DEVELOPMENT OF AN INDEX FOR WHEAT STRIPE RUST INFECTION

BY

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L. De Wet

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seven stations used were M = Moorreesburg, R = Rietrivier, W = Winterton, B = Bethlehem, S = Swellendam and T = Tygerhoek

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CHAPTER 1

INTRODUCTION

Stripe (yellow) rust caused by *Puccinia striiformis* Westend f. sp. *tritici* Eriks. is one of the most important fungus diseases of wheat (*Triticum aestivum L.)*. Wheat yield losses as high as 84 % have been recorded under severe epidemic conditions. The disease is most commonly found in cool wet areas and occurs regularly in NW Europe, the Mediterranean wheat growing region, Middle East, NW U.S.A., Australia, East African highlands, China, the Indian subcontinent, New Zealand and the Andean region of South America. (Boshoff, van Niekerk and Pretorius, 1998; Boshoff, 2000).

Historically, the disease has not been widespread in southern hemisphere countries. Stripe rust was first observed in Australia in 1979 and in New Zealand in 1982. In South Africa the disease was first detected in August 1996 (Boshoff *et al.*, 1998) in the winter rainfall area near Moorreesburg in the Western Cape (Table 1.1). The disease was well established by the time it was first detected, so the initial point and time of outbreak could not be determined. The epidemic (defined by dictionaries as a disease upon many, simultaneously, but not continuously and introduced from the outside) was most severe in the Western and Northern Cape. According to Boshoff *et al.* (1998) cool and wet conditions until flowering are favourable for the outbreak of an epidemic. The initial epidemic cost producers approximately R30 million in fungicides. The disease also spread to the southern part of the Western Cape where infection was low, presumably because weather conditions were hot and dry. The western and southern areas of the Eastern Cape were also affected by the disease. Later in the 1996 season it was also found at Rietrivier in the summer rainfall area near Kimberley (Boshoff *et al.*, 1998).

In 1997, Boshoff *et al.* (1998) recorded that stripe rust was first observed in July in the southern and western areas of the Western Cape. Epidemic conditions developed in the southern areas of the Western Cape and the western and southern areas of the Eastern

Table 1.1: Infection of *Triticum aestivum* by *Puccinia striiformis* f. sp. *tritici* from 1996 – 2001 in the main wheat growing areas of South Africa (Boshoff, van Niekerk and Pretorius, 1998). 1999 – 2001 data were obtained from W.H.P. Boshoff (Personal communication, *Small Grain Institute-Agricultural Research Council, Bethlehem, R.S.A.*, 2001)

Year	Occurrence of *Puccinia striiformis* f. sp. *tritici*	
	Epidemic	**Non-Epidemic**
1996	1. Western Cape. 2. Northern Cape.	1. southern Western Cape (low). 2. western and southern Eastern Cape.
1997	1. southern Western Cape. 2. western and southern Eastern Cape. 3. western, central and eastern Free State.	1. western Western Cape (low). 2. KwaZulu-Natal. 3. Gauteng. 4. North West. 5. Northern Province.
1998	eastern Free State (new race).	1. Western Cape. 2. Eastern Cape. 2. southern Western Cape. 3. KwaZulu-Natal.
1999	1. Western Cape. 2. northern and eastern Free State.	1.Western Cape. 2. southern Western Cape. 3.KwaZulu-Natal. 4.central and eastern Free State.
2000	northern and eastern Free State.	1. Northern Province. 2. Western Cape.
2001	eastern Free State.	1. Western Cape (low). 2. central Free State (low). 2. Northern Province.

Cape (Table 1.1). The western and northern areas of the Western Cape experienced low infection as resistant cultivars had been planted. Infection also occurred in the Free State, KwaZulu-Natal, Gauteng, North West and Northern Province. In the same year, 1997, infection in western, central and eastern Free State was high (Boshoff *et al.*, 1998).

In 1998 few stripe rust problems were experienced in the Western Cape and Eastern Cape due to resistant cultivars and unfavourable weather conditions. During this year, first symptoms were observed in the last week of July near Swellendam. In the summer rainfall area, first symptoms were observed in KwaZulu-Natal and the eastern Free State. Epidemic outbreaks occurred in the eastern Free State as a result of development of a

new pathogenic race. Indications are that the pathogen over summered in both the winter and summer rainfall regions during all the years of infection. (Boshoff and Pretorius, 1999).

During 1999, 2000 and 2001 epidemics developed in the northern and eastern Free State regions and in 1999 in the Western Cape. Outbreaks occurred in the Western Cape in all these years, but in KwaZulu-Natal only in 1999. Infections in the central and eastern regions of the Free State were observed in 1999 and 2001. In the Northern Province outbreaks occurred for the first time since stripe rust observation in South Africa (1996), in 2000 and 2001 (W.H.P. Boshoff, personal communication, *SGI-ARC, Bethlehem, R.S.A.*, 2001). According to Boshoff *et al.* (1998) favourable weather conditions, susceptibility of several cultivars, high costs of fungicidal applications and the high mutation potential of the stripe rust pathogen qualify stripe rust as a damaging disease with a strong impact on local wheat production.

From literature reports and experience in South Africa it is clear the occurrence of wheat stripe rust is strongly influenced by environmental conditions. The effect of fungicidal applications on the environment further highlights the seriousness of the disease. Therefore, the hypothesis for this project is that temperature, rainfall, relative humidity (RH), sunshine hours and wind speed data, together with disease data can be used to develop an index for use as an early warning system for *P. striiformis* f. sp. *tritici* in susceptible cultivars of *T. aestivum*.

The following objectives were formulated for this project:

> The main objective is to develop an early warning index for infection of stripe rust (*P. striiformis* f. sp. *tritici*) in susceptible cultivars of common (bread) wheat (*T. aestivum*) for the main wheat growing areas of Republic of South Africa.

a) To assess the influence of weather parameters (minimum and maximum temperature, minimum and maximum RH, sunshine hours, wind speed and rainfall) on infection of stripe rust on *T. aestivum*.

b) To conduct a laboratory experiment in order to collect disease infection information under constant conditions of high RH and set temperature range which could be useful in the development of an index.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

2.1 Life-cycle of *Puccinia striiformis*

The life cycle of *P. striiformis* f. sp. *tritici* (Fig. 2.1) consists of the uredinial and telial stages. Stripe rust populations can exist, change in virulence, and result in epidemics independent of an alternate host. Urediniospores are the only known source of inoculum for wheat (Roelfs, Singh and Saari, 1992). Wiese (1987) states that the teliospores and alternate hosts are required for the completion of pathogen life cycles in rust fungi but not for disease initiation. The sporulating uredinia can survive to a temperature of –4 °C (Roelfs *et al.*, 1992).

Urediniospores are produced in great numbers during the cropping cycle and are dispersed by wind to other plants, where they generate new infections and secondary urediniospores in intervals as short as seven days (Wiese, 1987).

2.2 Epidemiology

Stripe rust takes its name from the characteristic stripe of uredinia (or lesions or pustules) that produce yellow coloured urediniospores. Urediniospores are produced during the wheat season and initiate germination within one to three hours of contact with free water between the range of temperatures –2 °C to 23 °C. (Roelfs *et al.*, 1992). Wiese (1987) writes that germ tubes are used to penetrate the stomata on host leaves. Spread of urediniospores is favoured during rain by washing spores from the air onto the leaf surfaces. This moisture results in an increase in humidity near the leaf surface, which if high, can restrict spore movement from the surface. This creates favourable conditions for infection. On the other hand, rain can also wash spores from the plant surface causing spores to land on the soil. Wind can remove spores from the leaf surface and spread them

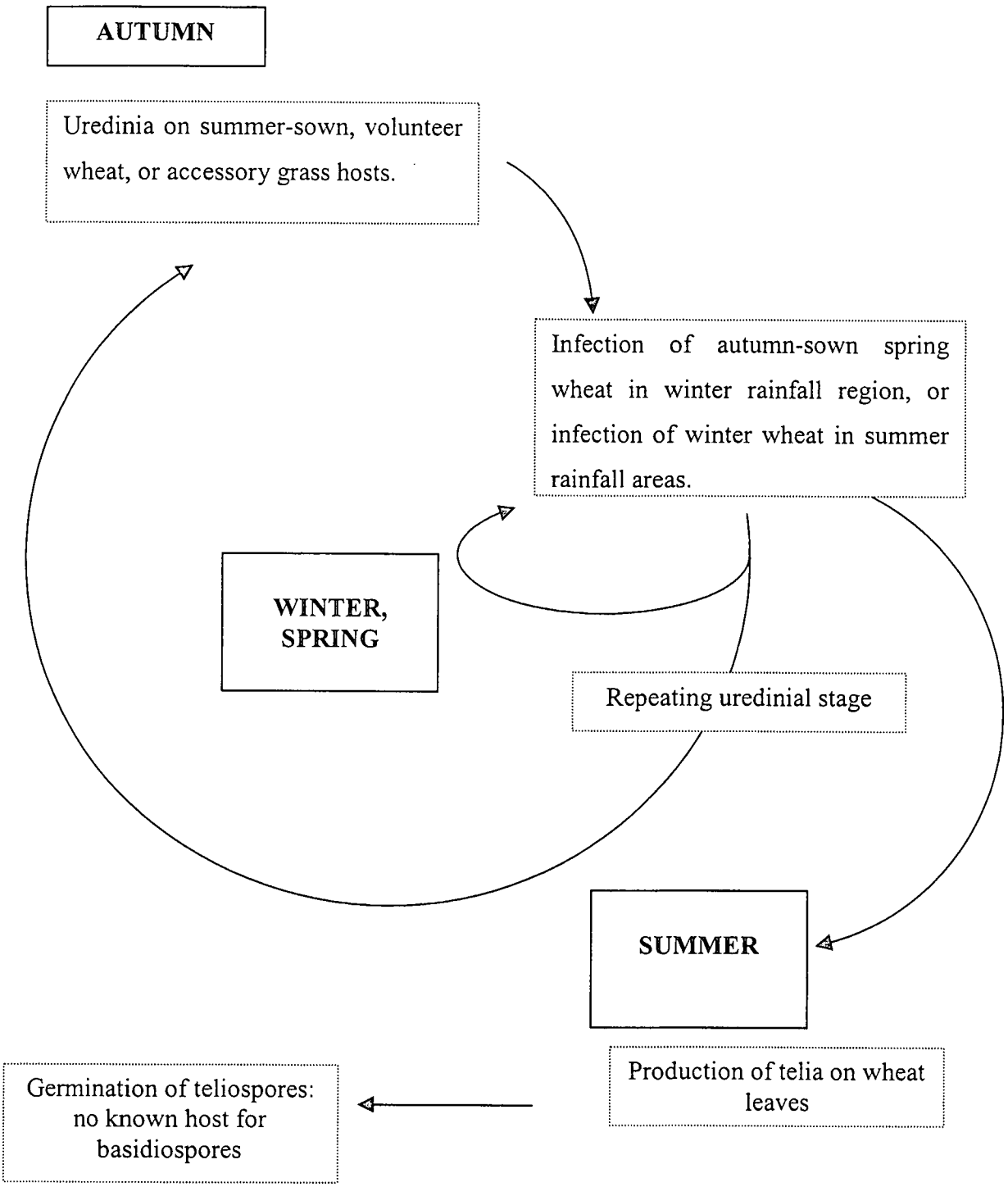


Fig. 2.1: The life cycle of *Puccinia striiformis* f. sp. *tritici* on *Triticum aestivum* in South Africa (adapted from Roelfs, Singh and Saari, 1992).

to other plants locally or carry them over large distances to other areas (Roelfs *et al.*, 1992). According to Wiese (1987) spores can survive more successfully year round on hosts (wheat, rye, barley or perennial grasses) in cooler climates than the spores of leaf or stem rusts. There is no known alternate host and spores can increase 10 000 fold per generation.

Roelfs *et al.* (1992) maintain that on a clear, hot, dry day with temperatures greater than 25 °C, RH< 30 %, wind speed approximately 5 ms^{-1} and no rain in the previous 24-48 h, spore numbers can total 10 000 cm^{-2}, even though the previous day numbers totaled only 500 – 1000/cm^2. The pathogen can spread without additional spores or infection periods. Thus it can be assumed that there is a constant presence of spores in the atmosphere. However, a change in air temperature will influence disease progress (Roelfs *et al.*, 1992). Optimum air temperatures for germination of spores under controlled conditions are between 7 °C and 11 °C (Dennis, 1987; Park, 1990). Park (1990) found that plants under field conditions can become infected even when air temperatures fluctuate within the range 19 °C - 30 °C as long as free water was present, whereas experiments conducted under controlled environments had lower maximum air temperatures under which infection took place. One could comment here that the field conditions quoted here are air temperatures most probably measured in a standard weather station. Even so, it is possible that a whole range of temperatures are present in the crop canopy. This can lead to possible infection even when high air temperatures are measured by standard weather stations as there are lower temperatures within the canopy. Positioning of instruments within the crop canopy would enable one to measure the actual temperature at the site of infection. This temperature would then be comparable to controlled environment temperature measured during laboratory experiment.

Park (1990) also found that spores produced in hotter environments needed a higher air temperature for infection. Environmental factors such as air temperature during urediniospore production greatly affect the requirements for subsequent germination. He quotes Straib (1940) who found the maximum air temperature for spore germination was 2 °C - 3 °C higher for spores produced at 20 °C – 25 °C than when produced at 8 °C –

Table 2.1: Cardinal temperatures, minimum (T_{mn}), maximum (T_{mx}) and optimum (T_{op}) for initiation of spore germination of *Puccinia striiformis* f. sp. *tritiici* from various literature sources.

Reference	T_{mn} °C	T_{op} °C	T_{mx} °C	Laboratory /field	Country
Dennis (1987)	<0	7 – 10	18	Lab.	Australia
Ling (1945); Manners (1950); Coakley (1974); Sharp (1965) and Rapilly, 1979 (quoted by Dennis, 1987)	-4	7 – 18	27	Lab. and Field	China Great Britain N. America
Park (1990)	- -	15.4 -	21 19 – 30	Lab. and Field	Australia
Pretorius (personal communication, 1999)	2	11	15	Field	South Africa
Roelfs, Singh and Saari (1992)	-2	9 – 13	23	-	Europe, America
Wiese (1987)	0	3 - 15	21	-	N. America
Average calculated from literature	-0.8	10.6	23.3	Lab. and field	-
Average from data analysis	4	13	15-31	Field	South Africa

12°C. The wide range of possible air temperatures (Table 2.1) for infection by this pathogen is therefore obvious. Fig. 2.2 illustrates the range of cardinal temperatures given in Table 2.1 and shows that the temperatures from the data analysis fall within the range given by literature. Agrios (1978) mentions that moist weather conditions (in the form of rain or dew) influences germination of spores, penetration of the host by spores, infection of the host, distribution and spread of spores. Disease progress is also influenced by the moist weather conditions by increasing susceptibility, through increasing succulence of host plant leaves.

Roelfs *et al.* (1992) state that differences in relative humidity, light, temperature and pollutants combined with adult plant resistance have made studies of differences in pathogen aggressiveness difficult. These are thus additional factors that could have an influence on disease progress under field conditions.

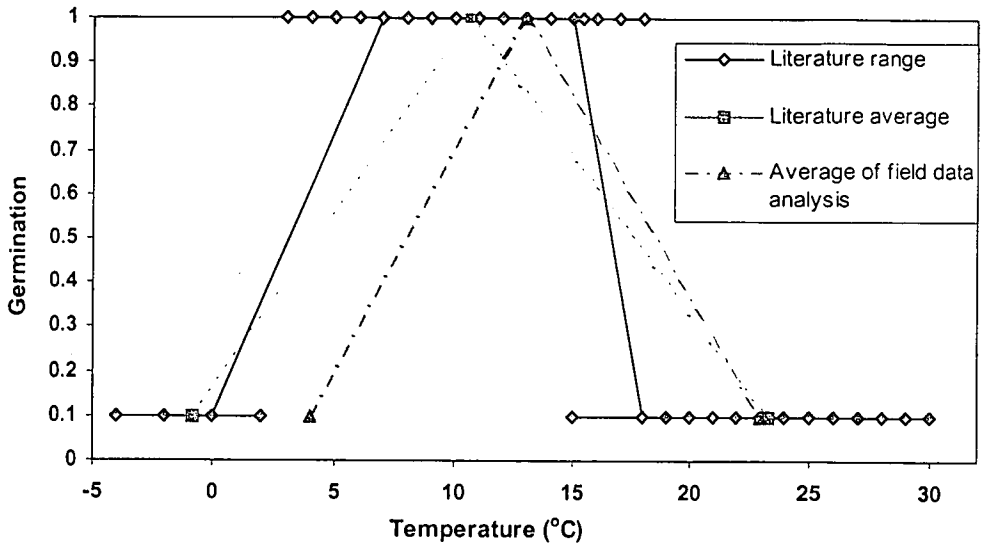


Fig. 2.2: Cardinal temperatures for initiation of spore germination of *Puccinia striiformis f. sp. tritici* taken from a) literature range, b) averages of literature range and c) averages from data analysis.

