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Factors Affecting the Levels of Eyespot and Fusarium Foot-Rot on Winter Wheat cv. Hereward in Cereal Monocrops and Wheat Clover Bicrops

M.J. Soleimani[1] and M.L Deadman[2*]

[1]Department of Plant Protection, Faculty of Agriculture, Bu-Ali Sina University, Hamadan, Iran,
[2]Department of Agronomy, Horticulture, Entomology and Plant Pathology, College of Agriculture, Sultan Qaboos University, P.O. Box 34, Al Khod 123, Sultanate of Oman

العوامل المؤثرة على مستويات مرض التبقع العيني وتعفن الجذور نتيجة فطر فيوزاريوم في القمح الشتوي صنف (هيوارد) كمحصول منفرد أو ثنائي مع البرسيم

الملخص: تم وصف العوامل المختلفة التي تؤثر على مدى المرض الذي يصيب سيقان القمح عند زراعته مع البرسيم الأبيض. لقد كانت كميات بقايا النبات على سطح التربة في نظام المحصول الواحد أعلى منها في حالة المحصولين. إلا أن تعفن البقايا كان أسرع في حالة المحصولين عنها في المحصول الواحد. وقد بلغت كثافة فطر سيدوسيركوبوريلا هيبوتركويدس (*Pseudocercosporella herpotrichoides*) وفطر فيوساريوم (*Fusarium* spp.) معدلا أعلى في بقايا النباتات في نظام المحصولين عنها في بقايا النبات في المحصول المنفرد. ولما كان تحلل النباتات في نظام المحصولين أسرع، فقد ظل لقاح الفطر فيه لمدة أقصر عن ما هو في نظام المحصول الواحد. كانت كثافة معدلات فطر *P. herpotrichoides* في التربة أعلى من كثافة معدلات فطر *Fusarium* spp. وأوضحت النتائج أن هناك تبادلا في المناخ المصغر داخل عروش النباتات، كما أن معزولات الفيوزاريوم في نبات البرسيم احتوت على مستويات مرتفعة من المرض في نبيتات القمح. وقد تمت مناقشة تأثير كل من هذه العوامل على تطور المرض وعلاقته بالتقارير المتوفرة مسبقا حول مستوى المرض في الحقل.

ABSTRACT: Various factors influencing the amount of disease on the stem-base of wheat, when grown as a component of a bicrop, with white clover, are described. The amount of crop debris on the soil surface remained higher in monocrop than bicropped plots. Furthermore, the rate of debris decay was faster in bicrops than in monocrops. Population levels of *P. herpotrichoides* and *Fusarium* spp. were higher on debris within bicrops than on debris within monocrop plots. However, because debris decomposition was more rapid in bicrops, inoculum availability was of shorter duration in bicrops than monocrop plots. In soil, populations of *P. herpotrichoides* were greater in bicropped plots than in monocrops, although no significant differences were observed for *Fusarium* spp. population levels. Pathogen cross-infection between bicrop components and changes in microclimate within crop canopies were shown to occur, and isolates of *Fusarium* from clover were shown to induce significant levels of disease on cereal seedlings. The effects of each of these factors on disease development are discussed in relation to previous reports of disease levels in the field.

A cereal-clover bicropping system, which utilises white clover (*Trifolium repens* L.) as a permanent understorey, has previously been shown to have considerable potential for growing winter cereals with greatly reduced inputs of agrochemicals, especially nitrogen (Jones and Clements, 1993). However, although Soleimani (1977) and Soleimani *et al.* (1999) have shown that the severities of fusarium foot-rot and eyespot respectively tend to increase with successive seasons at a faster rate in bicrops than in monocrops, the causes of this increase in disease have not been clarified.

Pseudocercosporella herpotrichoides (Fron) Deighton and *Fusarium* spp. are two of the pathogens commonly associated with the wheat foot-rot disease complex. Both pathogens are splash dispersed by rainfall. Spores are dispersed, in the case of *P. herpotrichoides*, generally less than 1 m from the

*Corresponding Author.

