

OPTIMISING ASPECTS OF A SOYBEAN BREEDING PROGRAMME

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ABSTRACT

Many aspects of a breeding programme can be manipulated and optimised to improve the overall success of the programme. Practically no research on soybean (*Glycine max* L. Merr) breeding programme improvement has been published in South Africa. Research from elsewhere in the world is relevant but the uniqueness of the environment, farming systems and germplasm, justifies local investigation. Expanding the influence of a programme, without the need to establish more breeding stations to cater for different environments is an important objective. The concept of a satellite selection site evolved from this objective, and the effectiveness thereof is demonstrated. Application of satellite selection sites for F_2 selection has significant implications for participatory breeding programmes.

Soybean rust (caused by *Phakopsora pachyrhizi*) has affected soybean production in regions of the country since 2001. The breeding station falls into one of the regions where soybean rust has occurred every season since to date (2001-2008) and this has had an influence on the breeding programme. The effect of genotype maturity group and planting date on yield loss to soybean rust are assessed using data generated from three seasons to establish strategies of how best to avoid this biotic stress. For soybean rust tolerance research, where consistently high rust pressure is required to evaluate genotypes, late planting is recommended. In a further study on soybean rust, pre-selected genotypes are evaluated for tolerance to soybean rust using a split-plot trial fungicide experiment conducted over three seasons. Variability in tolerance classifications over seasons resulted in the development of a novel statistic (W_iP_i) which facilitates the simultaneous selection of soybean genotypes for yield performance and yield stability under soybean rust pressure. Use of this statistic avoids the need for traditional split-plot procedures to evaluate soybean rust tolerance.

Selection methodology is a key topic in breeding, and it is dealt with in relation to the satellite selection sites and soybean rust tolerance. One of the final conclusions of this study is that pedigree selection has merit in both participatory style satellite selection and breeding for soybean rust tolerance.

DECLARATION

Research presented in this thesis represents original work by the author and has not been otherwise submitted in any form for a degree or diploma to any university.

Where use has been made of the work, data, pictures or graphs of others, this has been duly acknowledged in the text.

This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

- a. Their words have been re-written but the general information attributed to them has been referenced.
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John Antony Jarvie (Candidate)

As the candidate's Supervisor I agree/do not agree to the submission of this thesis.

.....

Dr Paul E. Shanahan (Supervisor)

PUBLICATIONS DECLARATION

Details of contributions made to publications emanating from work in this thesis:

Publication 1

Jarvie, J.A., and P.E. Shanahan. 2008. Investigating the use of satellite selection sites in soybean (*Glycine max* (L.) Merr.) breeding. *S. Afr. J. Pant Soil* 25:139-143.

The original idea of selecting at two different sites came from work done on peanuts in the 1980s by Wynne and Hildebrand, I believe, although no publication on this was traced. The thought process behind the satellite site concept was entirely my own, but I was considerably influenced by the work of N.W. Simmonds in the latter stages of this research. My co-author, Dr P.E. Shanahan, made significant contributions in data analysis and valuable editing suggestions. Anonymous reviewers made suggestions which improved the publication appreciably (General appendix: Publication 1). Aspects of this paper were presented at the VII World Soybean Research Conference, Foz do Iguassu, Brazil, 2004.

Publication 2

Jarvie, J.A. 2008. A review of soybean rust from a South African perspective. *Accepted S. Afr. J. Science* 105: 4 February 2009.

My supervisor, Dr P.E. Shanahan suggested that I publish my literature review from Chapters 2 and 3 with some modifications as a review article, because it captured some of the historical aspects of the soybean rust epidemic in South Africa which had not been formally documented before. Input into this publication, for the distribution and spread of soybean rust in particular, was made by Ms P. Kruger, Dr M. Craven and Dr F.J. Kloppe. The pathology aspect of this paper was verified by Mrs E. Dunlop and Dr P.E. Shanahan made editing suggestions (General appendix: Publication 2).

Publication 3

Jarvie, J.A., and P.E. Shanahan. 2008. Soybean yield loss trends associated with soybean rust in South Africa. *In preparation for submission to Crop Protection*

The need to quantify yield losses to soybean rust, a topic which was raised in the meetings of the soybean rust task team, ultimately provided the stimulus for this research. In my own breeding programme, it was important to establish how the trends in yield loss would affect breeding for soybean rust tolerance. Combining these needs with the routine evaluation of advanced lines was a logical progression, but perhaps if the end results could have been predicted, the trials may have been designed differently. The statistical analysis proved to be challenging and much discussion with my supervisor, Dr P.E Shanahan, was entered into before an analysis model was adopted (General appendix: Publication 3).

Publication 4

Jarvie, J.A., and P.E. Shanahan. 2008. Assessing tolerance to soybean rust in selected genotypes. *In preparation for submission to Field Crops Research*

The inspiration for choosing to breed for soybean rust tolerance at a time when many other researchers were concentrating on resistance probably came from many sources, and it would be hard to identify particular individuals. It is possible to say that my general perspective on soybean rust was considerably influenced by visiting international researchers with extensive experience such as: Dr S. Shanmugasundaram; Dr C. Levy; Dr G.L. Hartman; Dr M.R. Miles; and Dr R.D. Frederick. Pre-screening of genotypes for inclusion in the tolerance trials was done with the field support of Ms M. Govender and advice from Dr F.J. Kloppers and Dr. M.R. Miles. Analysis of the data was conducted with the assistance of Dr P.E. Shanahan and in discussions regarding the seasonal variability of this data, he suggested considering the use of stability measures. My choice of stability statistics and the development of the W_iP_i statistic were inspired by the comparative work of Flores and co-workers. Similarities to the calculation of Purchase's AMMI stability value were only discovered subsequent to the formulation of the statistic (General appendix: Publication 4).

.....
John Antony Jarvie (Candidate)

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UKZN

To my supervisor, mentor and research role-model Dr Paul Shanahan: thank you for your resolute moral support; your endless patience on matters statistical; and your thorough academic guidance.

External examiners

To the external examiners of this thesis (Dr A. James, CSIRO, Australia; Dr S. Shanmugasundaram, New Jersey, USA; and Dr M. Smit, SASRI, Mt. Edgecombe): thank you for taking time off your schedules to examine this thesis and for your kind words.

Family

Most importantly, I would like to thank my supportive family. To my mother and father, I know that if no one else reads this entire thesis, you will. Bless you. To my very dear wife Penny, thank you for allowing me to indulge myself at the expense of family time. The final year of writing up this thesis has been particularly tough on you. To my daughters Rachel and Erin, may the completion of this thesis inspire you to reach for your own dreams.