2.3 Interaction between host, pathogen and environment

Fig. 2.3 shows the interaction between the host, pathogen and environment. The host and environment have an influence on each other, as does host and agent. The environment influences the agent, but the agent has no influence on the environment, although theoretically it would, for example, if the agent defoliates the crop, then the microclimate within the canopy will be altered.

Interaction between the host, in this case, *T. aestivum*, and the environment, as well as on disease initiation and progression have the following possible reactions (Bourke, 1970):

- Density and distribution of the crop: A large area of densely planted wheat crop will be susceptible to disease.
- Condition of *T. aestivum* plants: Soft leaves (high water content) invite penetration. High relative humidity in the atmosphere following a dry spell also provides for easy penetration. An accumulation of untranslocated carbohydrate results in available substrate for the pathogen.

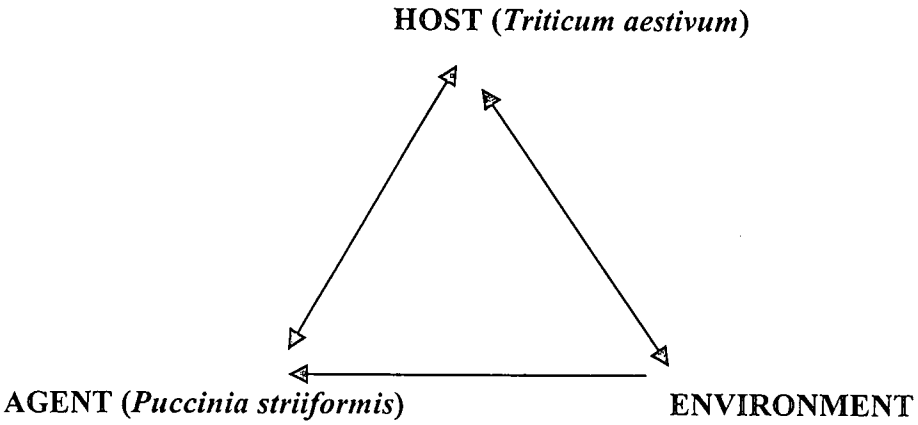


Fig. 2.3: Basic interaction between the environment, host and agent (Bourke, 1970).

- Soil water availability or content alters plant physiology and morphology, resulting in increased susceptibility with increase in water stress.
- Resistance to the disease differs from organ to organ and with growth stages.
- Planting dates and emergence of crops, as well as changes in cultivation practices, such as tilling can influence infection. For example, in order to avoid infection, winter wheat should be planted as early as possible, while spring wheat should be planted as late in the winter as possible(Wiese, 1987).
- Windbreaks, shelterbelts and aspect of fields could alter microclimate of crops, for example, south-facing versus north-facing slopes.
- Changes in irrigation practices. Irrigation at night for example could promote favourable conditions for infection, as the leaves are wet for a longer period of time and at lower temperatures than during irrigation applied in the day.

The viability of the pathogen is dependent on:

- Favourable weather conditions, in this case cool, wet conditions favour initiation of the disease. Wiese (1987) states that disease development is most rapid between 10 °C and 15 °C, in combination with intermittent rain or dew.

- Favourable weather conditions after initiation of germination. According to Wiese (1987) urediniospores rapidly lost viability at temperatures above 15 °C. They germinate optimally between 3 °C and 15 °C with an upper limit of 21 °C and lower limit of 0 °C. Night temperatures exceeding 18 °C, lead to reduced infection and resembles a resistant reaction (necrotic stripes). Xianming Chen (personal communication, *USDA-ARS, Wheat Genetics Unit, Washington State Univ., Pullman, WA, U.S.A.*, 2001) agrees with Wiese (1987).
- Roelfs *et al.* (1992) mention that urediniospores of stripe rust are susceptible to ultraviolet radiation and are therefore not transported in a viable state as far as those of leaf and stem rusts. They quote Madison and Manners (1972) as finding that stripe rust spores are three times more sensitive to ultraviolet radiation than those of stem rust. Zadoks (1961) however, reports that stripe rust was transported by wind in a viable state more than 800 km.

According to Bourke (1970) the environmental conditions in temperate climates inside and above the crop canopy depend largely on the nature and density of the crop and on the level of the underlying soil water. Differences are greatest on calm, sunny days and conditions are more similar in cloudy and wet weather. Wind in the boundary layer just above the crop helps to establish homogeneity. Relative humidity is high in the crop if canopy cover is complete and soil water is high. Differences between weather variables measured in the crop and those from a standard weather station nearby, would therefore be smaller during stable, homogenous weather conditions, which for example, occurs on cloudy or wet days. Larger differences in weather variables are observed in unstable weather, at high elevation or in mountain shelters. Fungus infections, such as stripe rust, would therefore be favoured by stable homogenous weather conditions, or cool and wet conditions, and this is confirmed by various authors, as discussed in Chapter 1.

It is therefore clear from the foregoing, that disease initiation and progression is the result of a complex interaction between host, pathogen and environment and it is therefore difficult to characterize these conditions if they are not monitored on an hourly or daily basis.

2.4 Indices

According to Roe (1984) empirical relationships identifying weather conditions favourable for disease development have been established for several of the most important fungal diseases, such as apple scab, barley mildew, potato blight, etc. Since stripe rust is a comparatively new fungus disease in South Africa it has only recently become the focus of attention (Boshoff and Pretorius, 1999). In the Pacific Northwest of U.S.A. it has been known to reduce yields since 1961 (Coakley, Line and Byod, 1983) and in Australia since 1979 (Park, 1990).

Dennis (1987) developed an equation relating temperature and wet duration period to infection, using one predominant Australian race, on wheat plants exposed to different temperatures for different time intervals up to a period of 48 h. The aim was to expose the plants to conditions favourable for maximum infection. It was found that infection increased with increase in temperature, but the rate at which this took place decreased as the temperature deviated further from the optimum (7 °C to 10 °C). The author states that this study should be applicable to most races of *P. striiformis* f. sp. *tritici* and is useful as a basis for predicting stripe rust infection from weather data. Application of the equation in this project was considered, but data required to apply the equation developed by Dennis (1987), namely the hourly values of leaf wetness duration, was not available in the wheat growing areas used in this project.

Gillespie and Sutton (1979) developed a predictive scheme for timing fungicide application to contol Alternaria leaf blight in carrots in Canada. They used temperature and duration of leaf wetness based on detailed regional forecasts of cloud cover and surface wind speeds for the development of the scheme. Criteria for the scheme were:
1) fungicide was only applied after 1 – 2 % of blight symptoms appeared on the leaves and 2) thereafter only when forecast weather for the forthcoming 36 h was favourable for infection. The scheme worked well for confined areas, as disease intensity data was required for each field. However, more widespread application of the scheme (in place of

implementing complex forecasting systems), was not possible due to a lack of specialized equipment and personnel required for detailed observations.

Coakley *et al.* (1983) improved on their own local statistical model for predicting stripe rust on winter wheat in the Pacific Northwest (Coakley, Boyd and Line, 1982) by the development of two regional models. One of these models was based on the relationship of:

a) disease intensity index to standardized negative degree days accumulated during the winter months of December and January.

b) the Julian day of spring (JDS = date when 40 or more positive degree days (PDD) had accumulated during the previous 14 days).

c) PDD for the 80 day period after the JDS.

According to the authors, the regional models have the advantage of application in regions where disease data is not available but where the following data is available: regional weather, susceptibility of cultivars and historical importance of the disease.

Ash, Brown and Rees (1991) developed a model for severity of stripe rust on wheat in Australia using regional weather data (1979 - 1989) for Horsham, Victoria. Stripe rust epidemics occurred in all these years and severity was recorded for the cultivar *Zenith* at growth stage 69 (Zadoks, Chang and Konzak, 1974). This model showed negative correlation of disease severity with maximum air temperature above 40 °C and its use in other regions for the prediction of probability of stripe rust epidemics (using historical data) was envisaged by the authors. This model used temperatures greater than 40 °C during the preceding calendar year to predict the probability of survival and epidemics of stripe rust for the season ahead. In its present form, therefore, the model is not applicable for R.S.A wheat production areas, as temperatures of greater than 40 °C are rarely experienced during the summer or autumn seasons.

Park (1990) investigated the role of temperature and rainfall on epidemiology (laboratory and field) of *P. striiformis* f. sp. *tritici* in the summer rainfall area of eastern Australia. He developed an equation for infection at constant temperatures. The equation shows that 50 % infection is expected at 18.2 ± 0.2 °C and no infection at 20.8 ± 0.2 °C, with the assumption of a 15 h moist period for infection in the laboratory study. The same tendency was not observed in the field study as moderate to severe infection (>50%) occurred in the field in the presence of free water even when temperature fluctuated between 19 °C and 30 °C.

2.5 General requirements for forecasting

There are several attributes necessary for a forecasting system to be successfully adopted and implemented by growers (Campbell and Madden, 1990):

- The forecast must be based on a reliable source of biological and environmental data and should be adequately tested for the area of distribution;
- simplicity of the index or model allows for greater acceptance by users;
- disease must be of sufficient economic importance for it to be considered worthy of forecast;
- disease should be easily detectable and management methods, such as use of indices combined with spray programs, for prevention of the disease should also be available and
- the forecast or early warning should be easily accessible to users, as well as cost effective.

In light of the previous paragraphs, there are a few specific requirements that need to be met for forecasting of plant diseases such as stripe rust in R.S.A. and so the following suggestions are made to make this possible:

- Provision of adequate data such as leaf wetness duration data so that equations such as the one by Dennis (1987) could be implemented and tested for South African conditions.

- The model used by Ash *et al.* (1991) could be adapted for South African conditions to use maximum temperatures other than 40 °C.

In conclusion, the development of an index for stripe rust in South Africa remains problematic given the limited length of the disease database. In regions where the above models have been applied successfully, the database (weather and disease) is longer than 10 years and the application of those models to other regions have yet to be validated. Nevertheless, an attempt has been made to develop a simple warning index for use by the wheat producer and other end-users in South Africa.

CHAPTER 3

ANALYSIS OF INCUBATION PERIOD OF STRIPE RUST INFECTION ON WHEAT

3.1 Introduction

Various authors are in agreement that temperature and moisture are the major climatic factors necessary for infection by stripe rust (Dennis, 1987; Wiese, 1987; Coakley, Line and McDaniel, 1988; Park, 1990; Ash *et al.*, 1991; Ellsion and Murray, 1992; Roelfs *et al.*, 1992; Boshoff *et al.*, 1998). The majority of these authors specify that lower temperatures than those required for leaf and stem rust must be present. Table 2.1 presents temperature limits for infection as between –4 °C and 30 °C, while RH should be high for a period of between one and three hours. This information was insufficient for the purposes of index development, as the specific critical or cut-off temperature and water requirement necessary for initiation of stripe rust were not known. An experiment was therefore planned to simulate favourable conditions for infection. It was run twice, the second time with modification and improvements to the setup. In the text they are referred to as Experiment 1 and Experiment 2.

The main objective of the experiment was to observe infection of stripe rust under conditions of high RH and a range of temperatures, so that the critical or cut-off temperature values could be found. High RH causes condensation on the leaves and so varying the length of exposure to high RH for a total period of 48 h was considered. Part of the experimental observation was to find the length of the incubation period, which is the time from infection to first symptom observation.

3.2 Method and Materials

3.2.1 Experiment 1

Eight-day-old seedlings of two wheat cultivars, one susceptible and one resistant (Karee and Pan 3349) were kept at four different temperature levels. Three replications of each cultivar were subjected to an exposure time period, starting from 0 h (control) after inoculation up to 48 h after inoculation. The aim was to keep RH high and the temperature as close as possible to the temperature level allocated. The experiment was run from 04-04-2001 to 06-04-2001. Use of the word *incidence* implies number of infected plants expressed as a percentage of the total number of plants and *severity* implies infected area of plant leaves as a percentage of total plant leaf area.

Five to ten wheat seedlings were planted in cones containing peatmoss soil in four trays and grown at 25 °C in a glasshouse (plate 3.1). Treatments in each tray consisted of 2 (cultivars) x 3 (replications) x 10 exposure time periods = 60 cones. After eight days of growth, small, recently emerged plants were clipped out so that they would not register zero with infection. Preparation of the containers was done one hour before start of the treatments (plate 3.2). The dry- and wet bulb sensors and *Hobo* data logger were placed on the inside of each container. Plants were removed from the glasshouse and inoculated with stripe rust pathotype $6E22A^{-}$ by spraying them with a suspension of freshly collected spores in sterile, distilled water containing a drop of Tween 20. About one litre of warm water at 35 °C was poured into the containers to generate vapour and thus a high RH as quickly as possible. For the control, three cones from the container for the 20 °C temperature level were moved to the glasshouse before insertion of the container into the growth chamber. The plants in these cones were inoculated, but not subjected to constant RH combined with a temperature level in a growth chamber. Trays were immediately placed within closed, plastic containers (sealed with masking tape) at different temperatures in a cold room and growth chambers. The temperature levels were 5 °C, 10 °C, 15 °C and 20 °C. Dennis (1987) did a similar experiment with different

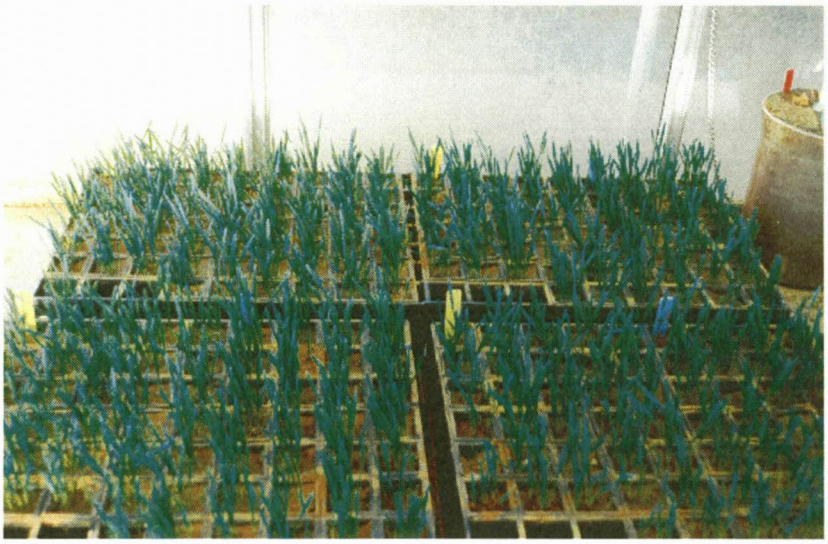


Plate 3.1: **Trays of wheat seedlings at the time of inoculation with *Puccinia striiformis* f. sp. *tritici***

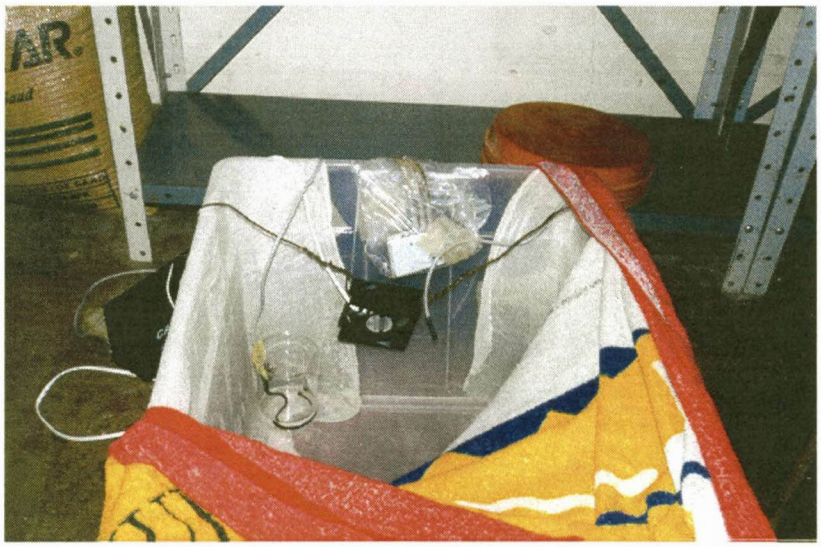


Plate 3.2: **One of the plastic containers as prepared for placement into a controlled temperature chamber before insertion of a seedling tray. The fan used in Experiment 2 is also shown.**

temperatures. Three cones from each cultivar and each plastic container were removed after 0, 1, 3, 6, 12, 18, 24, 30, 36, 42 and 48 h. Care was taken with removal of cones after each time period to keep the procedure consistent and was performed as quickly as possible to prevent disturbance of the humid environment in the containers. Upon removal, these cones were returned to the glasshouse, where the average temperature was in the range of 12 °C – 17 °C. The plants were checked each day to observe the first signs of infection. On day 14 disease readings were done.