infection source, although some can travel distances of up to 2 metres (Fitt and Nijman, 1983). When wheat is bicropped with an understorey of white clover, the number of spores dispersed by splash droplets is significantly reduced (Soleimani *et al.*, 1996). Under controlled conditions, using simulated rain, it was found that the number of splash droplets and splash dispersed spores caught at distances of up to 30 cm from the spore source were approximately halved in a bicrop compared to a wheat monocrop (Soleimani *et al.*, 1996). However, in the field, sufficient inoculum potential exists for eyespot and fusarium foot-rot levels to be greater in wheat-clover bicrops than in wheat monocrops (Soleimani, 1997; Soleimani *et al.*, 1999). Clearly, other factors are important in determining the rate of increase in disease severity within bicropping systems. Temperature and relative humidity at the site of infection, which is usually below the clover canopy, are likely to be less variable within a bicrop than in a wheat monocrop. Spore production on crop debris and the rate of decomposition of crop debris are also likely to vary significantly between cropping systems. In the case of *Fusarium* species, especially *F. avenaceum* which is a known pathogen of clover, there is the potential for the clover to act as a reservoir of wheat disease between and within cropping seasons. This report describes results from a series of experiments conducted in order to evaluate the effect of various environmental and biotic factors on wheat stem-base diseases when grown with white clover as a component of a bicropping system.

Materials and Methods

Field trials at the Institute for Arable Crops Research, Long Ashton, UK, were established and maintained, from 1993-1996, as described by Soleimani *et al.* (1999). During 1995/96 wheat straw debris from the previous season (when treatment locations had been identical) was collected from 1 m^2 quadrats laid at 3 locations within the 3 replicates of the 4 experimental treatments (Table 1). The collected straw was dried to a constant weight and the mean dry matter m^{-2} for each treatment was calculated.

Prior to drying, 10 g of the collected straw was cut to give straw lengths of 1-2 cm. The straw was washed in 100 ml sterile distilled water containing a small amount of surfactant (Tween 20) to aid spore removal. The numbers of *Fusarium* and *P. herpotrichoides* conidia g^{-1} straw (based on 3 separate counts from each replicate plot) were calculated for each treatment.

During the 1994/95 and 1995/96 growing seasons, soil samples (depth 0-15 cm) were collected from 3 randomly located sites within each replicate of the 4 treatments. Soil samples were air dried at 35°C and 10 g of each sample was sieved and used for assessment. The isolation of pathogen propagules from soil was done by the dilution plate method (Nelson *et al.*, 1983) on selective media for *P. herpotrichoides* (Sumino *et al.*, 1990) and *Fusarium* (Nash and Synder, 1962). The appropriate dilution factor was selected by choosing that which gave 10-20 colonies per Petri dish.

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
A	Donna	No
B	Milkanova	No
C	None	No
D	None	Yes

[a] cv Donna is a small leaved clover variety, cv. Milkanova has large leaves

The potential of clover to act as a reservoir of *Fusarium* infection was assessed during the 1993/94 season. During April and July, 20 clover plants were taken at random from a diagonal transect of the low input bicrop treatments (plots A and B, Table 1). Small segments (0.5 - 1.0 cm) of roots were surface sterilized in 10% sodium hypochlorite for 5 minutes followed by rinsing (3 times) in sterile distilled water. Clover samples were plated on a selective medium (Nash and Synder, 1962) and incubated at 25°C. The frequency of isolation (disease incidence) of *F. avenaceum* from root pieces was recorded as a percentage of the roots sampled.

To test the potential of *F. avenaceum* from clover to cause disease on wheat, two isolates from clover and a single isolate from wheat (cv. Hereward), taken from a sample originating from the same field trial, were used in cross inoculation tests. Seed of cv. Hereward was soaked for 1 min. in a suspension of *F. avenaceum* spores at a concentration of 2 x 10^5 conidia ml^{-1}. The seeds, including controls soaked in sterile distilled water, were sown in sterilized soil-based compost in 20 cm pots, with 8 seeds per pot. Immediately after sowing the pots were watered with 50 ml of the *F. avenaceum* spore suspension; control pots were watered with an equivalent volume of sterile distilled water. Four replicate pots were used for each treatment. Plants were assessed for disease symptoms after 10 weeks growth in a glasshouse at 20°C using the disease index rating (DIR) scale of Celetti *et al.* (1990). The number of tillers produced by each plant was also recorded.

During 1995/96, data loggers (Tinytalk II, RS Components Ltd, Corby, UK) with appropriate sensors for recording canopy air temperature and relative humidity, were installed within crop canopies in the wheat-clover bicrop and wheat monocrop experimental plots. Air temperature was measured with thermister probes placed 15 cm above ground level. Relative humidity (RH) within crop canopies was recorded with shielded RH probes placed 15 cm above ground level in replicate plots. Measurements of temperature and RH were monitored at 2.4 hour intervals from late December to the end of the growing season. Before harvest the data from the loggers was down-loaded and the mean daily air temperature and RH in each treatment was calculated.