LIST OF ABBREVIATIONS

Abbreviation	
AES	Agricultural experiment station
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
ASV	AMMI stability value
AUDPC	Area under the disease potential curve
AVRDC	Asian vegetable research and development centre
BS	Bulk selection
CIMMYT	International maize and wheat improvement centre
CSS	Control selection strategy
CV	Coefficient of variation
D	Determinate
dap	Days after planting
Dec	December
df	Degrees of freedom
DPI	Days post infection
EGT	Early generation testing
GxE	Genotype by environment
GxPD	Genotype by planting date
GxS	Genotype by site
HPI	Hours post infection
I	Indeterminate
IPCA	Interaction principal component analysis
KZN	KwaZulu-Natal
LSD	Least significant differences
masl	Metres above sea level
MG	Maturity group
MS	Mean squares
Nov	November

Abbreviation	
PD	Planting date
PS	Pedigree selection
RB	Red brown
REML	Residual maximum likelihood
rep	Replication
RLT	Relative life time
RR	Roundup Ready
RS	Recurrent selection
RSA	Republic of South Africa
SE	Standard error
SMS	Short message service
SP	Single pod
SS	Sum of squares
SSD	Single seed descent
SSS	Satellite selection strategy
Treat	Treatment
USA	United States of America
USDA-ARS	United States department of agriculture - agricultural research station
Vno	Genotype

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GENERAL INTRODUCTION

History of soybean variety development in South Africa

The question of whether to breed varieties locally or to rely on introductions from other countries is an on-going debate in South Africa. Early in the last century (1908, according to Smit (1987)), soybean genotypes were successfully introduced at various testing centres around the country. This precipitated an intensive plant introduction programme by the Department of Agriculture, which ultimately provided the stimulus required to start soybean breeding in South Africa. During the period 1925 to 1970, a number of South African breeding programmes were established at a time when virtually no demand for the crop (Van Niekerk, 1967) existed in the country. Public programmes were established at Bethlehem, Pretoria, Cedara and Potchefstroom. Apparently, a single adapted genotype of Chinese origin (Smit, 1987) formed the basis of the public breeding programmes' early success and probably also accounts for the certain uniqueness of southern African germplasm. Successful genotypes such as Geduld, Blyvoor, and Welkom (all named after South African gold mines) were the products of the public programmes during that era. Masterpiece was one of the first varieties released out of a local private programme and was reportedly (Van Niekerk, 1966) derived from a cross between a Manchurian line and a Hungarian line, the cross made in Portugal but selected in South Africa by Dr. Kreutzer of Gunson Seeds, Johannesburg.

South African germplasm was also instrumental in contributing to the development of the soybean industry in neighbouring Zimbabwe (formerly Rhodesia). Germplasm exchange between the South African breeding programmes and the Crop breeding Institute in Harare (formerly Salisbury) in the period 1960-1973 led to the development of key varieties in the history of Zimbabwean soybean production. Rhosa (after Rhodesia - South Africa) was a direct selection out of a genotype bred in the Potchefstroom programme and Impala was the progeny of a cross between Rhosa and a line out of the Cedara breeding programme (Tattersfield, personal communication).

The start of an organised seed trade in soybeans in South Africa was marked by the first 'certified' seed crop of the cultivar Welkom in 1967, when the total grain production in the country was approximately 4500 t (Vorster, 1968). During the period 1970-2000, many

varieties cultivated (Table 1) were accessed directly from public breeding programmes in the USA and elsewhere. These were supplemented by locally bred varieties such as Highveld Top (Bühmann Saad), Prima and PAN 660 (PANNAR). Identification of adapted varieties for the summer rainfall areas was done with the aid of the National Cultivar Evaluation Programme (Smit, 1987). The expansion of soybean grain production in South Africa was in part as a consequence of AMMI mediated pattern analysis (Smit and Piper, 1997) of the National Cultivar Evaluation data, which improved variety recommendations significantly. The defining of homogenous production regions facilitated the subsequent selection for specific adaptation eventually leading to improved genetic gains.

Table 1 Examples of commercial cultivars grown in South Africa during the period 1970-2000 which were direct introductions from other countries

Decade	Cultivar	Breeding Institution
1970-79	Williams	Illinois AES
	Oribi	Salisbury Breeding Institute, Rhodesia
	Essex	Virginia State University
1980-1989	Columbus	Kansas AES
	Ransom	North Carolina AES
	Forrest	Mississippi AES
	Hutton	Florida AES
	Impala	Salisbury Breeding Institute, Rhodesia
1990-99	A5409	Nidera, Argentina
	Hutcheson	Virginia State University

The advent of the transgenic era and the expansion of utility patents (Boerma, 1998) resulted in some restrictions to the free flow of germplasm, and changed the patterns of genotype sourcing in South Africa. The term ‘transgenic era’ is a broad reference to the release of varieties containing genetic material from other organisms, transferred via recombinant DNA techniques. Use of the Roundup Ready (RR) transgene technology (Padgett *et al.*, 1995) was initially licensed exclusively to commercial companies and this resulted in a global swing away from public varieties to proprietary varieties as the technology became more popular. The reaction of public breeding programmes (in the

USA in particular) to this development was to specialize in niche-market (often quality related) varieties in order to keep their breeding programmes alive. The combination of the fact that few public programmes had access to the RR transgene and that they had moved towards developing speciality food types effectively excluded public programmes as a source of direct introductions to South Africa. Introductions of varieties from countries such as USA, Argentina and Brazil still continued, but the source of these varieties had shifted towards proprietary genotypes from commercial companies with legal access to the transgenic trait.

A further potential restriction to the free flow of germplasm across the globe in the transgenic era has been brought about by new trait registrations. Owners of transgenic traits are only likely to release their traits in countries where their intellectual property can be protected (Roth, 1995) and they are able to recover the royalties due to them. Although South African farmers have embraced RR technology (Van der Walt, 2006), recovery of royalties due to owners of the technology has been problematic. A reluctance to register further transgenic traits in South Africa currently (2008) exists, which could effectively deny the country access to the latest cultivars from markets where these new traits have been released. If this reluctance continues, and there are no good local breeding programmes supporting the industry, then it is a matter of time before the crop will become uncompetitive and the soybean industry in South Africa will stagnate. It is widely accepted that breeding locally in a country will be more successful in the long term than relying on introductions from other countries. From a strategic perspective, it would appear to be important that there are local breeding programmes that can service the industry.

Variety adaptation

Most soybean productions in South Africa fall between the latitudes of 23-30°S, which means that the variation in photoperiod across the production area is relatively small. Altitude variation across the production region is quite dramatic, varying from 600 to 1900 masl. Altitude and temperature are inversely related and have a direct effect on genotype adaptation. The two primary drivers of genotype adaptation are photo-period and temperature and, since the photo-period variations across the region are relatively small, temperature (altitude) has the dominant influence on variety adaptation in South Africa. The AMMI location grouping patterns that Smit and Piper (1997) investigated were based

on genotype reactions at 63 localities in the National Cultivar Evaluation Programme, and these correlated strongly with altitude. Locations were thus classified as being in either the Cool, Moderate or Hot production region. In the USA variety adaptation is classified in bands dictated predominantly by latitude. In the flat Midwest of the USA, the range of adaptation of a single maturity group is roughly 4° in latitude. South Africa in contrast, has a dramatic variation in altitude across any 4° range in latitude which may require the deployment of varieties from up to five different maturity groups (IV to VIII). As a general rule, discounting the effects of planting date and moisture regime, maturity groups IV and V would be best adapted to the Cool region; maturity groups V-VII to the Moderate region; and maturity groups VII-VIII to the Hot region.

