Piotrowska M *et al*. Fungicide Sensitivity Monitoring in Cereals, Forest and Minor Crop Pathogens in the UK. In: Deising HB; Fraaije B; Mehl A; Oerke EC; Sierotzki H; Stammler G (Eds), "Modern Fungicides and Antifungal Compounds", Vol. VIII, pp. 135-140. © 2017 Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978 3 941261 15 0

Fungicide Sensitivity Monitoring in Cereals, Forest and Minor Crop Pathogens in the UK

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ABSTRACT

Fungicide sensitivity in three important fungal pathogens, *Ramularia collo-cygni, Dothistroma septosporum* and *Botrytis cinerea* was studied in the UK. The three pathogens have been subject to varying degrees of investigation. The first two examples are closely related *Mycosphaerella* species and are both considered moderate to high risk to the appearance of fungicide resistance. Our investigations indicate a slow decline in sensitivity to demethylation inhibitors in *R. collo-cygni,* but no change in sensitivity to organochlorines or succinate dehydrogenase inhibitors. We have established a baseline for sensitivity to fungicides for *D. septosporum. B. cinerea* is a relatively understudied pathogen of minor crops but our results indicate widespread resistance to quinone outside inhibitor fungicides.

INTRODUCTION

Barley (*Hordeum vulgare*) is one of the major world crops and the second most abundant component of UK cereal production. Ramularia leaf spot (RLS) is the common name given to late season necrosis attributed to the fungus *Ramularia collo-cygni* (*Rcc*) (Walters et *al.* 2007). RLS has been shown to reduce yield by up to 1.0 t/ha in susceptible varieties (Oxley & Havis 2004). RLS has moved from being an unreported disease to a major barley disease in the UK within 12 years. Symptoms usually appear post-flowering in the crop. Initial symptoms resemble small rectangular pepper spots on upper leaves. The spots often have a chlorotic halo and are bound by the leaf veins. Over time the symptoms coalesce to form large areas of necrotic tissue. Varieties have been shown to differ in their susceptibility to RLS (Havis et *al.* 2012), but there is no current effective varietal control available to growers and best control has to date been achieved with an effective late season fungicide spray (Havis et *al.* 2015).

Dothistroma septosporum is a causal agent of Dothistroma needle blight (DNB), a damaging disease of pines and other conifers worldwide. It causes the premature defoliation of needles, leading to reduced tree growth and in some cases to tree death (Fraser et *al*. 2015). Currently DNB is considered to be a threat to both exotic pine plantations and native Scots pine (*Pinus*

sylvestris) in the UK, the latter example raising concerns about the conservation of native Caledonian pinewoods (Brown et *al. 20*12).

Dothistroma needle blight is controlled by different management options including silvicultural methods aiming to reduce inoculum loads, use of alternative less susceptible hosts in the rotations and chemical control. Copper-based fungicides are known to be effective and widely used in DNB spread and prevention (Bulman et *al*. 2013). In addition to copper-based products, single site inhibitors are used on nursery stocks in the UK and their use may increase as copper containing fungicides are withdrawn. The effectiveness of such products against *D. septosporum* remains however unknown and therefore there was a need to establish the sensitivity profiles to major fungicide classes (Piotrowska et *al.* 2016b).

Botrytis cinerea is a necrotrophic fungus that causes symptoms *(*grey mould) on a wide range of food plants, especially grapevine, tomato, soft fruits and vegetables. This disease brings about important economic losses in both pre- and postharvest crops. (El Oirdi & Bourab 2007). Control programmes are based on the use of a limited range of active fungicides, including two groups identified as high risk in terms of resistance development, i.e. quinone outside inhibitors (QoIs) and succinate dehydrogenase inhibitors (SDHIs). Little is known about the current UK resistance status of *B.cinerea* or about the risk of resistance appearing to the newer fungicide groups.

