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Studies on the interaction between *Septoria tritici* and *Stagonospora nodorum* in wheat

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Abstract

Interactions between *Stagonospora nodorum* and *Septoria tritici* were studied. Results from a detached glume experiment indicated that the interaction may be isolate–dependent, as it was shown that the interaction between the two pathogens may be beneficial or antagonistic depending on the isolate of each pathogen present. The number of spores produced by both pathogens was significantly greater when an aggressive isolate of *S. tritici* was mixed with a non-aggressive isolate of *S. nodorum*, whereas the number of spores produced by both pathogens was significantly less when two non-aggressive isolates were mixed. There was a significant reduction in disease level when *S. tritici* was applied prior to *S. nodorum*, compared to vice versa in the growth chamber. Results from growth chamber and field studies showed that *S. nodorum* produced significantly more spores when both pathogens were present together. It is concluded that *S. tritici* has a stimulatory effect on spore production by *S. nodorum*. However, there was a reduction of *S. tritici* spores observed in the dual inoculation treatments, suggesting that *S. nodorum* inhibits *S. tritici*.

Introduction

Most cereal pathology research has involved the study of single pathogens in isolation. However, in natural environments microorganisms rarely grow in total isolation from each other and the success of a particular organism may be influenced by the metabolic activities of others. It has been shown that plants attacked by one pathogen may be predisposed to attack by a second unrelated pathogen (Yarwood, 1959). *Septoria tritici* Roberge in Desmaz. [teleomorph: *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn] and *Stagonospora nodorum* (Berk.) Castellani and E.G. Germano [teleomorph: *Phaeosphaeria nodorum* (E. Müller) Hedjaroude] share a common host; therefore situations may occur in which the two organisms come into direct contact with each other, thus providing an opportunity for an interaction to occur. There have been many reports showing interactions between *S. nodorum* or *S. tritici* and other organisms (Brokenshire, 1974; Madariaga and Scharen, 1986; Spadafora and Cole, 1987; Adee et al., 1990). However, reports on the possibility of an interaction occurring between *S. nodorum* and *S. tritici* have not been conclusive. It has previously been reported that *S. tritici* has a stimulatory effect on *S. nodorum* in terms of symptom expression and spore production (Jones and Odebunmi, 1971). However, the typical blotching symptoms caused by *S. nodorum* tended to mask the less obvious *S. tritici* symptoms. Harrower (1978) suggested that *S. tritici* compensates for its relatively weaker colonising and disruptive ability by increasing the number of propagules available for dispersal when *S. nodorum* is present, and claimed that *S. tritici* may have a greater colonisation ability when the leaf tissue is already necrotic as a result

of *S. nodorum* infection. Jenkins (1978) found no evidence for an interaction between *S. nodorum* and *S. tritici*, and recorded that the amount of disease on leaves inoculated with both *S. tritici* and *S. nodorum* was approximately equal to the amount of disease on leaves infected with either pathogen alone. However, Jenkins (1978) noted a decrease in the level of *S. nodorum* on plants previously inoculated with *S. tritici*, although the decrease was not significant. Jenkins and Jones (1981) reported no evidence for an interaction between *S. nodorum* and *S. tritici* in terms of symptom expression, although the yield reduction was less when both pathogens were present together compared to either pathogen alone. Suparman (1994) showed the incubation period of *S. nodorum* increased when the pathogen was mixed with *S. tritici*.

Interactions between organisms have been studied using a number of techniques, including tests of inhibition *in vitro* (Skidmore and Dickinson, 1976), microscopic observations of hyphal interactions (Skidmore and Dickinson, 1976; Lewis et al., 1989) and visual estimation of disease symptoms. The predominant sterol component of most fungi, ergosterol, has been used to measure the level of fungal biomass in plants by many researchers (Ride and Drysdale, 1972; Seitz et al., 1979; Griffiths et al., 1985; Parker and Royle, 1993; Gunnarsson et al., 1996). In the present study, the level of ergosterol in infected plant tissue together with several other tests were used to determine if an interaction can occur between *S. tritici* and *S. nodorum* under controlled experimental conditions.