Rationale for this research focus

In countries such as South Africa, with limited seed markets (Fig. 1), it is imperative that the breeding programmes are efficient and effective. Many aspects of a breeding programme can be manipulated and optimised to improve the overall success rate.

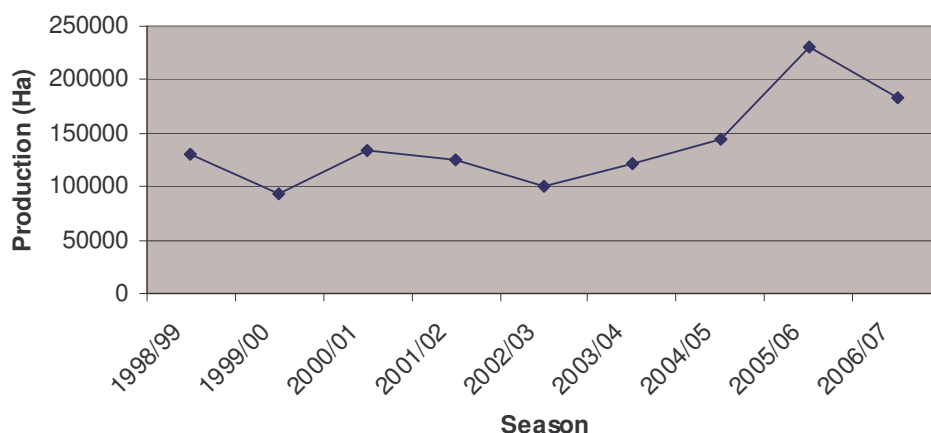


Fig. 1 South African soybean production (ha) in recent years (Anon, 2008)

Breeding has been described as a ‘numbers game’ where, if you have enough populations and progeny rows, some level of success is bound to be achieved. In a commercial programme, efficiency is important because the size of the programme will be capped by a budget (Witcombe and Virk, 2001). The importance of efficiency is accentuated in a small seed market where there are limited opportunities to recover research investment. The

breeder's goal thus is to make the most efficient use of the time, labour, land and financial resources available to achieve commercial success.

The soybean breeding programme at PANNAR has been running since 1988, and during this period some level of commercial success has been achieved (Table 3). The objective of this thesis is to investigate ways of improving the efficiency or success of this breeding programme. Any breeding programme aimed at producing commercial varieties will consist of a multitude of separate projects, each with an objective and a priority and all contained within the broader framework of the breeding programme structure. Improvements to the structure of the programme or to the separate projects within the programme will ultimately affect the overall success.

Practically no research on breeding programme improvement has been published in South Africa. Research from elsewhere in the world is relevant but the uniqueness of our environment, farming systems and our germplasm, justifies local investigation. The first area that receives attention in this thesis is the expanding of the influence and effectiveness of the programme. Within PANNAR, a single breeding station currently serves the South African requirements for cultivars in five maturity groups because the restricted total market size effectively excludes the establishment of more breeding stations on financial grounds. The concept of a satellite selection site evolved out of the need for more selection environments without further infrastructural costs, and the effectiveness thereof is evaluated in Chapter 1. Certain aspects of the adaptation acquired through a single season of F₂ selection were presented at the VII World Soybean Research Conference (Jarvie and Shanahan, 2004) and publication derived from Chapter 1 has been published in South African Journal of Plant and Soil (General appendix: Publication 1). The broader application of satellite site selection relevant to participatory style breeding programmes is demonstrated and discussed further in Chapter 1.

Soybean rust (caused by *Phakopsora pachyrhizi*) has affected soybean production in certain regions of the country since 2001. The breeding station falls into one of the regions where soybean rust has affected crops in every season (2001 to 2008) and this has had an influence on the breeding programme. In Chapter 2 the effect of soybean rust on yield loss is measured on a large number of genotypes, over two planting dates and three seasons. Conclusions relevant to the management of yield losses based on the interactions between

Table 3 Varieties released from the PANNAR programme for the ten year period 1996-2005. Varieties released under license were lines imported from other countries, all others emanate directly from the breeding efforts of the PANNAR programme

Year of Registration	Variety	Comments
1996	PAN 660	
1996	PAN 564	
1996	PAN 562 s	Under license
1997	PAN 779	
1997	PAN 872 ^a	
1997	PAN 875 ^a	
1998	PAN 589	
1998	PAN 780	
1999	PAN 891 ^a	
1999	PRIMA 2000	
2000	PAN 806 ^a	
2000	PAN 809	Under license
2001	PAN 513	
2001	PAN 510	
2002	PAN 626	
2002	PAN 421R	Under license
2002	PAN 520R	Under license
2002	PAN 522R	Under license
2003	PAN 737R	
2003	PAN 535R	
2003	PAN 538R	
2004	PAN 1643R	
2005	PAN 1652	

^a Varieties released in Zimbabwe

genotype maturity groups, planting date and season are presented. Yield loss trends associated with soybean rust, extracted from Chapter 2, have been prepared for publication in Crop Protection (General appendix: Publication 3).

Evaluation of tolerance to soybean rust is the research topic in Chapter 3. Genotypes pre-selected for tolerance to soybean rust are evaluated using conventional split-plot methodology. Due to variability in genotype reactions over seasons, a number of novel combinations of stability statistics are investigated for use in quantifying rust tolerance. Aspects of soybean rust tolerance assessment derived from Chapter 3 have been prepared for publication in Field Crops Research (General appendix: Publication 4). The literature reviews of Chapter 2 and Chapter 3 have been combined in a single review article submitted to South African Journal of Science (General appendix: Publication 2).

Chapter 4 is the general overview of the research contained in this thesis. Common themes that link the separate chapters of this thesis are discussed, and the key conclusions from this research are summarized.

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CHAPTER 1

INVESTIGATION OF THE USE OF SATELLITE SELECTION SITES IN SOYBEAN BREEDING

1.1 LITERATURE REVIEW: SOYBEAN SELECTION AND BREEDING STRATEGIES

1.1.1 Selection methods

Soybean cultivars are typically developed by hand cross-pollination (Walker *et al.*, 1979) of two parents followed by several generations of self-pollination, which occurs naturally. Homozygous or near homozygous (normally F_4 or later generation) lines are tested for performance over a series of increasingly comprehensive trials before the final selection of lines for commercialization is made. Selection methods differ in how the segregating F_2 - F_4 generations are handled. In soybeans, pedigree selection (PS), bulk selection (BS), single seed descent (SSD), early generation testing (EGT) and less commonly recurrent selection (RS) methods are employed.

