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Development of apothecia of *Tapesia yallundae* in contrasting populations selected by fungicides

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Abstract

Apothecia of the eyespot fungus, *Tapesia yallundae*, were found on 0–18% of straws in plots of wheat stubble in February–March 1994. The fungicides carbendazim, prochloraz or carbendazim plus prochloraz had been applied repeatedly to the same plots in each of the previous 9 years in which successive wheat crops had been grown. The factors most strongly correlated with the incidence of apothecia were the incidence and severity of eyespot in the preceding wheat crop and the frequency of carbendazim-resistant W-type fungus in populations recovered from that wheat crop. Plots treated with carbendazim, which had previously had more disease and more resistance to carbendazim in the pathogen population relative to untreated plots, therefore yielded most apothecia. Plots treated with prochloraz, which had selected for predominantly R-type fungus and decreased eyespot, yielded few apothecia. Single-ascospore isolates were all of the W-type and were more frequently carbendazim-sensitive than expected, except those from plots treated only with carbendazim. None showed decreased sensitivity to prochloraz. The implications of applying fungicides regularly for controlling eyespot on the capability of the eyespot fungus for genetic variation through sexual reproduction are discussed.

Introduction

Since the discovery of the apothecial stage of the cereal eyespot fungus, *Tapesia yallundae* (anamorph *Pseudocercosporella herpotrichoides*, in Australia [Wallwork, 1987] it has been found on straw stubble in several other countries, including England [Hunter, 1989; Dyer *et al.*, 1994b]. Its occurrence here and in other European Community countries may have increased because of the current policy of set-aside [Ansell and Tranter, 1992]. However, for apothecia to be produced, set-aside fields after cereals need to be left uncultivated, with standing straw stubble, through the main period of their production, which in England is from January to March [Dyer *et al.*, 1994a]. The use of fungicides may also influence apothecial production. This is because prochloraz, the fungicide used most widely for eyespot control [Polley, Slough and Jones, 1993], selects for the R-type fungus [Hoare, Hunter and Jordan, 1986; Bateman *et al.*, 1990], and sexual reproduction has been found only rarely in this type, occurring mainly between compatible strains of the W-type fungus [Dyer *et al.*, 1993]. The ability of a population of the fungus to produce apothecia is potentially of great importance in leading to greater variation, conferring the ability to respond to selection pressures, and allowing long range dispersal of airborne ascospore inoculum.

In a long term field experiment at Rothamsted, populations of the eyespot fungus selected by repeated applications of fungicides have been characterised by annual monitoring [Bateman *et al.*, 1990, 1995]. A need to put the experimental site into rotational set-aside for one year created a unique opportunity to study the development of apothecia, and to characterise the ascospore progeny. The relationships of these traits to the severity of infection in the preceding crop and to the population structure (in terms of pathogenicity

type and sensitivity to fungicides) of the pathogen as selected by fungicides were investigated.

Materials and methods

Meadow field at Rothamsted was put into set-aside after a twelfth successive wheat or barley crop. The previous wheat crop was harvested on 16 August 1993; the straw was then removed and the site left uncultivated. The experiment on the site, described previously [Bateman *et al.*, 1990; Bateman and Fitt, 1991] had two blocks of a split-plot design, with four whole-plots per block. The whole-plots were untreated or had been treated twice yearly with carbendazim (as Bavistin), prochloraz (as Sportak) or carbendazim plus prochloraz (as Sportak Alpha or Sportak plus Bavistin) in each of the preceding nine years, when winter wheat was grown. The same treatments were applied to the same plots in each year. Each whole-plot was split into six sub-plots (6 m × 4.5 m, separated by a minimum of 3 m of crop) that had been inoculated in autumn 1984 to achieve populations of *P. herpotrichoides* that had different proportions of W-type and R-type fungus and of carbendazim-sensitivity and carbendazim-resistance.Two sub-plots in each whole plot were uninoculated. By 1987, the populations in the sub-plots were unrelated to the inoculation treatments but there were large differences as a consequence of the fungicide treatments to the whole plots [Bateman *et al.*, 1990].

At least 100 standing straws were taken from random positions in each sub-plot between 28 February and 21 March 1994. They were stored at 4 °C in large plastic bags until assessed. Each straw was examined for the presence of apothecia of *T. yallundae* using a dissecting microscope. Those straws with apothecia and some without were returned to cold storage.