Materials and methods

Detached glume experiment

Nine glumes from winter wheat (*Triticum aestivum* L.) cv. Riband were placed with their adaxial surface facing the upperlid in 9 cm Petri dishes containing 0.5% water agar supplemented with 1.5 ml kinetin l^{-1}. Three replicates were used for each treatment. Each glume was inoculated with a 10 ml droplet of a spore suspension (10^6 spores ml^{-1}) of either *S. tritici* (T1 and T2), *S. nodorum* (N1 and N2) or an equal mixture of the two pathogens. Inoculum was produced on Czapek Dox V-8 (CDV-8) agar incubated at 18 °C for 14 days under a light cycle of 12 h near-ultraviolet (NUV) light/12 h dark (Cooke and Jones, 1970). Glumes were incubated at 18 °C under continuous white light. After 21 days, 9 glumes per treatment were macerated in 15 ml water, filtered through sterile muslin, and the number of spores of each pathogen counted using a haemocytometer. The de Wit replacement series (de Wit, 1960) described by Adee et al. (1990) was used to determine if there was an interaction between *S. nodorum* and *S. tritici*. The relative spore yield (RY) of each pathogen (yield in the mixture divided by yield when present alone) was calculated and then plotted against the input proportion (100, 50 or zero). The RY of each pathogen when present alone (100%) was equal to 1.0 and the other RYs were calculated with reference to this; at each input proportion, the sum of the RY gave the relative yield total (RYT). The shape of the RYT line reflected the interaction between the two pathogens in the mixture. The data were analysed by paired comparison *t*-tests, which compared observed RYs with expected RYs using a null-hypothesis model, in which interspecific and intraspecific competition were equal.

Growth chamber experiments

Wheat plants (cv. Riband) were maintained in a growth chamber (10 plants per pot) using a 16 h light cycle (16 °C, 60% RH) and 8 h dark cycle (12 °C, 85% RH) for 21 days (photosynthetic photon flux density was approximately 350 $\mu Em^{-2}s^{-1}$ measured by automatic light measure of a Ciras® Parkinson Leaf Chamber, PP Systems). Prior to inoculation, the youngest fully expanded leaf of each of the 10 plants was fixed horizontally onto bent polyacrylamide plates with unbleached cotton string according to the method of Jørgensen et al. (1996). Inoculum of *S. nodorum* (isolate N1) and *S. tritici* (isolate T1) was produced as described above. Four replicates were inoculated using a fine-mist atomiser with 20 ml *S. nodorum* (10^5 spores ml^{-1}) and 4 days later with either 20 ml *S. tritici* or water. Four replicates were also inoculated with 20 ml *S. tritici* (10^5 spores ml^{-1}) and 4 days later with either 20 ml *S. nodorum* or water. After each inoculation, the plants were covered with clear polythene bags for 48 h. The leaves were assessed for % disease severity 19 days post-inoculation (d.p.i.). As *S. tritici* did not produce typical necrosis in the growth chamber, the parameter used to assess disease severity for this pathogen was % area covered by pycnidia whilst % necrosis was assessed for *S. nodorum*. In this way, disease severity caused by both pathogens could be distinguished.

At the termination of the experiment, the leaves were macerated in 10 ml water which was filtered through sterile muslin prior to counting the spores of each pathogen using a haemocytometer. The experiment was repeated 3 times, and the mean data calculated.

Two infected leaves from each replicate were sampled per treatment on two occasions, 9 and 16 d.p.i., for quantification of ergosterol levels. Two leaves per replicate were cut and placed in teflon-lined screw-cap test tubes containing 2 ml methanol. The samples were stored at $-20\,^{\circ}C$ in the dark prior to ergosterol extraction from the leaves by refluxing and hydrolysation, followed by splitting of liquid phases (Gunnarsson et al., 1996). HPLC analysis was performed on a Spectraphysics liquid chromatograph (Spectra System P200) equipped with a rheodyne injector and a 20 µl loop. The ergosterol was separated on a 100 × 3 mm i.d. ChromSpher C18 (5 µm) column with a 10 × 2 mm ChromSep guard column (Chrompack), and detected using a Spectraphysics variable wavelength spectrophotometric detector (Spectra SYSTEM UV-2000) set at 282 nm. The mobile phase was acetonitrile/hexane/2-propanol (90/5/5, v/v/v) and isocratic elution took place at $1\,ml\,min^{-1}$. The individual ergosterol levels for *S. nodorum* and *S. tritici* were combined (Sn + Str) for comparison with the dual inoculation treatments (Sn–Str and Str–Sn). The results were analysed using analysis of variance (ANOVA) and the means separated using Fisher's protected least significant difference (pLSD) tests (STATVIEW v. 4.5).

