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Development of stem-base pathogens on different cultivars of winter wheat determined by quantitative PCR

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Abstract

The progress of development of stem-base pathogens in crops of second winter wheat was plotted in nine experiments in three years. The amount of each pathogen present was determined by quantitative PCR. Where *Tapesia yallundae* was present in quantifiable amounts, it usually developed earlier than the other eyespot pathogen, *T. acuformis*. Both species were usually present in greater amounts on cultivars which are more susceptible to eyespot. The sharp eyespot pathogen, *Rhizoctonia cerealis*, developed more erratically than either of the *Tapesia* spp. and there were no consistent effects on different cultivars. *Fusarium* spp., the cause of brown foot rot, were rarely present in quantifiable amounts, but *Microdochium nivale* was usually present as one or both of the varieties *nivale* and *majus*. Late-season (after anthesis) decreases in *M. nivale* suggest that any brown foot rot symptoms attributable to this fungus would have fully developed earlier. Cultivar differences in amounts of *M. nivale* were most clear in stems during internode extension and when relatively large amounts of DNA were present. Such differences approximately reflected eyespot susceptibility, cv. Soissons containing most and cv. Lynx containing least DNA. The results emphasise the difficulty in relating diagnoses, by quantitative PCR or other means, at early growth stages when decisions to apply fungicides against stem-base disease are made, to later disease severity.

Introduction

Effective use of fungicides against stem-base diseases of cereals depends on an accurate identification of the pathogens present and assessment of disease risk, especially from eyespot (*Tapesia* spp.), at the beginning of stem elongation. Identification of the diseases at this time is difficult because of poorly developed symptoms, a problem that may be exacerbated when they are caused by mixed infections. For example, early symptoms of eyespot may be obscured by those of the generally less-damaging pathogens that cause brown foot rot (*Fusarium* spp. and *Microdochium nivale* Samuels and Hallett) and sharp eyespot (*Rhizoctonia cerealis* van der Hoeven). However, PCR methods are available for positive diagnosis of the pathogens that might be involved. The major pathogens for which PCR procedures have been developed are *Tapesia* spp. (Nicholson et al., 1997), *F. culmorum* (W.G. Sm.) Sacc. (Nicholson et al., 1998), *M. nivale* (Nicholson et al., 1996) and *R. cerealis* (Nicholson and Parry, 1996). There are similar methods for the minor pathogens *F. avenaceum* (Corda) Sacc. (Turner et al., 1998) and *F. poae* (Peck) Wollenw. (Parry and Nicholson, 1996). Quantitative diagnosis of these pathogens was made possible by the development of competitive PCR for these pathogens (Nicholson et al., 1996; 1997). In this method the co-amplification of a known amount of 'competitor' DNA (containing primer annealing sites identical to those in the fungal target species) in

the sample permits estimation of the amount of the fungal DNA present.

This report describes results from nine field experiments, over three years and in three localities. Comparisons of different stem-base pathogens on different wheat cultivars are made, using quantitative PCR, to test for differences in infection time, rates of development in the shoots, and the extent to which the amount of early infection relates to the amount of late infection, when disease can affect grain-filling and hence yield. These results are considered in relation to the optimisation of fungicide applications and their timings.

Materials and methods

Field experiments

Three experiments, described in detail (Bateman et al., 2000) were done in each of three years, 1996/7, 1997/8 and 1998/9, on three sites in England. These were in the West Midlands at Harper Adams University College, in East Anglia at Morley Research Centre and in the South-east Midlands at Rothamsted Experimental Station.

Each experiment had four randomised blocks of 20 plots (minimum dimensions 10 m × 1.75 m) in which four cultivars of winter wheat were grown as second wheat crops. Fungicide treatments were applied to 16 plots per block at growth stage (GS) 31 (Zadoks et al., 1974). Results described here are from untreated plots only, that is all 20 plots before GS 31 but only four plots, one plot per cultivar, subsequently. The cultivars chosen had different susceptibilities to eyespot according to National Institute of Agricultural Botany (NIAB) resistance ratings. They were Lynx (the most resistant, rating 8 in NIAB trials; R.A. Bayles, NIAB, UK, pers. comm.), Brigadier (rating 5), Mercia (rating 5) and Soissons (rating 4). In 1998/9, Brigadier was replaced by cv. Abbot (rating 5) because of the former's susceptibility to yellow rust (caused by *Puccinia striiformis* Westend.). Epoxiconazole (86.5 g a.i. ha^{-1} as Opus) was applied during May (GS 37-55) where development of foliar diseases was observed; later fungicide applications were made as appropriate.

Husbandry (cultivations and applications of fertilisers and pesticides) was standard for the farms, except for sowing dates (which were moderately early when possible to encourage disease, but were sometimes delayed by adverse weather and soil conditions) and applications of the experimental fungicide treatments.

Sampling

Plant samples were taken from all plots on four or five occasions (Table 1). The first sample was taken at approximately the two-tiller stage (GS 22), usually in February. The second was at the beginning of internode elongation (GS 30–31), when fungicides would normally be applied to control eyespot. The

Table 1. Sowing dates, and dates and GS when samples were taken in nine experiments at three locations

Procedure	Harper Adams	Morley	Rothamsted
1996/7			
Sowing date	18 Oct.	4 Oct.	9 Oct.
Sample 1 (GS)	20 Mar. (22)	11 Feb. (12–22)	3–5 Mar. (22)
Sample 2 (GS)	10 Apr. (30)	15 Apr. (30–31)	7 Apr. (30–31)
Sample 3 (GS)	28 Apr. (32)	8 May (32–37)	24 Apr. (32–33)
Sample 4 (GS)	22 May (39)	20 May (37–55)	28 May (53–57)
Sample 5 (GS)	7 Jul. (75)	14 Jul. (77–83)	4 Jul. (75–77)
1997/8			
Sowing date	20 Oct.	29 Sep.	10 Oct.
Sample 1 (GS)	2 Mar. (24)	17 Feb. (22–26)	24 Feb. (23)
Sample 2 (GS)	30 Mar. (30)	6 Apr. (31)	1 Apr. (30)
Sample 3 (GS)	13 May (37)	11 May (33–45)	7 May (34)
Sample 4 (GS)	23 Jun. (69)	23 Jun. (71–75)	1 Jul. (73)
1998/9			
Sowing date	6 Oct.	9 Oct.	12 Oct.
Sample 1 (GS)	16 Feb. (22)	16 Feb. (12–22)	16 Feb. (22)
Sample 2 (GS)	18 Mar. (30)	7 Apr. (30)	8 Apr. (30–31)
Sample 3 (GS)	4 May (32)	11 May (33–41)	6 May (34)
Sample 4 (GS)	27 Jul. (85)	23 Jun. (71–73)	2 Jul. (73–77)

