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The Incidence and Severity of Eyespot on Winter Wheat Variety Hereward Grown as a Monocrop and as a Bicrop with White Clover

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معدلات ودرجة إصابة محصول القمح الشتوي (عينة هيرورد) بمرض التبقع العيني عند زراعتها كمحصول مفرد أو ثنائي مع البرسيم

الملخص : تم رصد مستويات إصابة محصول القمح الشتوي (عينة هيرورد) بمرض التبقع العيني، في تجربة حقلية لمدة ثلاث أعوام عند زراعتها كمحصول مفرد أو ثنائي مع البرسيم وذلك باستخدام أساليب الزراعة القياسية ومدخلات الإنتاج المنخفضة. زادت معدلات الإصابة (للأعوام الأول والثاني والثالث) وشدة الإصابة (للعامين الثاني والثالث فقط) مع المواسم في كل المعاملات. وبلغت المساحة داخل منحنى ارتقاء المرض، لمعدلات الإصابة في السنة الثالثة للقمح المفرد (متوسط كل المعاملات) ٣,٣٠ أضعاف السنة الأولى، بينما بلغت شدة الإصابة ١,٧٨ أضعاف السنة الثانية. وبلغت معدلات وشدة الإصابة في السنة الثالثة للمحصول الثنائي ١,١٢ و ١,٤٥ أضعاف الإصابة في المحصول المفرد على التوالي. كان الارتباط بين المساحة داخل منحنى ارتقاء المرض ومعدلات الإصابة ذات دلالة معنوية لكل المعاملات. تمت مناقشة النتائج على ضوء العوامل التي قد تؤثر على مستويات الإصابة، خاصة تغير المناخ المصغر وإنتاج الأبواغ على بقايا النباتات وتناثرها مع قطرات المطر.

ABSTRACT: In a three-year field trial, the level of eyespot on cv. Hereward winter wheat was recorded from cereal monocrops and cereal-clover bicrops receiving standard farming practice and reduced levels of input. Disease incidence (for years one, two and three) and severity (years two and three only), increased with each season in all treatments. In the third wheat crop the area under the disease progress curve (AUDPC) for disease incidence (mean of all treatments) was 3.30 times higher than that measured for the first wheat crop. The average AUDPC for disease severity in year three was 1.78 times greater than that for year two. In year three the eyespot incidence AUDPC for bicrops was 1.12 times greater than in monocrops. Disease severity AUDPC in year three was 1.45 times greater in bicrops than in monocrops. For all treatments there was a significant correlation between AUDPC and the rate of disease increase. The results are discussed in terms of those factors that are likely to affect eyespot levels within bicrops, especially microclimate changes, spore production on crop residues, and spore dispersal by rain-splash.

In the UK, the wheat stem-base disease complex is one of the major production constraints for winter wheat, with losses estimated to be $30 - $45 million, annually (Fitt, 1992). Data on the incidence of eyespot (*Pseudocercosporella herpotrichoides* [Fron] Deighton) and *Fusarium* spp. on winter wheat in England and Wales has indicated that in most years both diseases are widespread (Parry, 1990). *P. herpotrichoides* survives on straw from the previous crop and sporulates during cool weather in winter and early spring (Fitt and White, 1988). Conidia are splash dispersed by rainfall onto the stem-base of the new crop (Fitt and Bainbridge, 1983). Germination is followed by germ-tube extension, formation of appressoria and penetration of the

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coleoptile. Subsequently, runner hyphae are formed and sequential penetration of leaf sheaths occurs from differentiated mycelial plaques. Lesion development is encouraged by cool, wet weather (Higgins *et al.*, 1986).

Stem-base lesions produce a low proportion (about 10%) of the total spore number; the greater number of spores is produced on infected stubble (Rowe and Powelson, 1973). Spores are dispersed by rain-splash, generally to distances of less than one metre from the infection source, although some can reach distances of up to 2 m (Fitt and Nijman, 1983). As a consequence, infection tends to develop from isolated foci (Rowe and Powelson, 1973).

Benzimidazole fungicides were recommended for eyespot control during the 1970's. The advent of widespread resistance to benzimidazoles (Hollins *et al.*, 1985; King and Griffin, 1985; Sanders *et al.*, 1986) has led to increased usage of the more expensive ergosterol biosynthesis inhibitors, prochloraz, propiconazole and fluzilazole. Recently, reduced sensitivity to prochloraz has been observed in Europe (Birchmore *et al.*, 1994).