Field experiment

Winter wheat (cv. Riband) was sown in microplots, surrounded by $1\,m^2$ guard rows of oats. Inoculum of *S. nodorum* and *S. tritici* was produced on CDV-8 agar as described previously. In the case of *S. nodorum*, a 6 mm plug from the leading edge of a recently isolated *S. nodorum* colony (isolate N1) was placed in the centre of each CDV-8 plate. Inoculum of *S. tritici* was produced by streaking from a recently isolated *S. tritici* colony (isolate T1). After 14 days, the cultures were mixed with sterile distilled water (SDW) and the spore concentration of each pathogen adjusted to 5×10^5 spores ml^{-1}. One drop of Tween 20 was added per litre of inoculum to increase the wettability of leaf surfaces, prior to inoculation. At growth stage (GS) 49 (Zadoks et al., 1974) 4 replicates per treatment were inoculated with either 50 ml *S. nodorum* (N1), 50 ml *S. tritici* (T1), or 50 ml of an equal mixture of *S. nodorum* and *S. tritici*. Seven days later, plants previously inoculated with *S. nodorum* were inoculated with either 50 ml *S. tritici* or water, and those which previously received *S. tritici* were inoculated with either 50 ml *S. nodorum* or water. After each inoculation, the clumps were enclosed within large clear polythene bags for 72 h. Percentage disease severity was estimated on the flag leaves of 10 main tillers per replicate using the ADAS Septoria key (Anonymous, 1976) 14, 21 and 28 days after the first inoculation; no attempt was made to distinguish between symptoms of each pathogen. Five tillers per treatment were removed 33 days after the first inoculation to determine the number of spores of each pathogen present. Each tiller was cut into small pieces and placed into 250 ml flasks containing 50 ml water and 2 drops of cotton blue in lactophenol. The flasks were placed on an orbital shaker set at 140 rpm for 30 min, the suspension filtered through sterile muslin, and the number of spores of each pathogen determined using a haemocytometer. The mean count of 5 tillers was used per treatment. The results were analysed using ANOVA and the means separated using Fisher's pLSD test.

Results

Detached glume experiment

RY values and RYT lines for the interaction between *S. nodorum* (N1 and N2) and *S. tritici* (T1 and T2) are shown in Figure 1(a–d). An RYT line that remains at 1.0 results when the pathogen mixture is yielding in proportion to the input. The RYT lines obtained clearly indicate an interaction between the two pathogens. The observed total yield (i.e. RYT lines) for the following combinations: T1 and N1, T2 and N1 and T2 and N2 was significantly lower than the expected total yield (i.e. 1.0) calculated from single inoculations ($P < 0.05$), i.e. concave downward. Concave downward RYT lines (Figure 1a, c and d) indicate stronger interspecific rather than intraspecific competition, i.e. antagonism (Adee et al., 1990). However, the RYT line for the mixture of T1 and N2 was significantly greater than the expected value ($P < 0.05$). The concave upward RYT line (Figure 1b) is indicative of a beneficial effect of one or both pathogens on the other. The RY lines show that there was a significant decrease in the number of spores of *S. tritici* when T2 was mixed

