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Effet du stade de floraison du blé sur l'efficacité d'infection de l'*Ustilago tritici*

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Résumé de l'article

Dans l'ouest du Canada, la détermination du niveau de résistance de lignées de blé (*Triticum aestivum*) à l' *Ustiiago tritici* est habituellement faite par inoculation lorsque les plantes atteignent le stade mi-anthèse du développement de l'épi. Par contre, il n'est pas toujours possible de faire les inoculations à ce stade, ainsi des inoculations sont parfois faites quelques jours avant ou après la mi-anthèse. L'objectif de cette étude était de déterminer si l'inoculation, par la méthode du vide partiel, d'épis de blé avec l'*U. tritici* à différents stades du développement de l'épi pouvait avoir un effet sur le nombre de grains viables par épi et, ultérieurement, sur le pourcentage de plantes cariées issues de grains inoculés. À chaque année durant 5 ans, quatre lignées de blé ont été inoculées au champ à trois différents stades du développement de l'épi. Les stades du développement de l'épi étudiés étaient la pré-anthèse, la mi-anthèse (les anthères à chaque extrémité de l'épi sont déhiscentes alors que celles du centre sont jaunes) et la post-anthèse (toutes les anthères sont déhiscentes). Il y avait des différences significatives entre les quatre lignées de blé et les 5 années de l'étude quant au nombre de grains viables par épi et le pourcentage de plantes cariées. Un nombre de grains viables par épi plus grand et un pourcentage plus faible de plantes cariées ont été significativement obtenus avec l'inoculation post-anthèse. Nous concluons que l'inoculation de l'*Ustilago tritici* par la méthode du vide partiel doit être faite au stade préanthèse ou mi-anthèse du développement de l'épi de blé afin de minimiser les effets négatifs de la maturité sur l'efficacité d'infection.

The effect of flowering stage in wheat on the infection efficiency of *Ustilago tritici*

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In general, in western Canada, the inoculation of wheat (*Triticum aestivum*) lines to determine the level of resistance to *Ustilago tritici* occurs when the plants are at the mid-anthesis stage of spike development. However, it is not always possible to inoculate at this stage so occasionally inoculations are done a few days before or after mid-anthesis. The objective of this study was to determine if inoculation of wheat spikes with *U. tritici* at different stages of spike development using the partial-vacuum method affected the number of viable seed per spike and subsequent percentage of smutted plants grown from inoculated seed. Four lines of wheat were inoculated at three different stages of spike development in the field each year for 5 years. The stages of spike development studied were pre-anthesis, mid-anthesis (anthers at either end of the spike were dehisced while those in the middle of the spike were yellow), and post-anthesis (all anthers dehisced). There were significant differences among the four wheat lines and the 5 years of the study for the number of viable seed per spike and the percent of smutted plants. Inoculation at post-anthesis resulted in a significantly greater number of viable seed per spike and lower percentage of smutted plants. We conclude that the partial-vacuum method of inoculation with *Ustilago tritici* should be done at the pre- or mid-anthesis stage of wheat head development to minimize the negative effect of maturity on infection efficiency.

[Effet du stade de floraison du blé sur l'efficacité d'infection de l'*Ustilago tritici*]

Dans l'ouest du Canada, la détermination du niveau de résistance de lignées de blé (*Triticum aestivum*) à l'*Ustilago tritici* est habituellement faite par inoculation lorsque les plantes atteignent le stade mi-anthèse du développement de l'épi. Par contre, il n'est pas toujours possible de faire les inoculations à ce stade, ainsi des inoculations sont parfois faites quelques jours avant ou après la mi-anthèse. L'objectif de cette étude était de déterminer si l'inoculation, par la méthode du vide partiel, d'épis de blé avec l'*U. tritici* à différents stades du développement de l'épi pouvait avoir un effet sur le nombre de grains viables par épi et, ultérieurement, sur le pourcentage de plantes cariées issues de grains inoculés. À chaque année durant 5 ans, quatre lignées de blé ont été inoculées au champ à trois

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différents stades du développement de l'épi. Les stades du développement de l'épi étudiés étaient la pré-anthèse, la mi-anthèse (les anthères à chaque extrémité de l'épi sont déhiscentes alors que celles du centre sont jaunes) et la post-anthèse (toutes les anthères sont déhiscentes). Il y avait des différences significatives entre les quatre lignées de blé et les 5 années de l'étude quant au nombre de grains viables par épi et le pourcentage de plantes cariées. Un nombre de grains viables par épi plus grand et un pourcentage plus faible de plantes cariées ont été significativement obtenus avec l'inoculation post-anthèse. Nous concluons que l'inoculation de l'*Ustilago tritici* par la méthode du vide partiel doit être faite au stade pré-anthèse ou mi-anthèse du développement de l'épi de blé afin de minimiser les effets négatifs de la maturité sur l'efficacité d'infection.