3.2.2 Experiment 2

The experiment was repeated from 02-08-2001 to 04-08-2001. Preparation was similar to Experiment 1 with the following differences: The preparation of the containers was done the day before, so that a period of at least four hours for equilibration of temperature and RH could take place before insertion of the trays. The inside of the containers were lined with muslin cloth soaked in cold water and about one litre of cold water was poured into each of the containers to facilitate high RH conditions. The dry- and wet bulb sensors and *Hobo* data logger were placed inside each container, with the logger hanging in a plastic bag to prevent moisture absorption. A fan was placed in each container to face the sensors, blowing over the sensors only, so that a ventilated wet and dry bulb temperature reading could be obtained without disturbing the free water condensed on the leaves. A second dry bulb sensor was placed on the outside of the container to measure chamber temperature. The above set-up was ready to be placed in the growth chambers and cold room about four hours before the experiment started. The inside of each container was saturated with a squeeze bottle and covered with a very damp towel to maintain a high RH inside each container. The lids were placed over the towels to ensure extra precaution against a drop in RH. The squeeze bottle was used to saturate the air inside the container with water after each exposure time opening period so that a high RH could be maintained.

The results of both experiments were analyzed using *NCSS 2000 Statistical System for*

Windows (Hintze, 1998). The means of incidence and severity were calculated for each temperature level (5 °C, 10 °C, 15 °C and 20 °C), cultivar (Karee and Pan 3349), exposure time period (interval numbers 1 – 10) and the interactions between them.

3.3 Results and Discussion

During both experiments temperatures were set at 5 °C, 10 °C, 15 °C and 20 °C. However, the temperatures measured in the containers were not always the same as the temperature settings or levels. Therefore the results were analyzed according to the measured temperatures. Interval numbers correspond to increasing exposure time period, which is the number of hours after inoculation (Table 3.1).

After 7 – 14 days, the first signs of infection were observed as chlorotic flecking of leaves, gradually developing into sporulation lesions.

Table 3.1: Interval number corresponding with exposure time period for 48 h run of Experiments 1 and 2.

Interval Number	1	2	3	4	5	6	7	8	9	10
Exposure time period (h)	1	3	6	12	18	24	30	36	42	48

3.3.1 Measured temperature and temperature levels versus disease incidence for all intervals

According to Figs. 3.1 and 3.2 and Table 3.2 below, the disease incidence for each replication varies between 0 and 100 % for most of the temperature levels and time intervals. There were a few exceptions, however, for both interval numbers and temperature levels. For example, incidence varied from 10 % – 100 % and 9 % – 100 %, for interval numbers 3 and 4 and 5 – 10, for Experiment 2. At the 5 °C and 20 °C temperature levels, the full range of incidence was not observed. For example, at the 5 °C temperature level, only 60 % – 100 % incidence for Experiment 2 and 40 % – 100 %

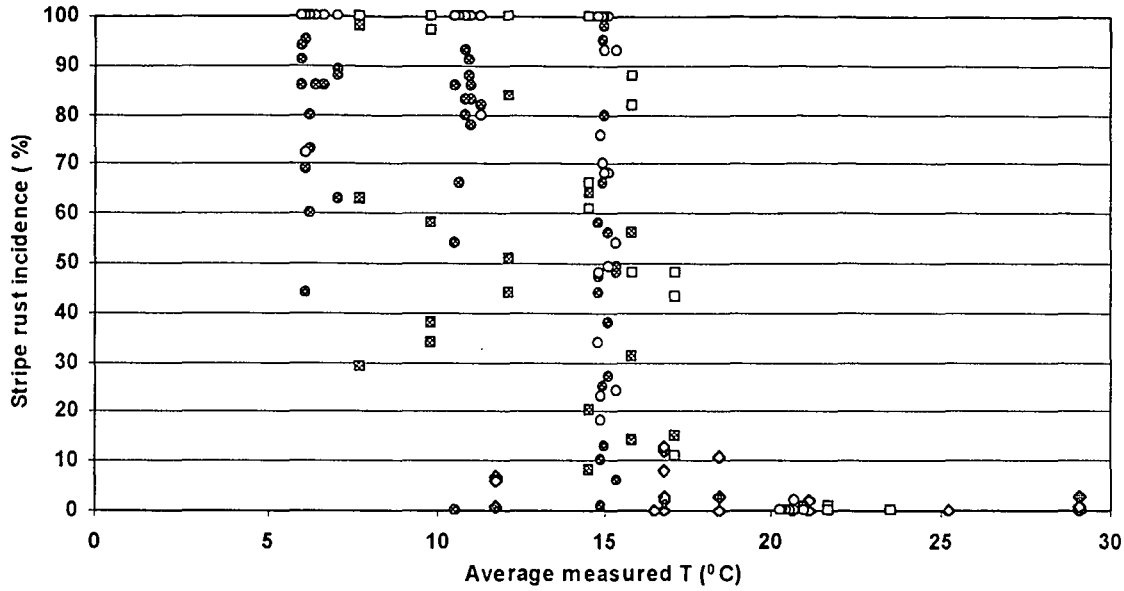
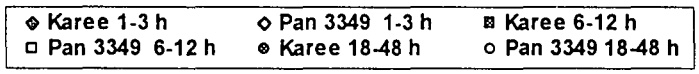


Fig. 3.1: Average measured temperature (T) for each interval number versus disease incidence for each replication of Karee (Cultivar 1) and Pan 3349 (Cultivar 2) for three time intervals: 1 - 3 h, 6 - 12 h and 18 - 48 h (Exp. 1).



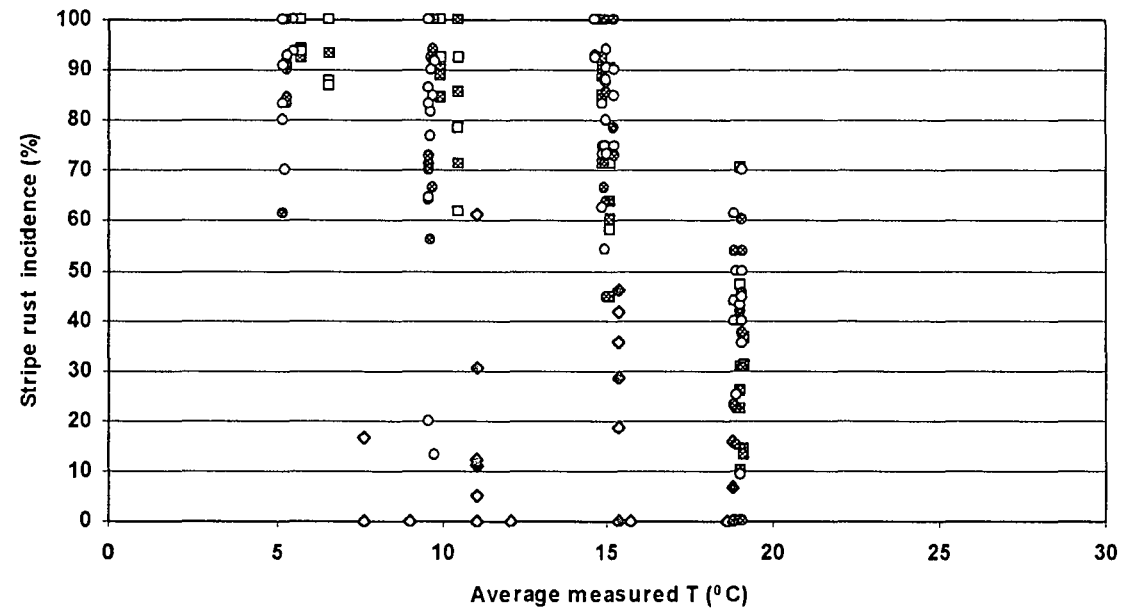
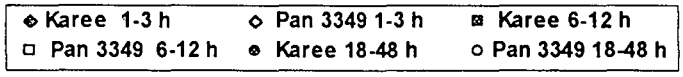


Fig. 3.2: Average temperature (T) for each interval number versus disease incidence for each replication of Karee (Cultivar 1) and Pan 3349 (Cultivar 2) for three time intervals: 1 - 3 h, 6 - 12 h and 18 - 48 h (Exp. 2).



incidence for Experiment 1 was observed. At the 20 °C temperature level only 0 % – 70 % incidence for Experiment 2 and 0 % – 10 % incidence for Experiment 1 was observed. At the 10 °C and 15 °C temperature levels, the full range of incidence for both experiments was observed, i.e. 0 % – 100 %. The maximum incidences for interval numbers 1 and 2 shown in Table 3.2 were also higher for Experiment 2 (46 % and 62 %) than in Experiment 1 (7 % and 13 %). This observation is explained by Fig. 3.3, which shows that actual measured temperatures for Experiment 2 were lower at each temperature level, allowing infection to take place.

Table 3.2: Comparison of disease incidence with all measured temperatures for three time intervals for replications of Karee and Pan 3349 in Experiments 1 and 2.

Interval number	EXPERIMENT 1		EXPERIMENT 2	
	Disease incidence (%)		Disease incidence (%)	
	Karee	Pan 3349	Karee	Pan 3349
1 and 2	0 – 7	0 –13	0 - 46	0 – 62
3 and 4	0 – 98	0 – 100	10 – 100	31 – 100
5 - 10	0 - 100	0 - 100	0 - 100	9 - 100

Table 3.3 gives the maximum temperatures for each temperature level measured during the first time interval. Here temperatures of above 24 °C for Experiment 1 and below 20 °C for Experiment 2 are shown for the 20 °C temperature level. The reason for the difference lies in the different setup of the two experiments. The period of equilibration time at a set temperature level and RH before implementation of Experiment 2, allowed for measured temperatures to be closer to set temperatures and to show less variations.

The 0 % infection for interval numbers 3 – 10 in both experiments occurs in the majority of cases for the 20 °C temperature level, which indicates that high temperatures did not favour infection. Table 3.4 shows the maximum and average incidences for all the temperature levels for interval numbers 1 and 2. The 0 % infection for interval number 1 can explained by the lack of sufficient exposure time to a high RH. For interval number 2

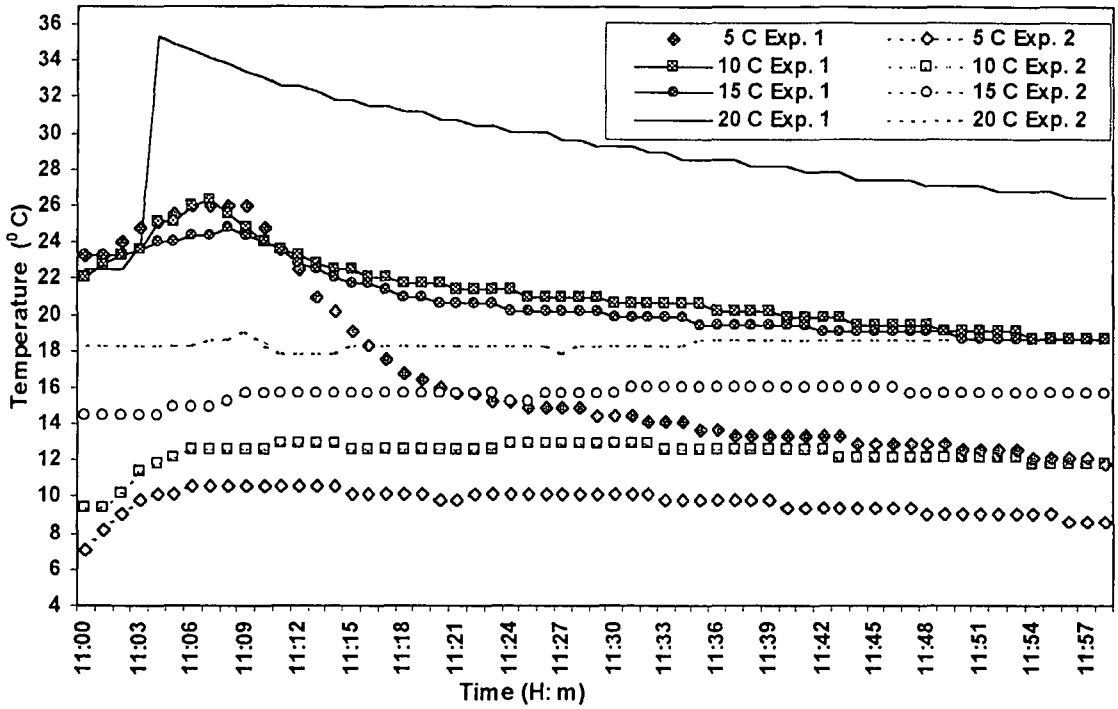


Fig. 3.3: Equilibration of dry bulb air temperature against time in containers for Experiments 1 and 2 for the first time interval one hour after inoculation.

Table 3.3: Comparison of maximum measured temperatures (T) for the first exposure time period (1 h) for Experiments 1 and 2.

Temperature level (° C)	Maximum T for Experiment 1 (° C)	Maximum T for Experiment 2 (° C)
5	26.0	10.6
10	26.3	12.9
15	24.8	16.0
20	35.3	19.0

disease incidence varies between 0 % and 80 % for Experiment 1 and between 0 % and 62 % for Experiment 2. Here the maximum incidence of 80 % incidence occurred in Pan 3349 at the 10 °C temperature level where a temperature of 16.8 °C (not shown) was measured. The maximum of 62 % incidence was observed, also at the 10 °C temperature

level, in Pan 3349 where a temperature of 11 °C (not shown) was measured. The average incidence for all the temperature levels for both cultivars was 1 % for interval number 1 and 20 % for interval number 2 in Experiment 1 and in Experiment 2 the average incidence was 0 % and 14 % (Table 3.4 and Fig. 3.4).

Table 3.4 **Maximum and average incidence at the different temperature (T) levels for interval numbers 1 and 2 in Experiments 1 and 2 for Karee and Pan 3349.**

Experiment 1								
T level	Interval number 1(1 h)				Interval number 2 (3 h)			
	Maximum incidence		Average incidence		Maximum incidence		Average incidence	
	Karee	Pan3349	Karee	Pan3349	Karee	Pan3349	Karee	Pan3349
5	0	0	0	0	75	71	11	56
10	0	11	0	4	29	80	13	57
15	0	0	0	0	9	38	3	12
20	0	11	0	4	0	13	0	4
AVERAGE	**0**	**6**	**0**	**2**	**28**	**50**	7	**32**
Average incidence for interval number for Karee and Pan 3349				**1**				**20**
Experiment 2								
T level	Interval number 1				Interval number 2			
	Maximum incidence		Average incidence		Maximum incidence		Average incidence	
	Karee	Pan3349	Karee	Pan3349	Karee	Pan3349	Karee	Pan3349
5	0	0	0	0	0	17	0	6
10	0	0	0	0	31	62	18	22
15	0	0	0	0	46	42	25	32
20	0	0	0	0	7	16	2	5
AVERAGE	**0**	**0**	**0**	**0**	**21**	**34**	**11**	**16**
Average incidence for interval number for Karee and Pan 3349				**0**				**14**

Infection was above 0 % at 5 °C, 10 °C and 15 °C temperature levels for an exposure time period of three or more hours (interval numbers 2 –10) for both experiments (Figs. 3.5 and 3.6). For one hour and more, but less than three hours (interval number 1), two

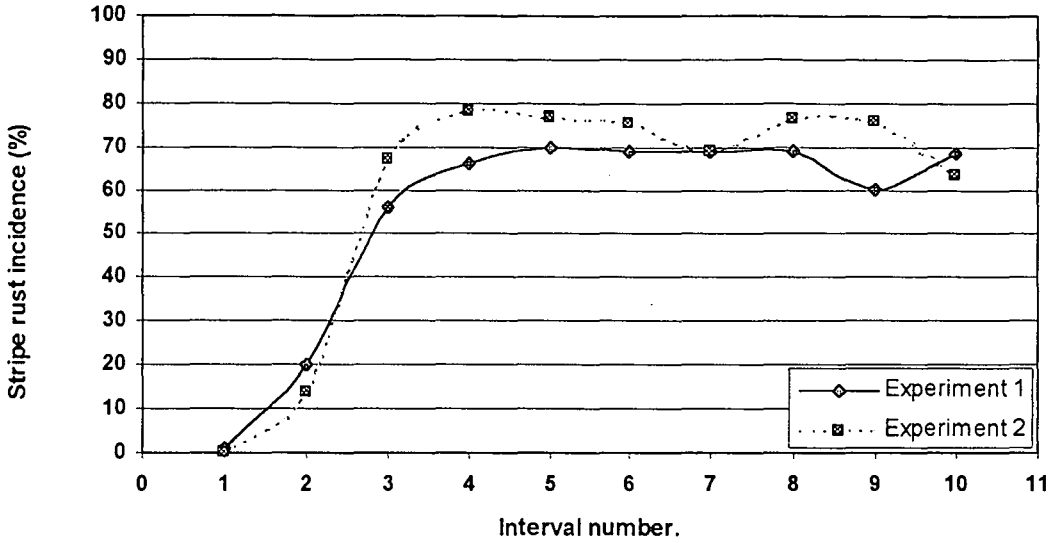


Fig. 3.4: Interval number versus average incidence (%) for all the temperature levels for Experiments 1 and 2 for Karee and Pan 3349.