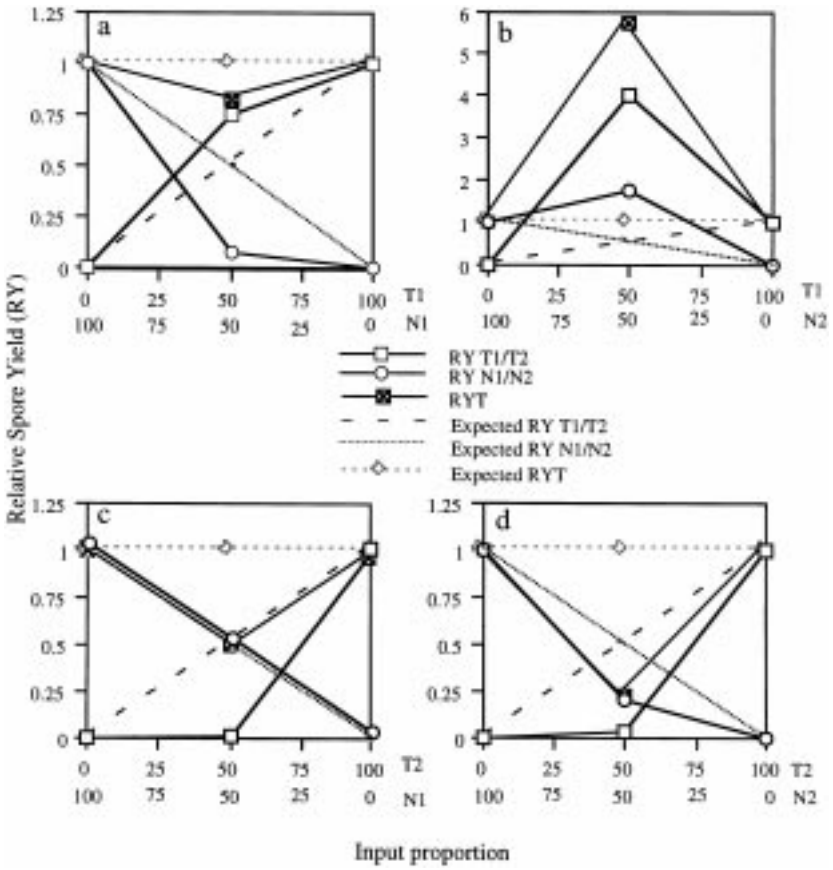

Figure 1. Detached glume experiment. Relative spore yields of *S. tritici* (T1 and T2) and *S. nodorum* (N1 and N2) on cv. Riband 21 d.p.i. The interactions between (a) T1 and N1, (b) T1 and N2, (c) T2 and N1 and (d) T2 and N2 are shown. The spore yield of each pathogen alone is given a value of 1.0 and the RY (relative yield) when the pathogens are mixed together (50% input) is expressed as a proportion of this value. The RYT (relative yield total) is calculated by adding the RYs of the two pathogens together. The pathogens are expected to yield in proportion to the input of each; therefore the expected RYT is 1.0 (i.e. when there is no interaction).

(50% input) with N1 (Figure 1c) and N2 (Figure 1d) compared to the expected value ($P < 0.001$). The number of spores of *S. tritici* was significantly higher when T1 was mixed (50% input) with N1 (Figure 1a) and N2 (Figure 1b) compared to the expected value ($P < 0.001$). The number of spores of *S. nodorum* was significantly less than the expected value when N1 was mixed (50% input) with T1 ($P < 0.001$) (Figure 1a); however, the number of spores of N1 was equal to the expected value when mixed with T2 (Figure 1c). The RY line for N2 shows that the number of *S. nodorum* spores was significantly greater than expected when N2 was mixed with T1 ($P < 0.05$) (Figure 1b) and significantly less than expected when mixed with T2 ($P < 0.01$) (Figure 1d).

Growth chamber experiment

Disease severity was estimated by assessing % necrosis (for *S. nodorum*) and % area of the leaf covered by pycnidia (for *S. tritici* as *S. tritici* did not always produce necrosis in the growth chamber) and the results are presented in Table 1. When *S. nodorum* was present alone (Sn) and when *S. nodorum* was applied prior to *S. tritici* (Sn–Str) there was significantly more necrosis compared to the other treatments ($P < 0.01$). There was less necrosis when *S. nodorum* was present alone (Sn) compared to the *S. nodorum* followed by *S. tritici* treatment (Sn–Str); however the difference was not significant. *S. nodorum* produced significantly more spores when *S. tritici* was applied prior to (Str–Sn)