1.1.1.1 Single seed descent

Single seed descent or variations of this method are by far the most popular breeding method currently employed by soybean breeders. With SSD, a single seed is selected from each individual in the population from F_2 through to F_4 (commonly in soybeans) without selection. At some point (F_5 normally) single plants are harvested and progeny rows are planted and evaluated (Fig. 1.1). Boerma and Cooper (1975) compared PS, SSD and EGT with each other and found no consistent yield benefits in any of the methods. Their recommendation was to use SSD, since it was the least costly of the methods. Snape and Riggs (1975) compared the gene distribution of the F_2 with the F_6 derived by SSD, considering various combinations of additive, complete dominance, complementary and duplicate gene interactions. They demonstrated that transgressive segregants were produced in all cases in the F_6 , and given the expense of EGT, concluded that SSD was the most efficient breeding method. Casali and Tigchelaar (1975) compared PS, BS and SSD methods and showed that with high heritabilities, PS was superior, but with low

heritabilities SSD was the most effective. This perhaps has been one of the more important factors influencing the choice of SSD as the default selection method in soybeans. Mechanization of plot planting and harvesting became more common place in the 1980s, followed by increased computerization and direct data capture. This resulted in a dramatic increase in the trial plot capacity of breeding programmes which further encouraged the change from PS to SSD. Single seed descent also suited soybean breeding as it entered the transgenic era. The major drive at this time was to convert or generate lines containing the RR gene (Padgett *et al.*, 1995) as fast as possible. Using SSD it was possible to advance three generations in a year using either glasshouse facilities or winter nurseries, because no selection besides that for glyphosate tolerance was required.

May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
		Field cross				F ₁ in Glasshouse						
Field F ₂ , SP						Winter Nursery F ₃ , SP				Winter Nursery F ₄ ,		
SP	F ₅ , single plant selections											
F _{5.6} Field planted progeny row												
F _{5.7} Yield trials 1 location												
F _{5.8} Yield trials, few locations												
F _{5.9} Yield trials, multi-locations												

(Adapted from an undated research brochure of the Jacob Hartz Seed Company, Inc.)

Fig. 1.1 A schematic example of a commercial soybean breeding programme in the Northern hemisphere, using a modified SSD selection method. SP = Single pod; Winter Nursery = Field planting in Belize, Central America.

1.1.1.2 Pedigree selection

Pedigree selection was arguably the most popular selection method used by soybean breeders in the past, but as labour has become more expensive and mechanization more widespread, breeders have moved to SSD. Tracing of pedigree information is held as one of the restrictions of PS; however with the increased computer power available today, keeping track of pedigrees and familial relationships is no longer laborious.

There are justified reasons for the continued use of this selection method, if the expense and logistics of the process can be controlled. As already discussed, it has been shown that PS is a superior selection method when heritabilities are high (Casali and Tigchelaar, 1975). One of the ways to ensure high heritabilities is to space-plant the segregating generations. This allows better phenotypic expression of the genotype without interference from neighbouring plants or plots (Donald, 1968). From a practical perspective it also means that the breeder is able to see the entire plant (important with visual selection techniques) which may not be possible when the population is planted at a high density. The implementation of a selection ideotype depends on the active recognition of the ideotype attributes and not on competitive ability of a plant with its neighbours. One of the disadvantages of space-planting is, if land is limiting, that large F_2 populations may take up a sizeable area. With improved heritabilities, selection for adaptation in the early generations can be very successful. Testing pure lines or near homozygous lines at commercial plant densities is done in the final testing stages, which eliminates the genotypes that display negative compensation as a response to increased competition in a pure stand. Some agronomic attributes such as lodging resistance are expressed differently at low plant densities, so although they can be selected for in space-planted conditions, these can only effectively be evaluated in the final testing phases at commercial plant densities.

Where parents differ greatly in the beneficial genes that they carry, the chances of combining many of these genes in one genotype is very low if no selection takes place (Burton, 1987). The chance of getting transgressive segregants in wide crosses is thus low with SSD, but using PS in the early generations the chances improve dramatically. Continual exposure to a selection pressure over the segregating generations will assist in fixing desired gene combinations in their homozygous state. Commercial soybean programmes concentrate largely on elite x elite crosses, which is conceivably also the reason that SSD serves their needs well. On the other hand, it needs to be borne in mind that genetic variation in soybean is relatively small compared to many crops (Sneller, 1994), which is why wide crossing is particularly important. For wide crosses, or even crosses between elite parents of different backgrounds, PS would appear to have substantial benefits.

1.1.1.3 Early generation testing

Early generation testing involves the evaluation of F_2 derived families in an attempt to identify F_2 plants that have a generous complement of favourable genes (Cooper, 1990). The identification of superior F_2 plants would then allow the dedication of resources to selecting from within those single plant families for the best performing lines. In practice, single plant selections are made in the F_2 , which could be a random sample of the F_2 or specific selections for adaptation. Families derived from single F_2 plants ($F_{2.3}$) would be evaluated in trials, followed usually by the evaluation of the surviving $F_{2.4}$ families. Seed of each $F_{2.3}$ family is derived from a single plant, so with the limited seed available, trials are usually restricted to small plots which are often un-replicated (Hegstad *et al.*, 1999). Increased precision in the $F_{2.4}$ trials is usually obtained because enough seed is normally available for larger plots, more replications and locations. The $F_{2.5}$ families surviving the two cycles of evaluation are subjected to intensive selection to produce lines that should have a generous complement of favourable gene combinations. Early generation testing relies on the assumption that the performance of a family at an early generation of selfing is predictive of its performance at homozygosity. Bernardo (2003) showed that the effects of residual heterosis in the F_2 have a very small effect on the correlation between the performance of an early generation family and a descendent homozygous line. When dominance is absent, the effectiveness of selection improves with increasing homozygosity. However, the theoretical correlation value for a F_2 derived line is high to begin with (0.707), which suggests that EGT is expected to be effective.

Hegstad *et al.* (1999) showed that the predictive power of EGT was better in some pedigrees than in others but, that in general, it was possible to identify populations from which the highest yielding lines were derived. Boerma and Cooper (1975) found that SSD was more effective and efficient than EGT. In practice, it is non-genetic factors that determine whether EGT is effective or not. The heritability of selections in the early generations is often low because of small plots and inadequate replication (St. Martin and Futi, 2000). Perhaps this could be improved by using more sophisticated trial designs (for example row x column designs) and more powerful computing procedures such as REML (Residual maximum likelihood). Early generation testing is still not widely practiced in soybean breeding because in theory it reduces the need for multi-location testing in the

advanced stages of variety development, but in practice it adds another tier to the testing structure.