MATERIALS AND METHODS

Ramularia collo-cygni isolates were produced by careful excision of conidiophores from the surface of infected barley leaves. Leaf samples, were collected from SRUC field trial sites during five cropping seasons. Single spore *Rcc* cultures were produced by careful isolation of conidia from infected lesions onto potato dextrose agar supplemented with the antibiotic streptomycin. Mycelial suspensions were produced from single spore isolates grown in alkyl ester broth for 10 days at 16°C on an orbital shaker. The suspensions were filtered and diluted to a concentration of 2.5 x 10^3 pieces of mycelium per ml. (Piotrowska et *al. 20*16a). The 96 well plate assay was conducted using final fungicide concentrations of 50, 10, 5, 1, 0.5, 0.1 and 0.05 ppm in a total volume of 200 µl (100 µl of mycelial suspension added to 100 µl of fungicide amended media). Plates were incubated at 16 ^{o}C, with continuous shaking, for one week before being read in FLUOstar Omega plate reader (BMG Labtech, Germany) at a wavelength of 400 nm. EC_{50} values were calculated by the MARS data analysis software (BMG Labtech). A number of technical grade fungicides were used in sensitivity assays. Succinate dehydrogenase inhibitors (SDHIs); isopyrazam, bixafen, fluxapyroxad, boscalid; demethylation inhibitors (DMIs); prothioconazole-desthio, epoxiconazole; organochlorine chlorothalonil; anilinopyrimidine cyprodinil.

In order to study the resistance status of *Dothistroma septosporum*, we tested selected active ingredients on isolates from forest stands, which had not been exposed to fungicides, to obtain the range of baseline sensitivity and isolates from nursery outbreaks (received from Alice

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Holt, Forest Research, UK) to evaluate if any shifts in sensitivity to major single site fungicide classes has already occurred in the nursery situation. In total we tested six fungicide classes: QoIs (azoxystrobin), DMIs (prothioconazole-desthio, propiconazole), phenylamides (PA, metalaxyl-M), anilinopyrimidines (cyprodinil), dicarboximides (iprodione) and SDHIs (boscalid) (Piotrowska et *al.* 2016b).

The resistance status of *B. cinerea* was studied by collecting a range of single spore isolates from vegetable, soft fruit and flower crops in the UK. Resistance to the QoI fungicides was tested by a molecular assay (restriction digest) to identify the presence or absence of the G143A mutation (Fountaine & Fraaije 2009). Sensitivity to a range of fungicides was examined using a recently developed multiwell plate assay (Mackenzie, unpublished).

RESULTS

Table 1 shows that *Rcc* sensitivity to SDHI fungicides between 2010 and 2015 appears stable and that the newer products (isopyrazam (iso), bixafen (bix) and fluxapyroxad (flux) appear more effective in *in vitro* testing per g of a.i than the older carboxamide, boscalid (bos). Results for prothioconazole-desthio (pro) suggest that *Rcc* isolates tested seemed to be less sensitive in 2015. In general the isolates were more sensitive to pro than the older DMI epoxiconazole (epo). The multisite fungicide chlorothalonil (chlor) was very effective against *Rcc* in the testing system.

Table 1 Mean EC_{50} values (µg/ml) for *Rcc* isolates to test fungicides (full names see text).

Year	iso	bix	bos	flux-	pro	chlor	cyp	epo
2010	0.03	0.02	0.08	0.11	*	*	*	*
2011	0.02	0.01	0.13	0.13	*	*	*	*
2012	0.02	0.02	0.15	0.04	0.03	*	*	0.40
2013	*	*	*	0.05	0.04	0.04	0.53	0.37
2015	*	*	*	0.15	0.21	0.06	0.79	1.44

* No testing done in this year

The sensitivity assay results in Table 2 suggest that QoIs, DMIs and SDHIs are effective against *D. septosporum* in *in vitro* tests, with low mean EC_{50} values for azoxystrobin of 0.009 mg/l, prothioconazole-desthio of 0.002 mg/l, propiconazole of 0.012 mg/l and boscalid of 0.236 mg/l. The remaining three classes of fungicides, PA, anilinopyrimidines and dicarboximides were ineffective in *D. septosporum* control *in vitro* as no reduction in pathogen growth was observed at the highest concentration tested. There were no detectable difference in sensitivity in nursery isolates as compared to forest isolates to fungicides classes tested *in vitro* (Piotrowska et *al*. 2016b).