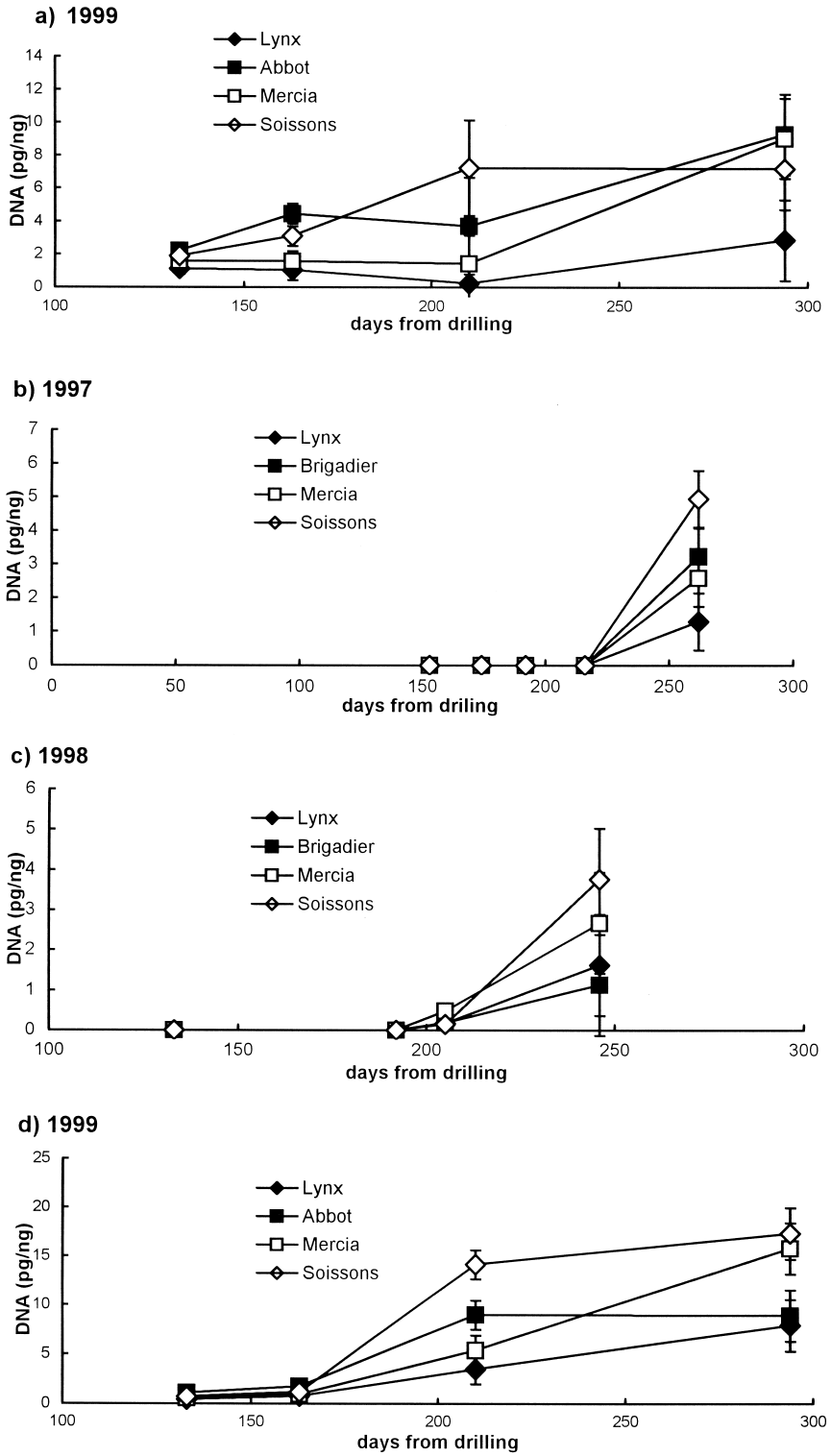


Figure 1. Development of eyespot pathogens on wheat plants at Harper Adams: (a) *Tapesia yallundae* in 1999; (b) *T. acuformis* in 1997; (c) *T. acuformis* in 1998; (d) *T. acuformis* in 1999.