1.1.1.4 Recurrent Selection

Recurrent selection has limited application in soybean breeding (Kenworthy and Brim, 1979) because of the difficulty in obtaining large numbers of crossed seed from intermating. Use of male sterility (St. Martin, 1981) to obviate the need to use laborious hand pollination techniques has failed to make RS more widely used in soybean breeding. From a historical perspective, however, soybean improvement can be viewed as many cycles of RS (St. Martin, 1982). Released cultivars are used as parents, reselected, followed by a new cycle of releases. These are recombined, reselected and the whole cycle is repeated. On a global scale this form of RS is concentrating beneficial genes in soybean and fears of narrowing the genetic base abound (Sneller, 1994; Gizlice *et al.*, 1996).

1.1.2 Breeding strategies

1.1.2.1 Population structure

In conventional breeding of self pollinated crops, breeders may deal with hundreds of crosses in a season. This could lead to the generation of many thousands of progeny rows and ultimately lines in the homozygous or near homozygous state that would require evaluation in trial plots. Assuming that the number of plots that can be evaluated in any given programme has a limit, it is in theory possible to determine the optimum number of crosses and the optimum F₂ population size per cross. Theory, however, is unsatisfactory (Witcombe and Virk, 2001) in determining the optimum number of crosses because it would require the knowledge of genetic complementarity between the two parents for the entire genome. Witcombe and Virk (2001) surveyed plant breeding companies to see what was done in practice. The results included field crops such as peas (*Pisum sativum*), oilseed rape (*Brassica napus*), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.). Typically about 200 F₂ populations were grown with a population size of between 1000 and 1500 individuals. The overriding strategy amongst commercial breeding programmes was to use a high number of crosses to achieve their genetic gain. Using the highest yielding genotypes as parents in crosses (based on the most recent trials) is likely to

involve many lines of which little is known about their parental ability. This approach would require that a high number of crosses are produced to ensure that some good combinations are made. The penalty of this approach is that the population size may need to be restricted to contain the size of the programme.

In Witcombe and Virk's survey, there were no commercial programmes that utilized low cross numbers (10 or less per year) combined with very large populations. There are very few other examples that utilise the low-cross number strategy, particularly not from commercial programmes. It has been favoured in the participatory plant breeding approach where the skills and facilities of the co-operators restrict the number of populations that can be handled successfully. The success of a low-cross number strategy is reliant on careful choice of parents and excellent predictive skills of the breeder. Strategies for choosing parents may differ dramatically between programmes, but where information from relatives is available, predicting the performance of a cross is greatly improved and the number of crosses needed for success can be reduced (Troyer, 1996).

1.1.2.2 Genotype x environment interaction

Understanding genotype by environment interaction (GxE) is an important element in determining which breeding strategy to follow (Annicchiarico, 2002). Genotype x environment interaction is defined as the differential response of genotypes to environments (Romagosa and Fox, 1993). Crossa (1990) stated that data collected from multi-location trials are likely to be comprised of three fundamental aspects: structural patterns; non-structural noise; and the joint effect of genotype, environment and their interaction. The function of experimental design and statistical analysis is to recognise and interpret the patterns and interactions displayed by genotypes and locations, and to minimise the unexplained noise.

Significant GxE interaction for a quantitative trait such as yield can reduce the correlation between genotypic and phenotypic values and negatively affect the response to selection (Comstock and Moll, 1963). Quantitative GxE occurs when there is a differential response to environments by genotypes, but no changes in rank. Qualitative GxE or crossover interactions occur where there are rank changes over environments, and these interactions are generally challenging to plant breeders. If the performance of a genotype is above the

mean over a wide range of environments, it could be classified as having general or wide adaptation. If on the contrary, it has good performance over a limited range of environments, then it would be classified as having specific or narrow adaptation (Annicchiarico, 2002).

If the GxE term from an analysis of variance (ANOVA) is partitioned into temporal and spatial components, and the genotype x location (GxL) is a significant part of the GxE, then the locations can be subdivided into homogenous groups that limit the GxE within the groups. If this subdivision of locations can be successfully done by a physical assessment of the site (by grouping according to rainfall, latitude or temperature) then this is the logical method. Often, environments cannot be characterized by a single factor, so the answer is to allow similar genotypic reaction to group the environments using multivariate statistical techniques. Pattern analysis techniques such as the additive main effects and multiplicative interaction (AMMI) model (Gauch and Zobel, 1996) have been successfully applied in many crops to identify homogenous sub-regions, for example: wheat (Annicchiarico *et al.*, 2005); and sunflower (*Helianthus annuus* L.) (De La Vega and Chapman, 2006).

The merits of strategies targeting specific adaptation versus those of wide adaptation in a breeding programme can be debated at length. Breeding for wide adaptation aims to achieve a variety that performs well in all or nearly all the sites within a target environment. Breeding for specific adaptation aims to produce a number of varieties which each perform well within a sub-region of the target environment. Achieving wide adaptation is arguably the Holy Grail in plant breeding, as it provides a basis for yield stability and food security across a broad range of conditions over many environments. Conversely, specific adaptation strategies promise higher site specific yields. The decision on adaptation strategy employed in a breeding programme is one based on the gains offered by each strategy.

1.1.2.3 Specific adaptation strategies

Specific adaptation strategies have been shown (Annicchiarico *et al.*, 2005) to provide greater yield gains albeit at increased cost. The higher cost is incurred through the increased field testing that is required and the larger number of cultivars that would need to

be maintained (Ceccarelli and Grando, 2007). Using AMMI analysis to cluster localities into sub-regions, Annicchiarico *et al.* (2005) were able to increase the heritability of yield by reducing GxS interaction. The resultant advantage of the specific adaptation breeding strategy over wide adaptation varied from no difference to nearly 40%, depending on the sub-region. Ceccarelli and Grando (2007) argued that unfavourable environments tend to differ widely from each other whilst favourable environments had many similarities. Consequently, in order to limit crossover interactions between genotypes, many target environments or sub-regions need to be declared particularly in unfavourable environments. One of the serious limitations of this strategy is that a large number of selection sites are required and a potentially large number of varieties would need to be released and maintained. Despite this, they concluded that selection for specific adaptation was fundamental to achieving good genetic gains particularly in unfavourable conditions. Atlin *et al.* (2000) recognized that a division of resources would inevitably be required if a large breeding programme was replaced with several smaller ones, and showed that the loss of precision associated with reduced test locations was likely to be greater than the gain in selection response. De La Vega and Chapman (2006) working on sunflower hybrids in Argentina found that with highly repeatable experiments, the division of resources did not affect their precision to a significant extent. Their conclusion was that testing for specific adaptation would lead to improved responses. This conclusion was based on evaluation of genotypic means only and did include the selection of segregating generations in the target environment.

The efficacy of specific adaptation strategies can be increased by using a pool of germplasm which has crucial adaptative traits for the sub-region in question (Annicchiarico *et al.*, 2005). Without substantiating the claim, the same author stated that early generation selection would benefit specific adaptation strategies even further. The specific adaptation strategy demanded less statistical manipulation of data and was well suited to breeding programmes of medium-sized countries or poorer resourced research systems.