Table 1. Growth chamber experiment. Mean % necrosis and number of spores of *S. nodorum* and *S. tritici* 19 d.p.i (standard deviation in brackets)

Treatment		% Necrosis	Number of spores 10 ml^{-1} (×50,000)	
Inoculation 1	Inoculation 2		*S. tritici*	*S. nodorum*
S. nodorum	Water	70.5 (4.2)	0.0 (0.0)	8.5 (0.6)
S. tritici	Water	26.4 (3.3)	55.8 (6.7)	0.0 (0.0)
S. nodorum	*S. tritici* (Sn – Str)	72.5 (13.3)	1.8 (0.9)	46.7 (4.1)
S. tritici	*S. nodorum* (Str – Sn)	45.5 (8.8)	8.5 (1.3)	12.6 (1.7)

or after (Sn–Str) *S. nodorum* compared to *S. nodorum* alone (Sn) ($P < 0.05$). However, there were significantly more *S. nodorum* spores produced when *S. nodorum* was present prior to *S. tritici*, compared to vice versa ($P < 0.01$) (Table 1). There were significantly more *S. tritici* spores produced when *S. tritici* was present alone (Str) compared to the other treatments ($P < 0.001$); however, there were significantly more spores produced by *S. tritici* when *S. tritici* was applied prior to *S. nodorum*, compared to vice versa ($P < 0.001$) (Table 1).

Following ergosterol extraction, the peak areas of samples were compared with those obtained by direct injection onto the column of standard ergosterol solutions (0–3.5 μg ml^{-1}). The concentration of ergosterol was expressed as μg per leaf and the results are presented in Figure 2. The level of ergosterol was significantly higher when *S. nodorum* was applied prior to *S. tritici* compared to vice versa ($P < 0.01$). The level of ergosterol increased significantly ($P < 0.001$) for all treatments at 16 d.p.i. (Figure 2).

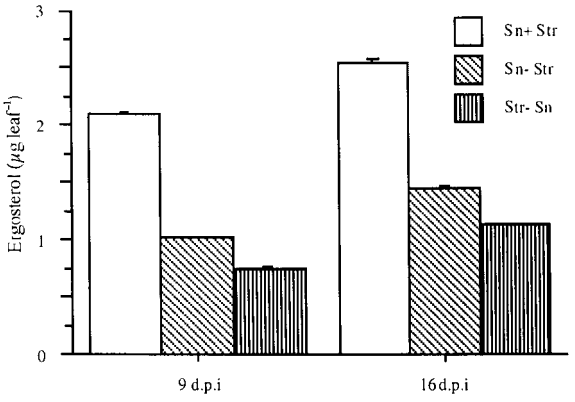

Figure 2. Growth chamber experiment. Mean ergosterol content (±standard error) in wheat leaves 9 and 16 d.p.i. with *S. nodorum* (Sn) and *S. tritici* (Str).

Field experiment

The mean % necrosis produced by the treatments at three assessment dates is presented in Figure 3. Overall, treatment 4 (*S. nodorum* applied prior to *S. tritici*) produced significantly more necrosis than the other treatments ($P < 0.05$). Dual inoculation treatments produced significantly more disease compared to either pathogen alone (($P < 0.05$). The results of tiller washings are presented in Table 2; *S. nodorum* produced significantly less spores when the pathogen was present alone compared to when *S. tritici* was also present ($P < 0.01$). The greatest numbers of *S. nodorum* spores were produced when *S. nodorum* was applied prior to *S. tritici*. There were significantly more *S. tritici* spores produced when *S. tritici* was applied prior to *S. nodorum* compared to the other treatments ($P < 0.001$); however, there were more *S. tritici* spores produced when *S. tritici* was applied prior to *S. nodorum*, compared to the other treatments ($P < 0.01$). However, there were significantly more *S. tritici* spores produced when *S. tritici* was present alone (treatment 3) compared to treatments 4 (*S. nodorum* applied prior to *S. tritici*) ($P < 0.05$) and 6 (a mixture of *S. nodorum* and *S. tritici* applied prior to water) ($P < 0.01$).