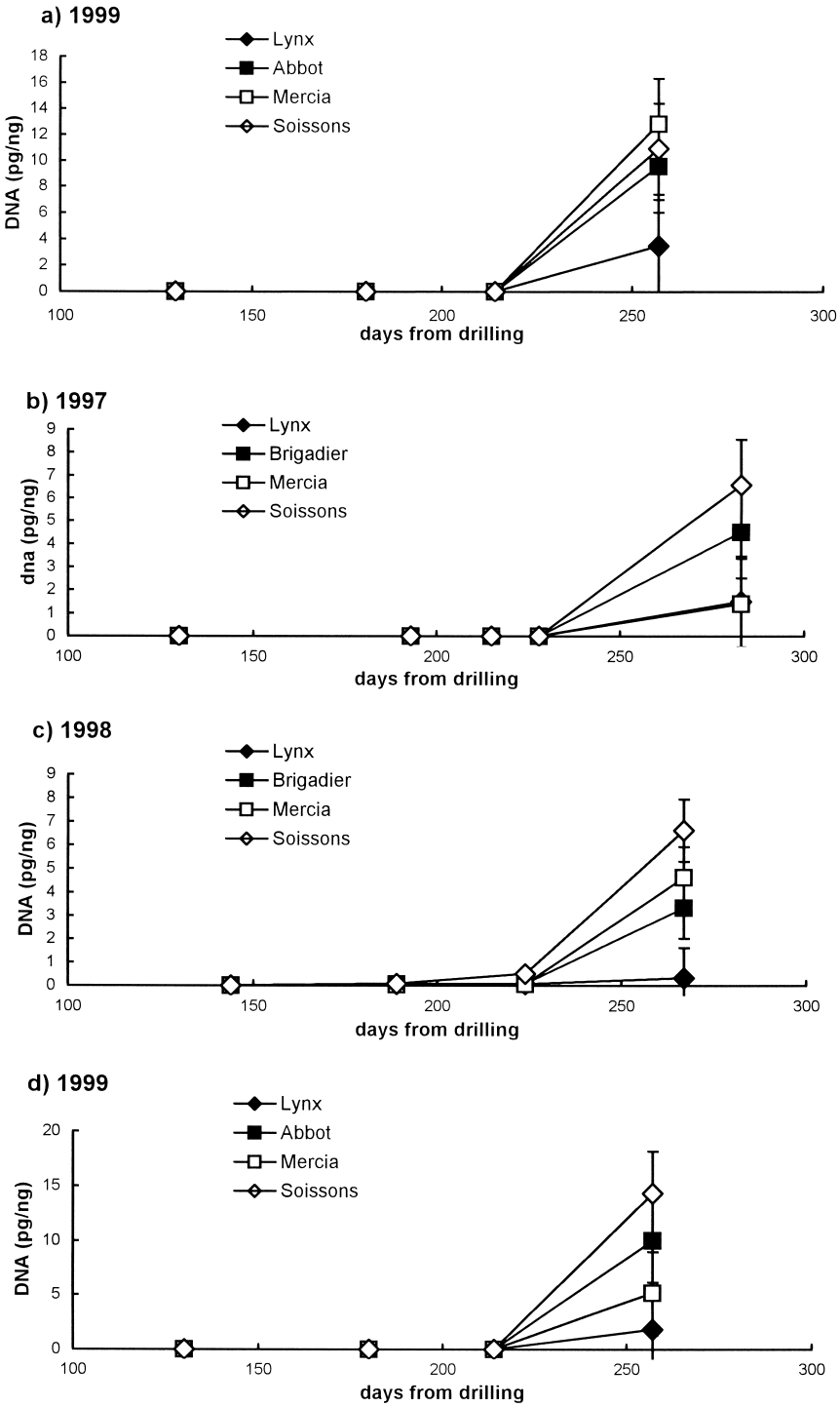


Figure 2. Development of eyespot pathogens on wheat plants at Morley: (a) *Tapesia yallundae* in 1999; (b) *T. acuformis* in 1997; (c) *T. acuformis* in 1998; (d) *T. acuformis* in 1999.

third was taken 2–3 weeks later. Two later samples were taken in 1997, one during May and one in July, during grain ripening. A single later sample, usually during late anthesis or the early ripening stages, was taken in 1998 and 1999. At each sample time, three plants were taken from each of 10 sampling positions in each plot. The sampling positions were located at random positions along two, approximately parallel,

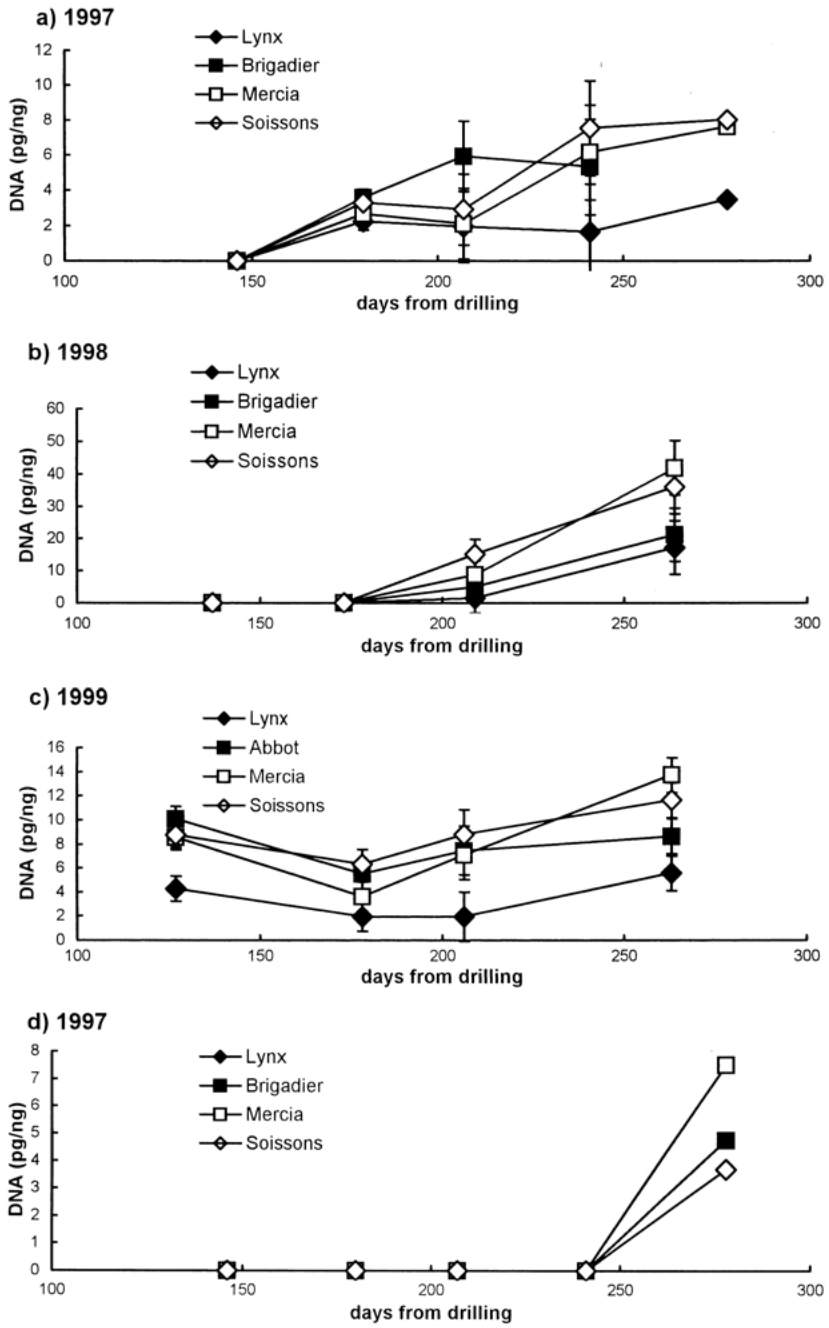
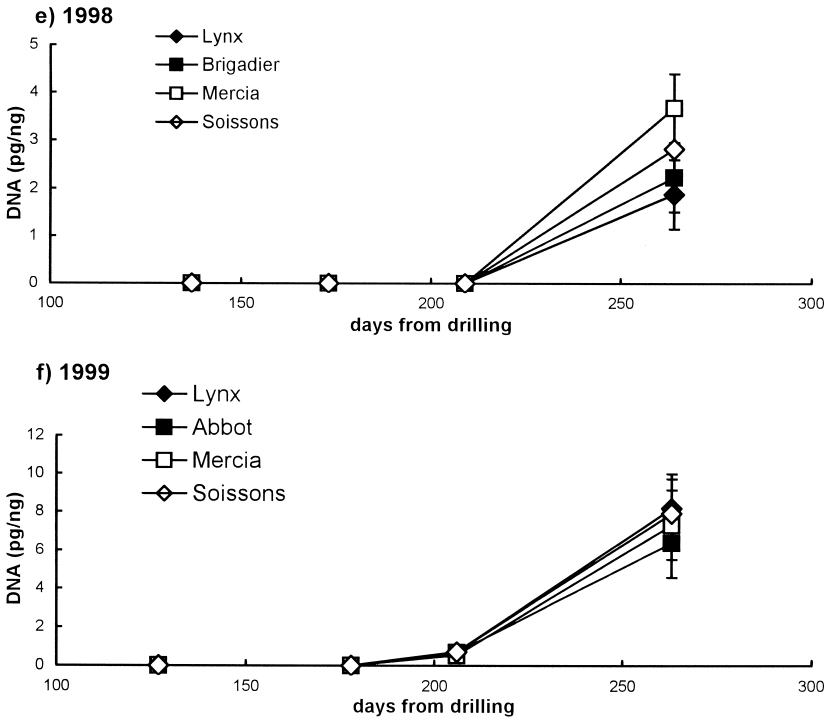


Figure 3. Development of eyespot pathogens on wheat plants at Rothamsted: (a) *Tapesia yallundae* in 1997; (b) *T. yallundae* in 1998; (c) *T. yallundae* in 1999; (d) *T. acuformis* in 1997; (e) *T. acuformis* in 1998; (f) *T. acuformis* in 1999.