1.1.2.4 Wide adaptation strategies

As an international organization, CIMMYT (International maize and wheat improvement centre) has been the champion of wide adaptation in plant breeding with the global success

of their wheat and maize (*Zea mays*) programmes firmly based on broad adaptation. The cornerstones of their strategy have been: targeting Mega Environments; the use of diverse germplasm; shuttle breeding (see par. 1.1.2.6); selection under optimum conditions; and multi-location testing (Braun *et al.*, 1996). A Mega Environment is defined as a broad area, not necessarily contiguous and often transcontinental, with similar biotic and abiotic stresses, having common cropping systems and consumer requirements. Germplasm bred for a particular Mega Environment would accommodate all the major stresses of that environment, but perhaps not all the secondary stresses. CIMMYT have defined 12 Mega Environments that classify the global wheat growing regions. Within a Mega Environment, millions of hectares are addressed with a certain degree of homogeneity with respect to their wheat breeding objectives. One of the benefits of selection for broad adaptation is that trial data can be pooled over many sites, thereby increasing the precision of estimating genotypic means (Atlin *et al.*, 2000). Extensive testing networks are likely to produce genotypes with broad adaptation because the gain in broad-sense heritability associated with this precision more than compensates for the inability to exploit local adaptation.

1.1.2.5 Participatory plant breeding strategies

Conventional plant breeding is generally performed by breeders skilled in the art and science of selecting superior genotypes for specific target objectives. This relies on their intimate knowledge of the breeding objectives, and the application of the best strategy to attain this objective. Participatory plant breeding strategies exploit genetic variability generated by plant breeders for selection in the target environments by farmers, extension officers and breeders (Ceccarelli and Grando, 2007). This process is similar to conventional breeding except that selection, testing and other key decisions are jointly taken by farmers and the breeders. Although the theory behind this is sound, Ceccarelli and Grando (2007) made sweeping statements regarding the rationale behind their use of this process, including but not limited to: conventional plant breeders act unilaterally without consultation; and conventional plant breeding has been unsuccessful in marginal environments. Clearly the plant breeding industry in particular and agriculture in general would be in a bad way if these statements were true. However exaggerated their statements may be, these are certainly areas of concern that require the breeder's constant attention in order to achieve success. Understanding all aspects of the breeding target, inclusive of farming practices and the socio-economic environment of those who will utilize the

breeding product is crucial to the success of any programme. This would not be limited to marginal environments, although it is possible that this is where breeders' understanding is most lacking.

With participatory programmes, testing and selection may be independently undertaken at a number of locations. On farm trials are very common, even in conventional programmes. The concept of on farm selection however, is one that has not had particularly wide acceptance in the breeding industry for mostly practical reasons. On farm selection has the practical restriction in that there is generally little or no infrastructure and expertise present, which means a great deal more breeder input is required. Compounding this is the fact that there are almost a limitless number of unique farming environments and rather finite breeding resources. In an attempt to compromise between selecting for local adaptation and dividing resources, Jarvie and Shanahan (2008) demonstrate the use of a satellite selection site to make gains in local adaptation without the traditional problems associated with multi-location selection.

Participatory programmes have been in existence for a crop such as dry bean (*Phaseolus vulgaris*) for a number of years in Africa, without many noteworthy results. The more opinions sought, often, the more difficult it is to reach consensus. Dry bean participatory programmes have also involved seed multiplication schemes. A common African example of this would be that a national breeding programme would (at great effort and cost) bulk up small quantities of a newly released variety. This would be given out to, say, 100 farmers at no cost. All they would be required to do in return is supply an equal quantity of seed from their harvested crop to another farmer, on the condition that he or she does the same. In theory the number of farmers who have access to the new variety doubles every season, and after a few seasons, the variety would be widely distributed and the poor would have cheap access to improved genetics. In an example from personal experience with a participatory seed programme in Malawi, the national programme released 2 t of seed in this fashion. Within three years there was less seed than they started with. Perhaps this is a cynical example, but it demonstrates that unless there is strong administrative control and commercial incentive, both in terms of the breeding and the multiplication thereafter, the system is doomed to failure.

1.1.2.6 Decentralized breeding

Simmonds (1984) stated that many breeding programmes practice some degree of decentralized breeding, where special sites have been used for achieving particular breeding objectives (such as drought or disease). Some programmes, such as the CIMMYT wheat programme, made use of shuttle breeding (Braun *et al.*, 1996) which utilized selection at sites other than a single central breeding station to cater for varied adaptation requirements. The selection sites referred to by Simmonds (1984) and Braun *et al.* (1996) differ to the participatory approach in that they would normally be associated with research stations where infrastructure and expertise is available.

Nobel laureate Norman Borlaug started the shuttle breeding approach in CIMMYT in 1944 to speed up the breeding for stem rust (*Puccinia graminis*) in wheat (Braun *et al.*, 1996). It is considered one of the cornerstones of CIMMYT's breeding methodology for an entirely different reason however (Wang *et al.*, 2003). The shuttling of selection generations between two key locations (Cd. Obregon and Toluca) in Mexico, lead to the broad adaptation of CIMMYT wheats. Without initially realizing it, the shuttle approach was responsible for the selection of photoperiodic insensitivity in wheat which permitted the widespread use of Mexican wheats. Key to the success of the shuttle approach was the choice of contrasting locations. Cd. Obregon is a low altitude arid site that uses irrigation whilst Toluca is a high altitude site which is favourable for disease development.

In a study of simulated responses to selection, Simmonds (1991) could show no clear benefit in adaptation by alternating contrasting environments. The strong differential response to selection in contrasting environments suggested separate specific adaptation programmes rather than a mixed-site compromise. It also supported the widely accepted lore that adaptation to an environment is best achieved by selecting in that environment. Acknowledging the scarcity of clear examples of selection responses to contrasting environments, Simmonds (1984) described the selection response to two contrasting South African environments (Shakaskraal and Pongola). The sugarcane (*Saccharum officinarum*) clones in this example performed best in the environment that they were selected in, and this response demonstrates the principle on which the decentralized selection strategy has been based. Simmonds (1991) complained that there was much data from trials conducted at contrasting sites, but very little which demonstrated the effects of systematic selection at

contrasting sites. Clearly there is still a need to demonstrate the response to selection in the early segregating generations, and it is out of this need that the concept of satellite selection sites evolved.

1.2 OBJECTIVES

Plant breeding lore for developing genotypes for a specific environment would advocate selection in that environment (Falconer, 1981). In practice, it is not always logistically possible to maintain a full breeding program in all target environments. The norm for soybean breeding would be to select the segregating generations in one environment (usually at the main breeding station) and the lines generated would then be tested in multiple target environments (Simmonds, 1991; Almeida, 2002; Orf, 2004). This strategy is generally effective in selecting superior genotypes if the genotypic reaction in the target environment is similar to the genotypic reaction in the selection environment. However, if there is a significant GxE interaction, this practice is unlikely to be the most effective method for developing varieties for that specific target environment.