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Table 2 EC_{50} (µg/ml) values for *D. septosporum* isolates to various fungicide groups.

Fungicide	Native forest (EC_{50})	Nursery isolates (EC_{50})
Azoxystrobin (QoIs)	0.003-0.023	0.004-0.042
Prothioconazole (DMIs)	0.001-0.006	0.001-0.004
Propiconazole (DMIs)	0.006-0.041	0.005-0.043
Boscalid (SDHIs)	0.102-0.514	0.100-0.598
Cyprodinil (AP)	8.398-100	-
Iprodione (Dicarb.)	100	-
Metalaxyl-M (PA)	100	-

The results summarized in Table 3 show that almost all of the *B. cinerea* isolates tested carried the G143A mutation which confers resistantance to the QoI fungicides. Only one isolate from trees did not carry the mutation. Initial results from the mutiwell assay show pyraclostrobin has higher EC_{50} value than boscalid or fludioxonil.

Table 3 Presence of G143A mutation in *B. cinerea* isolates by crop type and year.

Year	Crop type	No of isolates tested	Resistant isolates	Sensitive isolates
2013	Vegetable	6	6	0
	Flowers	10	10	0
	Tree	5	4	1
2014	Vegetable	10	10	0
	Fruit	5	5	0
	Flowers	6	6	0

DISCUSSION

The current reliance on fungicides for control of RLS makes the careful stewarding of active ingredients a pressing concern. Varietal resistance to RLS is moderate at best and not complete (AHDB 2016). The closely related pathogen *Zymoseptoria tritici* has seen a rapid evolution of resistance to QoI fungicides and a slower decline in the efficacy of DMI fungicides (Cools & Fraaije 2008). Resistance to QoIs in *Rcc* appeared at a similar time to the appearance of the same G143A genetic mutation in *Z. tritici* (Fountaine & Fraaije 2009). The results in this study indicate a slow decline in the sensitivity to the DMI fungicides, prothioconazole and

epoxiconazole. There have also been reports of *Rcc* isolates with reduced sensitivity to SDHI fungicides (FRAC 2016). UV mutations in the fungus have been produced and characterised in controlled conditions (Piotrowska et *al*. 2016a). The results presented indicate no shift in sensitivity to the SDHI fungicide, fluxapyroxad for Scottish isolates up until the 2015 season but ongoing monitoring of the resistance situation is required.

We established the range of baseline sensitivity of *D. septosporum* isolates to some of the major single site inhibitors classes *in vitro*, which can be used in the future monitoring programs. For the present moment there is no fungicide resistance development in nurseries in the UK. However, nurseries are using high risk fungicides such as QoIs or DMIs, and therefore fungicide resistance management guidelines should be adapted in regular disease programs. These could draw on experience from broad acre crops, where resistance management practices are well established and documented throughout the years (Piotrowska et *al*. 2016b).

The results presented here indicate that in *Botrytis cinerea* resistance to the QoI fungicides is widespread in the UK. The development of new assays will allow the testing of the sensitivity of *B. cinerea* isolates to a number of fungicides. Multiple resistances to fungicides have been reported in *B. cinerea* isolates from vineyards in Germany (Leroch et *al. 20*11). Establishment of the levels of resistance to fungicides in the UK will allow the design of more effective control strategies.

All three examples demonstrate the need for baseline sensitivity data and on-going monitoring so that changes in sensitivity can be rapidly intimated to relevant stakeholders and guidelines developed. Information on many pathogen-fungicide combinations is incomplete, particularly for minor crops or for pathogens affecting tree species.

