Development of a Greenhouse Screening Method for Adult Plant Response in Wheat to Stem Rust

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Abstract

Bender, C. M., Prins, R., and Pretorius, Z. A. 2016. Development of a greenhouse screening method for adult plant response in wheat to stem rust. Plant Dis. 100:1627-1633.

Screening for adult plant resistance in wheat to stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is generally conducted in field plots. Although such evaluations are successful if managed properly, field ratings are time consuming, expensive, weather dependent, and open to inoculum of unwanted races or other confounding diseases. The objective of this study was to develop a dependable system of screening the response of adult plants to stem rust under greenhouse conditions. A comparison of inoculation methods and incubation environments showed that plants inoculated with urediniospores suspended in water, followed by a 24 h dew period in a plastic chamber constructed in a greenhouse, gave the most consistent results. Measurements of response type, stem rust severity, and frequency in follow-up experiments indicated that the most reliable infection was

obtained when plants sprayed with 1.25 mg urediniospores per ml water were incubated in the plastic chamber. Using the optimized protocol, a Kariaga × Avocet S doubled haploid population was inoculated with two *P. graminis* f. sp. *tritici* races. Depending on the race, composite interval mapping showed flag leaf infection type to be significantly influenced by regions on chromosomes 6A, 6D, and 7D. Stem rust severity and reaction type mapped to chromosomes 6D and/or 6A. The *Lr34/Yr18/Sr57* gene derived from Kariaga on chromosome 7D affected the rust response on flag leaves but not on stems of greenhouse-grown plants. This study showed that phenotyping and genetic analysis of especially major effect stem rust resistance in adult wheat plants is possible and reproducible under controlled conditions in a greenhouse.

Stem rust of wheat (*Triticum aestivum* L.), caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., is regarded as one of the most damaging diseases of wheat because of its pathogenic variability, adaptation to long-distance wind dispersal, epidemic potential, and ability to cause significant losses (Dean et al. 2012). The importance of wheat stem rust was re-emphasized by the detection of African race “Ug99” (North American race code TTKSK) in Uganda (Pretorius et al. 2000b). This discovery, in particular the occurrence of virulence for the commonly used *Sr31* resistance gene, has since initiated a global effort to combat the disease (McIntosh and Pretorius 2011). At present, 12 variants within the Ug99 race group are known, differing in virulence for *Sr9h*, *Sr21*, *Sr24*, *Sr30*, *Sr31*, *Sr36*, and *SrTmp* (Fetch et al. 2016; Patpour et al. 2015; Pretorius et al. 2012b; Singh et al. 2011, 2015). Distribution of Ug99 variants has been confirmed in Egypt, Eritrea, Ethiopia, Iran, Kenya, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Uganda, Yemen, and Zimbabwe (Nazari et al. 2009; Pretorius et al. 2012b; Singh et al. 2008; Visser et al. 2011; <http://rusttracker.cimmyt.org> [accessed January 26, 2016]).

Although fungicides can be used to control wheat stem rust (Wanyera et al. 2009), chemical protection is expensive, requires expertise, the availability of spraying equipment, and a supply of registered products. Thus, the deployment of resistant cultivars is widely accepted as the best way to reduce inoculum levels and to prevent or control the disease, especially for resource-poor farmers. Since rust pathogens have the ability to adapt and acquire virulence for resistance genes, the ongoing identification, characterization, and utilization of sources of resistance are needed.

Resistance to stem rust is broadly classified as all stage resistance, which can be detected in wheat seedlings and all subsequent growth stages, and adult plant resistance (APR), which is most effective in mature plants in the field (Ellis et al. 2014; Lagudah 2011). However, the efficiency of stem rust phenotyping can be improved by scoring adult plant responses in a greenhouse where the racial identity of inoculum and uniformity of application can be controlled. The objective of this study was to develop and validate a dependable screening system for assessing APR for stem rust in the greenhouse. Procedures were optimized using sets of wheat lines differing in their response to stem rust and the system was verified with a doubled haploid population known to carry *Sr5*, *Sr26*, and *Lr34/Yr18/Sr57* (Prins et al. 2011).