Financial considerations may prevent the establishment and maintenance of separate breeding stations for all target environments, but may still allow the operation of satellite selection sites. A satellite selection site could be established at any facility (such as a remote testing site with little or no infrastructure) that would allow for effective selection of a segregating population. The breeding activities at a satellite selection site should be restricted to the minimum required to achieve a significant adaptation to that site; however, this has not yet been quantified. This study was designed to establish whether it is possible to secure site-specific adaptation to a target site by a single generation of selection in that environment.

1.3 MATERIAL AND METHODS

1.3.1 Breeding strategy

The data used in this study was generated in the soybean breeding programme of PANNAR, a private seed company headquartered in Greytown, South Africa. The breeding station is located at Greytown (Table 1.1), which has a moderate climate allowing

the evaluation of a wide range of maturity groups; IV through to VIII (Smit and de Beer, 1994; 1995). The limited size of the total South African soybean production area, however, does not justify a second breeding station. The target environment chosen for the establishment of a satellite selection site was Delmas, Mpumalanga, located within an important soybean production area. Unlike at the breeding station, genotypic adaptation at the satellite site is more specific and is restricted to maturity groups IV and V (Smit and de Beer, 1994; 1995).

Table 1.1 Selection site details

	Breeding Station	Target Environment
Location	Greytown	Delmas
Altitude (masl)	1012	1580
Latitude (°S)	29.04	26.08
Annual Rainfall (mm)	776	562
Crop rotation	Maize/soybean	Maize/soybean/maize/sunflower
Soybean planting season	1 November – 15 December	1 – 30 November
Trial planting dates:	5.11.2001	2.11.2001
	8.12.2001	27.11.2001
Best suited maturity group	Mid VI	Late IV

A conventional pedigree breeding procedure was used in the study, where visual selection was carried out up to the F₅ generation. Five populations were generated in 1996, all utilizing Forrest (Hartwig and Epps, 1973) as a female parent (Table 1.2). Forrest was a direct introduction from Mississippi to South Africa that was successfully planted across all production regions for a period of more than 10 y. Forrest has resistance to *Meloidogyne incognita* and *M. javanica* and showed good tolerance to the drought stress conditions that characterize dry-land soybean production in South Africa. Five male parents were chosen from a diverse range (Table 1.2) of well adapted, locally bred genotypes. PAN 430 was chosen as a parent because of its short stature and excellent resistance to lodging, a characteristic that was problematic in Forrest. Prima was a variety that dominated South African production for more than 15 y with adaptation across all the production environments. Wilge and PAN 556 had minor agronomic improvements

compared to Prima, but lacked the overall yield stability that characterized ‘the grand old lady of South African soybean production’. Hennops was an upright maturity group VI variety with an indeterminate growth habit. All six parents had good yield performance records at both localities (Smit and de Beer, 1994; 1995). The female parent Forrest had white flowers and all the male parents had purple flowers. Confirmation of the F₁ was done using flower colour as a marker.

Table 1.2 Characteristics of the six parental soybean varieties

	Female parent	Male parents				
	Forrest	PAN 430	PAN 556	Hennops	Prima	Wilge
Growth habit	D ^a	D	I ^b	I	I	I
MG ^c	V	IV	V	VI	V	V
Lodging (1-9) ^d	6	1	3	2	3	2

^a D= determinate

^b I= Indeterminate

^c MG= Maturity Group

^d(1-9) = rating scale, where 1 is good and 9 is poor

Each of the five F₂ populations was split in two equal quantities containing 900 seeds, in order to impose the two selection strategies. In the control selection strategy (CSS), all segregating generations were selected at the breeding station by the breeder. In the satellite selection strategy (SSS), F₂ plant selections were made at the satellite site in the target environment by a trial technician, after which all subsequent generations (F₃ through to F₅) were selected at the breeding station by the breeder. All breeding generations were space planted (0.15 m intra-row, 0.9 m inter-row) to enhance selection efficiency. Thirty F₂ plants from all of the five F₂ populations at each of the selection sites were selected. Pedigree selection was followed through to the F₅ generation, where three of the best lines from each population and for each selection strategy were individually bulked for testing in the F₆ generation. A selection index that considered all important agronomic criteria at harvest (including but not limited to: lodging; shattering; pod height; and green stem) in a single rating was used to determine the three best lines per population. In cases where the

index alone failed to discriminate the three best lines, progeny row yields were used to aid selection.

1.3.2 Evaluation of breeding lines

The effect of the two selection strategies (15 lines per selection strategy, represented by three $F_{4,6}$ lines from each of five different pedigrees) on yield adaptation was evaluated in trials set out in a randomized block design with two replications, planted in both the target environment and at the breeding station. The trials were planted early in November and repeated again at both locations at a later planting date in the same season (Table 1.1). The net plot size was two rows of 4.4 m, planted at an inter-row spacing of 0.9 m and seeded at a plant population of 380 000 plants ha^{-1} . Plots were harvested with a plot combine and the plot grain yields were converted to t ha^{-1} at 12.5% moisture.

The plant and lowest pod height (cm) per plot were obtained using a measuring stick placed upright in the centre of a row to measure a representative plant. Days to maturity (R8) were recorded, lodging and 'visual appeal' at harvest was rated on a scale of 1-9; where 1 is good and 9 is poor. Visual appeal is a selection index that considers all important agronomic criteria at harvest including (but not limited to) lodging, shattering, pod height and green stem in a single rating.

1.3.3 Statistical analysis

After F tests showed homocedasticity of the error variances derived from the analyses of the individual planting dates (Appendix 1.1), the data from the two planting dates at each of the locations were combined (Appendix 1.2). A three factor ANOVA was performed using Genstat (Version 4.2) to estimate the main effects of selection strategy, pedigree, trial location and their interactions. Least significant differences ($P = 0.05$) based on t -values were determined by Genstat.

1.4 RESULTS

1.4.1 Yield

The effect of using the satellite selection site to improve adaptation to the target environment can be quantified by comparing yields of the 15 lines developed using SSS with those of the 15 CSS lines (Table 1.3). The positive yield difference between SSS and CSS, as measured in the trials conducted in target environment, represents the beneficial effects of applying a single generation (F_2) of selection in the target environment. Although there was some variation in the effect of the two selection strategies across pedigrees, the mean difference between SSS and CSS of $+0.306 \text{ t ha}^{-1}$ was significant ($P = 0.05$).

In contrast the negative yield difference between SSS and CSS, as measured in the trials conducted at the breeding station, represents the detrimental effects that a single generation of selection at the satellite site had on yield adaptation to the breeding station location. Again the effect of selection strategy varied across pedigrees, with the mean effect of -0.164 t ha^{-1} not significant ($P = 0.05$).

Standardizing the effect of selection strategy by expressing it as a percentage of the control strategy (CSS) allows for the comparison of these responses across the two trial environments. The mean standardized effect of the SSS in the target environment was 11.9%, compared to -5.3% in the breeding station environment. In the target environment yield trials, the difference in the mean effect between the two selection strategies is attributable solely to selection of the F_2 in the target environment (Table 1.3). In contrast, in the yield trials at the breeding station, the difference in the mean effect of selection strategies resulted from the negative effect of selecting the F_2 in the target environment not being completely moderated by the positive effects of selection at the breeding station in the later filial generations ($-F_2 + [F_3 \text{ to } F_5]$).