Discussion

When two plant pathogens are present on the same host, the interaction between them can be expressed as predisposition, antagonism and/or predation. Many of the effects of interactions between plant pathogens are mediated by the host plant; following infection by one organism, the host plant tissue becomes more or less susceptible to another organism (Yarwood, 1959). The objective of the present study was to determine if the presence of *S. nodorum* affects disease development by *S. tritici* and vice versa.

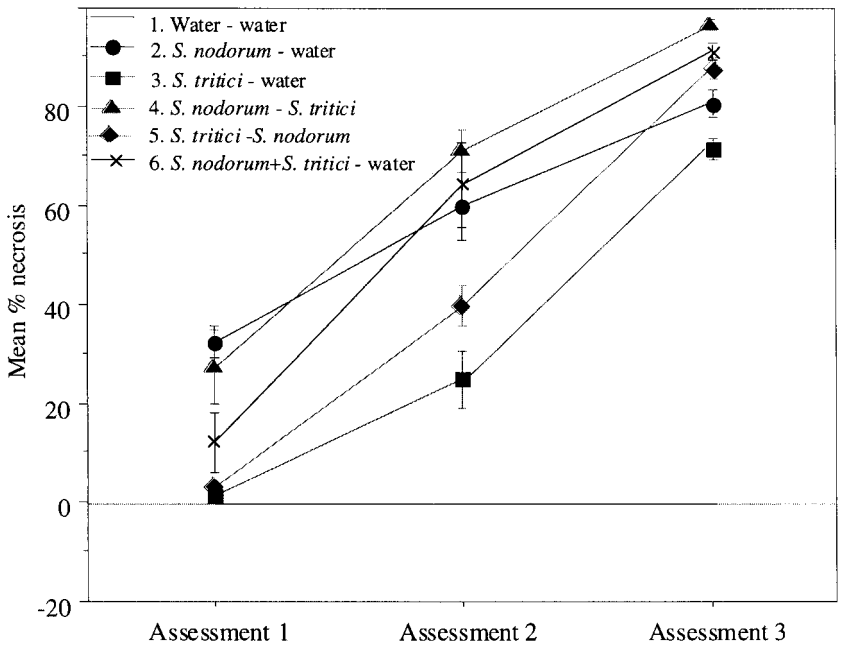

Figure 3. Field experiment. Mean % disease severity (±standard error) at the first (14 d.p.i.), second (21 d.p.i.) and third (28 d.p.i.) assessment on cv. Riband leaves inoculated with either *S. tritici*, *S. nodorum*, a mixture of *S. nodorum* and *S. tritici* or water and after 7 days, inoculated with either *S. tritici*, *S. nodorum*, a mixture of *S. nodorum* and *S. tritici* or water (d.p.i. = days after first inoculation).

Table 2. Field experiment. Mean number of spores of *S. nodorum* and *S. tritici* 33 d.p.i (standard deviation in brackets)

Treatment		Number of spores 10 ml^{-1} (×50,000)	
Inoculation 1	Inoculation 2	*S. tritici*	*S. nodorum*
Water	Water	0.0 (0.0)	0.0 (0.0)
S. nodorum	Water	0.0 (0.0)	1.8 (0.6)
S. tritici	Water	4.1 (1.3)	0.0 (0.0)
S. nodorum	*S. tritici*	2.8 (0.9)	7.3 (1.7)
S. tritici	*S. nodorum*	9.5 (1.3)	5.0 (0.8)
S. nodorum + *S. tritici*	Water	2.4 (0.5)	4.0 (1.8)

There have been several recent reports where de Wit plots have been used to describe interactions between pathogens (Adee et al., 1990; Newton et al., 1998). It has also been reported that spore production represents the total effect on pathogen multiplication of all components of host resistance and is therefore considered to be a more useful estimate of interactions (Johnson and Taylor, 1976). The de Wit analysis was used here to describe the results of spore counts from the detached glume experiment. The majority of the spore yield total (RYT) lines were significantly less than the expected value, i.e. concave downward, indicating a strong interspecific interaction. However, one of the RYT lines was significantly greater than the expected value i.e. concave upward. A concave upward RYT line is reported to be indicative of a beneficial effect of one or both pathogens on the other (Adee et al., 1990). The number of spores produced by both pathogens was significantly greater when T1 and N2 were mixed and significantly less when T2 and N2 were mixed. This suggests that the interaction between *S. nodorum* and *S. tritici* may be isolate-dependent. Previous experiments (not reported here) have shown that T1 is more aggressive than T2 and N1 is more aggressive than N2. An aggressive isolate of one pathogen mixed with a non-aggressive isolate of the other pathogen, such