Materials and Methods

Experiment 1. The feasibility of infecting adult wheat plants with *P. graminis* f. sp. *tritici* was investigated on cultivars Inia 66 (*Sr2*), Hartog (*Sr2*), Kariaga (susceptible), and Morocco (susceptible). Wheat plants were grown in sterilized soil in tapered plastic cones (4 × 4 × 10 cm) (three plants per entry per cone) at 18 to 25°C in a greenhouse. Cones were positioned in a 14 × 7 unit tray, supported by a corresponding, raised grid of nylon line to prevent plants from lodging (Pretorius et al. 2007). After emergence, plants were fertilized twice with Multifeed water soluble fertilizer (19:8:16 NPK plus micronutrients), followed by a water suspension of Wonder 3:2:1, a slow nitrogen release granular fertilizer, at 7-day intervals for the duration of the trial.

Plants were inoculated with race TTKSP (UFS isolate UVPgt59, avirulent for *Sr9h*, *26*, *27*, *31*, *36*, *Kiewiet*, *Satu*, and *Tmp* and virulent for *Sr5*, *6*, *7b*, *8a*, *8b*, *9a*, *9b*, *9d*, *9e*, *9g*, *10*, *11*, *17*, *21*, *24*, *30*, *38*, and *McN*) 51 days after planting when the majority were flowering (Zadoks growth stage 58). Three inoculation methods, two incubation environments, and two moisture duration periods were tested. In the first inoculation method, fresh urediniospores were suspended in light mineral oil (5 mg/ml) in gelatin capsules and sprayed onto plants using the system primarily developed for applying rust spores to seedlings (Browder 1971). Secondly, spores were suspended in distilled water (2.5 mg/ml) and Tween 20 (0.03% v/v) and applied

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure is available online.

Accepted for publication 2 March 2016.

to stems using a compressed air sprayer, similar to the procedures for inoculating adult wheat plants with stripe rust (Pretorius et al. 2007). Thirdly, dry spores (0.008 g) were applied for 3 min to horizontally positioned plants in a settling tower (Negussie and Pretorius 2005). The same source of freshly collected urediniospores of TTKSP, increased in bulk on LCSr24Ag seedlings, was used in the respective experiments.

Plants from all entries and inoculation methods were incubated either in the dark in a condensation dew chamber or in natural light/darkness in a plastic chamber in a greenhouse. Treatments were randomized within each chamber. The condensation chamber, manufactured from metal sheeting and measuring 180 cm wide × 120 cm high × 100 cm deep, functioned in an air-conditioned room at 20°C. Dew was formed by vapor released from two water baths set at 40°C and recessed in the bottom of the chamber. The plastic chamber measured 100 × 85 × 60 cm and consisted of an aluminum frame covered with 200 µm greenhouse sheeting (Evadek [green tint], Gundle API Plastics, Springs, South Africa). The plastic-covered frame was placed in a tray filled with water to a depth of approximately 5 cm. Inside the frame plants were placed on a metal grid raised above the water line. Plants were incubated for either 12 or 24 h. The temperature ranges in the greenhouse cubicle housing the plastic chamber and condensation dew chamber were 20 to 28°C and 20 to 24°C, respectively. Each treatment was replicated three times, with three cones per replicate.

When the dew cycles ended, plants were dried at 20°C before placing them in a greenhouse set to an 18 to 25°C night/day schedule. Stem rust infection response types (resistant R, moderately resistant MR, moderately susceptible MS, and susceptible S) (Stubbs et al. 1986) were recorded and the total number of pustules on a 5 cm stem section of the second last internode was counted 14 days post-inoculation.