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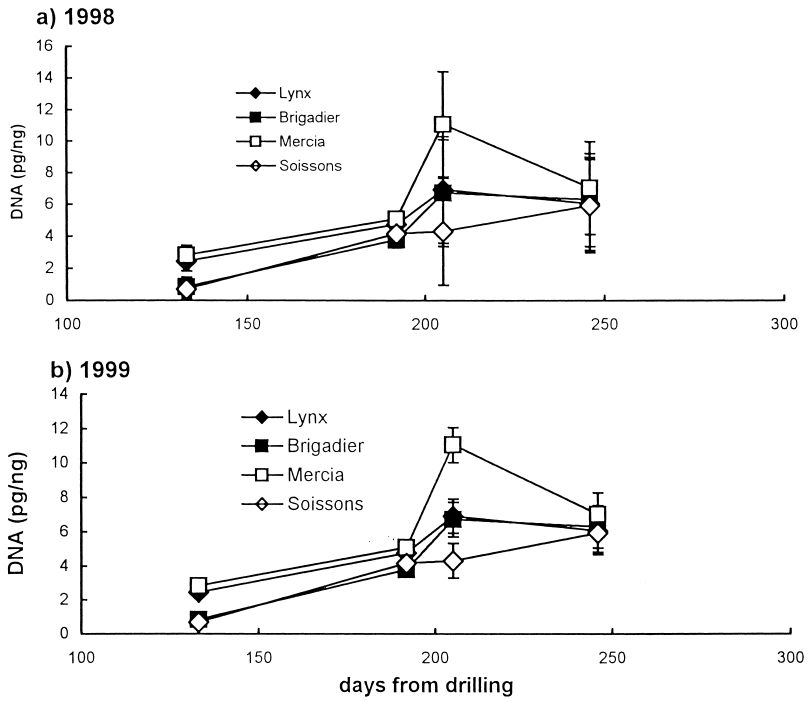

Figure 4. Development of the sharp eyespot pathogen, *Rhizoctonia cerealis*, on wheat plants at Harper Adams: (a) in 1998; (b) in 1999.

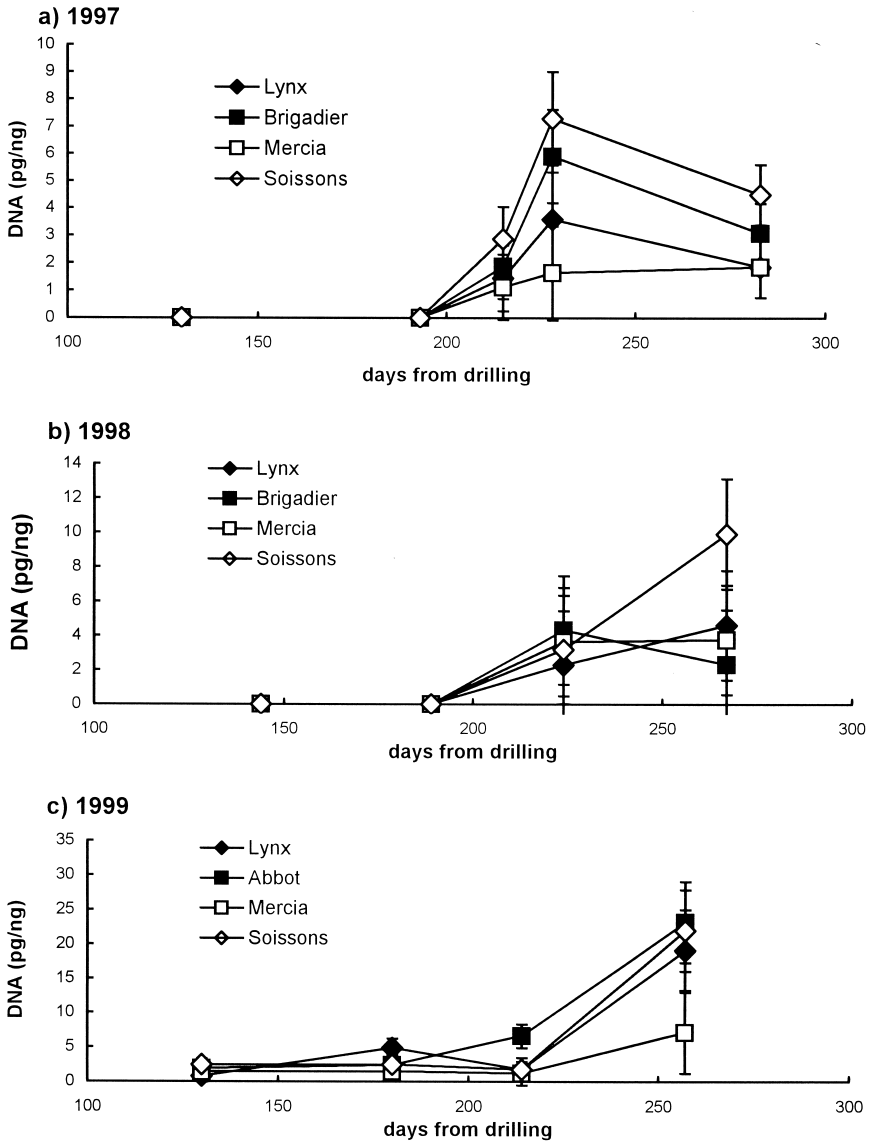

Figure 5. Development of the sharp eyespot pathogen, *Rhizoctonia cerealis*, on wheat plants at Morley: (a) in 1997; (b) in 1998; (c) in 1999.

zig-zag transects in each plot. This sampling pattern is recommended for eyespot (Parker et al., 1997).

PCR quantification of fungal pathogens

DNA was extracted from the bases of whole shoots (before stem elongation) or stems of the main shoots, after assessing diseases, and processed as described previously (Bateman et al., 2000). Diagnosis of pathogens was by 'touchdown' PCR in which the annealing temperature is decreased throughout the programme to reduce spurious priming of the primers, thus enhancing specificity of the reaction (Don et al., 1991). The pathogen DNA was quantified using competitive PCR. Data were analysed by factorial analysis of variance using Genstat.

