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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- Fungal isolates were obtained from orchards experiencing difficulties in controlling scab and these are being tested for their sensitivities to three fungicides.

Background and expected deliverables

One consequence of fungicide use is the selection of fungal strains less sensitive to the fungicide used and so disease management strategies have been developed in order to reduce the risk of emergence and spread of these insensitive fungal strains. Overuse of DMI fungicides has led to the emergence of scab strains less sensitive to DMI fungicides in the USA and Canada. In the long-term this may lead to loss of disease control. There is some anecdotal evidence that isolates from DMI-sprayed orchards in the UK appear to have an overall reduced sensitivity to myclobutanil (Systhane), which is commonly observed in other regions. However, failure in scab control is often due to poor spray timing and/or cover, rather than other factors such as reduced sensitivity.

A few recent studies showed that cross-resistance of the scab fungus to fungicides may or may not exist, depending on the particular fungal populations concerned. If reduced sensitivity to one fungicide exists, then care is needed to select alternative products without jeopardising disease control and resistance management. However, necessary knowledge on cross-resistance that is required to make such rational selection decisions is not yet available.

Recent Canadian research suggests independent resistance mechanisms to myclobutanil (Systhane) and kresoxim-methyl (Stroby) but a positive correlation in resistance to myclobutanil (Systhane) and flusilazole (various products not approved on tree fruit). This result does not agree with results obtained in New York State. More recent research on other pathogens also suggests that the presence and the extent of cross-resistance depends on the particular fungal populations and fungicides concerned. It is therefore necessary to carry out research for each particular fungal population to understand the potential of cross-resistance to particular fungicides. In any anti-resistance strategy, information on cross-resistance is critical to devising control strategies in cases where reduced sensitivities to one fungicide have been observed. There has been no published information on the baseline sensitivity and the current status of sensitivity to common scab fungicides in the UK scab population.

Recently, HDC-funded EMR to conduct a preliminary investigation on the cross-resistance of apple scab (with a limited number of isolates) to all scab fungicides registered in the UK (HDC project TF 190). Most of the observed cross-resistance to fungicides is expected on the basis of the chemistry. However, the strong correlation in the sensitivity to dithianon (a multi-site action fungicide) with DMI fungicides (e.g., myclobutanil and fenbuconazole) was unexpected, and is also worrying since dithianon and DMI fungicides are often used in the same spray programmes.

Understanding scab population structure with particular reference to its virulence (race) is critically important for a breeding programme and effective deployment of current varieties with different resistance genes. Recently, the nomenclature of scab races and the corresponding resistance genes has undergone extensive revision, culminating in the proposition of a new nomenclature system in 2011. Consequently, a new set of indicator genotypes is proposed to differentiate scab races.

Scientists plan to establish a plot at EMR with 19 proposed indicator genotypes for future monitoring of scab race structure, joining a world-wide monitoring programme. This monitoring will generate valuable information, not only for growers in terms of cultivar deployment but also for breeders in terms of breeding for resistance and for pathologists in terms of predicting the spread of new virulence.

Expected deliverables and benefits

- We will establish a plot at EMR with 16-19 indicator genotypes for monitoring of scab race structure, joining a world-wide monitoring programme.
- Monitoring of the scab race structure will generate valuable information not only for growers in terms of cultivar deployment but also for breeders in terms of breeding for resistance and for pathologists in terms of predicting spread of new virulence.
- This project will generate information to confirm whether there is significant correlation in the scab response (sensitivity) to dithianon and myclobutanil.
- This correlation, if confirmed, will have significant impact on our current scab control programme.

Summary of the project and main conclusions

EMR scientists have collected 30 single spore isolates from several orchards where scab control was problematic in recent years. These isolates are currently being tested for their sensitivities to myclobutanil, fenbuconazole and dithianon. It is too early to report any substantiated conclusions or results.

The scientific team has obtained graft wood of all indicator genotypes for testing scab races from Switzerland and has successfully grafted them to rootstocks. There are four or five plants per indicator genotype. They have been re-potted and will be planted out in an orchard at East Malling Research. These plants will be regularly monitored to assess the race profile of the scab fungus which will contribute to global research on understanding the scab race structure over time.

Financial benefits

Growers will benefit from the project results in the following ways:

1) Use of correct products as alternations to control scab and minimise the establishment and subsequent spread of scab strains that are insensitive to fungicides.
2) Maintaining a good range of effective fungicides against scab to achieve effective control.
3) Planting apple cultivars with an appropriate resistance background, selected partially on the basis of scab monitoring results on the indicator genotypes in the future.

Action points for growers

There are insufficient results yet to list any actions points for growers.