Experiment 2. The water/spore suspension method was refined in a second experiment that was conducted twice. The same entries as above and an experimental line (ZA2006-28 EXP.3) with *Sr24* and *Sr31* were used (Pretorius et al. 2012a). Growth conditions before and after inoculation were as described above. Using a spray gun and compressor, 100 ml of each of three spore concentrations (0.67, 1.25, and 2.5 mg/ml) of race TTKSP were applied as uniformly as possible to approximately 100 adult plants per concentration. Plants were incubated as described earlier in either a dark dew chamber or in plastic tents in a greenhouse for 24 h. A CR10X data logger (Campbell Scientific, Inc., Somerset West, South Africa) monitored temperature, humidity, and leaf wetness through HMP45C probes and a dielectric leaf surface wetness sensor (Decagon Devices, Inc., Stellenbosch, South Africa). Stem rust incidence (percentage infected plants) and infection response types were determined 14 days after inoculation. The coefficient of infection (C.I.), where severity according the modified Cobb scale is multiplied with a constant for host infection response ($R = 0.2$, $MR = 0.4$, $MRMS = 0.6$, $MS = 0.8$, and $S = 1$; Roelfs et al. 1992), was calculated.

Validation of infection procedure. The water/spore suspension method was first tested by inoculating 20 South African spring wheat

cultivars, and secondly, two sets of a Karioga × Avocet S doubled haploid (DH) mapping population of 254 entries with *P. graminis* f. sp. *tritici* races BPGSC (UFS isolate UVPgt57, avirulent for *Sr5*, *6*, *7b*, *8b*, *9e*, *17*, *21*, *24*, *26*, *30*, *31*, *36*, *38*, and *Tmp* and virulent for *Sr8a*, *9a*, *9b*, *9d*, *9g*, *9h*, *10*, *11*, *27*, *Kiewiet*, *Satu*, and *McN*) and TTKSP, respectively. BPGSC is a South African race with virulence to triticale (Olivera et al. 2013) whereas TTKSP is a member of the Ug99 race group. Plants (two replications with three to five plants per cone), grown and fertilized as before, were inoculated at anthesis with 1.25 mg spores/ml and incubated for 24 h from 13:00 to 13:00 in plastic dew chambers in a greenhouse cubicle.

Stem rust incidence, severity, and infection response type were recorded 14 days after inoculation. Severity ratings considered the whole stem whereas the infection response type was noted on the last internode. C.I. was calculated as described above. The cultivar experiment was repeated in the greenhouse. Field data of the same entries evaluated in an unreplicated rust nursery infected with TTKSP at the PANNAR Research Station, Greytown, KwaZulu-Natal, South Africa in 2009 were compared with greenhouse data. Data for pustule number and C.I. were analyzed for variance according to a completely randomized design. Standard deviations were calculated as a measure for distinguishing treatment means. The relationship between greenhouse and field-derived stem rust scores was qualified using linear regression analysis.

Flag leaf infection type was included with stem rust incidence, severity, infection response type, and C.I. when scoring the DH population as adult plants in the greenhouse. The rating of flag leaf infection followed the standard 0 to 4 scale described by McIntosh et al. (1995). For mapping purposes, it was necessary to convert the scores to a 0 to 9 scale (Table 1). Using the published Karioga × Avocet S maps (Prins et al. 2005, 2011) and stem rust traits, composite interval mapping (CIM) was performed with Windows QTL Cartographer V2.5 to i) verify whether *Sr5* (6D) and *Sr26* (6A) could be detected, and ii) determine whether *Lr34/Yr18/Sr57* (7D) contributes toward the stem rust resistance phenotype observed in the population.

The DH mapping population and control plots of Karioga and Avocet S were evaluated at Greytown, South Africa in 2012 for comparison with the greenhouse study. Field trials were planted, maintained, and inoculated with race PTKST (UFS isolate UVPgt60, avirulent for *Sr9h*, *21*, *26*, *27*, *36*, *Kiewiet*, *Satu*, and *Tmp* and virulent for *Sr5*, *6*, *7b*, *8a*, *8b*, *9a*, *9b*, *9d*, *9e*, *9g*, *10*, *11*, *17*, *24*, *30*, *31*, *38*, and *McN*) as described by Prins et al. (2011). Similar to TTKSP, PTKST is a member of the Ug99 race group. Stem rust responses were recorded for each entry on 18 October as severity (percentage stem area infected) and reaction type (R, MR, MS, and S including interpolations).