Results

Concentrations of DNA of pathogenic fungi in shoot or stem bases of plants not treated with fungicides, when present in amounts sufficient to quantify, were plotted

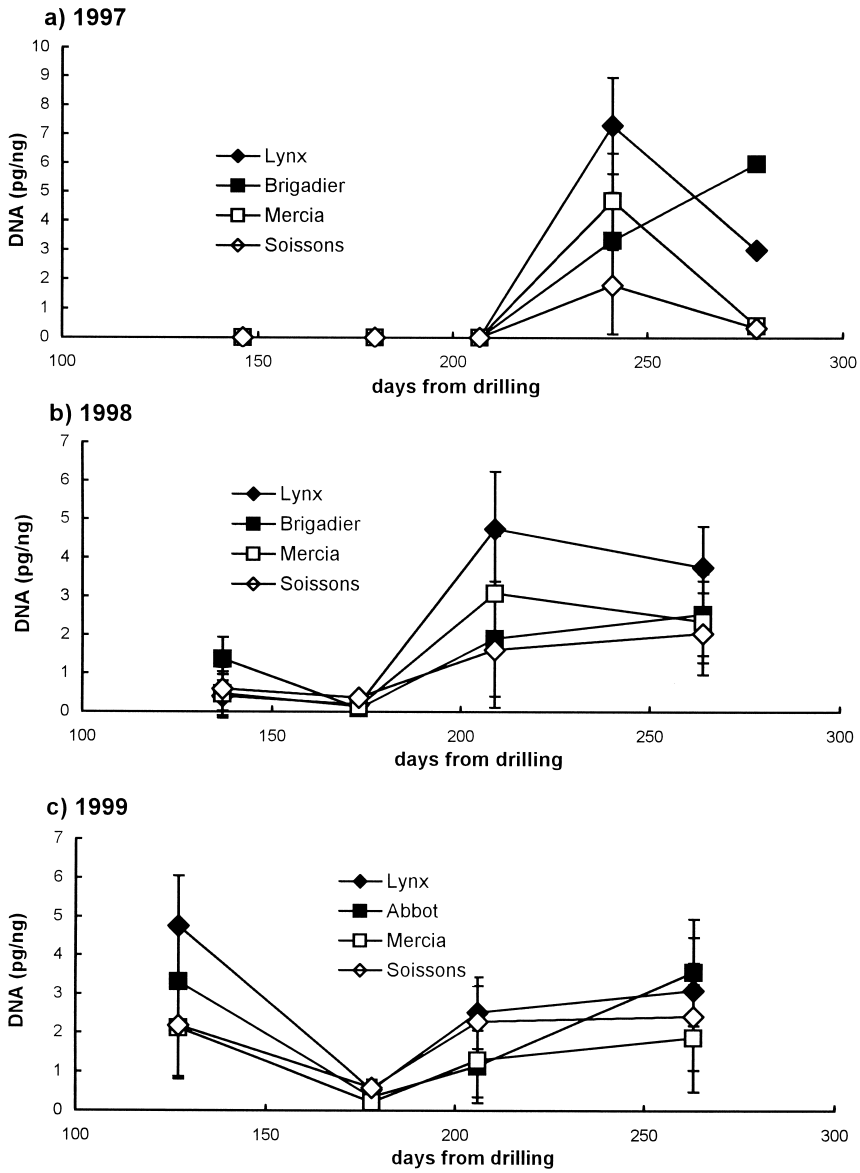

Figure 6. Development of the sharp eyespot pathogen, *Rhizoctonia cerealis*, on wheat plants at Rothamsted: (a) in 1997; (b) in 1998; (c) in 1999.

against time. The DNA concentrations for each cultivar are means of 20 plots in samples taken before fungicide treatments were applied and means of four plots (untreated only) in later samples. SEDs for comparing cultivars are taken from factorial analyses of variance that tested the effects both of cultivars and fungicides, except where the data were inadequate for analysis (e.g. because of missing plot samples). Decreases in DNA between samples sometimes occurred and were sometimes the result of loss of senescent outer leaf sheaths, which each fungus would normally have colonised before it reached the stem.

Tapesia *spp.*

At Harper Adams, *Tapesia yallundae* Wallwork and Spooner was present only in 1999, when it occurred throughout the sampling period (Figure 1a). Cultivar

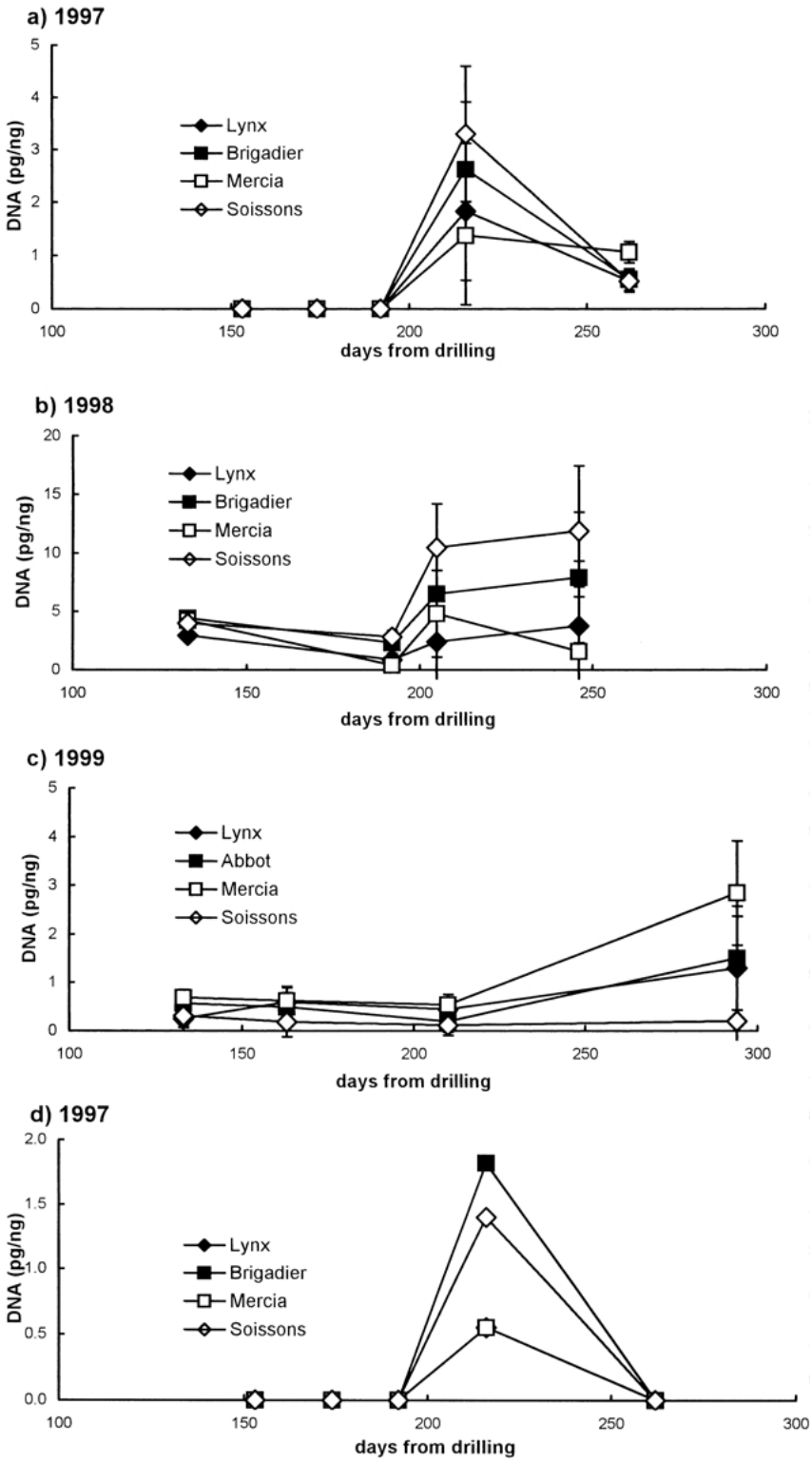

Figure 7. Development of varieties of *Microdochium nivale* on wheat plants at Harper Adams: (a) var. *nivale* in 1997; (b) var. *nivale* in 1998; (c) var. *nivale* in 1999; (d) var. *majus* in 1997; (e) var. *majus* in 1998; (f) var. *majus* in 1999.