INTRODUCTION

The Canadian Food Inspection Agency (CFIA) is responsible for the registration of all cultivars of wheat (*Triticum aestivum* L.) to be grown commercially in Canada. The CFIA makes its decision on registration of new wheat cultivars based on the recommendations of various committees, such as the Prairie Registration Recommending Committee for Grain (PRRCG). These committees review all potentially new cultivars to ensure that they meet certain guideline recommendations for agronomic, quality and disease performance. The data used by the PRRCG to make its recommendations of new wheat lines are obtained from various cooperators involved in the evaluation process.

One of the criteria used in the evaluation of the wheat lines is resistance (or susceptibility) to loose smut caused by the fungus *Ustilago tritici* (Pers.) Rostrup. Each yr in western Canada, approximately 800 of these wheat lines are evaluated at the Cereal Research Centre (CRC), Agriculture and Agri-Food Canada, Winnipeg, Manitoba. Seed from each line is planted in the field in the spring, inoculated at mid-anthesis (Nielsen 1983) with mature heads harvested in late summer. Inoculated seed is sown in soil beds in the greenhouse in the fall, and evaluations of the percent infection of mature plants by *U. tritici* are made. Normally, 3 yr of testing is needed for effective evaluation of wheat lines for their level of resistance to loose smut, but 1 yr of testing is sometimes sufficient, *i.e.*, if the line is highly susceptible. The process of evaluation is labor and time consuming.

The inoculation of wheat lines for loose smut testing in Manitoba occurs generally over a period of 2 to 3 wk in July of each yr. If environmental conditions are favorable, flowering of the different wheat lines is evenly distributed over this 2- to 3-wk period and there is sufficient time to inoculate the lines at mid-anthesis (Nielsen 1983). However, if environmental conditions are not favorable, such as hot temperatures during the flowering period, many wheat lines may reach mid-anthesis at the same time, making it difficult to inoculate all the heads at this stage because of time restrictions. This results in some heads being inoculated at a stage of development other than mid-anthesis. This is a concern because reliable data for each inoculated line is necessary for cultivar registration process.

The flowering stage of cereals at the time of smut inoculation can influence infection levels. Some reports showed that optimum infection of wheat can be obtained by inoculating wheat ears as they emerge from the sheath, when the anthers are green and the stigma immature (Beniwal and Karwasra 1991, Gothwal 1972), while others demonstrated that optimum infection levels arise from inoculation around anthesis (Batts 1955, Jones and Dhitaphichit 1991, Loria *et al.* 1982, Mishra and Jain 1970). Unfortunately, most of these studies are hard to compare because

different inoculation procedures were employed and environmental conditions or the experiments were either not repeated or not statistically analyzed.

The objective of this study was to determine if inoculation of wheat heads at different stages of development using the partial vacuum method (Nielsen 1983) influenced the number of viable seed (seed which can germinate and grow to produce mature plants) from inoculated spikes and the subsequent infection levels of mature plants grown from inoculated seed.

MATERIALS AND METHODS

The four wheat lines 79 CBW"A"#72 (cbwa), Laura, HY-320 and HY-355 were used in these experiments. All four lines are considered susceptible to *U. tritici* using the standard inoculation procedures (Nielsen 1983, Thomas and Menzies, unpublished data).

