2006) and are obvious arenas of potential endophyte action. The presence of endophytes under water stress conditions causes differential accumulation of many metabolites (e.g. cytosine, diethylene glycol, galactinol, glycerol, heptadecanoate, mannose, oleic acid, proline, rhamnose, succinate and urea). Accumulation of these metabolites suggests that fungal endophytes influence plants to accumulate certain metabolites under water-stress (Dastogeer et al., 2017). Future work is required to establish the principal mechanisms involved in the endophyte associated barley yield increases that we have described in this study.

Application of these endophytes on a commercial scale will lead to significant benefits for the farmer, the consumer and the environment, with far-reaching consequences for the future sustainable production of barley crops. The potential to extend the technology by targeting endophytes recovered from other crop wild relatives growing under stressed conditions is vast, and represents a bright and exciting opportunity for developing microbial inoculants in other important crops.

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Cover Image
Source: Backweston field trial site in Mid-July 2017 (Carrigtwohill in Ireland)

Note
A patent for the use of these endophytes has been filed by Trinity College Dublin and University College Dublin (patent holders), and the use of these endophytes for biofertilisation and biocontrol purposes in cereal crop plants by third parties is subject to negotiated agreement with the patent holders and they may not be used without such permission.

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