as T1 and N2, may give a different result to a mixture of isolates of equal aggressiveness. The interaction between the two pathogens may be either antagonistic or beneficial depending on the isolate of each pathogen present. A criticism of this study is that only a single mixture (50 : 50) was used; however, other experiments (not reported here) using different proportions of each pathogen in the mixture also showed an interaction between the two pathogens, although it was shown that the interaction depends on the proportion of each pathogen in the mixture. *S. tritici* was shown to have a stimulatory effect on sporulation by *S. nodorum* when both pathogens were present in equal proportions or there was a greater proportion of *S. tritici* in the mixture. However, a negative effect of *S. tritici* on sporulation by *S. nodorum* was observed when a greater proportion of *S. nodorum* was present.

de Wit curves have been criticised for the fact that they do not take into account the expected density-dependence of competition (Snaydon, 1991). It has been reported that changes in the density of one organism (strain) are confounded by effects of changes in the density of the other, making it impossible to fully describe reproductive dynamics as a function of both organisms (strains) (Snaydon, 1991). However, Newton et al. (1998) concluded that deviations from expected null RYT straight lines are produced only by differences between intra- and interstrain competition; thus a significant deviation from the null RYT line is good evidence that intra- and interstrain competition differ significantly for at least one of the competing strains. Thus, the significant deviation from the null straight lines observed in this study can be taken as a good indicator that there is an interaction between *S. nodorum* and *S. tritici*. However, the de Wit plots do not offer substantial insight into the mechanisms behind observed relative reproductive outputs in co-existing pathogen strains (Newton et al., 1998).

In the growth chamber experiments there was significantly less disease when *S. tritici* was applied before *S. nodorum*. It was also shown that there were significantly more *S. nodorum* spores produced when both pathogens were present together. This suggests that *S. tritici* has a stimulatory effect on *S. nodorum* in terms of spore production, especially when *S. nodorum* is applied prior to *S. tritici*. However, it was observed that *S. nodorum* has an inhibitory effect on spore production by *S. tritici*, as there were significantly fewer *S. tritici* spores produced when both pathogens were present together. Gunnarsson et al. (1996) recorded great variation in levels of ergosterol and pointed out the limitations of this method for quantitative fungal biomass analysis in natural samples. However in the present study, single-spore isolates were used, inoculations were carried out at the same time, and the conditions were identical for all treatments; therefore the levels of ergosterol found represent accurate values for treatments relative to each other. The level of ergosterol for Sn + Str was significantly higher than in the other treatments at both sample times. The lowest level of ergosterol was found in the *S. tritici* followed by *S. nodorum* treatment. This result suggests that less fungal mycelium is produced when *S. tritici* is applied prior to *S. nodorum* and that the presence of *S. tritici* in the tissue inhibits the growth of *S. nodorum*. This suggestion would be in agreement with Jenkins (1978), who found that there was a decrease in the level of *S. nodorum* on plants previously inoculated with *S. tritici*, although the decrease was not found to be significant.

In the field experiment, there was significantly more disease when *S. nodorum* was applied prior to *S. tritici* compared to the other treatments. The results show that there are higher levels of necrosis when the two pathogens are present together compared to either alone, particularly when *S. nodorum* is present prior to *S. tritici*. It was shown that *S. tritici* has a stimulatory effect on *S. nodorum*, as there were significantly more *S. nodorum* spores produced in the presence of *S. tritici*; these results are in agreement with the growth chamber experiment. In the field experiment it was found that the presence of *S. nodorum* stimulates spore production by *S. tritici* as there were more *S. tritici* spores produced when *S. nodorum* was applied after *S. tritici* compared to *S. tritici* alone. Jenkins and Jones (1981) reported that *S. nodorum* was the more efficient leaf coloniser; therefore *S. tritici* may be stimulated by *S. nodorum* only if it has had time to colonise the tissue prior to the arrival of *S. nodorum*. If *S. nodorum* has already colonised the tissue prior to *S. tritici*, *S. tritici* may be inhibited.