Results

DEBRIS DECOMPOSITION: Wheat debris persistence in the crop canopies during the growing season, expressed as total dry matter (DM) m^{-2}, is shown in Table 2. The amounts of debris remained higher in monocrop direct drilled plots than in other plots. In December the amount of debris on the soil of bicropped plots (25.5g m^{-2}), was 35% of that on the soil of monocrops (73.3g m^{-2}, Table 2). By June the amount of stubble in bicrop plots (7.5g m^{-2}) was 17.8% of the amount on monocrop plots (42.1g m^{-2}, Table 2). The amount of debris within plots of monocrop wheat sown following ploughing was low throughout the season (Table 2).

FUSARIUM SPP. AND *P. HERPOTRICHOIDES* CONIDIAL PRODUCTION ON CROP DEBRIS: Numbers of conidia (g^{-1} wheat debris) of *Fusarium* spp. and *P. herpotrichoides* are shown in Table 3. On all sample dates *Fusarium* spore production was higher on stubble within bicrops than on stubble from monocrops. The results were similar for *P. herpotrichoides* with the exception of the final sample date (Table 3). There were no consistent significant differences between plots with different clover varieties for either pathogen. Similarly, there were no consistent differences on spore production levels, of either pathogen, from stubble collected from ploughed and non-ploughed monocrop treatments.

PATHOGEN ISOLATION FROM SOIL: Table 4 shows mean data on pathogen population levels in soil taken from the four experimental plots. Population levels for both pathogens were, with the exception of plot B, higher in 1995/96 (third wheat) than 1994/95 (second wheat). Population levels of *P. herpotrichoides* in bicropped plots (6.7 and 7.3 propagules g^{-1} in 1994/95, and 10.0 and 4.3 propagules g^{-1} in 1995/96) were significantly higher ($P < 0.05$) than in monocropped plots (2.8 and 0.7 propagules g^{-1} in 1994/95, and 3.7 and 4.0 propagules g^{-1} in 1995/96).

TABLE 2

Amounts of wheat stubble (gm^{-2}) persisting in wheat mococrop and cereal-clover bicrop plots at various dates during the growing season of wheat cv. Hereward.

Date	Plot A/B[a]	Plot C	Plot D
13.12.95	25.5	73.3	1.1
11.02.96	21.8	42.5	1.2
01.03.96	15.6	48.1	2.1
08.04.96	10.3	46.4	0.6
14.06.96	7.5	42.1	0.4

[a] Amounts of stubble collected from treatments A and B (see Table 1) were combined

TABLE 3

Mean number (thousands) of P. herpotrichoides conidia and Fusarium spp. spores g^{-1} of wheat stubble in wheat monocrop and cereal-clover bicrop plots of winter wheat cv. Hereward.

	Sample Date				
Treatment[b]	13.12.95	11.02.96	01.03.96	28.04.96	14.06.96
P. herpotrichoides					
A	6.1	110.0	60.0	90.0	850.0
B	111.0	45.5	89.0	13.5	105.0
C	5.5	23.5	20.0	31.5	50.0
D	22.0	27.0	28.5	46.5	0.0
LSD	62.0	100.0	50.0	33.5	39.0
Fusarium Spp					
A	61.5	71.5	138.0	121.5	95.0
B	221.5	26.5	227.0	153.5	98.5
C	28.0	55.0	109.5	61.0	50.0
D	50.0	16.5	59.5	65.5	0.0
LSD	131.5	32.5	132.0	55.5	44.0

Mean of 3 replicates; [b] see Table 1

There were no consistent significant differences in *P. herpotrichoides* population levels between plots with different clover varieties, or between monocrop plots sown by direct drilling or sown following plough cultivations. There were no consistent significant differences in *Fusarium* spp. levels between monocrop and bicrop treatments, or between treatments with different clover varieties, or between monocrops established following direct drilling and plough cultivations (Table 4).