Single ascospore isolates were obtained as described previously [Dyer *et al.*, 1994a] from a minimum of three separate groups of apothecia per fungicide treatment. Each straw piece bearing apothecia was fixed with petroleum jelly to the inverted base of a 5 cm Petri dish over 3 ml 0.01% (v/v) Tween 80 solution in another 5 cm Petri dish base. The two Petri dish bases were sealed together with tape. After at least 48 h at 15 °C, an aliquot (0.5 ml) of each discharge was transferred to a 9 cm Petri dish containing water agar with antibiotics. After a further 48 h at 15 °C, germinated ascospores were transferred to water agar and incubated under white light or near-ultraviolet light at 15 °C. *Tapesia yallundae* was identified by its characteristic conidia. All cultures from single ascospores were prepared before the end of April 1994.

Table 1. Effects of repeated applications of fungicides to winter wheat on development of apothecia of *Tapesia yallundae* on standing straw stubble

Treatment to plots	Logit % straws with apothecia (back-transformed %)	
None	−1.691	(3.3)
Carbendazim	−1.064	(10.6)
Prochloraz	−2.756	(0.4)
Carbendazim + prochloraz	−2.710	(0.4)
S.E.D. (D.F. = 3)	0.2840	
P	0.02	

Isolates were characterised by transferring 4 mm plugs from colonies on water agar to one-fifth-concentration potato dextrose agar (PDA) [Creighton and Bateman, 1991], unamended or containing carbendazim at 2 mg l^{-1}. After 10–14 days at 20 °C, colonies were identified as W-type or R-type by colony morphology and as sensitive or resistant to carbendazim according to whether or not they grew on the carbendazim-amended agar. Further subcultures were made onto one-fifth PDA containing prochloraz at 2 mg l^{-1} to identify isolates with decreased sensitivity to this fungicide [Bateman, 1990].

The effects of fungicides applied to the plots in the preceding years on the incidence of apothecia were determined by analysis of variance. The inoculation treatments were included as a factor in the analyses but they had no effects and no further reference is made to them. Regression analyses were made of the incidence of straws with apothecia in the 1994 stubble on the incidence of eyespot and the proportions of different types of the eyespot fungus in the 1993 crop.

Results

Apothecia of *T. yallundae* were found on a greater proportion of straws from plots that had been treated with carbendazim than were untreated (Table 1). The smallest proportions of straws with apothecia were from plots treated with prochloraz, with or without carbendazim.

Regression analyses showed significant associations between the percentages of stems with apothecia

Table 2. Regressions of percentage straws with apothecia of *T. yallundae* in standing straw stubble (y) on percentage of stems with eyespot and with different types of the eyespot fungus in the preceding wheat crop (x)

x variate*	Regression equation	*P*	Variance accounted for (%)
Stems with eyespot	y = 0.986x−3.25	<0.001	53.8
Stems with moderate-severe eyespot	y = 1.293x−2.16	<0.001	44.9
Stems with Ws	y = −0.067x−2.12	>0.05	–
Stems with Wr	y = 0.714x−1.92	<0.001	45.8
Stems with Rs	y = −0.511x−2.49	<0.01	13.0
Stems with Rr	y = 0.191x−2.10	>0.05	–
R in population	y = −1.332x−1.80	<0.001	36.3
r in population	y = 0.501x−2.35	<0.01	13.4

* All data are from the 1993 crop [Bateman *et al.*, 1995]. The variates (x and y) are percentages transformed to logits. W = W-type; R = R-type; s = carbendazim-sensitive; r = carbendazim-resistant.

and the incidence of eyespot, especially moderate and severe eyespot, in the preceding wheat crop (Table 2). The incidence of apothecia was also associated with the frequency of infection by carbendazim-resistant W-type fungus in the preceding crop and, negatively, with the incidence of infection by carbendazim-sensitive R-type fungus. Greatest numbers of apothecia were associated with populations in the preceding crop that had most W-type or most carbendazim-resistant fungus. However, only a relatively small proportion of the total variance was accounted for by each of the regressions. Multiple regression analyses (not shown) were made of percentage straws with apothecia on combinations of three of the variates shown to be most strongly correlated by the single regressions, i.e. % stems in the preceding crop with eyespot, % stems with carbendazim-resistant W-type fungus and % R-type in the fungus population. The percentages of variance accounted for were increased little or not at all over those for the single regressions.