Ten to 12 seeds of each wheat line were sown per hill plot in May of 1991, 1992, 1993, 1994 and 1995 at the CRC field station at Glenlea, Manitoba. At heading, 30 spikes of each hill were selected for inoculation. Ten of the spikes were in the pre-anthesis stage of development with green anthers, 10 spikes were at mid-anthesis (the anthers at either end of the spike were dehisced, while those in the middle were yellow) and 10 spikes were in post-anthesis (all anthers dehisced). In 1991 and 1992, all three stages were inoculated on the same day for a particular wheat line. However, it was occasionally difficult to get 10 heads at each stage of development. Therefore in 1993, 1994 and 1995, each stage of floral development was inoculated as it became ready, so that some spikes of the same wheat line were inoculated on different days. About 1 cm was cut off the tips of each inoculated spike with scissors to mark the inoculated heads and the spikes were tagged for identification of inoculation stage.

The partial-vacuum method described by Nielsen (1983) was used for inoculation. With this method, three spikes are placed in a inoculation cylinder and immersed under vacuum in a suspension of water and teliospores of *U. tritici* at a concentration of about 4 g teliospores per L of water. The vacuum is maintained for 2-3 s and then released, allowing the teliospore suspension to drain into a reservoir. Without removing the spikes from the inoculation cylinder, this procedure is immediately repeated once.

The loose smut races T2, T9, T10 and T39 (Nielsen 1987) were employed in the inoculum suspension; each at 1 g teliospores per L of water. These four races represent the common races of *U. tritici* in western Canada (Thomas and Menzies, unpublished data). A fresh mixture of inoculum was prepared each day of inoculation.

Each spike was harvested and thrashed individually. The number of seed from the inoculated spikes were recorded and sown in a soil bed in the greenhouse during the following winter. At heading, the number of healthy and smutted plants were recorded and the percentage of smutted plants was determined.

The data were analyzed using an ANOVA and where the F test was significant at $P < 0.05$, the means were compared using a LSD test. Comparisons were made among different stages of inoculation, the wheat lines, yr and yr by line interactions. Because a residual analysis showed a relationship between the residual and predicted number of viable seed from each inoculated spike, ANOVA and LSD were applied to log transformed data; both log transformed and back transformed data are presented. It was not necessary to transform percent smutted plants.

RESULTS AND DISCUSSION

There were significant differences among the four lines for the number of viable seed from an inoculated spike and the percent of smutted plants (Table 1). HY-320 produced significantly more viable seed per spike than the other three lines and cbwa produced significantly less viable seed per spike

Table 1. The effect of inoculation with *Ustilago tritici* on the number of viable seeds and percent smutted plants in four wheat lines inoculated at different stages of anthesis, 1991-1995

Treatment	Log e (1+number of viable seed from an inoculated spike)[a]	Number of viable seed from an inoculated spike	Percent smutted plants[b]
Wheat line			
HY-320	3.0 a	18.9	68.0 a
HY-355	2.8 b	15.6	63.5 a
cbwa	2.6 c	12.9	64.5 a
Laura	2.8 b	15.4	47.1 b
Year			
1991	2.8 b	16.1	53.2 b
1992	3.3 a	27	70.7 a
1993	2.4 c	9.7	37.9 c
1994	2.7 b	14.1	67.6 a
1995	2.8 b	15.1	74.5 a
Stage of anthesis			
Pre	2.7 b	13.3	66.2 a
Mid	2.7 b	13.5	67.1 a
Post	3.1 a	20.9	49.1 b

[a] The data were analyzed using an ANOVA and where appropriate, the means were compared using a LSD test. Within treatment groups, means followed by the same letter are not significantly different ($P < 0.05$). The standard error for the means of the wheat lines was 0.063, for yr, 0.056 and for stages of anthesis, 0.048.

[b] The data were analyzed using an ANOVA and where appropriate, the means were compared using a LSD test. Within treatment groups, means followed by the same letter are not significantly different ($P < 0.05$). The standard error for the means of the wheat lines was 3.76, for yr, 3.37 and for stages of anthesis, 2.92.

than the other three lines. These differences likely represent cultivar differences rather than effects of the inoculation procedure. The mean percentage of smutted plants ranged from 68.0 % for HY-320 to 47.1 % for Laura, with Laura having a significantly lower percentage of smutted plants than HY-320, HY-355 and cbwa.