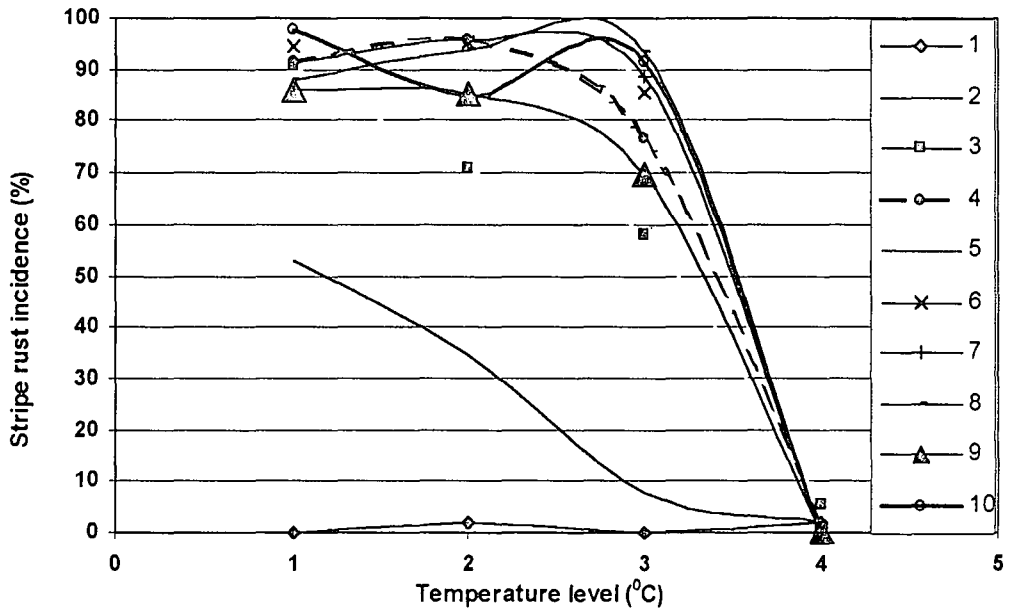


Fig. 3.5: Temperature level (1 - 4) for each interval number (1 - 10) versus incidence (%) for Experiment 1 for Karee and Pan 3349.

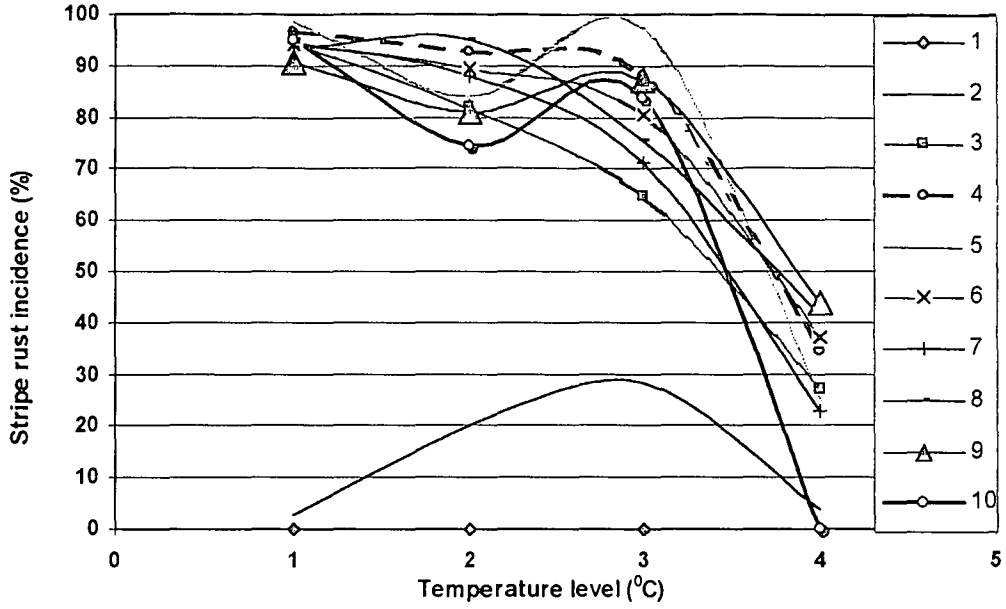


Fig. 3.6: Temperature level (1 - 4) for each interval number (1 - 10) versus incidence (%) for Experiment 2 for Karee and Pan 3349.

incidences of observation of 4 % each (average for three replications, of which the maximum incidence was 11 % - Table 3.4) at the 10 °C and 20 °C temperature levels for Pan 3349 for Experiment 1 was observed. The possible cause for observed infection was that during removal of cones at the 10 °C and 20 °C temperature levels, a drop in temperature was experienced by the plants, which were at 21.1 °C and 25.2 °C in the growth chambers (measured temperatures for Experiment 1 were higher than the four set temperature levels occurred, especially during the first interval). In the glasshouse temperatures varied between 12 °C and 17 °C.

For the 20 °C temperature level there are varying values of incidence observed in Experiments 1 and 2 (Table 3.5). Infection of 4 – 11 % occurred in Experiment 1 for interval numbers 1 – 3 in Pan 3349, but not in Karee. Temperatures noted here were greater than 20 °C for at least a few minutes within each interval. However, infection of 0 % – 4 % for interval numbers 4 – 5 occurred in both cultivars. In Experiment 1 no infection occurred for interval numbers 6 - 9 where temperatures below or equal to 20 °C prevailed, except in interval number 7 where temperatures were above 23 °C. These

temperatures (>23 °C) only prevailed for a period of 13 minutes. It was found (not shown) that temperatures above 20 °C for a period of an hour or longer inhibited infection and gave incidence of 0 %. In contrast, only interval number 1 of Experiment 2 showed no infection. During all the other intervals, where temperatures stayed below 20 °C, infection was observed. These observations prove that infection is not inhibited by short rises in

Table 3.5: Comparison of disease incidence (average % for each interval) for the 20 °C temperature level in Experiments 1 and 2. Temperature in °C for each interval number is the mean temperature occurring over the whole period (t = 0) for that interval. Ranges (in brackets) of temperatures (°C) indicate a range of temperatures above 20 °C for a few minutes within the interval.

Interval number	EXPERIMENT 1			EXPERIMENT 2		
	Karee (%)	Pan 3349 (%)	Temperature (°C)	Karee (%)	Pan 3349 (%)	Temperature (°C)
1	0	4	28.6 (25.3 - 35.3)	0	0	19.0
2	0	4	25.0 (21.8 - 35.3)	2	5	19.0
3	0	11	23.4 (20 - 35.3)	22	33	19.0
4	3	0	20.0	19	50	18.7
5	0	4	19.7	12	48	18.7
6	0	0	19.7	26	48	19.0
7	0	0	20.5 (23.5 – 35.3)	14	14	19.4
8	0	0	20.0	38	45	19.4
9	0	0	19.7	38	50	19.4

temperature above 20 °C, but by prolonged rises in temperature and also depends on other factors such as cultivar susceptibility, to be discussed in the next paragraph.

3.3.2 Cultivar and interval number versus disease incidence

A comparison was made between the two cultivars and the interval numbers for both experiments (Table 3.6). Standard deviation (STDEV) for cultivars for all intervals was the lowest at interval number 1 in both experiments (1). STDEV for cultivars for interval number 2 was the highest (9.06). STDEV between cultivars for both experiments was higher for Experiment 2 (29.93 and 35.31) and total STDEV between experiments was

low at 3.80. Table 3.6 shows that Pan 3349 exhibits higher incidence. This could be attributed to the broader leaves of Pan 3349.

Table 3.6 Comparison of average incidence (%) in Karee and Pan 3349 for all intervals and STDEV for cultivars, interval numbers and Experiments 1 and 2.

Experiment	Cultivar	Average incidence for intervals (%)			STDEV
		1	2	3 - 10	
1	Karee	0	17	62	
	Pan 3349	2	32	70	29.93
2	Karee	0	11	72	
	Pan 3349	0	16	76	35.31
STDEV		1	9.06	5.89	3.80

3.3.3 Cultivar and measured temperature versus disease incidence

First of all, the actual measured temperatures were used here to try and find the actual cut-off values for incidence. Although Pan 3349 is more resistant to stripe rust according to its infection type, incidence was in all cases mostly higher for this cultivar in this experiment. When incidence is compared for the various temperatures in Experiments 1 and 2 then it can be seen that there was a definite decrease in incidence as the temperature increased from 15 °C to 20 °C. (Fig. 3.7). The incidence remained constant at a high value (>60 %) for both cultivars at temperature levels below 20 °C. This gives a clear indication that temperatures of 15 °C and lower are conducive to infection and that there is a critical temperature somewhere between 15 °C and 20 °C which inhibits infection. According to Fig. 3.7, temperatures greater than 16 °C result in reduced incidence, and at a temperature of 22 °C incidence was 0 % for Karee in Experiment 1, but 2 % incidence in Pan 3349. In Experiment 2 incidence was 17 % for Karee and 30 % for Pan at a temperature of 19 °C. These results show significantly lower incidence at only slightly higher temperatures than those by Park (1990). Although Park (1990) does not clarify whether infection was incidence or severity, the results from his laboratory experiment differ from the results found in this project. He found that 50 % infection

occurred at temperatures between 18 °C and 18.4 °C and 0 % infection at temperatures between 20.6 °C and 21 °C. These temperatures differ from the results found in this project by 1 °C – 1.7 °C (no incidence at 22 °C and no severity at 22.3 °C).

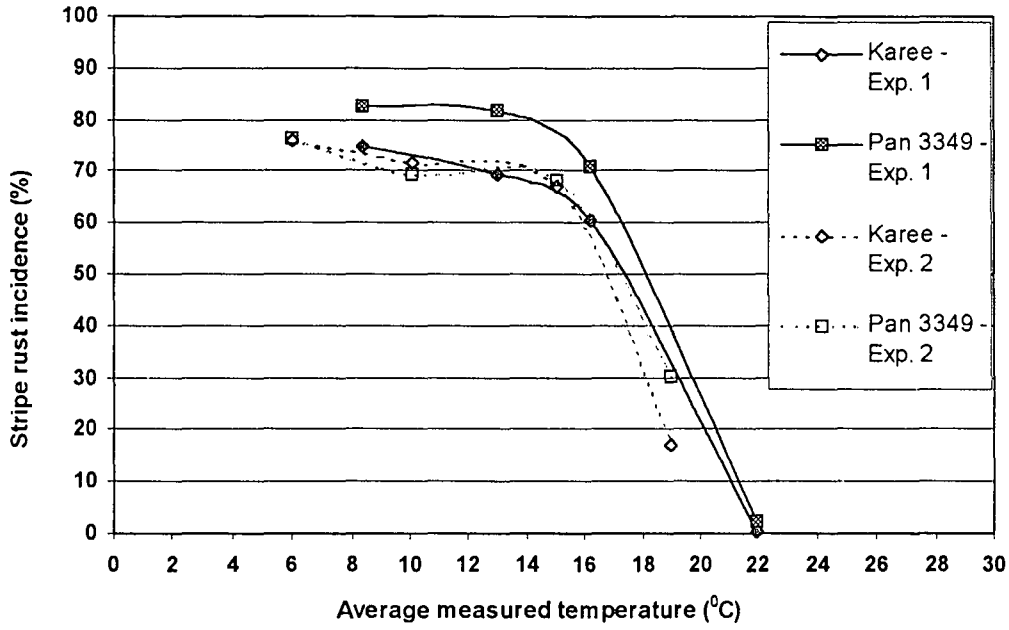


Fig. 3.7: Average measured temperature for all intervals for each temperature level (1 - 4) versus incidence (%) in Experiments 1 and 2 for Karee and Pan 3349.

He also refers to other authors such as Dennis (1987) whose results (maximum infection at 18 °C) differed from those acquired in his experiment. His explanation for this difference was that temperatures during urediniospore production differed and this could have affected the requirements for subsequent germination.

3.3.4 Temperature versus severity

In general, stripe rust severity was shown to be statistically higher in Experiment 1 than in Experiment 2 (Appendix 1 and 2 and Fig. 3.8). Severity was also higher in Karee than Pan 3349 (as opposed to incidence, where the opposite was true). According to Coakley

and Line (1981a), Coakley and Line (1981b) and Coakley *et al.* (1983) frequency (incidence) and severity of stripe rust on certain winter wheat cultivars was associated with daily above-average winter and below-average spring temperatures. The overall higher temperatures in Experiment 1 (especially in the first few hours of the exposure time period) (Fig. 3.3) could explain higher severity observed, but it is more probable that the presence of dew could be the cause. According to Fig. 3.8, severity of stripe rust declines as the temperature increases, so that infection is more severe at the lower temperatures.

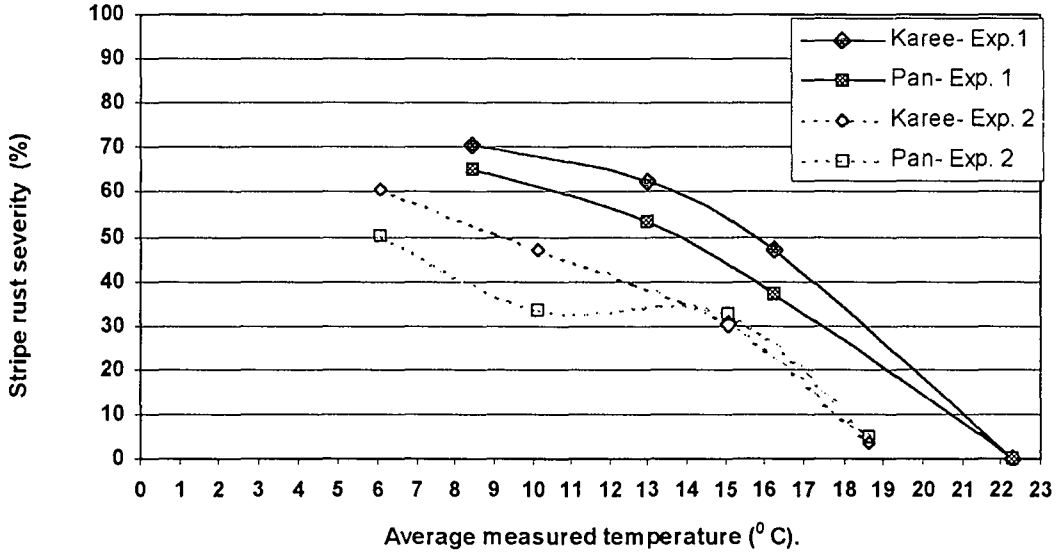


Fig. 3.8: Average measured temperature for all intervals for each temperature level (1 - 4) versus average severity (%) for Experiments 1 and 2 for Karee and Pan 3349.

To try and identify cut-off values for severity, the actual measured temperatures were used here as for incidence. For example, in Experiment 1 severity >60 % at 8.4 °C; > 50 % at 13 °C and > 30 % at 16 °C. In Experiment 2 where measured temperatures were lower, however, severity was also lower, being >50 % at 6 °C and > 30 % at 10.1 °C and at 15 °C. At the 20 °C temperature level, where an average temperature of 18.6 °C was

measured in Experiment 2, only 4 % severity was observed in Karee and 5 % in Pan 3349. In Experiment 1, however, a severity of 0.2 % was observed for both cultivars at a measured temperature of 22.3 °C.

3.3.5 Cultivar and intervals versus severity

A comparison was made between the two cultivars and over all the intervals for both experiments for severity. The severity of infection was low when only exposed to high humidity for a short exposure time period (1 –3 h) (Fig. 3.9). The severity then increased steadily to above 50 % until the inoculated plants were exposed to at least 12 h (interval number 4) of high humidity.