Cereal-clover bicropping has been suggested as an alternative, reduced input cultivation method for intercropping winter wheat in a permanent clover understorey (Jones and Clements, 1993). The clover, once established, can survive as a perennial ground cover into which successive wheat crops are directly drilled. By using this method, N input levels can be reduced from 150-200 kg N ha^{-1} for a second and subsequent conventional wheat crop to 50 kg N ha^{-1}. In cereal-clover bicropping systems the build-up of available N has been shown to increase threefold by the end of the second year of cultivation (Jones and Clements, 1993). Furthermore, pest numbers appear to be greatly reduced in the intercrop system (Clements *et al.*, 1995).

There is little quantitative data on the effects of intercropping on disease development. The reports that are available are largely from results of research on tropical crops such as cassava, bean, maize and cowpea (for example, Sumner *et al.*, 1981). The current report describes results from a three-year, field-based trial established to determine the effects of cereal-clover bicropping and input level on the incidence and severity of eyespot on winter wheat.

Materials and Methods

At the Institute for Arable Crops Research, Long Ashton, UK, a fine, firm seed-bed was prepared on land previously under oil seed rape. The soil received 75 kg ha^{-1} of each P and K fertiliser, but no N was added. White clover (*Trifolium repense* L.) seed was sown at 10 kg seed ha^{-1} on 10 June 1993. The clover (cv. Donna) was established over most of the field; cv. Milkanova was established for areas designated for treatments C and D (Table 1). The developing swards of clover were topped in August and cut for silage in early October. A randomised complete block experimental design was imposed with three replicates. In late October 1993, plots (13 x 60 m) were marked out for each of the treatments (Table 1). For treatments G and H, plots were ploughed and sown with winter wheat cv. Hereward. Plots for other treatments were left unploughed and winter wheat cv. Hereward was direct drilled into the clover understorey (treatments A,B,C and D) or directly into the soil (treatments E and F), using a Hunter Rotary strip-seeder, following chemical removal of the clover. Clover cvs Donna (small leaves) and Milkanova (large leaves) were chosen because of their contrasting leaf sizes. Leaf size has the potential to affect the pattern of rain-splash dispersal of *P. herpotrichoides* (Soleimani *et al.*, 1996).

During the growing season, the conventional input treatments (Table 1) received a standard farm management regime of 140 kg N fertiliser, the growth regulators chlormequat and choline chloride, the herbicides diflufenican + isoproturon and fluroxypyr, the fungicides tebuconazole, chlorothalonil and cyproconazole and the insecticide pirimicarb. The low input treatments received only one fungicide (propiconazole at 0.25 the recommended rate) and the insecticide primicarb. Unless otherwise stated all chemical applications were applied at manufacturers recommended rates and at the appropriate stage of cereal growth. All disease infections arising in the different treatments were initiated from natural inoculum sources.

TABLE 1

Summary of treatments used to evaluate the effect of cereal-clover bicropping on eyespot development in winter wheat cv. Hereward

Treatment	Clover cv.[a]	Ploughed	Input level
A	Donna	No	SFP[b]
B	Donna	No	Reduced
C	Milkanova	No	SFP
D	Milkanova	No	Reduced
E	None	No	SFP
F	None	No	Reduced
G	None	Yes	SFP
H	None	Yes	Reduced

[a]Clover cv. Donna is a small leaved variety, cv. Milkanova is a large leaved type

[b]SFP, standard farming practice

TABLE 2

Sampling dates and associated plant growth stage (GS) used to evaluate the level of eyespot on cv. Hereward in wheat monocrops and cereal-clover bicrops

1993/94		1994/95		1995/96	
Sample date	GS	Sample date	GS	Sample date	GS
26/01/94	17	15/01/95	18	13/12/95	15
01/03/94	24	08/02/95	21	11/02/96	22
25/03/94	28	22/03/95	25	01/03/96	26
29/04/94	32	26/04/95	33	28/04/96	31
27/05/94	35	-	-	-	-
09/06/94	45	16/06/95	43	14/06/96	41
01/07/94	75	-	-	-	-
18/07/94	85	26/07/95	83	19/07/96	87

From November to the time of harvest 20 plants were taken at random from a diagonal transect of each plot; the main tiller was considered for disease assessment. Cereal growth stage (Zadoks *et al.*, 1974) was noted and for each leaf sheath/stem-base, disease incidence was noted for each sample and disease severity was recorded using the scale of Scott and Hollins (1974). Where necessary *P. herpotrichoides* was isolated from stem base material using the method of Sumino *et al.* (1990). Eyespot was assessed on 6-8 occasions each year (Table 2). The samples were taken from all plots in 1994 and 1996, and all plots except the high level input in wheat-clover bicrop plots in 1995. The high level input bicrop plots were lost in 1995 due to destruction of clover as a consequence of misapplication of fluroxypyr in April of that year.