Results

Experiment 1. The adult plant infection response type ranged from S for Morocco and Karioga to MRMS for Hartog, and MS for Inia 66. In successful infections, the first pustules on susceptible entries were visible 6 days after inoculation. The number of pustules on a 5 cm stem section was significantly ($P < 0.05$) influenced by inoculation and incubation methods used. Very little stem rust developed on plants incubated in the condensation dew chamber in the first experiment (Fig. 1A). In this chamber, only trace amounts of rust were observed on plants inoculated with dry spores or with a spore/water suspension. Some stem rust occurred on plants inoculated with oil, with those incubated for 24 h showing marginally more pustules than the 12 h dew period (Fig. 1A). High levels of stem rust developed on Karioga and Morocco plants inoculated with spores suspended in water and incubated in the plastic chamber for 24 h (Fig. 1B). In this treatment, the rust levels on stems of the more resistant Hartog and Inia 66 were proportionally lower than on the susceptible entries. Some stem rust occurred in the dry spore and spore/oil treatments with indications of higher infection levels after a 24 h incubation period (Fig. 1B).

Experiment 2. In the second experiment, cultivar responses were similar to those reported above. The *Sr24+31* line showed R to MR

Table 1. Conversion of flag leaf stem rust infection types to a 0 to 9 scale

Infection type ^{a,b}	Scale
0; to ;	1
;c to ;cn	2
Z;1	3
;1c to ;1+	4
Z2 to 2 =	5
2 to 2+3	6
Z3 to 3 =	7
3 to 3++	8
4	9

^a A “Z” indicates the predominant distribution of uredinia toward the leaf base as observed for a particular infection type class.

^b The converted scale interval 5 included infection types associated with chlorosis and necrosis.

infection response types to race TTKSP. Results of the two repeats were not significantly ($P < 0.05$) different and data were pooled. The percentage of infected plants was significantly ($P < 0.05$) influenced by genotype and incubation method used. Stem rust incidence on plants incubated in the condensation chamber was low and inconsistent (Fig. 2A). In contrast, a high incidence (65 to 100%) was observed on plants incubated in the plastic chamber (Fig. 2B). High temperatures were reached inside the chamber in the greenhouse with a maximum of 38°C shortly after incubation commenced. Night temperatures were stable at 19 to 20°C during 22:40 to 06:40, again rising to 38°C when the incubation was terminated at 13:00. Condensation chamber temperatures ranged between 21 and 24°C. Here, relative humidity was 42% at the onset of the experiment but increased to 98% 1 h later, which was maintained for the duration of the cycle. Relative humidity in the greenhouse chamber started at 35%, rising to 75% after 1 h, reached a maximum of 97% at 02:00 before decreasing to 75% when the plants were removed. The quantification of leaf wetness showed a slower onset and lower levels of moisture on plants in the plastic chamber compared with the condensation chamber (data not shown). Spore concentration did not significantly ($P < 0.05$) influence the percentage of plants showing stem rust symptoms. However, pustule size on the susceptible entries was restricted in the 2.5 mg/ml treatment. Severity values as high as 80% were observed on Morocco plants. C.I. data supported the expected response range from resistant to highly susceptible (Fig. 3).

Validation of infection procedure. Response of South African cultivars inoculated with races BPGSC and TTKSP varied from resistant (0R) to susceptible (80S) with mean stem rust incidences of 93 and 98% recorded for the first greenhouse experiment and its independent repeat (Table 2). An $R^2 = 0.42$ ($P \leq 0.05$) was obtained for the relationship between greenhouse and field C.I. scores but was lower for severity ($R^2 = 0.35$, $P \leq 0.05$). The 0R response of several entries to BPGSC was typical of the presence of *Sr5* for which this race is avirulent.