A total of 311 single ascospore isolates were recovered. All had W-type morphology. Seven of the 12 untreated plots yielded, respectively, 3, 10, 16, 18, 18, 19 and 20 isolates, of which 0, 0, 3, 0, 5, 9 and 6 were resistant to carbendazim. Eleven carbendazim-treated plots yielded, respectively, 3, 5, 5, 6, 9, 13, 16, 17, 19, 20 and 45 isolates, all of which were resistant to carbendazim. Isolates were usually obtained from at least three different apothecial groups from each plot with one of these treatments. Expected numbers of carbendazim-resistant isolates (Table 3) were calculated from the proportions of W-type isolates from the 1993 crop that were carbendazim-resistant in those plots that yielded the single ascospore isolates. Comparing the expected numbers with the observed numbers showed that apothecia from plots that were untreated or treated with carbendazim plus prochloraz yielded significantly fewer carbendazim-resistant single ascospore isolates than expected, whilst those from plots treated only with carbendazim yielded more than expected (Table 3).

None of the isolates showed any growth on agar amended with prochloraz.

Discussion

Apothecia of *T. yallundae* were found in appreciable numbers on straw stubble in plots on Meadow field, Rothamsted. This does not necessarily indicate that sexually compatible isolates [Dyer *et al.*, 1993] were already present in the field population because isolates were applied to some plots in 1984 and their mating types were not determined. Other work suggests that sexual reproduction may occur frequently in fields of standing stubble left after an eyespot-infected wheat crop [Dyer and Lucas, 1995]. This is likely to become increasingly common with the establishment of rotational set-aside [Ansell and Tranter, 1992], a procedure likely, therefore, to increase genetic recombination and opportunities for variation in the pathogen.

Analysis of the ascospore progeny showed that all had growth characteristics of the W-type of *T. yallundae*. This is further evidence that sexual repro-

Table 3. Frequency of carbendazim-resistance among single ascospore isolates of *Tapesia yallundae* from apothecia from plots that had been treated with different fungicides in the preceding years

Fungicide treatment to plots	No. plots (of 12) yielding isolates	No. single ascospore isolates	No. isolates resistant to carbendazim	Expected no. isolates resistant to carbendazim*	χ^2 (*P*)
None	7	104	23	53.6	36.1 (<0.001)
Carbendazim	11	158	158	150.2	8.2 (<0.01)
Prochloraz	1	15	0	1.4	1.5 (>0.02)
Carbendazim + prochloraz	2	34	22	29.6	15.1 (<0.001)

* Calculated from population data from the preceding wheat crop [Bateman *et al.*, 1995], but using data only from those plots that yielded single ascospore isolates.

duction in the field is almost entirely restricted to the W-type (although the contribution of the inoculated isolates to the mating population is not known); there are only two reports of ascospores producing colonies characteristic of the R-type [King, 1990; Dyer *et al.*, 1994b]. The reason for the rarity of sexual reproduction in the R-type is unclear, but there may be a lack of compatible isolates in the field or of a particular environmental trigger.

Fungicide use has been shown to influence the population structure of *T. yallundae* in the growing crop by differential selection [Bateman *et al.*, 1990]. Frequency of apothecia in 1994 was associated with the amount and severity of eyespot in the preceding crop and with the population structure of the eyespot fungus in that crop. By controlling disease, as well as by selecting strains, the fungicides carbendazim and prochloraz therefore influenced the amount of sexual reproduction, as indicated by the incidence of apothecia. Prochloraz suppressed infection and selected for the R-type, which failed to produce apothecia. Applying prochloraz regularly is therefore likely to decrease the ability of the eyespot fungus to produce genetic variation, perhaps contributing to stability in the population that it selects. In contrast, carbendazim increased disease (after repeated applications) relative to that in untreated plots, thereby favouring the development of apothecia. It also selected for carbendazim-resistant fungus. Therefore the effects of carbendazim-sensitivity type and of the pathogenicity type (W or R) on the development of apothecia are likely to be confounded. The interdependence of characteristics in the fungus population and incidence of disease are further supported by the small percentages of the variance accounted for by the single regressions and the failure of multiple regressions to increase these.

Fungicide treatment has also been shown to affect sexual reproduction in the barley powdery mildew fungus, *Erysiphe graminis* f. sp. *hordei* [Hayter, 1990]. In that fungus, numbers of cleistothecia produced differed according to the host barley cultivar, but fungicide application masked this effect with cleistothecial production reduced greatly in all cultivars.