The area under the disease progress curve (AUDPC) for disease incidence and severity was calculated for each treatment for all growing seasons, using the method of Shaner and Finney (1977). The rate of disease increase (r) was similarly computed for each treatment and for each season. In this way comparisons were made between monocrop and bicrop treatments, between treatments receiving different levels of agrochemical inputs and between bicrops treatments incorporating different clover varieties. Correlation coeficients for the relationship between AUDPC and rate of disease increase were also calculated. Where required, statistical analyses were performed using the SAS computer package (SAS, 1988).

Results

DISEASE INCIDENCE: The incidence of eyespot (as measured by the AUDPC) in all direct drilled, monocrop and bicrop, plots increased each year. In 1993/94 the clover-wheat bicrop and wheat monocrop treatments, at equivalent levels of N input, did not differ significantly in eyespot incidence (Table 3). However, disease levels were generally higher in bicrop plots (treatments B and D) than in monocrop direct drilled plots (treatment F) and ploughed plots (treatment H). Assessment of the incidence of eyespot during 1994/95 indicated that direct drilling had a significant and reducing effect on disease incidence in monocrops (treatments E and F) compared with conventional cultivations (treatments G and H; Table 3). The effect of bicropping was to increase eyespot incidence relative to the monocrop direct drilled treatment, although differences were not significant between bicropped plots, and plots which had been conventionally cultivated. In monocrop treatments, the level of input had no effect on the incidence of eyespot. In 1995/96 the incidence of eyespot in low level input bicropped plots (B and D) was significantly ($P > 0.01$) higher than the direct drill monocrop treatment (F), but less so than the monocrop ploughed treatment (H). Within the wheat monocrop plots, although the rate of disease incidence increase was higher in direct drilled cultivated plots than those sown following ploughing (G and H), these differences were not statistically significant (Table 3).

TABLE 3

Area under the disease progress curve (AUDPC) for disease incidence in treatments evaluating the effects of wheat monocrops and cereal-clover bicrops on eyespot disease development in cv. Hereward winter wheat

Treatment[a]	1993/94	1994/95	1995/96
A	2297	N/A[b]	3387
B	2255	4483	11288
C	2112	N/A	4836
D	2120	6330	11475
E	2487	3672	8159
F	1920	2703	7770
G	2643	5573	6937
H	2232	7668	5288
LSD	838.8	2810.0	3126.0

[a]see Table 1; [b]results not available - see text for details

The rate of disease incidence increase was generally higher for each successive season. There were no significant differences between the rate values in monocrop or bicropped plots in 1993/94. The rate values were higher in wheat-clover bicropped plots than wheat monocrop plots during year 1994/95. Within the wheat monocrop plots the rates of increase in plough drilled plots were greater than direct drilled plots at equivalent levels of input (Table 4). In 1995/96 the rate

TABLE 4

Rate of disease increase in treatments evaluating the effects of wheat monocrops and cereal-clover bicrops on eyespot development in cv. Hereward

Treatment[a]	1993/94	1994/95	1995/96
A	0.015	N/A[b]	0.050
B	0.012	0.017	0.117
C	0.012	N/A	0.043
D	0.013	0.049	0.095
E	0.014	0.016	0.059
F	0.009	0.002	0.034
G	0.010	0.034	0.073
H	0.011	0.041	0.048
LSD	0.008	0.043	0.034

[a]see Table 1; [b]results not available - see text for details

of disease incidence increase for wheat-clover bicrop plots at low levels of input ($r > 0.095$ units/day) was three times greater than that estimated for wheat monocrop plots ($r = 0.034$) at the same level of input. There were positive correlations between the AUDPC and the rate of disease increase in the three growing seasons over the period of investigation ($r = 0.52$, P = 0.008, d.f. = 22; $r = 0.51$, P = 0.028, d.f. = 22; $r = 0.68$, P = 0.0001, d.f. = 22; for 1993/94, 1994/95 and 1995/96 respectively).

DISEASE SEVERITY: An analysis of the severity of eyespot infection (assessed using the method of Scott and Hollins, 1974, and expressed as the AUDPC) in 1994/95 showed that treatments G and H (conventional input and reduced input wheat monocrops sown following ploughing) had the lowest proportion of severe lesions. The treatments with the highest AUDPC values were B and D (low-input levels of bicrop plots). The rate of eyespot disease severity increase in 1994/95 was greater in wheat-clover bicrop plots than the wheat monocrop direct drilled plots. In plough drilled wheat monocrop plots, the rate of increase in disease severity was higher than direct drilled monocrop plots (Table 5).