The flag leaves and stems of greenhouse-grown DH Karioga $\times$ Avocet S plants were uniformly infected with *P. graminis* f. sp. *tritici*. Flag leaves of both the parental lines, Karioga (IT 1) and Avocet S (IT 0); were resistant to race BPGSC, whereas a predominantly susceptible reaction was expressed on the stems of Karioga (70MSS). Flag leaves of Karioga (IT Z2++) and Avocet S (IT 1) inoculated with race TTKSP expressed intermediate levels of resistance. Similar results were obtained for TTKSP on the stems of Karioga (60MSS), but not for Avocet S where a high severity of small pustules was observed (50MR). The reaction/infection types of the DH lines ranged from highly resistant to highly susceptible on stems and flag leaves. Lower flag leaf infection types were noticed on those with tip necrosis. Pictures of infection levels and response types are attached as Supplementary Figure S1. Segregation ratios between resistant (flag leaf infection types 0; to Z3) and susceptible (flag leaf infection types 3 to 4) DH lines did not deviate significantly from a three gene model (*Sr5*, *Sr26*, *Sr57*) for BPGSC ($\chi^2_{7,1} = 1.81$) ($P = 0.179$) and two gene model (*Sr26*, *Sr57*) for TTKSP ($\chi^2_{3,1} = 0.54$) ($P = 0.463$). Using CIM, flag leaf infection types of BPGSC were significantly influenced by regions on chromosomes 6A, 6D, and 7D, whereas genes controlling infection types of TTKSP mapped to chromosomes 6A and 7D (Table 3). As expected, a low percentage of variance was explained by *Sr57* (7D) for leaf infection types i.e., 1.4 and 6.1% for BPGSC and TTKSP, respectively. Stem severity and reaction type mapped to chromosomes 6A and 6D for BPGSC as opposed to only 6A for TTKSP. The results corresponded with the resistance genes postulated to occur in the population. BPGSC is avirulent for *Sr5* and *Sr26* on the Avocet S-derived chromosomes 6D and 6A, respectively, while TTKSP is avirulent only for *Sr26*. Neither severity nor reaction type on the stems of the DH lines detected *Sr57* in the greenhouse whereas the 7D region was shown to have a minor effect in reducing severity ($R^2 = 6.5\%$), and the related C.I. ($R^2 = 2.4\%$), in the field (Table 3). The 7D QTL interval also varied for the two leaf infection type scores and the field stem severity score that were significantly detected, with either *cssrf6* (*Sr57*-diagnostic) or *csLV34*

(linked marker) being the most significant associated marker. Additional unknown minor QTL were not consistently detected with BPGSC and TTKSP across the different scores in the greenhouse, and similar to *Sr57*, the phenotypic variance explained by them were $\leq 1.7\%$. Minor QTL of unknown identity were more consistently detected in the field score with PTKST. The chromosome regions of the minor resistances were not the same across the three pathotypes tested and were not considered further.

Discussion

APR to the rust pathogens is a priority in most programs breeding for disease resistance in wheat (Agenbag et al. 2012; Lagudah 2011; Njau et al. 2013; Singh et al. 2011, 2015; Tsilo et al. 2014; Yu et al. 2014). Accurate assessment of partial resistance, which by definition includes many APR phenotypes, is essential for developing varieties carrying this type of resistance (Ayliffe et al. 2013). Lowe et al. (2011) mentioned that a definitive disease phenotype, whether quantified macroscopically or microscopically, is the starting point for understanding partial resistance in context with its molecular mechanisms, eventually leading to gene cloning and application in breeding. Likewise, Ellis et al. (2014) stated that a detailed characterization and understanding of APR is required to advise rust resistance breeding. Screening for APR classically involves assessment of rust infection in field plots that could be costly, time consuming, and its expression dependent on unwanted biotic and abiotic stress factors (Hickey et al. 2012). Thus, a suitable greenhouse screening method

Fig. 1. Mean number of stem rust pustules counted 14 days after inoculation on a 5 cm stem section of adult plants of four cultivars inoculated with *Puccinia graminis* f. sp. *tritici* race TTKSP and incubated in the dark in a condensation dew chamber (A) and in natural light/darkness in a plastic chamber in a greenhouse (B). Error bars represent the standard deviation. Legend for both graphs displayed only in (A).

presents an additional prospect for studying stem rust in adult plants provided that such a system is reliable, accurate, and efficient in terms of high-throughput capacity.