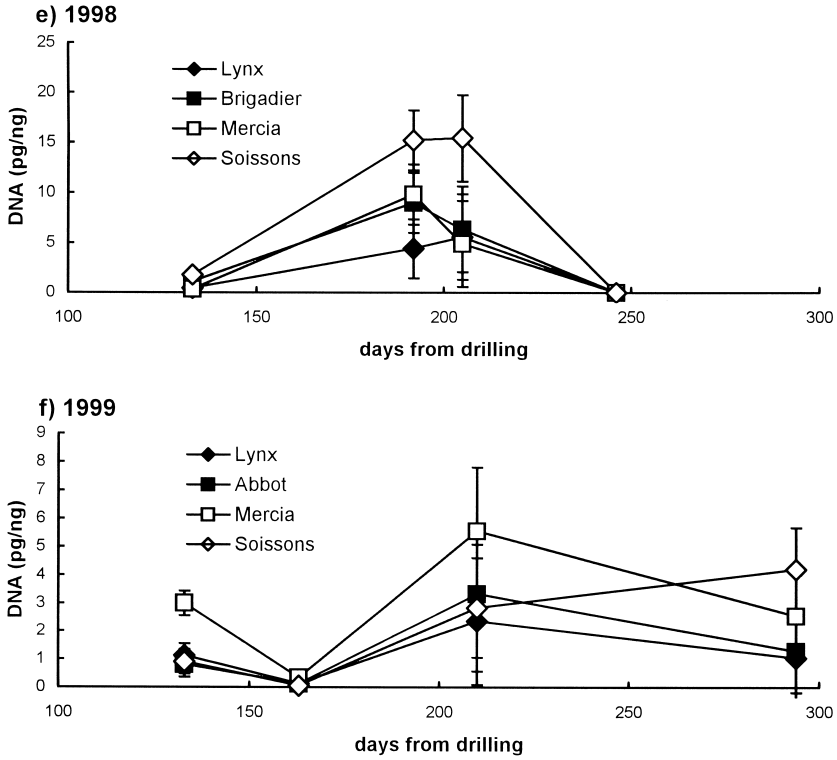

Figure 7. Continued.

differences were closer to those expected from NIAB ratings at GS 32 (210 days) than at GS 85 (294 days), when Abbot and Mercia had greatest amounts of DNA of this fungus. *T. acuformis* (Boerema, Pieters and Hamers) Crous was present in all years (Figure 1b–d). This fungus began to develop only after 200 days (after fungicide treatments had been applied to other plots) in 1997 and 1998. In 1999, *T. acuformis* was present throughout the sampling period. It occurred in smaller amounts than *T. yallundae* in the early samples but in greater amounts in the later samples, especially in cvs Mercia and Soissons.

The development of *Tapesia* spp. at Morley (Figure 2) was similar to that at Harper Adams except that both species appeared late in 1999 and were quantifiable only at GS 71–73 (257 days). In the last sample in each year, cv. Soissons contained most DNA of *T. acuformis* (Figure 2b–d), consistent with NIAB ratings for eyespot; the cultivar differences were less clear for *T. yallundae*. At Rothamsted in all years, *T. yallundae* (Figure 3a–c) developed earlier and was present in greater amounts than *T. acuformis* (Figure 3d–f). Cultivar Mercia often contained more DNA of each fungus than did other cultivars at the final samples although not significantly more than cv. Soissons.

Rhizoctonia cerealis

DNA of *R. cerealis* was not found in 1997 at Harper Adams (Figure 4) and was present only after 200 days on stems at Morley (Figure 5) and Rothamsted (Figure 6). It was present in small amounts in early samples at Harper Adams and Rothamsted in 1998 and at all sites in 1999. It tended to appear on stems soon after stem extension (GS 31 onwards), in May, and occasionally declined in the summer. There were no consistent cultivar differences although Mercia tended to become most infected at Harper Adams and Soissons at Morley.

Microdochium nivale

The development of *M. nivale* on shoot and stem bases showed little consistency over sites or years

(Figures 7–9). *M. nivale* var. *nivale* sometimes decreased on young plants, before stem extension, as the leaf sheaths senesced, as at Harper Adams in 1998 (Figure 7b) and Rothamsted in 1998 (Figure 9b). *M. nivale* var. *majus* sometimes behaved similarly, as at Harper Adams (Figure 7f) and Rothamsted (Figure 9f) in 1999. When there was a relatively large amount of either fungus on stems in May, a decrease usually followed as the plants matured. This occurred with var. *nivale* at all sites in 1997 (Figures 7a–c, 8a–b, 9a–c), and with var. *majus* in all years at Harper Adams (Figure 7d–f) and in 1997 at Rothamsted (Figure 9e). A late-season increase in var. *nivale* in 1999 at Morley (Figure 8c) was accompanied by a decrease in var. *majus* (Figure 8f).

Effects of cultivar were usually most apparent when there was most DNA present in the stems: cv. Soissons often contained most DNA while Lynx contained least. The cultivar effects were similar for each of the varieties of the fungus.

Discussion

Where *T. yallundae* was present in quantifiable amounts, it usually developed earlier than *T. acuformis*.