In 1995/96, both low input level bicropped plots (B and D) had significantly greater AUDPC values than other treatments. Although lower AUDPC values were recorded for wheat monocrop, ploughed plots (G and H) than direct drilled plots (E and F), these differences were not statistically significant (Table 5). In this year severity increase rates for high level input bicropped plots (A and C) were the highest among all treatments. In low level input plots, wheat monocrop plough drilled (H) had the highest rates of disease severity increase followed by the wheat-clover bicrop (B and D) plots (Table 5).

TABLE 5

Area under the disease progress curve (AUDPC) for disease severity, and rates of disease severity increase in treatments used to evaluate the effects of wheat monocrops and cereal-clover bicrops on eyespot development in winter wheat cv. Hereward

	AUDPC		Infection rate	
Treatment[a]	1994/95	1995/96	1994/95	1995/96
A	N/A[b]	7300	N/A	0.0076
B	9917	22202	0.0036	0.0041
C	N/A	8033	N/A	0.0062
D	8916	23317	0.0041	0.0032
E	8377	12950	0.0032	0.0023
F	7403	12420	0.0030	0.0026
G	4198	9101	0.0034	0.0047
H	3863	7407	0.0038	0.0047
LSD	3009	5829	0.0024	0.0021

[a]see Table 1; [b]results not available - see text for details

Discussion

Results for disease incidence in 1993/94 indicate that bicrop and monocrop treatments, at equivalent levels of N, did not differ significantly in the AUDPC values. During the 1994/95 season, eyespot disease incidence AUDPC values in direct drilled wheat monocrops were less than in crops sown following ploughing. These results are similar to those of Pratley (1995) who found that eyespot lodging was greater in conventionally cultivated treatments than in direct drilled cultivation systems. Prew *et al.* (1985 and 1995) and Jenkyn *et al.* (1995) have also reported eyespot to be more prevalent after mouldboard ploughing than after tined cultivation. The results are also similar to those of Herrman and Wiese (1985) who found that a reduced tillage regime lessened eyespot by 50% compared with a conventional cultivation; disease in no-tillage treatments was reduced by a further 50%. Herrman and Wiese (1985) suggest that the decrease in the level of disease under reduced and no-tillage treatments could be due to straw at the soil line separating the plant's lower stem from the pathogen located in the soil.

The results indicate that for all years there were generally higher levels of disease incidence in the bicrop treatments compared to the monocrop treatments. Results from the current study indicate that, among the direct drilled plots, eyespot was more severe in wheat-clover bicropped plots than in wheat monocrops. Soleimani (1997) has shown that spore production by *P. herpotrichoides* on wheat straw is greater within bicropped plots compared to wheat monocrops, presumably because environmental conditions are more conducive to spore formation. Furthermore, Soleimani (1997) has shown a significant positive correlation between spore production (g^{-1} wheat straw) and disease levels in the monocrop and bicrop treatments. Higgins and Fitt (1984) have reported an increase in the severity of eyespot lesions with increasing numbers of spores applied to wheat seedlings. More severe eyespot lesions under a clover canopy, where an increased soil moisture content has been reported (Clements, 1995), supports the results of Higgins *et al.* (1986), who demonstrated that increasing soil moisture content favoured an increase in the severity of eyespot lesions. Jordan and Tarr (1977, 1978) have reported that the incidence and severity of lesions on wheat plants exposed to inoculum in the field is increased by incubation under conditions of higher relative humidity. Higgins and Fitt (1984) have suggested that eyespot lesions, which start in the outer cells of stems and only become more severe as the mycelium spreads to inner cells and to adjacent cells to girdle the stem, would be particularly sensitive to the water potential of the outer cells. Thus, it is possible that, in bicropped plots, higher moisture levels in the upper layer of soil keeps the outer cells of infected stems moist and so provides a more conducive environment for eyespot lesion development.

In the field, it is likely that eyespot lesions are initiated by groups of spores that probably reach plants in splash droplets containing many spores (Fitt and Nijman, 1983). It has also been reported that eyespot is chiefly important in cool, moist climates (Higgins and Fitt, 1984), presumably because of the environmental requirements for sporulation, dispersal and infection. Under simulated rainfall, bicropping has been shown to restrict *P. herpotrichoides* spore dispersal relative dispersal in monocrops (Soleimani *et al.*, 1996). However, it would appear that, in the field, sufficient spores are being produced and transferred to uninfected host tissues for increased disease severity levels to develop within bicrops.

In conclusion, the results suggest that bicropping wheat with white clover increases the incidence and severity of eyespot. The level of disease appears to increase with the duration of the treatment. Further work is required to examine the causes of this increase and to examine whether similar disease level increases are indicated for other wheat stem base and root diseases, especially take-all.

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