SCIENCE SECTION

Introduction

Apple scab, caused by *Venturia inaequalis* (Cke.) Wint., is an economically important disease of apples worldwide. The disease attacks leaves, flowers, fruits, shoots and bud scales. Scab control is usually achieved by the use of fungicide sprays applied routinely from bud burst at 7-10 d intervals until the risk of scab ceases. Demethylation inhibitor (DMI) fungicides are probably the most important group of fungicides for scab control in the UK because of their curative action against scab and their control of powdery mildew, which in the UK is the next most important disease of apples. Overuse of DMIs in some countries has led to selection and subsequent establishment of *V. inaequalis* strains that are less sensitive to DMIs (Stanis & Jones, 1985, Thind *et al*., 1986, Hildbrand *et al*., 1989, Braun & McRae, 1992, Köller *et al*., 1997, Jobin & Carisse, 2007). Resistance to DMIs is believed to be quantitative (Georgopoulos & Skylakakis, 1986, Köller & Scheinpflug, 1987, Smith *et al*., 1991); consequently, the loss of sensitivity by the pathogen tends to be gradual.

Information on cross-resistance is critical for devising control strategies where reduced sensitivity to one fungicide is observed. Previously, for *V. inaequalis* there appeared to be a lack of cross-resistance between dodine, the benzimidazoles and the DMIs. The efficacy of scab control achieved with benzimidazoles in orchards with fungal strains resistant to dodine was high (Jones, 1981) and DMIs controlled scab effectively in orchards located within a region known for widespread dodine and benzimidazole resistance (Wilcox *et al*., 1992). Recent research indicated, however, that the mechanisms of resistance to DMIs and dodine fungicides might not be entirely independent (Köller & Wilcox, 1999, Köller & Wilcox, 2000). Isolates of *V. inaequalis* selected for phenotypic traits of resistance to dodine or benomyl remained as sensitive to other classes of fungicides; but isolates that were already resistant to dodine were prone to accelerated adaptations to other fungicides.

In the UK most apple growers apply spray programmes made up of combinations of fungicides which differ in mode of action, to achieve better control and minimise the risk of fungicide resistance. The use of DMI fungicides in the programme has increased in recent years, mainly because of the lack of alternative products to control powdery mildew. Two common DMI fungicides used in the UK to control scab are Systhane (a.s. myclobutanil) and Indar (a.s. fenbuconazole); the former is more widely used because of its efficacy against powdery mildew. There is some evidence for reduced sensitivities to myclobutanil in the UK (Roberts & Crute, 1994, Gao *et al*., 2009); nevertheless, poor scab control in sprayed orchards can usually be attributed to poor spray timing and/or extended spray intervals due to unfavourable weather (Berrie, pers comm.). Due to the lack of alternatives in controlling

scab and powdery mildew, growers sometimes continue to use DMI fungicides, even in orchards with reduced sensitivity to DMIs.

Recently, HDC-funded EMR to conduct a preliminary investigation on the cross-resistance of apple scab (with a limited number of isolates) to all scab fungicides registered in the UK (HDC project TF 190). Most of the observed cross-resistance to fungicides is expected on the basis of the chemistry. However, the strong correlation in the sensitivity to dithianon (a multi-site action fungicide) with DMI fungicides (e.g. myclobutanil and fenbuconazole) was unexpected and also worrying, since dithianon and DMI fungicides are often used in the same spray programmes.

Understanding scab population structure with particular reference to its virulence (race) is critically important for a breeding programme and effective deployment of current cultivars with different resistance genes. Recently, the nomenclature of scab races and the corresponding resistance genes has undergone extensive revision, culminating in the proposition of a new nomenclature system in 2011. Consequently, a new set of indicator genotypes is proposed to differentiate scab races.

Defra is funding a project on scab epidemiology and genetics at EMR. In the Defra project, we shall collect many isolates from different orchards for molecular characterisation. These isolates can also be used for testing insensitivities to fungicides, thus saving cost.

We propose to establish a plot at EMR with 19 proposed indicator genotypes for future monitoring of scab race structure, joining a world-wide monitoring programme. This monitoring will generate valuable information, not only for growers in terms of cultivar deployment but also for breeders in terms of breeding for resistance and for pathologists in terms of predicting spread of new virulence.

Materials and methods

Collection of samples

Samples of leaves or fruit with actively sporulating lesions of apple scab were obtained from various orchards in the summers of 2010 and 2011 via several consultants; these orchards all experienced problems in controlling scab. In total we have samples from seven orchards where scab control was difficult in 2010 or 2011. Once delivered to the lab at EMR, actively sporulating lesions were cut out from the leaves or fruit using a cork borer. The lesions were then laid out on paper towels and allowed to air dry for 48 h. The scab lesions were individually placed into a micro-centrifuge tube and stored at -18°C until required.