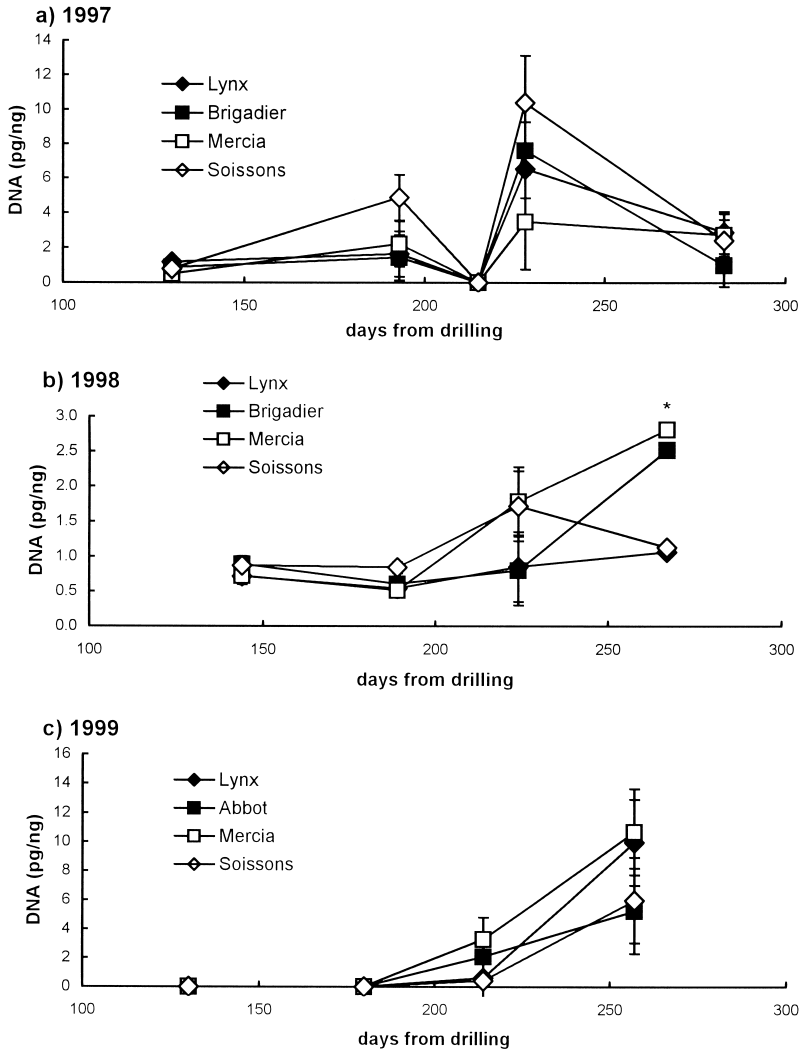

Figure 8. Development of varieties of *Microdochium nivale* on wheat plants at Morley: (a) var. *nivale* in 1997; (b) var. *nivale* in 1998; (c) var. *nivale* in 1999; (d) var. *majus* in 1998; (e) var. *majus* in 1999.

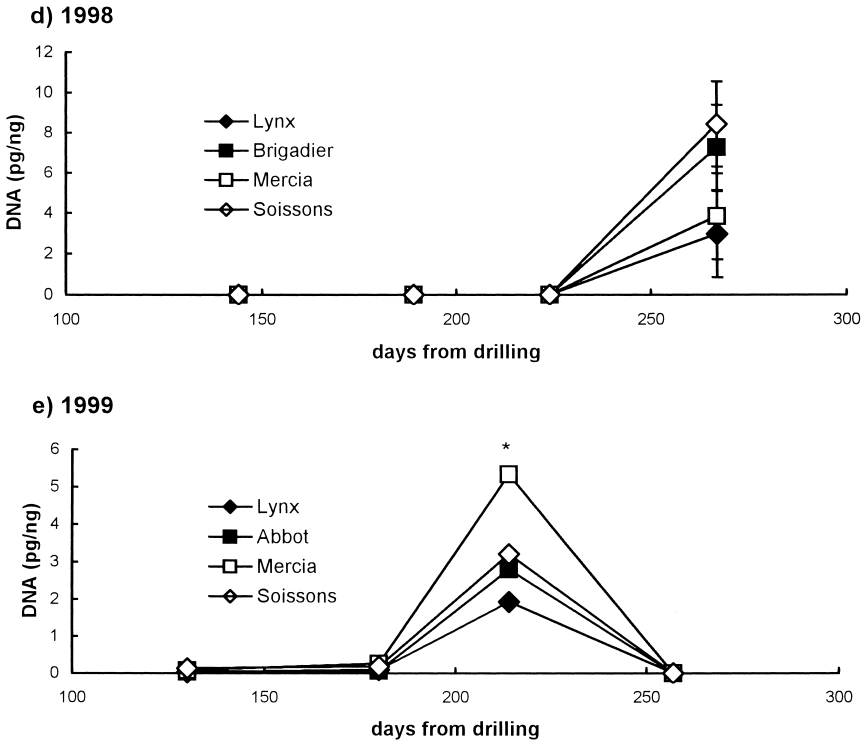

Figure 8. Continued.

These results using PCR confirm the results usually obtained by disease assessments after inoculum had been applied artificially (Goulds and Fitt, 1990; Bateman et al., 1990). Overall, there was most DNA during the main period of grain-filling (after GS 69) when there had also been significant amounts of DNA, usually of *T. yallundae*, during the early stem elongation period (GS 31–34). The results provide further evidence that *T. acuformis* is potentially the less damaging pathogen (Bateman and Jenkyn, 2000; 2001; Bateman et al., 2000). Effects of cultivar were usually as expected from NIAB resistance ratings for both *Tapesia* spp. Evidence from Rothamsted, the only site at which both species were quantifiable in all years, or from the other sites in 1999, was insufficient to confirm that cultivar differences were smaller for the slower-developing *T. acuformis*, a suggestion that emerged from controlled environment studies (Bateman et al., 1990).

Rhizoctonia cerealis developed more erratically than *Tapesia* spp. and, as expected, was less consistently affected by cultivar. It sometimes failed to develop aggressively on stems following leaf-sheath infection, as indicated by occasional decreases in amounts of DNA before the final sample.

Cultivar differences in amounts of *M. nivale* were most clear in stems during internode extension and when relatively large amounts of DNA were present. In these circumstances, the cultivar differences approximated to the NIAB ratings for eyespot susceptibility, Soissons containing most and Lynx least DNA. This suggests a relationship between genetic resistance to eyespot and *M. nivale*, which may result from a facility for the latter to invade tissues already damaged or weakened by other pathogens (Bateman, 1993; Bateman and Munnery, 1995). This seems not to have been reported before and, subject to further research to understand the role of *M. nivale* in yield losses, may have relevance to cereal breeding programmes. The late-season decreases in *M. nivale* suggest that brown foot rot symptoms attributable to this fungus had become fully developed earlier; this was supported by regressions of the extent of disease symptoms on amounts of DNA at successive samples (Turner et al., 2002).

The development of a particular pathogen may have been suppressed by the presence of other pathogens.

Such suppression has been demonstrated on wheat shoots and may be influenced by the sequence of infection by the different fungi (Bateman and Munnery, 1995). More frequent sampling than in the present experiments would have been necessary to demonstrate clearly the sequence of infections.