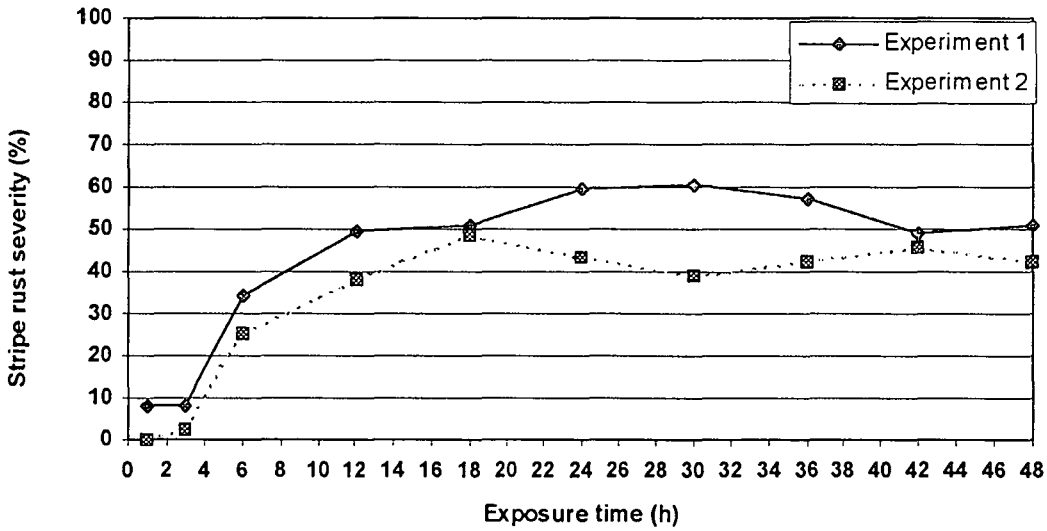


Fig. 3.9: Exposure time versus severity (%) for all temperatures for Experiments 1 and 2 with mean values for Karee and Pan 3349.

Exposure to high humidity for longer periods of time (>12 h) did not increase the overall average severity of the disease significantly and values remained between 40 % and 60 %.

A comparison was made between the two cultivars and the interval numbers for both experiments for severity (Table 3.7). Standard deviation (STDEV) for cultivars for interval numbers 2 and 3 - 10 was lower at interval number 2 (3.3). STDEV between cultivars for both experiments were similar for Experiments 1 and 2 (4.95 and 4.24) and total STDEV between experiments was low at 0.5. Table 3.7 shows that Karee exhibits higher severity than Pan 3349. This result is opposite to that found in the case of incidence.

Table 3.7 Comparison of average severity (%) in Karee and Pan 3349 for interval numbers and STDEV for cultivars, interval numbers and Experiments 1 and 2.

Experiment	Cultivar	Average incidence for interval numbers (%)			STDEV
		1	2	3 – 10	
1	Karee	0	9	55	4.95
	Pan 3349	0	7	48	
2	Karee	0	2	44	4.24
	Pan 3349	0	3	38	
STDEV		0	3.3	7.14	0.5

3.3.6 Comparison between Experiment 1 and Experiment 2

According to the statistical analysis, there is no significant differences between the disease incidence means for the two experiments, but severity showed significant difference, with Experiment 1 showing greater severity than Experiment 2. This could be attributed to the fact that there was probably more dew present during Experiment 1 in the absence of the fan. In Experiment 2 the fan promoted airflow and probably higher evaporation of free moisture from the plants.

3.3.7 Measurement of temperature and RH

When water continually evaporates into the air, a point is reached when the number of water molecules evaporating from the surface is equal to the number of molecules returning to the water, in the form of condensation. When this point is reached, i.e.

saturation, the relative humidity is 100 % and a state of equilibration is present. The equilibration times for T_a and T_w are shown in Figs. 3.10 and 3.11. Temperature measurements clearly indicate that equilibration times were faster during Experiment 2. In Experiment 1 it took approximately 18 h for the temperature to reach equilibration after setting up the system.

Therefore during Experiment 2 the containers were allowed to equilibrate at the set temperature level from the day prior to the start of the inoculation and experimental run. During Experiment 2 the equilibration time was reduced to four hours at temperatures of 15 °C and 20 °C and approximately 6 and 12 h for 10 °C and 5 °C respectively. The reason for the shorter equilibration times was because the containers were set up the previous day, and then only briefly opened four hours before the experimental run to check battery, etc. The addition of the fan in Experiment 2 most probably also contributed to shorter equilibration times, as the circulation of air would have promoted constant air flow within the containers, as well as evaporated any possible condensation on the temperature sensors.

3.3.8 Standard Deviation (STDEV) and Average Deviation (AVEDEV)

STDEV is the deviation of a value from the average and AVEDEV is the average of the STDEV. Both values were calculated for Experiments 1 and 2 so that the difference in results between the two experiments could be calculated. The *Excel* computer program was used and the values are shown in Table 3.8.

Table 3.8: STDEV and AVEDEV values for T_a and T_w for Experiment 1 (Exp. 1) and Experiment 2 (Exp. 2) for the whole time period (48 h) during each experiment.

	AVEDEV for T_a and T_w (Averages of STDEV)				STDEV for Ta			
	5 °C	10 °C	15 °C	20 °C	5 °C	10 °C	15 °C	20 °C
Exp. 1	0.33	0.16	0.09	0.20	1.99	1.98	1.13	1.65
Exp. 2	-	-	-	0.10	1.42	0.82	0.99	1.92

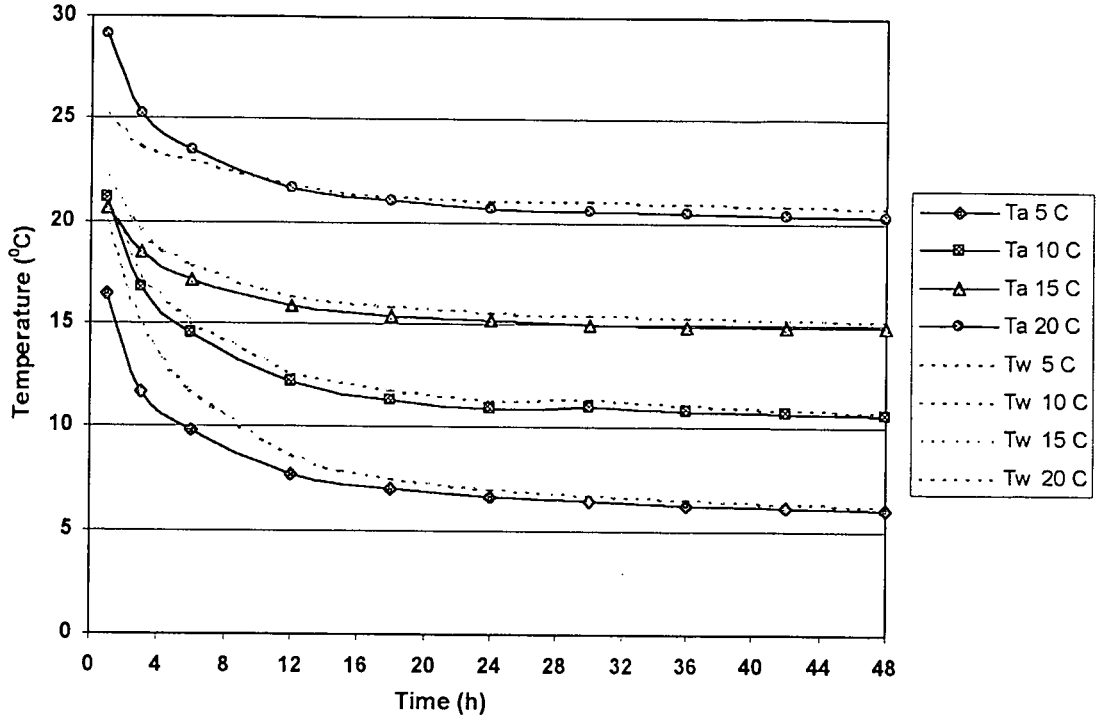


Fig. 3.10: Comparison of T_a and T_w (averages of 1 min readings of every hour) at the different temperature levels for 48 hours for Experiment 1.

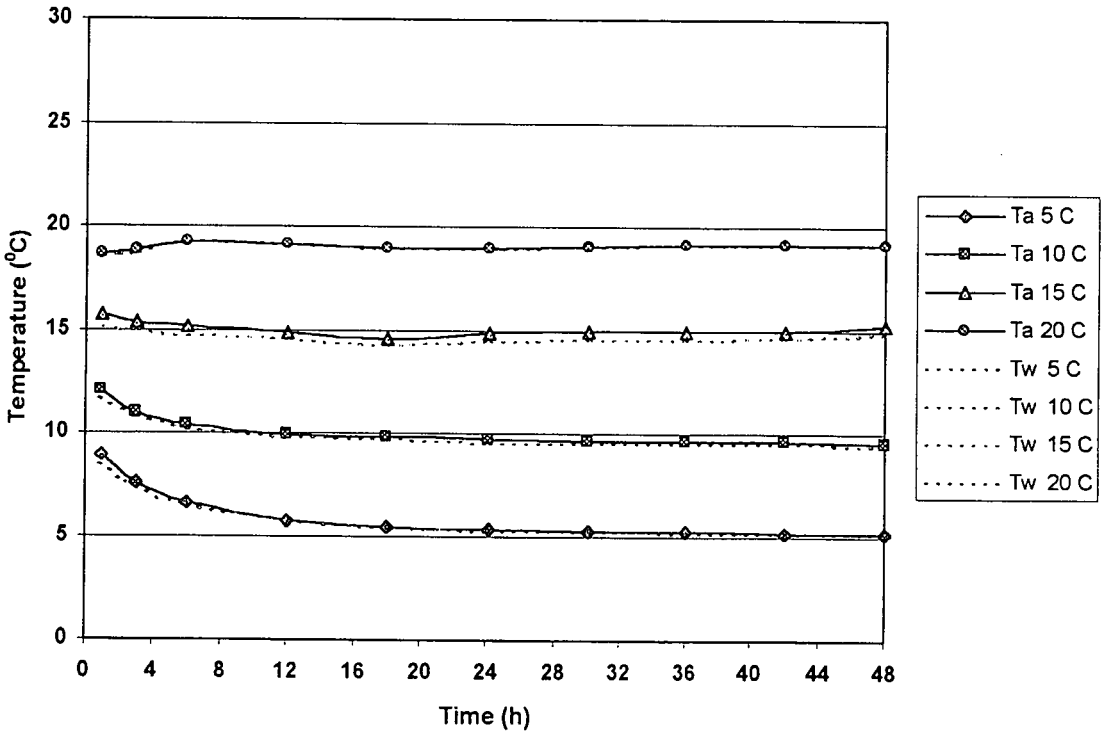


Fig. 3.11: Comparison of T_a and T_w (averages of 1 min readings of every hour) at the different temperature levels for 48 hours for Experiment 2.

The 5 °C, 10 °C and 15 °C temperature sensors (T_a and T_w) for Experiment 2 were disconnected for some time during the running of Experiment 2, resulting in a loss of data for the periods between 14:25 – 22:50 on 02-08-2001; 22:57 on 02-08-2001 to 4:56 on 03-08-2001, and between 14:25 and 16:59 on 02-08-2001. No temperatures were therefore measured during these time periods and the STDEV was calculated by omitting these time periods for the temperature levels concerned. The AVEDEV could not be calculated.

The T_a temperatures in Experiment 1 were constantly lower than the T_w temperatures indicating the presence of dew on the dry bulb sensors. This resulted in incorrect RH readings. The fan in Experiment 2 caused the air in the container to circulate and so evaporation of condensed air on the Ta sensors prevented the incorrect readings observed in Experiment 1.

3.3.9 Relative humidity (RH)

The main objective of the experiment was to observe stripe rust infection under conditions of high RH. In Experiment 1, RH was not able to be calculated as explained above, so the results for Experiment 2 will be discussed here. The values in Table 3.9 were taken from the detailed data set where temperatures were measured every minute by the Hobo temperature sensors. RH remained high and only decreased (not lower than 65 %) during interval numbers 6, 8 and 9 for the 5 °C and 20 °C temperature levels. For the most part it varied between 83 % – 100 % and always equilibrated within a few minutes. During Experiment 2 the plants in the chambers were always wet with dew, which is a good indication that RH remained at 100 % during most of the experimental period. RH for Experiment 1 could not be compared with Experiment 2, as mentioned previously.

3.3.10 Statistical Analysis

According to the analysis of variance (Appendix) stripe rust incidence for Experiments 1 and 2 was significantly ($P<0.01$) influenced by temperature, time and the interaction between temperature and time (Figs. 3.1, 3.2, 3.4, 3.5 , 3.6 and Table 3.6). Differences between the two experiments were that cultivar influenced incidence in Experiment 1, but

Table 3.9: Relative humidity (RH) values for Experiment 2 and equilibration periods for interval numbers 1 - 10.

Interval number	Temperature level (°C)	RH value (%) before equilibration	RH value (%) at equilibration	Equilibration time (minutes)
1	5	86	95	11
	10	90	91	13
	15	92	92	Equilibrated
	20	96	100	3
2	5	106	95	3
	10	100	100	Equilibrated
	15	104	96	96
	20	86	100	3
3	5	-	-	Tw disconnected
	10	100	100	Equilibrated
	15	-	-	Ta disconnected
	20	86	100	2
4	5	-	-	Tw disconnected
	10	86	100	9
	15	100	96	12
	20	83	100	4
5	5	94	100	1
	10	-	-	Tw disconnected
	15	96	100	6
	20	93	100	3
6	5	67	94	3
	10	95	95	Equilibrated
	15	100	96	7
	20	76	100	2
7	5	94	100	1
	10	100	100	Equilibrated
	15	100	96	2
	20	86	100	3
8	5	94	100	1
	10	90	100	1
	15	92	96	5
	20	65	100	3
9	5	94	94	Equilibrated
	10	90	100	3
	15	85	96	4
	20	70	100	4
10	5	106	100	5
	10	92	96	6
	15	92	96	6
	20	83	100	3

interaction between temperature and cultivar influenced incidence in Experiment 2. These results show that incidence is high in plants which, at the 5, 10, 15 and 20 °C temperature levels, were exposed to high RH for a period of 3 or more hours (Intervals 3 – 10). Pan 3349 exhibited higher incidence than Karee in Experiment 1 than Pan 3349 in Experiment 2 (Table 3.6), showing the influence of cultivar on incidence. In Experiment 2 interaction between temperature and cultivar was significant (Fig 3.7).

Stripe rust severity for Experiments 1 and 2 was significantly ($P<0.01$) influenced by temperature, cultivar, time and interaction between temperature and time (Figs. 3.8, 3.9 and Table 3.7). For Experiment 2, there was however, significant interaction between temperature and cultivar (Fig 3.8), and between cultivar and time (Table 3.7). These results show that severity followed the same pattern as observed in incidence, with the difference that severity was higher in Experiment 1 than in Experiment 2 (Fig. 3.9). Table 3.7 shows values of 4.95 and 4.24 relatively similar, indicating significance for interaction between cultivar and time.

3.4 Summary and Conclusions

During the course of the experiments and for a period of 7 days following, the plants were checked for symptoms of stripe rust, which are chlorotic flecking of the leaves or the appearance of yellow coloured urediniospores, also on the leaves. It was found that on day 7, the first symptoms had appeared and this is confirmed by observations made by Wiese (1987). As infection could develop up to 14 days after inoculation, disease readings were done once only, on day 14 after inoculation. In this summary, infection refers to incidence plus severity.

3.4.1 Similarities for Experiments 1 and 2 are summarized as follows:

- No or reduced infection occurred at the 20 °C level, which confirms the necessity of low temperature for infection of stripe rust on wheat.

- No or reduced infection occurred for interval number 1 (one hour), which shows that the inoculated plants must be exposed to moisture for longer than one hour for infection to take place. The presence of moisture is essential as stated by various authors at the beginning of this chapter, therefore plants would not be infected if exposed to the same temperature in a dry atmosphere.
- Infection was greater than 0 % for 5 °C, 10 °C and 15 °C for interval numbers from 2 to 10 showing that infection is highly likely at these temperatures if the RH conditions are maintained high for three or more hours. Exceptions to the above occurred if there was a sudden drop in temperature on removal of cones from the constant conditions of the growth chambers to the glasshouse (12 °C – 17 °C).
- For both incidence and severity, it was found that temperatures greater than 20 °C inhibited or prevented infection. Figs. 3.7 and 3.8 show that at a temperature of 22 °C (Experiment 1) infection was as low as 0 to 2 %
- According to the statistical analysis, incidence showed no significance for Experiments 1 and 2, but severity showed statistical significance. This could have resulted from the presence of the fan in Experiment 2, evaporating some moisture from the leaves. Amount of moisture could therefore have influenced severity of infection.

3.4.2 Differences for Experiments 1 and 2 are summarized as follows:

- Measured temperature in Experiment 2 was overall lower and closer to the set temperatures because of prior equilibration and continual circulation of air. This would be an important factor to consider in future experiments.
- Incidence was observed for 20 °C temperature level for interval numbers 2 – 9 in Experiment 2. Here average measured temperatures were below 20 °C. In Experiment 1, however, average measured temperatures were at 22 °C and higher incidence was observed. This confirms that temperatures constantly below 20 °C are more likely to result in infection than temperatures higher than 20 °C.