Examination of the single ascospore progeny showed a tendency for selection of sensitivity to carbendazim except where carbendazim-resistance was abundant, i.e. after treatments with carbendazim alone. However, this observation may have arisen because the ascospore progeny came from relatively few apothecia, which may have resulted predominantly from crosses between carbendazim-sensitive isolates. Sampling a large number of straws was intended to ensure against this. Also, that ascospores were obtained from seven and 11 different plots that had been untreated or treated with carbendazim respectively, and from several different apothecial groups in many of these plots, shows that they were the progeny of a number of mating events for those treatments. Although a slight decrease in sensitivity to prochloraz has been detected in the R-type fungus in populations that had been exposed to repeated applications of prochloraz in this experiment [Bateman *et al.*, 1995] and in other experiments in France [Migeon, Mathon and Chudzicki, 1993], no isolates with effective resistance to prochloraz was found during screening of the ascospore progeny. However, W-type ascospore isolates with a five-fold range in sensitivity have been found elsewhere [Dyer and Lucas, 1995]. Sexual reproduction between field strains with decreased sensitivity to prochloraz may result gradually in decreased sensitivity in populations of *T. yallundae*.

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References

Ansell DJ and Tranter RB (1992) Set-aside: in Theory and Practice. University of Reading Department of Agricultural Economics, Reading

Bateman GL (1990) Comparison of the effects of prochloraz and flusilazole on foot rot diseases and on populations of the eyespot fungus, *Pseudocercosporella herpotrichoides*, in winter wheat. Zeitschrift für Pflanzenkrankheiten und Planzenschutz 97: 508–516

Bateman GL and Fitt BDL (1991) Effects of fungicides on eyespot in five successive crops of winter wheat in plots with different initial or fungicide-selected populations of *Pseudocercosporella herpotrichoides*. Crop Protection 10: 479–484

Bateman GL, Fitt BDL, Creighton NF and Hollomon DW (1990) Changes in populations of *Pseudocercosporella herpotrichoides* in successive crops of winter wheat in relation to initial populations and fungicide treatments. Crop Protection 9: 135–142

Bateman GL, Landau S and Welham SJ (1995) Sensitivity to prochloraz in populations of the eyespot fungus, *Pseudocercosporella herpotrichoides*, in relation to fungicide treatments and their efficacy in continuous winter wheat. Annals of Applied Biology 126: 235–247

Creighton NF and Bateman GL (1991) Problems in identifying pathogenicity types of *Pseudocercosporella herpotrichoides* by colony morphology arising from the source and concentration of the nutrient agar. Mycological Research 95: 253–254

Dyer PS, Bateman GL, Lucas JA and Peberdy JF (1994a) Seasonal development of apothecia of the cereal eyespot pathogen *Tapesia yallundae* on straw. Annals of Applied Biology 125: 489–500

Dyer PS, Nicholson P, Rezanoor HN, Lucas JA and Peberdy JF (1993) Two-allele heterothallism in *Tapesia yallundae*, the teleomorph of the cereal eyespot pathogen *Pseudocercosporella herpotrichoides*. Physiological and Molecular Plant Pathology 43: 403–414

Dyer PS, Papaikonomou M, Lucas JA and Peberdy JF (1994b) Isolation of R-type progeny of *Tapesia yallundae* from apothecia on wheat stubble in England. Plant Pathology 43: 1039–1044

Dyer PS, Lucas JA (1995) Incidence of apothecia of *Tapesia yallundae* at set-aside sites in England and sensitivity of the ascospore offspring to the fungicides benomyl and prochloraz. Plant Pathology (in press)

Hayter JBR (1990) The Genetics and Molecular Biology of *Erysiphe graminis* DC. f.sp. *hordei* Marchal. PhD thesis, University of Cambridge

Hoare FA, Hunter T and Jordan VWL (1986) Influence of spray programmes on development of fungicide resistance in the eyespot pathogen of wheat, *Pseudocercosporella herpotrichoides*. Plant Pathology 35: 506–511

Hunter T (1989) Occurrence of *Tapesia yallundae*, teleomorph of *Pseudocercosporella herpotrichoides*, on unharvested wheat culms in England. Plant Pathology 38: 598–603

King AC (1990) First record of *Tapesia yallundae* as the teleomorph of *Pseudocercosporella herpotrichoides* var. *acuformis*, and its occurrence in the field in the Federal Republic of Germany. Plant Pathology 39: 44–49

Migeon JL, Mathon MP and Chudzicki AM (1993) Le prochloraze dans le nord de la France. Premiers cas de résistance pratique du piétin-verse du blé (*Pseudocercosporella herpotrichoides*). Mededelingen de Faculteit Landbouwwetenschappen Rijksuniversitat, Gent 58: 1401–1409

Polley RW, Slough JE and Jones DR (1993) Winter Wheat Disease Survey Report 1993. CSL/ADAS, 44 pp

Wallwork H (1987) A *Tapesia* teleomorph of *Pseudocercosporella herpotrichoides*, the cause of eyespot of wheat. Australasian Plant Pathology 16: 92–93