Obtaining single-spore isolates

In order to produce reliable test results, we have isolated single spore isolates from sampled leaf discs. We aimed to obtain 10-15 single spore isolates for sampling site. Water agar (WA, 15 g/L), amended with rifamycin (50 mg/L) (Sigma-Aldrich, Poole, UK) after autoclaving, was used to obtain single-spore isolates. An infected leaf disc (stored at -18°C) was added to deionised water and agitated thoroughly to release conidia. Conidial suspensions were adjusted to $8x10^3$ per ml and 200 µl of suspension pipetted onto the rifamycin-amended WA plates and spread evenly. After incubation at 15°C for 24 h, individual germinated spores were excised using a needle under a microscope in a laminar flow cabinet, and placed on potato dextrose agar (PDA) plates. Plates were incubated at 15°C and, when large enough, each colony was transferred to a fresh PDA plate.

In vitro testing for fungicide sensitivity

Fungal isolates were tested for sensitivity to three fungicides (Table 1). For those fungicides with two active substances, we obtained formulated products with each active substance (a.s.) for ease of data interpretation. The concentrations for testing were determined from the previous HDC-funded project (TF 190) and for each fungicide, five concentrations were used. A stock solution for each fungicide was prepared and an appropriate amount of the stock solution was used to amend water agar to produce agar plates with the required concentrations of each fungicide. Rifamycin was also added to the agar (0.05 µg/ml) to reduce bacterial contamination.

Table 1. List of fungicides and their a.s. and testing concentrations (ppm, mg/L) used for testing scab isolates

Product and a.s.	Mode of action	FRAC code	Concentration					
			C1	C2	C3	C4	C5	C6
Systhane - myclobutanil	Curative (DMI)	G1(3)	0	0.01	0.1	0.5	2	5
Indar - fenbuconazole	Curative (DMI)	G1(3)	0	0.01	0.05	0.25	0.5	1
Dithianon - dithianon	Both (?) (MSC)	M9	0	0.01	0.1	0.5	2	5

Curative effect

Mycelial plugs of 4 mm size mycelial were cut from each isolate and placed on to the agar plates with each concentration of specific fungicide (Table 1). For each combination of concentration, isolate and fungicide there were two replicate plates. On each plate there were three mycelial plugs that were 5 cm from each other. The plates were incubated at 20°C in the dark. The length and width of each colony were measured 4-5 weeks after incubation.

Data analysis

In several cases, where the fungal growth/development was much greater in fungicide-amended plates than in untreated plates, we substituted the fungal development without fungicides with the larger development value. ED_{50} values were estimated by fitting an exponential or a linear by linear quotient model to the fungal growth data (adjusted for initial plug size). It is not possible to fit a single model type to all the data sets; thus, the better of the two models (on the basis of the percentage of variance accounted for) was chosen for each sample to estimate the ED_{50} value. For a few data sets, both models failed to fit the data but the fungal development was clearly reduced by more than 50%; we used the simple linear interpolation to estimate the ED_{50} value. In several other cases it was not possible to estimate the ED_{50} value and fungal growth was only reduced slightly within the fungicide concentration tested; for these cases, we simply indicate that the ED_{50} value was greater at the largest concentration tested. Both normal and Spearman correlation coefficients were calculated between ED_{50} values for each pair of fungicides. Genstat (Payne, 2006) was used in all analyses.

Establishing a plot with scab race indicator genotypes

Graft bud wood of all indicator genotypes was obtained from Switzerland, grafted onto MM106, potted up and maintained in a sand-bed.

Results

Isolate collection and testing

We collected 30 single spore isolates from several orchards where scab control was problematic in recent years. We have just started testing them for their sensitivities to myclobutanil, fenbuconazole and dithianon. We have already completed the testing for three isolates.

Establishing a plot for monitoring scab race structure

We have obtained graft wood of all 19 indicator genotypes for testing scab races from a Swiss researcher Dr Andrea Patocchi (Agroscope Changins-Wädenswil Research Station ACW, Schloss, PO Box 8820, Wädenswil, Switzerland) and have successfully grafted them onto rootstocks. There are four or five plants per indicator genotype. They have been re-potted and maintained in a sand-bed. We plan to plant them out in 2014. These plants will be regularly monitored to assess the race profile of the scab fungus and contribute to global research on understanding the scab race structure over time.

Conclusions

- Sufficient numbers of isolates were obtained from orchards experiencing difficulties in controlling scab. These are being tested for their sensitivities to the three fungicides.
- Indicator genotypes were successfully grafted to the rootstocks and will be planted out in 2014 at EMR.

Technology transfer

Because of the sensitivity of this work, we purposely did not actively involve ourselves with technology transfer activities with growers until we have obtained clearer results.

- We held in-depth discussions of the work with BASF researchers and agreed to exchange isolates for testing sensitivities to fungicides in March-April 2013

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