ISOLATION OF *F. AVENACEUM* FROM CLOVER: The frequency of isolation of *F. avenaceum* from clover cvs Donna and Milkanova in April and July 1994 is shown in Table 5. The isolation frequency of *F. avenaceum* increased greatly from low levels in April (3.6% and 6.0% of clover plants yielding isolates in treatments A and B respectively) to high levels in July (53.0% and 61.0% of clover plants yielding *F. avenaceum* isolates). There were no significant differences in disease incidence between the two clover varieties.

FUSARIUM CROSS INFECTION FROM CLOVER TO WHEAT: The mean DIR scores resulting from inoculation by each *F. avenaceum* isolate is shown in Table 6. The DIR was greatest (90.63) in wheat inoculated with the wheat isolate of *F. avenaceum*. Wheat inoculated with *F. avenaceum* from clover caused less severe symptoms (DIR = 74.38 and 69.38 for the two isolates) than the wheat isolate but had significantly ($P < 0.05$) more severe symptoms than the control (DIR = 10.00). Wheat tiller number was shown to be significantly ($P < 0.05$) lower in plants inoculated with *F. avenaceum* isolated from wheat (1.80 tillers per plant) than in wheat inoculated with *F. avenaceum* isolated from clover (3.70 and 3.50 tillers per plant for the two isolates). Tiller number in the control plants (3.68 tillers per plant) was not significantly different from tiller number per wheat plant inoculated with the clover isolates of *F. avenaceum*.

TABLE 4

Soil population levels[a] (propagules g^{-1} soil) of P. herpotrichoides and F. avenaceum in wheat monocrop and cereal-clover bicrop plots of winter wheat cv. Hereward.

	P herpotricoides		*F. avenaceum*	
Treatment[b]	94/95	95/96	94/95	95/96
A	6.7	10.0	2.0	4.1
B	7.3	4.3	4.1	3.0
C	2.8	3.7	2.5	2.6
D	0.7	4.0	1.2	3.0
LSD	2.5	2.8	1.7	4.1

[a] Mean of 3 replicates; [b] see Table 1

TABLE 5

Mean incidence (%)[a] of F. avenaceum infection of clover from wheat-clover bicrops.

	Treatment[b]	
Date	A	B
29.04.94	3.6	6.0
01.07.94	53.0	61.0

[a] Mean of 3 replicates; [b] see Table 1

TEMPERATURE AND RELATIVE HUMIDITY DIFFERENCES BETWEEN MONOCROP AND BICROP CANOPIES: During the early part of the growing season, when the clover plants were still close to the ground, temperature differences between the treatments were small. As the season progressed the increased clover growth produced shading at the base of the wheat stems and consequently temperatures were lower than those in the monocrop treatments. As a result, between the period 20 March to 5 May, the total number of days when the mean air temperature was optimum for eyespot infection (7-8°C, Fitt and White, 1988) was 33 days in the wheat clover bicrop, and 19 days in the wheat monocrop.

Similarly, during the early part of the season when wheat and clover growth was slow and the crop canopies were less than 10 cm in height, there was no significant difference between the in-crop humidity levels. As the season progressed, humidity at the wheat stem-base was higher (>85%) in the wheat-clover bicrop compared to the wheat monocrop. The air within the clover canopy remained saturated for prolonged periods. This occurred during the time when the clover canopy was developing rapidly, eventually providing complete ground cover between the wheat plants. During the 45 day period from 20 March to 5 May the mean daily RH in the bicrop was greater than 85% on 30 days, the level necessary for eyespot infection (Fitt and White, 1988). In the wheat monocrop, over the same period, the number of days with RH > 85% was 17.

Discussion

Within any intercropping system, including the wheat-clover bicropping system, different factors are likely to cause increased or decreased disease pressure. Soleimani *et al.* (1996) have shown that within the

wheat-clover system, the sieving effect of the clover results in fewer spores of wheat pathogens, such as *P. herpotrichoides*, being effectively splash dispersed from sporulating lesions, or crop debris, to healthy plant material. Similar results have also been reported for foliar pathogens of wheat such as *Septoria tritici*, when the cereal is grown as an intercrop component (Bannon and Cooke, 1998). Thus, under the controlled conditions employed by Soleimani *et al.* (1996), disease pressure in an intercrop may be less than in a monocrop. Under field conditions, however, there is likely to be redistribution, by rain splash, of pathogen spores sieved out by the clover. A proportion of these redistributed spores will be deposited onto wheat plants. Indeed, Soleimani *et al.* (1996) have shown that such redistribution is greater from clover foliage than from soil.