It is important to note that the dual inoculations in the field and growth chamber experiments allow different time intervals for disease development. As there were more *S. nodorum* spores produced when *S. tritici* was applied prior to *S. nodorum* compared to *S. nodorum* alone, which had a longer time to develop, it can be concluded that an interaction occurs between the two pathogens. Other experiments (not reported here) in which the two pathogens were applied

simultaneously in different proportions also showed an interaction between the two pathogens. The growth chamber results differ from the field experiment results for spore production by *S. tritici*, and this may be because *S. tritici* did not always behave in the field in the same way as under artificial conditions. There was also a shorter time interval between inoculations in the growth chamber which may have affected the results for *S. tritici*. The effect of *S. nodorum* on *S. tritici* was more apparent in the detached glume experiment compared to tiller washings from the field experiment. The tiller washings represented the number of spores from the head and leaf, whereas in the detached glume experiment the effect of the pathogen mixtures on the heads only was determined. Mielke (1980) found that leaf and head infection by *S. nodorum* was not always correlated, and Jones and Odebunmi (1971) reported that *S. nodorum* was more pernicious than *S. tritici* on the heads; it is therefore likely that the observed interaction could have been affected by these factors.

The results presented here suggest that *S. tritici* may have a stimulatory effect on *S. nodorum* in terms of symptom expression and spore production, and confirm the results of Jones and Odebunmi (1971). However, Suparman (1994) showed that the incubation period of *S. nodorum* was longer when the pathogen was mixed with *S. tritici*, suggesting inhibition. It was shown that *S. nodorum* also inhibited spore production by *S.tritici*, and there were fewer spores of both pathogens when *S. tritici* was applied before *S. nodorum*. These results contradict those of Harrower (1978) who suggested that *S. tritici* produces more propagules in the presence of *S. nodorum*; however, the current study shows that this is only true if *S. tritici* has had time to colonise the leaf prior to invasion by *S. nodorum.* The level of ergosterol significantly decreased when *S. tritici* was applied prior to *S. nodorum* compared to vice versa, indicating that the level of fungal biomass in the leaves was less; this suggests competition occurred between the two pathogens and is in agreement with the data from the growth chamber and field experiments, where the level of disease for the *S. tritici* followed by *S. nodorum* treatment was significantly less than in the other treatments.

Overall, the results suggest that there is an interaction between *S. nodorum* and *S. tritici*. However, it is not possible to state conclusively the nature of this interaction. It is possible, however, that the presence of *S. nodorum* prior to *S. tritici* reduced the latent period of *S. tritici*. Geuting (1984) observed a promotion of *S. nodorum* by *E. graminis*, indicated by a reduced latent period. It has been suggested that *S. tritici* has a hemi-biotrophic phase to its life-cycle initially (Royle, pers. comm.), therefore it is also possible that the previously necrotic tissue as a result of the presence of *S. nodorum* hastens the necrotrophic phase of its life-cycle. Brokenshire (1974) suggested that *E. graminis* hastened the onset of the necrotrophic phase of the *S. tritici* life-cycle. Blakeman and Brodie (1977) showed that two fungi, *Colletotrichum herbarum* and *Phoma betae* which possess a temporary saprophytic phase on the surface of the leaf prior to penetration, were adversely affected by microbial competition. Blakeman and Brodie (1977) suggested that conidia with a relatively long lag phase before commencement of germination would be more sensitive to nutrient competition than rapidly germinating conidia. Therefore, it is also possible that *S. nodorum* and *S. tritici* compete for nutrients on the leaf surface. Many other factors, including the aggressiveness of the pathogen isolates involved and the susceptibility of the host, may also affect the interaction between the two pathogens. The majority of experiments discussed here involved aggressive isolates and a susceptible host plant. It could be postulated that an interaction between the two pathogens may also occur under natural field conditions; however, further research using more isolates and cultivars is now required.

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