The concept of improving and accelerating adult plant rust phenotyping in a controlled environment was presented by Pretorius et al. (2000a) who showed that APR to leaf rust and stripe rust was clearly expressed in flag leaves of plants grown under continuous light and inoculated 28 days after sowing. When the same approach was followed in a mapping study, not all stripe rust resistance QTL were expressed in a Karioga × Avocet S DH population (Ramburan et al. 2004). However, Pretorius et al. (2007) showed that improved APR results with stripe rust were obtained when more vigorous plants were grown in a greenhouse as opposed to the mini-adults raised under constant light in a growth chamber. This upgraded greenhouse technique was validated with an extended Karioga × Avocet S population and all three major stripe rust resistance QTL were detected (Prins et al. 2011). In the latter study, adult plant leaf rust responses were also successfully mapped in plants grown in a greenhouse.

Hickey et al. (2012) found that APR to stripe rust could be reliably detected from leaf reactions of wheat varieties inoculated at the stem elongation stage 3 weeks after planting. When plants were grown under continuous light, both Ramburan et al. (2004) and Hickey et al. (2012) mentioned that younger leaves were more susceptible than older leaves, indicating an environmental effect on the expression of stripe rust resistance in young plant tissues. Working with leaf rust, Singh (1992) reported that low infection types were best

recognized in older leaves, which supports the approach of phenotyping sturdy plants at an appropriate growth stage. Less research has been done on greenhouse assessment of stem rust-infected adult wheat plants. Sunderwirth and Roelfs (1980) determined anthesis as the best growth stage for reliable greenhouse observations of *Sr2* expression. They found that the number and size of uredinia of *P. graminis* f. sp. *tritici* were significantly reduced on adult plants containing *Sr2*. Typically, susceptible type lesions on *Sr2*

Fig. 3. Comparison of coefficient of infection of three different spore concentrations of *Puccinia graminis* f. sp. *tritici* race TTKSP on five cultivars calculated 14 days after inoculation. Error bars represent the standard deviation.

Fig. 2. Stem rust incidence (percentage plants infected) 14 days after inoculation on adult plants of five cultivars inoculated with *Puccinia graminis* f. sp. *tritici* race TTKSP and incubated in the dark in a condensation dew chamber (A) and in natural light/darkness in a plastic chamber in a greenhouse (B). Error bars represent the standard deviation. Legend for both graphs displayed only in (A).

plants were situated above the nodes. Eaton et al. (1984) determined stem rust infection at two mature growth stages in a greenhouse but concluded that the prediction of field infection levels using greenhouse variables was not possible.

Although a reliable greenhouse method was developed in the present study, marked differences occurred in the successful outcome of inoculation methods combined with incubation environments. In general, the lowest infection levels were achieved in the condensation dew chamber, which maintains free moisture, but without runoff, on plant surfaces. This facility, in which plants are incubated in darkness, has been successfully used for consistent infection of seedlings in several rust pathosystems, including wheat stem rust, for over 25 years. Although temperature inside the chamber was within the boundaries of 15 to 24°C for optimal germination of *P. graminis* f. sp. *tritici* urediniospores (Sharp et al. 1958), low levels of stem infection occurred. This is particularly true for plants either inoculated with dry spores or with a water-spore suspension

containing a wetting agent. Similarly, low infection levels under controlled conditions were experienced by Rowell et al. (1958), who found that even with 98 to 100% and 50 to 60% germination and appressorium formation rates, respectively, only 15 to 25% successful infections occurred.

The best and most dependable results were obtained with adult plants incubated in the plastic chamber. The obvious differences between the two incubation environments were moisture and light, with heavy dew and no light prevailing in the condensation chamber, as opposed to less moisture and several hours of natural, yet filtered daylight in the plastic chamber. Rowell (1984) mentioned that many appressoria remain quiescent when plants are left in dark, moist conditions but will develop penetration structures if the temperature is raised to 30°C, either in darkness or in light. Staples and Macko (1984) stated that germination of urediniospores of *P. graminis* f. sp. *tritici* is inhibited by continuous irradiation, even before the germ tubes emerge. Burrage (1970) reported that light inhibited germination of urediniospores on wheat leaves but several studies have pointed out that *P. graminis* f. sp. *tritici* is rather unusual since substomatal vesicle formation requires exposure to light (Rowell et al. 1958; Sharp et al. 1958; Yirgou and Caldwell 1968). Given viable urediniospores and their effective deposition on plants, it appears that prolonged dew formation on stems within the required temperature window is not necessarily the only requirement for successful infection of adult plants. From our results, the initial stem wetness provided by the water-based inoculum spray, together with the water tray in the bottom of the plastic chamber, provided enough humidity for spore germination. According to our side-by-side comparison of dew chambers, an incubation period of 24 h, including a natural light and dark phase, and/or less free moisture within a plastic chamber, were conducive to the stem infection process.