Eyespot is recognised as the most important stem-base disease of wheat and the principal target for fungicides applied at the beginning of stem extension. *T. acuformis* was the only eyespot pathogen that occurred in quantifiable amounts in all nine of the field experiments described. This fungus tends to develop late, as it did in most of the experiments described here, and so was not detectable in many of the samples taken before GS 31. Its late development also results in smaller yield losses than the earlier developing *T. yallundae* (Bateman and Jenkyn, 2000; 2001). Consequently, early infection by the pathogens that would indicate risk and a need to apply fungicides was not often encountered (Turner et al., 2001). Where *T. acuformis* is the predominant species, as has recently been the case in much of the UK (West et al., 1998),

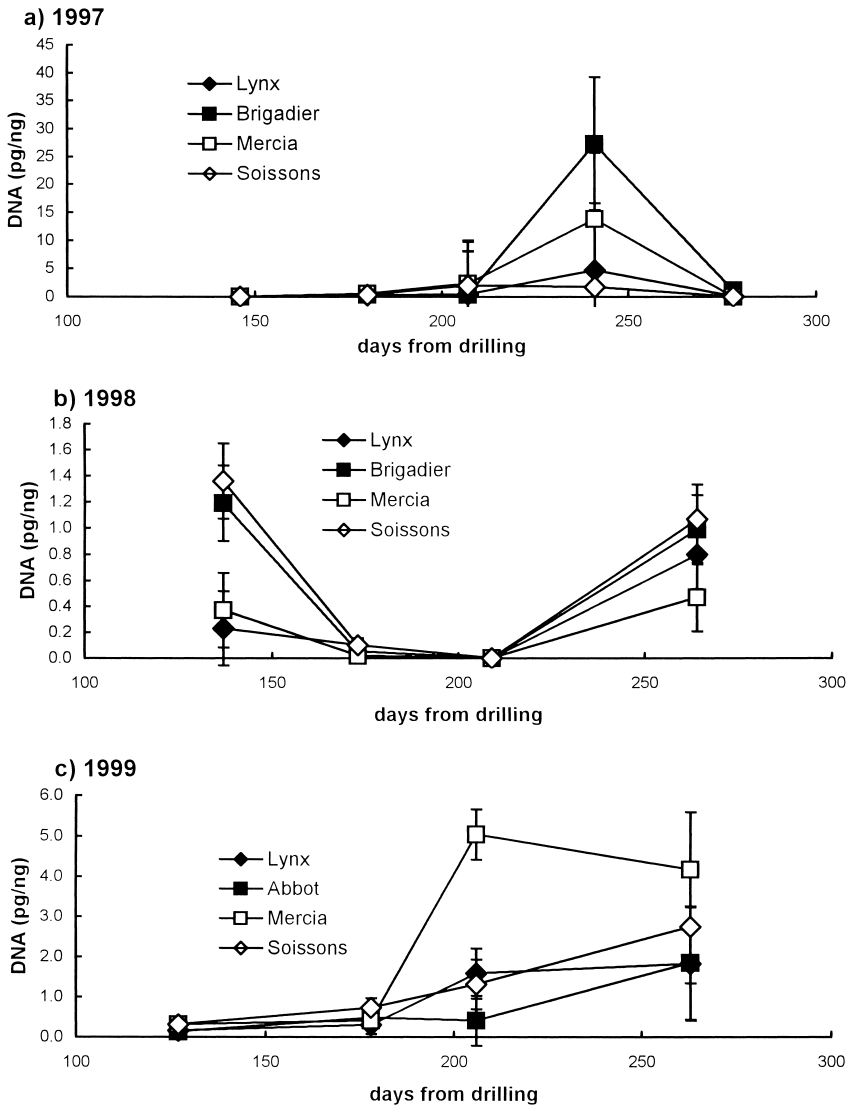

Figure 9. Development of varieties of *Microdochium nivale* on wheat plants at Rothamsted: (a) var. *nivale* in 1997; (b) var. *nivale* in 1998; (c) var. *nivale* in 1999; (d) var. *majus* in 1997; (e) var. *majus* in 1998; (f) var. *majus* in 1999.

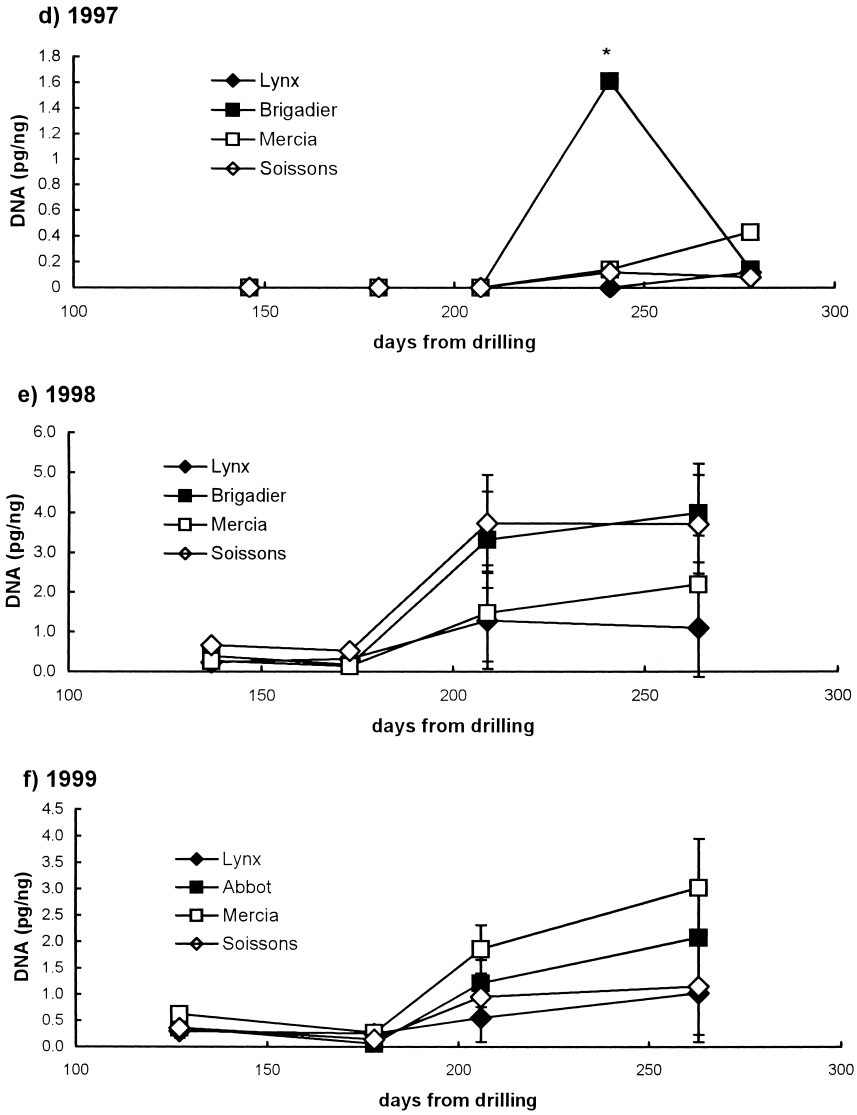

Figure 9. Continued.

quantification of the pathogens at GS 31 is unlikely to be an adequate means of assessing risk or to provide information on which to base a decision to apply fungicide. Extensive lesion development, often indicating the presence of *T. yallundae* at this stage, appears to remain the best indication of risk.

Acknowledgements

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