The general conclusion is that incidence was influenced by temperature, length of exposure to free water, cultivar and various interactions between them. Temperatures of

between 5 °C and 15 °C, in the presence of high RH (free water) for a period of three or more hours, are favourable for infection of wheat by *P. striiformis* f. sp *tritici*. Infection is reduced or absent at temperatures higher than 20 °C, or when exposed for a period of one hour or less.

The cut-off temperature for incidence observed from the results of Experiments 1 is 18 - 22 °C, in the case of Karee. For Pan 3349 it must be slightly higher, as 2 % infection was found at a temperature of 22 °C. For severity, a temperature of 22.3 °C still resulted in infection of 0.2 %, so the conclusion is made with relative certainty that temperatures greater than 22.3 °C, will prevent infection by stripe rust.

It is well known that conditions in the laboratory are not the same as in the field and that possibilities for infections at higher temperatures in the field have been reported (Park, 1990), but it is also known that while the T_a measurements in a standard weather station are higher than the actual T_a measurement on the leaves (in the crop), then the results from the laboratory experiment could be extrapolated to conditions in the field.

CHAPTER 4

DEVELOPMENT OF AN INDEX FOR STRIPE RUST INFECTION

4.1 Introduction

Environmental disease indices are tools that can use current weather data to assist in giving an early warning of conditions that would be conducive for infection by a certain disease. To develop such an index, information about the ideal weather conditions for disease infection, together with historic field data of actual infection observations, are required. From a theoretical perspective it is known that cool and wet weather conditions are favourable for the infection of *Triticum aestivum* by the fungus *Puccinia striiformis* f. sp. *tritici* (Dennis, 1987; Wiese, 1987; Park, 1990; Ellison and Murray, 1992; Roelfs *et al.*, 1992; Boshoff *et al.*, 1998 and Boshoff and Pretorius, 1999). Weather factors to consider therefore would be temperature and the presence of moisture, in the form of rainfall, dew or mist. The terminology that will be used in this dissertation to represent a day on which either rain, dew or mist occurs is a "wet day." Acquisition of disease occurrence observations and weather data was necessary so that a relationship between weather parameters and disease presence could be developed. As information on the critical temperatures was non-existent, it was decided to use the results of the laboratory experiment (Chapter 3) as a basis for the development of an index for stripe rust infection.

It was found that under controlled laboratory conditions of high humidity and constant low temperature (5 °C – 15 °C), infection was high, while constant temperatures of 20 °C and above, caused a lower incidence or absence of the disease. Incidence is the number of infected plants as a percentage of the total number of plants and will be referred to as "incidence". Exposure to free water for less than three hours resulted in an absence of disease incidence, therefore a period of three or more hours was found necessary for infection to take place.

The field data was used to validate the index developed from the experiment, and because a wet day is necessary for infection, dew days were used if rain was absent. If dew point (T_{dp}) was greater than or equal to the minimum temperature T_{mn} then it was taken as a day on which dew could have formed, and called a dew day. The dew point temperature was calculated using the following formulae (Preston-Whyte and Tyson, 1988):

$$T_{dp} = -273.16 + \left[\frac{273.16 - \{2.076 * \ln(e_a / 0.6108)\}}{1 - \{0.0579 * \ln(e_a / 0.6108)\}}\right]$$

$$RH = \frac{e_a}{e_s} x100$$

$$\text{Therefore } e_a = \frac{e_s}{100} xRH$$

Where e_a is the average e_a during a 24 h period

$$e_a = [½(e_a(T_{mn}) + ea(T_{mx})]$$

When assuming a diurnal curve where RH_{mn} and T_{mx} occur at midday and T_{mn} and RH_{mx} in the early morning, then

$$e_a = \frac{e_s(T_{mn}) * RH_{mx}/100 + e_s(T_{mx}) * RH_{mn}/100}{2}$$

where $e_s(T_{mn})$ is saturated vapour pressure at minimum temperature T_{mn} and

$$e_s(T_{mn}) = 0.6108 * EXP\left[\frac{(17.2694 * T_{mn})}{(237.3 + T_{mn})}\right]$$

where $e_s(T_{mx})$ is saturated vapour pressure at maximum temperature T_{mx}

$$e_s(T_{mx}) = 0.6108 * EXP\left[\frac{(17.2694 * T_{mx})}{(237.3 + T_{mx})}\right]$$

If no dew was present, then mist days were also considered to provide sufficient moisture for infection. A mist day is determined by using daily $RH_{mx} \geq 95$ % (personal communication Jana Olivier, *UNISA, Pretoria, R.S.A.*, 2001). A wet day is classified as a

day when one of the following three conditions occurs: either it rains or the $T_{mn} \leq T_{dp}$ or the $RH_{mx} \geq 95$ %.

Two indices were developed. TDD_{14} was developed using total degree days for a period of 14 days as the incidence values for the experiment were only recorded on day 14. These incidence values were related to TDD_{14}. TDD_{14} cannot be useful to the producer as an alert or early warning, as he could observe the infection himself after 14 days. To make the index useful one must be able to warn the producer before the 14 d period has elapsed. Therefore TDD_7 was calculated for seven days and related to incidence, resulting in TDD_7. The use of TDD_7 could result in timely action (such as chemical control measures) being taken, as the disease is extremely infectious and spreads quickly once it has been established, especially in the presence of moisture. Development of TDD_{14} will be discussed first.

4.2 Criteria and requirements

According to Bourke (1970), the relationship between the weather and the development of the disease or the life-cycle of the pathogen must be the core of the working model. For the purpose of index development therefore, it was necessary to use historical weather data and disease observation data to find the combination of weather factors necessary for the initiation of the disease. The index would then be validated on historical data (from a different data set) and could then be applied to the medium term weather forecast to validate or test it further.

4.3 Assumptions

Complicated interactions between many variables seem to influence disease incidence, but not all were addressed in this project, so the following assumptions were made so that some other variables can be checked.

4.3.1 Inoculum is assumed to always be present in the atmosphere in sufficient amounts for disease infection to occur.

4.3.2 Standard weather station data (daily) and disease data (weekly) used were assumed to have been accurately recorded.

4.3.3 The observation of the disease in the field must have resulted from ideal conditions for infection some 7 to 14 days previously. Therefore, the first possible wet day during that time period (7 – 14 d) was taken to be the starting date for the index calculation.

4.4 Method and Materials

The following data was available for index development:

4.4.1 Disease observation data consisting of field data and expressed as incidence (%) for the years 1996 – 2000. Observations were made on a weekly basis and included information on growth stage and whether diseased crops were from commercialized or experimental plots (*SGI-ARC, Bethlehem, R.S.A.*; 1999).

4.4.2 Corresponding daily weather data was supplied from various automatic weather stations around the country representing each wheat growing region, for the years 1996 – 2000 and consisting of daily maximum temperature (T_{mx}), minimum temperature (T_{mn}), maximum relative humidity (RH_{mx}), minimum relative humidity (RH_{mn}), rainfall, average temperature (T_{av}), wind speed and sunshine hours (*Institute for Soil, Climate and Water, ARC, Pretoria, R.S.A.*, 2000).

4.4.3 Experimental data from the laboratory experiment was divided into two groups:

- Disease data expressed as incidence.
- Temperature and RH measured throughout laboratory experiment for plants in the growth chambers and the glasshouse.

4.4.4 Data analysis

In order to find the most probable combination of weather variables for disease initiation, seven stations were chosen from the main wheat growing areas in which high infection (50 % – 100 %) occurred for the years 1996 to 1998 (*SGI- ARC, Bethlehem, R.S.A.*, 2000). The data set was split so that the years 1999 – 2000 could be used to validate the index. The months July to November were used as disease infection was usually observed during this time period. The seven stations were taken from the five main wheat growing areas across South Africa, namely, Rûens-Western Cape, Swartland-Western Cape, Northern Cape, Free State and KwaZulu-Natal as listed in Table 4.1.

The *Excel* computer program (Microsoft) was used to manipulate the weather data from the seven stations. The relationship between the different weather variables (monthly and

Table 4.1: Details of the seven weather stations chosen from the five main wheat growing areas across South Africa with data period indicating years in which infection was observed. (Latitude = Lat., Longitude = Long. and Altitude = Alt., meters above sea level = Masl).

Region	Weather station	Data period (for infection observation)	Lat. (° 'S)	Long. (° 'E)	Alt. (masl)
Rûens - W. Cape	Tygerhoek	1996 - 1998	34 08	19 54	168
		1998 - 2000	34 13	19 90	168
	Swellendam	1997 - 1999	34 02	20 27	125
Swartland - W. Cape	Moorreesburg	1996,1998 - 2000	33 09	18 41	158
	Augsburg	1996	32 10	18 54	9
Northern Cape	Rietrivier	1996 - 1997	29 04	24 37	1140
		1997 - 2000	29 07	24 62	1140
Free State	Bethlehem	1997 - 2000	28 10	28 18	1631
KwaZulu-Natal	Winterton	1997 - 1998	28 52	29 31	1067

daily) was investigated to find the most significant combinations for disease incidence. The results from Experiment 2 were used on the grounds of greater constancy of RH and temperature measurements compared to those found in Experiment 1. They were used as a basis from which to develop an index. Combinations of total degree days (TDD),

accumulated DD (defined below), accumulated $T_{mx} - T_{mn}$, accumulated T_{mx} and accumulated T_{mn} were calculated and the relationship between them and disease incidence was investigated. TDD was calculated using the following equation for degree days, DD:

$$DD = [(T_{mx} + T_{mn})/ 2 - T_b] \Delta t \qquad \text{(McMaster and Wilhelm, 1997)}$$

In the equation Δt denotes time period between measurements measured in hours or days. The sum of DD for each interval number for the first two days of the experiment gave a total degree hours (TDH). The sum of DD for each day over a period of 14 days after a wet day gave total degree days (TDD_{14}). T_b is the base temperature (taken as 0 °C) for initiation of spore germination (Wiese, 1987; Roelfs *et al.*, 1992). TDD_{14} is used throughout to refer to the total DD for a period of 14 days and in the case of the experiment, TDH is the total DD for a period of 48 h.

Firstly, TDH for each interval (Table 4.2) and each temperature level was calculated and plotted against average incidence of three replications taken at the end of the 14 day period of the experiment. For the first two days of the experiment TDH for each interval number was calculated by using the T_{mx} and T_{mn} experienced by the plants in the cold room or growth chamber and glasshouse. The T_{mx} and T_{mn} depended on where the plants were and the time of day during the experiment. The average temperature minus the base temperature $[(T_{mx} + T_{mn}) \div 2 - T_b]$ for each interval number was then multiplied by Δt, the time difference elapsed during that exposure time period (Table 4.2).

The sum of TDH for the first two days of the experiment, divided by 24 h gave the TDD for days 1 and 2, i.e. Σ °Ch $\div$ 24 hd^{-1} = °Cd. Secondly, TDD for days 3 – 14 were calculated using the glasshouse T_{mx} and T_{mn}. Incidence was only observed once on day 14 of the experiment. Average incidence was calculated for each interval for each temperature setting from disease readings taken on day 14 of the experiment.

Thirdly, the *Curve Expert 1.3* (Hyams, 1995) program was used to try and find the best

Table 4.2 Calculation of Δt for each interval number for the 48 h run of Experiment 2.

Interval number	1	2	3	4	5	6	7	8	9	10
Exposure time period (h)	1	3	6	12	18	24	30	36	42	48
Δt (h)	1	2	3	6	6	6	6	6	6	6

curves for the laboratory experimental data by plotting the various weather variables against incidence.

4.4.5 Statistical Analysis

Multiple regression using the program *Essential Regression and Experimental Design* (Steppan, Werner and Yeater, 1998) was used after data collection and formed part of the data analysis. Various complicated interactions between weather variables were done using the 1996 – 1998 data.

4.4.6 The cut-off value from TDD_{14} calculated from the experimental data was used to validate TDD_{14} using the weather station data. TDD_{14} was calculated 14 days before observed infection in the following way:

- A wet day was identified.
- DD was calculated and summed for 14 days from the wet day to give TDD_{14}.
- TDD_{14} calculated was compared with the cut-off value to give a prediction of less than 67 % incidence or greater than 67 % incidence for low or high risk of infection.
- This was then compared to the actual observed disease incidence for corresponding periods during 1996 – 1998 and 1999 – 2000.

4.4.7 TDD_{14} was not useful as an early warning for the producer, as mentioned earlier, so TDD_7 for 7 days was calculated.

4.5 Results and Discussion

4.5.1 Use of the "linear and interaction" option for multiple regressions (*Essential Regression and Experimental Design*), and relating field disease data (1996 – 1998) to various weather variables, resulted in a model consisting of eight terms:

- RH_{mx}
- RH_{mn}
- Rainfall
- RH_{mx}*Rainfall
- RH_{mn}*Rainfall
- TDD
- RH_{mx}*TDD
- RH_{mn}*TDD

The correlation for all the terms was poor ($r^2 = 0.69$), and as it was decided that a simple index was the aim of the project, other avenues needed to be explored.

4.5.2 Using the *Excel* program, it was found that T_{mn}, RH_{mn} and rainfall gave the best relationships with disease incidence. Several combinations of these and other weather variables were related to field incidence, but no significant correlation could be found.

4.5.3 Use of the laboratory experiment data

TDH plotted against stripe rust incidence for the first 48 h run of Experiment 2 (Fig. 4.1, Tables 4.3 and 4.4) show the results from the experiment for interval numbers 1 – 10. Each interval number represents TDH calculated for the 48 h run of the experiment. A definite increase in stripe rust incidence occurred at the 5 °C, 10 °C and 15 °C temperature levels for an increase in TDH. At a TDH of between 70 – 90, the incidence remained at constant values, but increased from 90 to about 150 (180 for the 20 °C setting), resulting in a variety of values in incidence. Incidence was low for the 20 °C temperature level and did not rise above 50 % incidence. This increase in incidence points to favourable conditions at the 5 °C, 10 °C and 15 °C temperature levels. The glasshouse temperature varied between 12 °C and 17 °C and was as a result often below

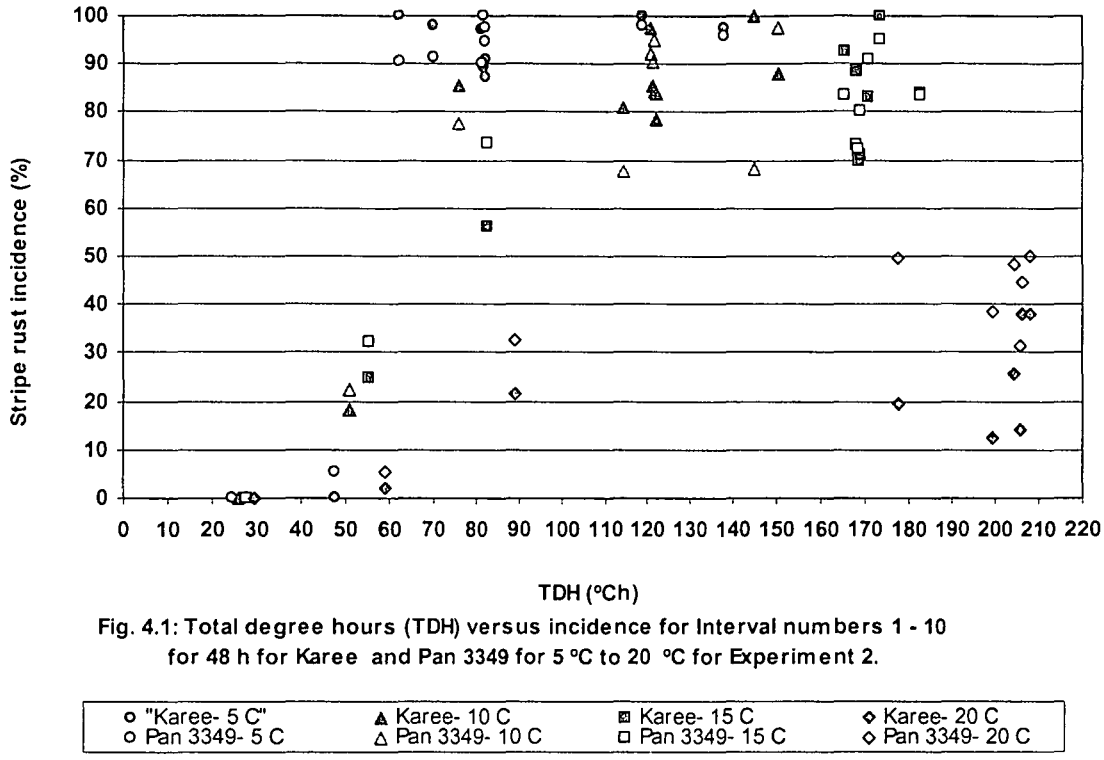


Fig. 4.1: Total degree hours (TDH) versus incidence for Interval numbers 1 - 10 for 48 h for Karee and Pan 3349 for 5 °C to 20 °C for Experiment 2.