The possibility of closed stomata as a physical factor in reduced rust infection was regarded as unlikely by Rowell (1984), but elevated respiratory CO₂ levels in the dark may play a role in poor infection. In addition, a variety of organic and inorganic substances in water may inhibit germination of rust spores (Rowell 1984). Based on our experience of successful seedling infection using this system, it is unlikely that water quality was responsible for the poor infection obtained in the dew chamber. Moisture is deposited on plant surfaces through condensation when vapor produced by the heated water baths, presumably without impurities, is cooled by the chamber walls. Furthermore, only water purified through reverse osmosis and sterilization was used in water-spore suspensions and successful infection in the plastic chamber ruled out water as a restraining factor.

In our study, it was possible that spores germinated in the dark chamber but that the adult plant infection process could not be completed due to an unfavorable light-temperature-moisture interaction.

Table 2. Stem rust incidence, severity, and infection response type, produced by races BPGSC and TTKSP on adult plants of 20 South African cultivars in the greenhouse and in the field^a

Cultivars	Greenhouse				Greytown field
	BPGSC		TTKSP		
	Rep 1	Rep 2	Rep 1	Rep 2	
Pan3434	20MRMS	20MRMS	50S	70MSS	20MSS
SST57	0R	0R	40MS	40MR	30MSS
Baviaans	30MS	20MSS	70MSS	50MS	20S
SST825	40MRMS	30MRMS	70S	80S	60MSS
SST88	tR	5R	70S	80S	50S
SST835	20R	20MRMS	60MS	60MS	30S
SST822	0R	0R	80MS	50MS	30MSS
SST027	0R	0R	5MS	30MSS	40MSS
CRN826	0R, 5S	0R, tS	10S	80S	70MSS
SST015	30MR	50MR	40MRMS	50MRMS	5R
Steenbras	0R	0R	0R	0R	0R
Kariega	50MSS	40MSS	50S	60S	70S
SST047	0R	0R	30R	0R	5R
Pan3492	10R	5R	20MSS	60S	30MS
Marico	10R	5R	50S	50S	80S
Pan3408	30MS	30MSS	70S	70S	70MSS
SST876	tMR	5MR	50MSS	80MSS	90S
Olifants	0R, 40MR	0R	60S	50MS	60MSS
Krokodil	10RMR	20RMR	40MRMS	60MR	60MS
Tankwa	0R	0R	10R	10R	0R
Morocco	70S	80S	80S	70S	80S

^a Severity (Roelfs et al. 1992) and infection response types (Stubbs et al. 1986) were determined 14 days after inoculation in the greenhouse.

Table 3. Adult plant stem rust resistance QTL detected by CIM in a DH wheat mapping population evaluated in the greenhouse and field

BPGSC ^a Chr ^c	QTL interval ^d	Associated marker	Leaves		Stems						Gene origin	Resistance gene
			Infection type	R ²	Severity		Reaction type		Coefficient of infection			
					LOD	R ²	LOD	R ²	LOD	R ²		
6A	wPt6951- wmc163.2	Sr26.43	13.1	7.7	6.2	3.9	24.9	21.7	9.0	7.6	AvS	<i>Sr26</i>
6D	cfid75- wPt3879	psp3200	62.4	60.8	32.2	26.1	20.9	17.5	17.7	16.0	AvS	<i>Sr5</i>
7D	barc352- cssrf6	cssrf6	2.7	1.4	NS	NS	NS	NS	NS	NS	Kariega	<i>Lr34/Yr18/Sr57</i>
TTKSP ^a Chr												
6A	wPt6951- wmc163.2	Sr26.43	8.6	7.4	14.4	22	29.5	15.2	37.5	46.9	AvS	<i>Sr26</i>
7D	cssrf6-gwm111.1	csLV34	8.0	6.1	NS	NS	NS	NS	NS	NS	Kariega	<i>Lr34/Yr18/Sr57</i>
PTKST ^b Chr												
6A	wPt6951- wmc163.2	Sr26.43			19.5	23.2	44.7	52.7	31.7	39.1	AvS	<i>Sr26</i>
7D	cssrf6- gwm295	csLV34			6.3	6.5	NS	NS	2.6	2.4	Kariega	<i>Lr34/Yr18/Sr57</i>

^a BPGSC and TTKSP *Puccinia graminis* f. sp. *tritici* races were used for greenhouse inoculation.