ACKNOWLEDGEMENTS

The authors would like to thank Carolyn Riddell, Richard Ennos, Peter Hoebe, Kalina Gorniak and Jeanette Taylor for their help in completing this work. The *Ramularia* research reported in this paper was partially funded by the Rural & Environment Science & Analytical Services Division of the Scottish Government. *Dothistroma septosporum* research was funded jointly by a grant from BBSRC, Defra, ESRC, the Forestry Commission, NERC and the Scottish Government, under the Tree Health and Plant Biosecurity Initiative. The *Botrytis cinerea* research was carried out as part of a DEFRA funded project (PS2726).

REFERENCES

AHDB (2016). Spring barley Recommended list 2016. Available online *[http://cereals.ahdb.org.uk/varieties/ahdb-recommended-lists/spring-barley-2016.aspx]*

Brown A V; Stone C; Clayden H (2012). Dothistroma needle blight: GB strategy. Edinburgh, UK: Forestry Commission.

Bulman LS; Dick MA; Ganley RJ; McDougal R; Schwelm A; Bradshaw RE (2013). Dothistroma needle blight. In: *Infectious Forest Diseases*. Nicolotti, G. and Gonthier, P (Eds.). Wallingford, UK: CAB International.

Cools HJ; Fraaije B (2008). Are azole fungicides losing ground against Septoria wheat disease? Resistance mechanisms in *Mycosphaerella graminicola*. *Pesticide Management Science* 64, 681-684.

El-Oirdi M; Bourab K (2007). Plant signalling components EDS1 and SGT1 enhance disease caused by the necrotrophic pathogen *Botrytis cinerea*. *New Phytologist* 175, 131-139.

FRAC (2016). Available online [*www.frac.info/.../sdhi.../sdhi.../minutes-of-the-2015-sdhi-meeting-recommendations-for-2016.pdf]*

Fraser S; Martin-Garcia J; Perry A; Kabir MS; Owen T; Solla A; Brown AV; Bulman LS; Barnes I; Hale MD; Vasconcelos MW; Lewis KJ; Dogmus-Lehtijarvi HT; Markovskaja S; Woodward S; Bradshaw RE (2015). A review of Pinaceae resistance mechanisms against needle and shoot pathogens with a focus on the *Dothistroma-Pinus* interaction. *Forest Pathology* 46, 453-471.

Fountaine J; Fraaije BA (2009). Development of QoI resistant alleles in populations of *Ramularia collo-cygni*. *Aspects of Applied Biology* 92, 123–126.

Havis ND; Oxley SJP; Burnett FJ; Hughes G (2012). Epidemiology of *Ramularia collo-cygni. Proceedings Crop Protection in Northern Britain* 2012, 119-124.

Havis ND; Brown JKM; Clemente G; Frei P; Jedryczka M; Kaczmarek J; Kaczmarek M; Matusinsky P; McGrann GRD; Pereyra S; Piotrowska M; Sghyer H; Tellier A; Hess M; (2015). *Ramularia collo-cygni* - an emerging pathogen of barley crops. *Phytopathology* 105, 895-904.

Leroch M; Kretschmer M; Matthias M (2011). Fungicide resistance phenotypes of *Botrytis cinerea* isolates from commercial vineyards in South West Germany. *Journal of Phytopathology* 159, 63-65.

Oxley SJP; Havis ND (2004). Development of *Ramularia collo-cygni* on spring barley and its impact on yield. Proceedings Crop Protection in Northern Britain 2004, 147-152.

Piotrowska MJ; Fountaine JMF; Ennos RA; Kaczmarek M; Burnett FJ (2016a). Characterisation of *Ramularia collo-cygni* laboratory mutants resistant to succinate dehydrogenase inhibitors. *Pesticide Management Science* DOI: 10.1002/ps.4442.

Piotrowska MJ; Ennos RA; Riddell C; Hoebe PN (2016b). Fungicide sensitivity of *Dothistroma septosporum* isolates in the UK. *Forest Pathology* DOI: 10.1111/efp.12314**.**

Walters DR; Havis ND; Oxley SJP (2008). *Ramularia collo-cygni*: the biology of an emerging pathogen of barley. *FEMS Microbiology Letters* 279, 1–7.