Table 4.3 **Correlation values for linear equations for Karee and Pan 3349 for TDH, and TDD_{14} related to average stripe rust incidence for the 48 h run of Experiment 2.**

Temperature level (°C)	r^2 for Karee		r^2 for Pan 3349	
	TDH	TDD_{14}	TDH	TDD_{14}
5	0.5	0.73	0.53	0.75
10	0.79		0.75	
15	0.88		0.79	
20	0.5		0.78	
AVERAGE	**0.67**	**0.73**	**0.71**	**0.75**

15 °C. This caused many of the TDH points to cluster on the graph between TDH 165 and 208. Longer exposure times also favoured incidence, but then incidence was constant from a certain point, i.e. TDH greater 70 for the 5 °C and 10 °C temperature levels. Table 4.3 gives the correlation values for TDH and TDD_{14} relating to incidence for Experiment 2 and it can be seen that the lowest r^2 value is 0.5 for TDH at the 5 °C and 20 °C levels.

The values for TDD_{14}, however were higher than those for TDH and so it was logical to use TDD_{14} for further calculations. TDD_{1+2} is the sum of TDH for all the intervals (Table 4.4).

Table 4.4: Total degree hours for interval numbers 1 - 10 (TDH), total degree days for days 1 and 2 (TDD_{1+2}) and average stripe rust incidence for Karee and Pan 3349 at all the temperature levels for the 48 h run of Experiment 2.

Interval number	TDH at 5 °C	Incidence Karee (%)	Incidence Pan 3349 (%)	TDH at 10 °C	Incidence Karee (%)	Incidence Pan 3349 (%)
1	24.59	0.00	0.00	26.11	0.00	0.00
2	47.78	0.00	5.56	51.17	18.13	22.18
3	70.16	97.78	91.39	75.95	85.71	77.59
4	137.82	97.44	95.96	150.42	87.83	97.44
5	118.86	100.00	97.92	144.67	100.00	68.33
6	82.26	91.11	97.62	121.58	84.36	95.00
7	81.89	89.28	100.00	121.15	85.42	90.61
8	81.32	96.97	90.00	120.83	97.44	92.31
9	82.61	87.18	94.44	122.10	78.57	83.69
10	62.28	100.00	90.30	114.36	80.91	67.78
TDD_{1+2} for Day 1 and 2	**32.90**	-	-	**43.68**	-	-
Interval number	**TDH at 15 ° C**	**Incidence Karee (%)**	**Incidence Pan 3349 (%)**	**TDH at 20 ° C**	**Incidence Karee (%)**	**Incidence Pan 3349 (%)**
1	27.94	0.00	0.00	29.41	0.00	0.00
2	55.54	24.91	32.04	59.00	2.22	5.33
3	82.96	56.03	73.41	89.07	21.49	32.79
4	165.18	92.44	83.44	177.86	19.44	49.47
5	173.67	100.00	95.05	199.72	12.41	38.33
6	168.02	88.33	73.06	204.58	25.64	48.43
7	168.63	69.84	72.47	205.88	13.89	31.60
8	169.11	71.19	80.28	206.43	37.95	44.81
9	170.94	83.12	90.94	208.25	37.91	49.91
10	182.43	83.77	83.33	229.59	-	-
TDD_{1+2} for Day 1 and 2	**56.85**	-	-	**67.07**	-	-

4.5.4 Daily TDD_{14} showed a different pattern to that observed for TDH (Fig. 4.2 and Table 4.5). A decrease in stripe rust incidence was observed for an increase in TDD_{14}, and according to Figure 4.2, the cut-off TDD_{14} is at 227.

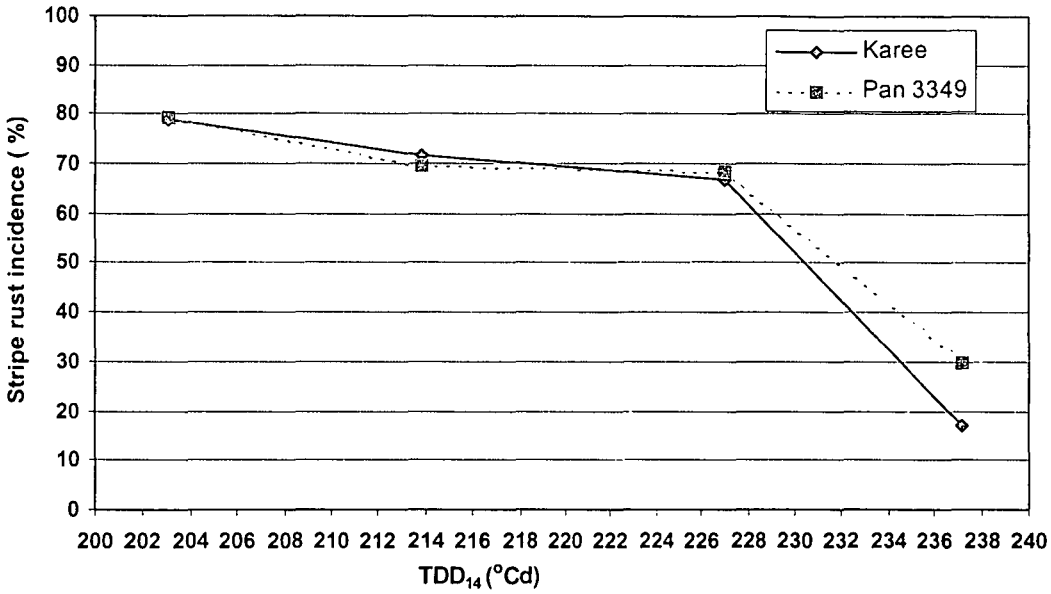


Fig. 4.2: Total degree days (TDD_{14}) for Days 1 - 14 versus average incidence observed in Experiment 2 for all the temperature levels for Karee and Pan 3349.

Table 4.5 Total degree days (TDD_{1+2}) for days 1 and 2, total degree days (TDD_{14}) for days 1 – 14 and average stripe rust incidence for Karee and Pan 3349 at the four temperature levels in Experiment 2.

Temperature level (° C)	TDD_{1+2} for days 1 and 2	TDD_{14} for days 1 - 14	Ave incidence Karee (%)	Ave incidence Pan 3349 (%)
5	33	203	79	79
10	44	214	72	70
15	57	227	67	68
20	67	237	17	30

4.5.5 The weather data measured in the laboratory experiment, where conditions were kept constant, could be very different from the field data, in that plants in the field are exposed to other influences, for example, wind, radiation, etc. To predict a range of incidence values, rather than a specific incidence would therefore be more useful, for example a value between 50 % and 100 % could indicate high risk and less than 50 % a low risk of infection. The decision was therefore made to use a range of TDD values related to a range of incidence values, rather than specific values.

Fig. 4.2 indicates the cut-off values for stripe rust incidence from Experiment 2. The cut-off value is a value at which the direction of relationship between two variables changes and in Fig. 4.2 this value is at a TDD_{14} of 227. TDD_{14} critical = 227, when TDD_{14} is calculated from a wet day for 14 days. TDD_{14} is calculated by summing the DD for the 14 days. If TDD > 227, then there will be a low risk (< 67 %) of disease infection as this high TDD_{14} value can only be achieved if reasonably high temperatures are experienced to accumulate to a value above the critical TDD_{14}. Likewise the $TDD_{14} < 227$ can only be achieved under conditions of low temperatures which is in accordance with the temperature requirements for disease incidence stated at the beginning of the project.

Table 4.6 shows the results from six regression lines fitted for TDD_{14} related to stripe rust incidence for Karee and Pan 3349 (Fig. 4.2) at two different temperature level ranges (5 °C – 10 °C and 15 °C – 20 °C). The results confirm an increase in stripe rust incidence between temperatures 5 °C and 15 °C ($r^2 = 0.91$) and a decrease in stripe rust incidence for temperatures between 15 °C and 20 °C ($r^2 = 0.96$). When the equations are solved using $TDD_{14} = 227$, the values for y are 66.56, 67, 66.79 and 68 for Karee 5 °C – 15 °C, Karee 15 °C – 20 °C, Pan 3349 5 °C – 15 °C and Pan 3349 15 °C – 20 °C. When only two regression lines are drawn, one for the 5 °C – 15 °C temperature level (combining Karee and Pan 3349) and one for the 15 °C – 20 °C temperature level, (also combining the two cultivars), then the y values are 66.66 and 67.5. The average of these last two values is 67.08. This proves that the cut-off value for $TDD_{14} = 227$ is valid and that the cut-off value for incidence is 67 %.

Table 4.6 **Equations from regression lines drawn for temperature level ranges of 5 °C – 15 °C and 15 °C – 20 °C, for TDD_{14} related to stripe rust incidence for Experiment 2 for Karee and Pan 3349. Y-values for the equations K + P solved for X = 199 are given.**

Cultivar	5 °C – 15 °C	r^2	15 °C – 20 °C	r^2
Karee	y = -0.4965x + 179.26	0.98	y = -5x + 1202	1
Pan 3349	y = -0.4492x + 168.76	0.85	y = -3.8x + 930.6	1
Karee + Pan 3349	y = -0.4729x + 174.01	0.91	y = -4.4x + 1066.3	0.96
Y-values for Karee +Pan 3349	66.66	-	67.5	-

4.6 Validation of TDD_{14}

4.6.1 Only wet days related to infection (Table 4.7).

TDD_{14} was calculated for observation dates during 1996 – 1998 and gave 9 out of 18 correct predictions (50 %) for highest incidence related to TDD_{14}. Highest incidence was the maximum incidence observed out of all cultivars present. Average incidence was the mean incidence observed for all the cultivars for a specific date. For average incidence observed and related to TDD_{14} prediction, 4 out of 18 correct predictions (29 %) were found. On further validation, using the 1999 – 2000 data (Table 4.8), 6 out of 15 correct predictions (40 %) for highest incidence observed and 5 out of 15 correct predictions (33%) for average field incidence were observed. The lowest incidence thus obtained was 29 % and the highest incidence was 50 %.

For several of the infection observation dates at the seven chosen stations irrigation (as confirmed by Coakley *et al.*, 1982) caused infection when there was no record of wet day present therefore they were omitted from the table. The first example is Rietrivier where infection was observed on 01-10-99 and 26-10-2000. The second example was Bethlehem (05-10-2000) where infection was observed which could have been caused by irrigation, but unfortunately these irrigation dates were not readily available. There was rain and possibly mist only three days prior to infection being observed. Theoretically, infection was initiated, although it can only be observed from seven days after infection initiation (Wiese, 1987) and this was also confirmed in Experiment 2, but data is not

Table 4.7: **Predicted TDD_{14} for highest and average field incidence (%) with TDD_{14} summed from 14 days before incidence observation (1996 – 1998). The seven stations used were M = Moorreesburg, R = Rietrivier, W = Winterton, A = Augsburg, B = Bethlehem, S = Swellendam and T = Tygerhoek.** Predictions are marked by high (H) and low (L), meaning high and low risk of incidence. Observed incidence > 67 % = high risk and < 67 % = low risk. Hits are correct predictions.

	Station Symbol	Calculated TDD_{14} for 14 days before observation	TDD_{14} prediction for field incidence	Highest observed field incidence (%)	Hit or miss predicted for highest observed	Average observed field incidence (%)	Hit or miss predicted for average observed
1	M	190	**H**	100	hit	39	½ hit
2	M	196	**H**	60	½ hit	60	½ hit
3	M	233	**L**	80	hit	50	hit
4	R	280	**L**	80	½ hit	80	½ hit
5	R	300	**L**	20	hit	20	hit
6	W	279	**L**	100	½ hit	100	½ hit
7	W	283	**L**	5	hit	5	hit
8	A	186	**H**	100	hit	43	½ hit
9	B	182	**H**	10	½ hit	10	½ hit
10	B	228	**L**	80	½ hit	80	½ hit
11	B	247	**L**	100	½ hit	70	½ hit
12	B	213	**H**	40	½ hit	25	½ hit
13	S	224	**H**	100	hit	65	½ hit
14	S	200	**H**	60	½ hit	55	½ hit
15	T	181	**H**	80	hit	80	hit
16	T	185	**H**	100	hit	65	½ hit
17	T	166	**H**	10	½ hit	10	½ hit
18	T	192	**H**	100	hit	53	½ hit
Total number of possible hits					18		18
Number of hits					9		4
Number of ½ hits					9		14
Number of misses					0		0
% of correct predictions					**50**		**29**

shown here. The third example is Bethlehem on 06-11-2000 where there was no rain, dew or mist and the possibility exists that irrigation could have been applied, as infection was recorded.

Table 4.8: **Predicted TDD_{14} for highest and average field incidence (%) with TDD summed from 14 days before incidence observation (1999 – 2000). The seven stations used were M = Moorreesburg, R = Rietrivier, W = Winterton, B = Bethlehem, S = Swellendam and T = Tygerhoek.** Predictions are marked by high (H) and low (L), meaning high and low risk of incidence. Observed incidence > 67 % = high risk and < 67 % = low risk. Hits are correct predictions.

	Station Symbol	Calculated TDD_{14} for 14 days before observation	TDD_{14} Prediction for field incidence	Highest observed field incidence (%)	Hit or miss predicted for highest observed	Average observed field incidence (%)	Hit or miss predicted for average observed
1	M	179	**H**	30	½ hit	30	½ hit
2	M	191	**H**	20	½ hit	20	½ hit
3	R	234	**L**	100	½ hit	70	½ hit
4	R	310	**L**	10	hit	10	hit
5	W	206	**H**	5	½ hit	5	hit
6	W	204	**H**	40	½ hit	40	½ hit
7	B	106	**H**	60	½ hit	60	½ hit
8	B	226	**H**	30	½ hit	18	½ hit
9	B	223	**H**	20	½ hit	12	½ hit
10	B	240	**H**	100	hit	53	½ hit
11	B	221	**H**	80	hit	55	½ hit
12	B	284	**L**	50	hit	50	hit
13	T	175	**H**	100	hit	100	hit
14	T	168	**H**	40	½ hit	40	½ hit
15	T	187	**H**	100	hit	100	hit
Total number of possible hits					15		15
Number of hits					6		5
Number of ½ hits					9		10
Number of misses					0		0
% of correct predictions					**40**		**33**

4.6.2 A few interesting observations can be made and possible explanations for non infection under favourable conditions of rain and low TDD_{14} and are as follows:

- Planting dates only start on 1 June (except southern Western Cape where planting starts from 15 April to 31 May), therefore infection not possible before emergence or the seedling stage (Ellison and Murray, 1992), which is 0 - 15 days after planting. Even though there were infection observations at Tygerhoek in July and August (1997) it was

decided to use the months September, October and November, as infection was mostly observed in these months at all the stations used in this project.

- Weather conditions in the field must be favourable for a period of longer than one hour for infection to take place, if results of the experiment are taken into consideration. As mentioned before, however, conditions in the field differ from those in the laboratory. This is in agreement with Roelfs *et al.* (1992) who stated that germination occurs within one to three hours of contact with free water, while Dennis (1987) agrees that a period of three hours is necessary for germination. Use of hourly data (not widely available) would make these observations and the TDH calculations possible.
- Cultivar resistance would influence susceptibility to infection (Park, 1990).
- Daily observation of disease presence would make it possible to better define the precise combination of weather variables necessary for disease initiation. Only weekly observations of incidence were provided by the present data set.

4.7 TDD_7 as an early warning index

TDD_{14} was developed for 14 days by using TDD_{14} from the experiment and relating this to stripe rust incidence. The index was then validated using the field data (previous section), but could not be useful for the producer in this form, as the incidence would already have developed into a full-blown infection if TDD_{14} was calculated for 14 days from a wet day. If the TDD values for the experiment are calculated for 7 days instead of 14 days, then the values for TDD_7 as observed in Table 4.9 are approximately half the values of TDD_{14}. TDD_7 critical is 1.77 times less than 227, which is 128. The assumption made is that the same type of weather would continue for the second week.

Table 4.9: Comparison between TDD_{14} and TDD_7 calculated at temperature levels (1 – 4) for Experiment 2.

Temperature level	5 °C	10 °C	15 °C	20 °C
TDD_{14}	203	214	227	237
TDD_7	104	115	128	138

Table 4.10: Predicted TDD_7 for highest and average field incidence (%) with TDD summed from 7 days before incidence observation (1999 – 2000). The seven stations used were M = Moorreesburg, R = Rietrivier, W = Winterton, B = Bethlehem, S = Swellendam and T = Tygerhoek. Predictions are marked by high (H) and low (L), meaning high and low risk of incidence. Observed incidence > 67 % = high risk and < 67 % = low risk. Hits are correct predictions.