^b PTKST *P. graminis* f. sp. *tritici* race was used for field inoculation.

^c Chromosome.

^d QTL significantly present with LOD score >2.2 ($P = 0.05$) after 1,000 permutations.

Although our investigation did not resolve which factors were critical for stem infections, it provided development of a successful assay for mature plants. In the present study, plant height (~65 cm), flag leaf length (~20 cm), and spike length (~6 cm, excluding awns) confirmed more robust plant growth (actual data not shown), thus avoiding the sensitive tissues of light-accelerated mini-adult plants. The quick maturation of flag leaves encountered previously (Pretorius et al. 2007) was not seen in the current work and we concluded that plant development was suitable for obtaining quality stem rust data. Using 100 ml inoculum for 30 entries provided adequate stem coverage and a concentration of 1.25 mg/ml spores suspended in water and Tween 20 was sufficient. The high density of pustules on the susceptible control Morocco suggested that lower concentrations, allowing larger uredinium growth, should be considered. Inoculum pressure through polycyclic uredinium production in the field versus a single infection phase in the greenhouse might explain most variation observed in severity rather than reaction type. Nevertheless, these data demonstrated that a single infection cycle in the greenhouse can either supplement, or, depending on the objective, substitute for field scoring.

The controlled status of the greenhouse method resulted in clear observations of when signs of *P. graminis* f. sp. *tritici* infections commenced. The first signs of uredinia were visible 6 days after inoculation and it would thus be possible to use this technique for assessment of macroscopic components of APR such as latent period, pustule size, and pustule density. The stems of greenhouse-grown material were uniformly infected by rust, confirming that a reliable level of infection can be achieved using the conditions as described. In terms of microscopic resistance components, the procedure is particularly adapted to the method described by Ayliffe et al. (2013) for quantification of fungal biomass. While their study used seedlings for wheat stem rust and flag leaves from field plots for leaf rust and stripe rust, the wheat germ agglutinin chitin (WAC) assay can equally well be applied to greenhouse-grown leaf sheath tissue to quantify *P. graminis* f. sp. *tritici* in adult plants.

In conclusion, this study describes a functional system for controlled infection of adult wheat plants with *P. graminis* f. sp. *tritici* for phenotyping fixed lines and populations, and for further application in germplasm characterization, inheritance studies, resistance mapping, and refined host-pathogen interaction studies. It also lays a foundation for improving moisture, temperature, and light requirements for application in epidemiological studies. All-stage resistance genes such as *Sr5* and *Sr26* were clearly visible but a seedling assay will be more efficient if major gene confirmation is the only objective. Should the interaction between resistance genes in adult plants be a priority of investigation, the currently described system will be more supportive. It is unlikely that greenhouse phenotyping will replace field testing as a certain type of partial resistance, e.g., that conferred by *Sr57*, was not reliably detected. Interestingly, when *Sr57* was detected in the adult plant leaf infection type scores versus the field scores, using different pathotypes, the significant QTL region varied slightly, with the same interval not being consistently detected. Lower C.I.'s were observed on varieties such as Inia 66 and Hartog, which contain the APR gene *Sr2*, providing evidence that infection of adult plants in a greenhouse will be an additional instrument for stem rust resistance assessments.

Acknowledgments

This research was supported in part by funds provided through a grant from the Bill & Melinda Gates Foundation to Cornell University for the Borlaug Global Rust Initiative (BGRI) Durable Rust Resistance in Wheat (DRRW) Project. The authors thank Dr. Gordon Cisar and Prof. Neal McLaren for comments during manuscript preparation.

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