There were significant differences among the yr for the number of viable seed from an inoculated spike and the percent of smutted plants (Table 1; $P < 0.05$). The number of viable seed from inoculated spikes was significantly lower in 1993 and significantly higher in 1992 than in the other 3 yr. Differences were not significant among 1991, 1994 and 1995. These differences among the yr may have been associated with the incidence and severity of Fusarium head blight (FHB) in southern Manitoba. In 1993, the epidemic of FHB was the most severe on record in southern Manitoba (Gilbert *et al.* 1994); conversely, FHB was not severe in 1992 (Gilbert *et al.* 1993). The FHB severities in 1991, 1994 and 1995 were similar and less severe than 1993 and more severe than 1992 (Gilbert *et al.* 1993, 1994, 1995, 1996; Wong *et al.* 1992). The smut inoculation procedure utilized at the CRC would aid in helping the causal agent of FHB to become established on inoculated heads, because spikes become throughly soaked during the procedure. Fusarium head blight was noted on the inoculated heads in all 5 yr of the study, but favourable conditions for FHB spread and development in 1993 and poor conditions in 1992 may have interacted with the inoculation procedure to create differences observed among the

5 yr for the number of viable seed per inoculated spike.

The mean percent smutted plants ranged from 74.5 % for 1995 to 37.9 % for 1993; this value was lower in 1993 than the other 4 yr (Table 1; $P < 0.05$). The percent of smutted plants was significantly lower in 1991 (53.2 %) than 1992, 1994 and 1995, and there were no differences among 1992, 1994 and 1995. The differences among the yr are interesting because the percent smutted plants does not follow the same yearly pattern as the number of viable seed from an inoculated spike. The combination of both FHB and *U. tritici* in our experiments may have resulted in a reduced germination and growth of the *U. tritici* infected seed, resulting in a lower percentage of smutted plants.

Changes in inoculum viability from yr to yr was not a factor in this study since the viability of the inoculum used in these tests was tested each yr by determining the teliospore germination rate on water agar. The inoculum had a greater than 80% germination rate in each yr of the study. High inoculum viability and the high concentration of teliospore in the inoculum (4 g L^{-1}) makes it unlikely that the differences observed among yr in this study are caused by differences in inoculum.

It is possible that the differences observed among yr may be a reflection of the environmental conditions during the inoculation period. Tapke (1931) observed a large reduction in the percentage of smut infection when flowers of wheat (cv. Little Club) inoculated with *U. tritici* were subjected to ranges of low relative humidity (RH). Under low RH, the smut teliospores may fail to germinate or germinate too slowly to enable the infecting hyphae to reach the ovary during the short period of susceptibility. Excessive heat will also lower teliospore germination and germ-tube growth, delay penetration of the ovary and preclude the fungus from reaching the growing point (Nielsen and Thomas 1996). The maximum and minimum daily temperatures and the mean daily temperature ranges are presented for 1991-1995 at Glenlea, in Table 2. When comparing the data for the percent smutted plants in Table 1 and the temperature data in Table 2 for 1991 to 1995, it does not appear that differences in the percent smutted plants from yr to yr can be attributed to extremes in temperature. The highest temperatures during the period of July

Table 2. Maximum, minimum and daily range mean of temperature at Glenlea, Manitoba, during wheat flowering which occurred from 1 July to 21 July in 1991-1995[a]

Year	Maximum daily temperature (°C)	Minimum daily temperature (°C)	Mean daily temperature range (°C)
1991	31	9	10.4
1992	27	4.5	10.8
1993	26.5	5	11.1
1994	26.5	8	10.4
1995	31.5	6	12

[a] Data obtained as part of a co-operative agreement between Agriculture and Agri-Food Canada and the Atmospheric Environment Service of Environment Canada for scientific research. Temperatures were measured in a Stevenson screen about 1.5 m above a short grass surface using glass thermometers or electronic sensors and data-loggers installed and maintained according to Atmospheric Environment Service standards.

1 to July 21 occurred in 1995, and the percent smutted plants was the highest in this yr. As well, it appears as if 1992 and 1993 had similar temperature environments but the percent smutted plants were different among these 2 yr with 1993 being the yr in which there were significantly lower percent smutted plants than any of the other yr of the study.