	Station Symbol	Calculated TDD_7 for 7 days before observation	TDD_7 Prediction for field incidence	Highest observed field incidence (%)	Hit or miss predicted for highest observed	Average observed field incidence (%)	Hit or miss predicted for average observed
1	M	95	**H**	30	½ hit	30	½ hit
2	M	98	**H**	20	½ hit	20	½ hit
3	R	120	**H**	100	hit	70	hit
4	R	167	**L**	10	hit	10	hit
5	W	116	**H**	5	½ hit	5	½ hit
6	W	84	**H**	40	½ hit	40	½ hit
7	B	59	**H**	60	½ hit	60	½ hit
8	B	131	**L**	30	hit	18	hit
9	B	98	**H**	20	½ hit	12	½ hit
10	B	116	**H**	100	hit	53	½ hit
11	B	115	**H**	80	hit	55	½ hit
12	B	138	**L**	50	hit	50	hit
13	T	94	**H**	100	hit	100	hit
14	T	78	H	40	½ hit	40	½ hit
15	T	99	H	100	hit	100	hit
Total number of possible hits					15		15
Number of hits					8		6
Number of ½ hits					7		9
Number of misses					0		0
% of correct predictions					**53**		**40**

Table 4.10 is similar (same years) to Table 4.8, but TDD_7 values were used instead of TDD_{14} values. Predictions of 53 % and 40 % correct were observed, with only two stations (numbers 6 and 9) giving predictions different to Table 4.8, i.e. "½ hits" for TDD_7 instead of "hits". This makes it possible to recommend using TDD_7 rather than TDD_{14}, as the calculations are shorter and the index more useful.

To summarize, a simple index was developed from daily T_{mx} and T_{mn} to predict high and low risk of infection using a critical TDD value of 227, after the accumulation of degree days starting with a wet day. The latter could be in the form of rain, dew or mist and intermittent wet periods could result in new infection cycles. The index developed predicted incidence observation at 14 days starting from a wet period. The purpose of the dissertation however, was to predict the first incidence observation and so TDD_7 was developed using a TDD_7 value of 128.

TDD_{14} critical = 227, if $y < 227$ then high risk and if $y > 227$, then low risk of infection by *P. striiformis* f. sp. *tritici.*

TDD_7 critical = 128, if $y < 128$ then high risk and if $y > 128$, then low risk of infection by *P. striiformis* f. sp. *tritici.*

4.8 Conclusion

The difficulty with the development of an index based on experimental data is that there are a host of additional factors in the field that also need to be taken into consideration. Conditions in the field are very different to conditions in the laboratory, where conditions are kept constant. The success rate of the TDD_{14} developed in this dissertation is in theory very low, ie. 29 – 50 % (Tables 4.7 and 4.8). TDD_7 shows values of 40 – 53 %, (Table 4.10) which are also low, but TDD_7 can be recommended, because it serves as an early warning and is designed to be simple and practical to use.

The model developed for severity of stripe rust on wheat in Australia by Ash *et al.* (1991), which showed negative correlation with the disease for temperatures over 40 °C, would not be able to be used here in South Africa, as such high temperatures would not be experienced during the summer preceding the wheat season. Their model also requires regional weather data, which may be more difficult to acquire on a regular basis than local weather station data. Dennis (1987) used an equation relating temperature and leaf

wetness duration, which is another weather variable not available from the majority of weather stations.

To conclude, further research would have to be done, especially field work so that infection could be measured daily and compared with the hourly and daily weather data. This would make it possible to relate the exact percentages of incidence to the weather data and in this way a new improved model could be developed to predict infection more accurately. This research would have to be producer-driven if the susceptible cultivars are still being planted and *P. striiformis* f. sp. *tritici* is still a problem. In addition to this, cost of and application of fungicides would also be strong motivation for introduction of a reliable warning system for stripe rust, among other diseases.

CHAPTER 5

SUMMARY

The main objective of this project was to develop an early warning index for infection of stripe rust (*Puccinia striiformis* f. sp. *tritici*) in susceptible cultivars of wheat (*Triticum aestivum*) for the main wheat growing areas of the R.S.A.

Various authors are in agreement that temperature and moisture are the major climatic factors necessary for infection by stripe rust. Temperature limits from the literature vary between –4 and 30 °C and no cut-off values were available. It was therefore decided to run an experiment to observe infection of stripe rust under conditions of high relative humidity and a range of temperatures, namely 5 °C, 10 °C, 15 °C and 20 °C.

Results from the experiment indicated that little or no infection occurred for the 20 °C temperature level and for exposure time periods of one hour or less. Exceptions occurred when sudden drops in temperature with removal of plants from growth chambers to the glasshouse took place. For both incidence and severity it was found that temperatures greater than 22 °C inhibited infection. The Pan 3349 cultivar was found to exhibit higher incidence than Karee. The statistical analysis shows significance for severity for Experiment 1 and 2, but not for incidence. Temperatures for Experiment 2 were slightly lower than those in Experiment 1 and could be explained by the presence of the fans in Experiment 2, to allow evaporation of dew from the sensors, allowing the sensors to register correct readings. The resulting higher moisture conditions in the still air in Experiment 1 could have contributed to the higher severity observed in Experiment 1.

Conditions in the laboratory are not the same as in the field and possibilities for infections at higher temperatures in the field have been reported by Park (1990). He warned against extrapolation of results from the laboratory to the field, but it was

nevertheless decided to use the results from the experiment as a basis for the development of an index for stripe rust infection of susceptible wheat cultivars in South Africa.

Values of 14 day total degree days (TDD_{14}) were calculated from total degree hours (TDH) acquired from the experiment. TDD_{14} for the experiment was calculated by summing degree days (DD) from inoculation until 14 days and relating them to average incidence observed on day 14. Two linear regression lines were obtained, one for 5 °C – 15 °C temperature level and the other for the 15 °C – 20 °C temperature levels. A cut-off TDD_{14} value of 227 where the two lines crossed indicated 67 % cut-off value for incidence. TDD_{14} was developed from this information and reads as follows:

If $TDD_{14} > 227$, then risk of incidence is low at < 67 %.
If $TDD_{14} < 227$, then risk of incidence is high at > 67 %.

TDD_{14} was validated by testing on 1996 – 1998 and 1999 – 2000 data. Correct prediction values for highest and average incidence observation were 50 % and 29 % for 1996 – 1998 data (Table 4.7) and 40 % and 33 % for 1999 – 2000 data (Table 4.8). TDD_{14} however, was thought to be impractical, so TDD_7 was developed by using TDD_7 for 7 days from a wet period. $TDD_7 = 128$ was found to be 1.77 times less than the value for $TDD_{14} = 227$ and so this value of 128 was used as the cut-off value. TDD_7 thus reads as follows:

If $TDD_7 > 128$, then risk of incidence is low at < 67 %.
If $TDD_7 < 128$, then risk of incidence is high at > 67 %.

TDD_7 was validated using the 1999 – 2000 data, with total correct predictions of 53 % and 40 % (Table 4.10). It was therefore decided that TDD_7 could be used by the producer as an early warning index, although the index would have to be tested in the field so that necessary improvements could be made.

It is recommended that research on stripe rust-environment interaction be continued, also to include other diseases and the recommendations made in Chapter 2. Another possible recommendation would be to plan a refined laboratory experiment with a mechanistic approach to use a constant temperature data determined model in real life situations. Temperature and RH would vary and the results should be useful to producer, as well as the researcher. A model suitable for various other diseases as well, could be of great benefit when the cost of pesticides and fungicides are taken into account. The successful application of such a model would be of great benefit to all. After all, food is our fuel and sustainable production of high quality foodstuffs is essential to our survival.

OPSOMMING

ONTWIKKELING VAN 'n INDEKS VIR KORING STREEPROES

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Die hoofdoel van hierdie projek is om 'n waarskuwingsindeks te ontwikkel vir vatbare kultivars van koring (*Triticum aestivum*) vir infeksie deur *Puccinia striiformis* f. sp. *tritici* (streeproes of geelroes) in R.S.A.

Volgens die literatuur is outeurs grotendeels eens dat temperatuur en vogtigheid die hoof klimaatsfaktore vir infeksie deur streeproes verteenwoordig. Temperatuurgrense vir infeksie lê tussen –4 en 30 °C, maar daar is streng gesproke geen afsnywaardes beskikbaar nie. Om hierdie rede is besluit om 'n eksperiment uit te voer om infeksie waar te neem, waar plante onderworpe is aan toestande van hoë relatiewe humiditeit en 'n reeks van temperature, nl. 5 °C, 10 °C, 15 °C en 20 °C.

Resultate verkry deur die eksperiment het geen of verminderde infeksie aangetoon by die 20 °C temperatuur vlak of by inkubasie-tydperke van minder as een uur blootstelling aan vogtige toestande. Uitsonderings op die reël het plaasgevind met skielike verminderings in temperatuur met verwydering van die plante na die glashuis. Vir beide voorkoms en siektegraad het geen infeksie voorgekom by temperature hoër of gelyk aan 22 °C nie en die Pan 3349 kultivar het 'n hoër voorkoms as Karee getoon. Volgens die statistiese analises, was daar beduidende verskille vir siektegraad tussen Eksperiment 1 en 2, maar nie vir voorkoms nie. Temperature vir Eksperiment 2 was effens laer en kan verduidelik word deur die aanwesigheid van waaiers wat gekondenseerde water op die temperatuur sensors verdamp het. Dus kon die hoër waterinhoud in Eksperiment 1 hoër siektegraad tot gevolg gehad het.

Toestande in die laboratorium is nie dieselfde as in die veld nie en moontlikhede vir infeksie by hoër temperature in die veld kom volgens Park (1990) algemeen voor. Hy

waarsku teen ekstrapolasie van laboratorium resultate na die veld, maar hierdie studie het nogtans voortgegaan om die resultate vanaf die eksperiment as basis vir die ontwikkeling van 'n indeks vir streeproesinfeksie te gebruik.

Waardes van 14 dae totale graaddae (TDD_{14}) is bereken vanaf totale graad-ure (TDH) van die eksperimentele waardes. TDD_{14} is bereken deur die gradedae (DD) bymekaar te tel vanaf inokulasie tot 14 dae later en die verwantskap dan met gemiddelde voorkoms waargeneem op dag 14, te verkry. Twee liniêre regressies is verkry, een vir die 5 °C – 15 °C temperatuur vlak en die ander vir die 15 °C – 20 °C temperatuurvlak. 'n Afsny-waarde van 227 vir TDD_{14} waar die twee lyne mekaar kruis, het 'n voorkoms waarde van 67 % getoon. TDD_{14} is ontwikkel vanaf hierdie inligting en lees soos volg:

Indien $TDD_{14} > 227$, dan is die risiko van voorkoms laag by < 67 %.
Indien $TDD_{14} < 227$, dan is die risiko van voorkoms hoog by > 67 %.

TDD_{14} is geëvalueer deur toetsing op 1996 – 1998 en 1999 – 2000 data. Korrekte voorspellingswaardes van hoogste en gemiddelde voorkoms waarneming was 50 % en 29 % vir 1996 – 1998 data (Tabel 4.7) en 40 % en 33 % vir 1999 – 2000 data (Tabel 4.8). TDD_{14} was onprakties vir gebruik en dus is TDD_7 ontwikkel deur gebruik te maak van TDD_7 vir 7 dae vanaf 'n nat periode. $TDD_7 = 128$ was 1.77 maal minder as die waarde vir $TDD_{14} = 227$, d.w.s. 'n waarde van 128. Hierdie waarde is toe gebruik as die afsnywaarde en TDD_7 lees dan soos volg:

Indien $TDD_7 > 128$, dan is die risiko van voorkoms laag by < 67 %.
Indien $TDD_7 < 128$, dan is die risiko van voorkoms hoog by > 67 %.

TDD_7 is bevestig deur gebruik te maak van die 1999 – 2000 data en die aantal totale korrekte voorspellings is 53 % en 40 % (Table 4.10). Daar is dus besluit dat TDD_7 deur die produsent gebruik kan word as 'n vroeë waarskuwingsindeks, alhoewel die indeks in die praktyk getoets sal moet word sodat die nodige verbeterings aangebring kan word. Daar word voorgestel dat navorsing op streeproes-omgewingsinteraksie voortgesit word,

ook op ander siektes en dat die aanbevelings gemaak in Hoofstuk 2 ondersoek word. Ander voorstelle sluit in die beplanning van 'n ingewikkelde laboratorium eskperiment met 'n meganistiese benadering om konstante temperatuur data model in die praktyk te gebruik. Temperatuur en relatiewe humiditeit sal natuurlik varieer en die resultate sal voordelig wees vir beide produsent en navorser. A model toepasbaar op ander siektes kan waardevol wees indien die kostes van gifstowwe in ag geneem word. The suksesvolle toepassing van so 'n model sal van groot waarde wees. Per slot van rekening, kos word benodig om aan die lewe te bly en dus is volhoubare produksie van hoë kwaliteit kossoorte noodsaaklik vir die voortbestaan van die mens.

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APPENDIX 1

ANALYSIS OF VARIANCE REPORT

EXPERIMENT 1 - INCIDENCE

Analysis of Variance Table

Source Term	DF	Sum of Squares (Alpha=0.05)	Mean Square	F-Ratio	Prob Level	Power
A: Temp	3	238820.9	79606.98	465.05	0.000000*	1.000000
B: Cult	1	4111.6	4111.6	24.02	0.000002*	0.979438
AB	3	962.9098	320.9699	1.88	0.135933	0.337736
C: Time	9	119962.1	13329.13	77.87	0.000000*	1.000000
AC	27	49823.83	1845.327	10.78	0.000000*	1.000000
BC	9	2265.232	251.6925	1.47	0.163109	0.504897
ABC	27	5060.654	187.4316	1.09	0.351867	0.724593
S	160	27388.93	171.1808			
Total (Adjusted)	239	448396.2				
Total	240					

* Term significant at alpha = 0.05

EXPERIMENT 1 - SEVERITY

Analysis of Variance Table

Source Term	DF	Sum of Squares (Alpha=0.05)	Mean Square	F-Ratio	Prob Level	Power
A: Temp	3	160474.8	53491.58	209.79	0.000000*	1.000000
B: Cult	1	2172.017	2172.017	8.52	0.004022*	0.664057
AB	3	931.15	310.3833	1.22	0.305267	0.227684
C: Time	9	98731.23	10970.14	43.02	0.000000*	1.000000
AC	27	40258	1491.037	5.85	0.000000*	1.000000
BC	9	3367.4	374.1555	1.47	0.164234	0.503926
ABC	27	4539.433	168.1272	0.66	0.898254	0.440224
S	160	40796	254.975			
Total (Adjusted)	239	351270				
Total	240					

* Term significant at alpha = 0.05

APPENDIX 2

ANALYSIS OF VARIANCE REPORT

EXPERIMENT 2 - INCIDENCE

Analysis of Variance Table

Source Term	DF	Sum of Squares (Alpha=0.05)	Mean Square	F-Ratio	Prob Level	Power
A: Temp	3	105531.7	35177.24	167.80	0.000000*	1.000000
B: Cult	1	577.9027	577.9027	2.76	0.098810	0.273358
AB	3	2062.703	687.5678	3.28	0.022514*	0.559051
C: Time	9	173364.7	19262.74	91.88	0.000000*	1.000000
AC	27	34040.41	1260.756	6.01	0.000000*	1.000000
BC	9	1343.694	149.2993	0.71	0.697185	0.242400
ABC	27	4344.207	160.8966	0.77	0.787623	0.518493
S	160	33542.5	209.6406			
Total (Adjusted)	239	354807.8				
Total	240					

* Term significant at alpha = 0.05

EXPERIMENT 2 - SEVERITY

Analysis of Variance Table

Source Term	DF	Sum of Squares (Alpha=0.05)	Mean Square	F-Ratio	Prob Level	Power
A: Temp	3	84383.41	28127.8	165.85	0.000000*	1.000000
B: Cult	1	1455.781	1455.781	8.58	0.003888*	0.667376
AB	3	2885.408	961.8027	5.67	0.001023*	0.817121
C: Time	9	68010.88	7556.764	44.56	0.000000*	1.000000
AC	27	28254.09	1046.448	6.17	0.000000*	1.000000
BC	9	3401.174	377.9082	2.23	0.022754*	0.723970
ABC	27	4253.916	157.5524	0.93	0.570350	0.627587
S	160	27135.29	169.5956			
Total (Adjusted)	239	219780				
Total	240					

* Term significant at alpha = 0.05