In the current study, wheat debris remaining from the previous cropping season was removed, by decomposition, at a faster rate in the bicrop than in the wheat monocrop established by direct drilling of seed (Table 2). However, Table 3 shows that pathogen spore production (*Fusarium* spp. and *P. herpotrichoides*) was greater on debris within the bicrop compared to the monocrop. Conditions under the clover canopy, within the bicrop, were presumably more conducive, in terms of RH, for spore production than in a wheat monocrop. An analysis of the relationship between spore production on crop debris in wheat monocrops and wheat-clover bicrops, at different levels of input, has indicated a positive relationship between *P. herpotrichoides* spore numbers produced and disease levels expressed as the area under the eyespot disease progress curve (Soleimani, 1997).

Table 4 indicates that the soil propagule population level of *P. herpotrichoides* was higher in bicrop plots than monocrop plots. This may be a reflection of the higher levels of spore production on crop residue within the bicrop system. Rowe and Powelson (1973) have shown that crop debris represents the major source of inoculum for eyespot infection. It is, therefore, unlikely that the raised level of propagule population in soils within bicropped treatments contributed to the increased level of disease observed in this treatment by Soleimani (1997).

The observation that *F. avenaceum* disease incidence on clover can increase to 50% (Table 5) may have serious implications for an intercropping system containing crops which share common pathogens. *F. avenaceum* is a severe pathogen of wheat in the UK (Jenkinson and Parry, 1994). It is also recognised as a serious pathogen of clover and other legumes (Booth, 1971). In a field survey, most of the *Fusarium* species isolated from common broad-leaved weeds were pathogenic on winter wheat (Jenkinson and Parry, 1994).

TABLE 6

Mean disease index rating (DIR) and tiller number on wheat (cv. Hereward) inoculated with F. avenaceum isolated from wheat and clover.

Treatment	Mean DIR[a]	Tiller number
F. avenaceum (clover 1)	74.38	3.70
F. avenaceum (clover 2)	69.38	3.50
F. Avenaceum (wheat)	90.63	1.80
Control	10.00	3.68
LSD	8.78	0.86

[a] Mean of 5 replicates

In low input systems, weeds may cause problems where herbicide doses are reduced. Within wheat-clover bicrops, *F. avenaceum* has been shown to cause significant levels of disease on the wheat crop (Soleimani, 1997). In the current study, isolates of *F. avenaceum* from clover caused significant levels of disease on the wheat seedlings (Table 6). Although the broad host range of this pathogen is well established (Booth, 1971), the degree of host specialisation within individual isolates is unclear. However, the results suggest that within bicrops there could be a significant risk to the wheat crop from inoculum originating from the clover component. Furthermore, *F. avenaceum* might be able to maintain pathogenicity and virulence on the legume crop between wheat crops. Further investigations are needed to determine the rate of *F. avenaceum* build-up in the soil of bicropping systems through a single growing season, and the role of clover in maintaining a potential reservoir of inoculum between seasons.

Data obtained from the temperature sensors indicates that during the part of the season when the risk of eyespot infection was highest (November - March in the UK, Hollins and Scott (1980)), the temperature within the bicrop was lower and the number of days when conditions were favourable for infection was greater. Eyespot infection is greatly influenced by factors affecting sporulation on infected straw, dispersal of conidia and latent period before symptoms become visible (Fitt and White, 1988). Temperature is one of the main factors which influences sporulation on debris, production being particularly abundant at temperatures of 0-15°C (Scott, 1971; Higgins and Fitt, 1984).

Infection of wheat by *P. herpotrichoides* is most effective when RH is greater than 85% (Fitt and White, 1988). In the current study, during the 45-day period from 20 March–5 May, RH conditions were favourable for eyespot infection on 30 days in the bicrop but only 17 days in the monocrop. When favourable

temperature and RH conditions are combined, the current results suggest that during the same period 53% of days were suitable for eyespot infection in the bicrop compared to 20% of days in the monocrop. Furthermore, Hollins and Scott (1980) have suggested that increased evaporation, leading to reduced moisture on and in the host tissue, is an important cause of reduced rates of eyespot infection in summer relative to spring months. In the current study, the humidity at the wheat stem-base was little different in monocrops and bicrops until April; subsequently the RH was significantly higher in the bicrop. It is likely therefore that new infections could be initiated over a longer period within the bicrop.

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