Inoculation at post-anthesis gave significantly greater numbers of viable seed from an inoculated spike than at the pre- or mid-anthesis (Table 1). The inoculation of the wheat spikes at the late stage of anthesis resulted in approximately 50% more viable seed from the spikes, than inoculation at the pre- or mid-anthesis stage. There was no difference between the pre- and mid-anthesis stages. Similar results were also reported by Mishra and Jain (1970), but their data was not statistically analyzed, so it is difficult to make comparisons. Mishra and Jain (1970) suggested that inoculation prior to anthesis may disturb the normal development of the ovary resulting in poor grain set. Our data does not support their findings, but their early inoculations were made at an earlier stage of floral development than our pre-anthesis stage. The higher seed set observed in both studies following post-anthesis inoculation probably reflects the advanced stage of pollination and seed development prior to inoculation and that inoculation had less of an impact on seed development than pollination.

Inoculation at post-anthesis produced significantly lower percentages of smutted plants than at the pre- or mid-anthesis stages (Table 1; $P < 0.05$). There was no significant difference between the pre- and mid-anthesis stage for the percent smutted plants. These results are contrary to those of Batts (1955) who observed that infection percentages of *U. tritici* were fairly consistent when inoculation occurred at pre-anthesis, anthesis and late anthesis. Gothwal (1972) and Beniwal and Karwasra (1991), using dry spore inoculation in the field observed maximum disease expression when inoculation occurred just as the wheat ears were emerging out of the leaf sheath; the anthers at this stage were green and the stigma immature. Mishra and Jain (1970) inoculated wheat plants in the field using 15- to 20-d-old cultures in 0.5 % aqueous malt extract and concluded that the best time for inoculation was when the anthers were just starting to emerge from the wheat head, prior to mid-anthesis. Jones and Dhitaphichit (1991) inoculated wheat plants (cv. Chinese spring) using a rubber bulb-hypodermic syringe technique and a teliospore suspension at 4 g L^{-1} and observed maximum infection around anthesis. Unfortunately, none of the studies cited analyzed their results statistically, making comparisons difficult. Loria *et al.* (1982) conducted replicated studies on the best stage of inoculation for optimum infection of *U. tritici* using a dry inoculation of spikelets with a talc and teliospore mixture. They found that inoculation at anthesis gave a significantly greater percentage of smutted plants than inoculation before or after anthesis. We also found that optimum levels of smut infection occurred at mid-anthesis but did not obtain significant differences between pre- and mid-anthesis stages of floral development. The differences between our study and that of Loria *et al.* (1982) may be the result of different levels of infection; the infection levels in their study being < 3 %.

It is interesting to note from our studies and others (Beniwal and Karwasra 1991, Gothwal 1972) that dry inoculation techniques tended to result in maximum infection levels when inoculation was prior to anthesis, while the use of inoculum suspended in a liquid medium resulted in maximum infection levels when inoculation was during anthesis (Table 1; Jones and Dhitaphichit 1991, Mishra and Jain 1970). It may be that the use of a liquid inoculum source has an adverse effect on smut infection prior to anthesis.

Significant ($P < 0.05$) line by yr interactions were also noted in the present experiments for the number of viable seed from an inoculated spike, but not for the percent smutted plants (means not presented). It appears that the significant interactions may be caused by

FHB rather than any other factor in these experiments. In 1993, when FHB was severe in southern Manitoba (Gilbert *et al.* 1994), the four lines had a very low number of viable seed from inoculated spikes, while in 1992, when FHB was not severe in the area (Gilbert *et al.* 1993), the four lines had significantly higher numbers of viable seed from an inoculated spike. Differences among lines for the number of viable seed from an inoculated spike became apparent in 1992.

In conclusion, the partial-vacuum method of inoculation of *U. tritici* (Nielsen 1983) should be used at the pre- or mid-anthesis stage of wheat head development to obtain optimum levels of infection. In general, inoculation at pre- and mid-anthesis resulted in higher infection levels compared to those observed when inoculation occurred at the late stage of head development. Therefore, under infection conditions that occur in Manitoba, if one cannot inoculate wheat spikelets at mid-anthesis, it is desirable to inoculate prior to mid-anthesis rather than after the mid-anthesis stage of wheat head development. This is important information to those trying to inoculate large numbers of wheat plants to screen for smut resistance, *e.g.*, a smut screening nursery for plant breeder's lines. The window of opportunity for inoculation of wheat lines with *U. tritici* to obtain accurate assessments of the lines' smut resistance is larger than the mid-anthesis stage of development. This allows a greater flexibility in inoculation of the wheat lines with *U. tritici*, which will lead to a more accurate assessment of smut resistance.

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