Although three individual lines selected using the CSS were well ranked at the satellite site (Table 1.4), the frequency of SSS lines within the top 10 rankings (7/10) far exceeded those of the CSS, which demonstrates the effectiveness of the SSS strategy. All three CSS lines that ranked within the top 10 (JV933; JV942; JV910) at the satellite site, were also well ranked at the breeding station. In contrast at the breeding station, the domination of

the CSS strategy was less well defined based on individual line rankings. There were two individual SSS lines (JV919 and JV951) that ranked within the top 10 at both locations.

Table 1.3 Analysis of the effect of selection strategy on yield performance of soybean lines at two locations

Trial Location	Pedigree ^a	Selection strategy		Effect of SSS (SSS-CSS)	Standardised effect of SSS	Filial generation responsible for selection effect
		SSS ^b	CSS ^c			
		Yield (t ha ⁻¹)			% ^d	
Target Environment ^e	Forrest/PAN430	2.596	2.310	0.286	12.4	
	Forrest/PAN556	3.033	2.684	0.349	13.0	
	Forrest/Hennops	2.626	2.602	0.024	0.9	
	Forrest/Prima	3.169	2.446	0.723*	29.6	
	Forrest/Wilge	2.922	2.770	0.152	5.4	
	Mean ^g	2.869	2.563	0.306*	11.9	F ₂
Breeding Station ^f	Forrest/PAN430	3.023	2.834	0.189	6.7	
	Forrest/PAN556	3.075	3.021	0.054	1.7	
	Forrest/Hennops	2.855	3.065	-0.210	-6.9	
	Forrest/Prima	2.683	3.128	-0.445*	-14.2	
	Forrest/Wilge	2.920	3.327	-0.350	-10.5	
	Mean ^g	2.911	3.075	-0.164	-5.3	-F ₂ + [F ₃₋₅]

* Significant ($P = 0.05$)

^aEach pedigree is represented by the mean yield of three individual lines

^bSSS = Satellite selection strategy, F₂ selected in Target Environment, further selection of F₃₋₅ at Breeding Station

^cCSS = Control selection strategy, selected at Breeding Station from F₂₋₅

^d% = Difference between the selection strategies, expressed as a percentage of the yield of CSS

^eTarget Environment = Delmas

^fBreeding Station = Greytown

^gMean = mean derived from 15 lines x 2 reps x 2 planting dates, SED = 0.1073

Table 1.4 Yield ranking of individual lines tested at the satellite site and the breeding station, pooled over planting dates

Satellite site (Delmas)				Breeding station (Greytown)			
Rank	Selection strategy	Line code	Pedigree	Rank	Selection strategy	Line code	Pedigree
1	SSS ^a	JV963	Forrest/Prima	1	CSS ^b	JV930	Forrest/Wilge
2	SSS	JV951	Forrest/Wilge	2	CSS	JV933	Forrest/Wilge
3	CSS	JV933	Forrest/Wilge	3	SSS	JV917	Forrest/PAN556
4	SSS	JV916	Forrest/PAN556	4	CSS	JV910	Forrest/PAN556
5	SSS	JV919	Forrest/PAN556	5	CSS	JV936	Forrest/Prima
6	SSS	JV952	Forrest/Wilge	6	SSS	JV980	Forrest/Hennops
7	CSS	JV942	Forrest/Hennops	7	CSS	JV943	Forrest/Hennops
8	CSS	JV910	Forrest/PAN556	8	SSS	JV919	Forrest/PAN556
9	SSS	JV967	Forrest/Prima	9	SSS	JV994	Forrest/PAN430
10	SSS	JV983	Forrest/Hennops	10	SSS	JV951	Forrest/Wilge
11	SSS	JV966	Forrest/Prima	11	CSS	JV942	Forrest/Hennops
12	SSS	JV997	Forrest/PAN430	12	SSS	JV996	Forrest/PAN430
13	CSS	JV945	Forrest/PAN430	13	SSS	JV953	Forrest/Wilge
14	SSS	JV981	Forrest/Hennops	14	CSS	JV911	Forrest/PAN556
15	CSS	JV911	Forrest/PAN556	15	CSS	JV938	Forrest/Prima
16	SSS	JV996	Forrest/PAN430	16	CSS	JV935	Forrest/Prima
17	CSS	JV930	Forrest/Wilge	17	CSS	JV928	Forrest/Wilge
18	SSS	JV917	Forrest/PAN556	18	CSS	JV948	Forrest/PAN430
19	CSS	JV939	Forrest/Hennops	19	SSS	JV981	Forrest/Hennops
20	CSS	JV938	Forrest/Prima	20	CSS	JV939	Forrest/Hennops
21	CSS	JV935	Forrest/Prima	21	SSS	JV967	Forrest/Prima
22	CSS	JV936	Forrest/Prima	22	SSS	JV997	Forrest/PAN430
23	CSS	JV948	Forrest/PAN430	23	SSS	JV916	Forrest/PAN556
24	CSS	JV928	Forrest/Wilge	24	CSS	JV947	Forrest/PAN430
25	CSS	JV943	Forrest/Hennops	25	CSS	JV945	Forrest/PAN430
26	CSS	JV912	Forrest/PAN556	26	SSS	JV966	Forrest/Prima
27	SSS	JV994	Forrest/PAN430	27	CSS	JV912	Forrest/PAN556
28	SSS	JV980	Forrest/Hennops	28	SSS	JV952	Forrest/Wilge
29	SSS	JV953	Forrest/Wilge	29	SSS	JV963	Forrest/Prima
30	CSS	JV947	Forrest/PAN430	30	SSS	JV983	Forrest/Hennops

^aSSS = Satellite selection strategy, F₂ selected in target environment, further selection of F_{3.5} at breeding station

^bCSS = Control selection strategy, selected at breeding station from F_{2.5}

1.4.2 Agronomic characteristics

For simplicity, data of agronomic characteristics measured or rated were combined over all pedigrees, both locations and both planting dates and only the main effects of the two selection strategies were then compared (Table 1.5). No significant differences between the strategies were found for lodging, plant height and visual appeal. The lines developed using SSS matured on average 2.9 d earlier than those developed using the CSS, which

was significant ($P = 0.05$). Pod height was also significantly lower ($P = 0.05$) in the SSS lines.

Table 1.5 Effect of selection strategy on agronomic characteristics, combined over all pedigrees, planting dates and locations

Characteristic	SSS ^a	CSS ^b	Difference
Lodging (1-9) ^c	2.7	2.9	-0.2
Maturity (days)	139.8	142.7	-2.9 *
Plant height (cm)	90.3	95.0	-4.7
Pod height (cm)	17.7	22.3	-4.6 *
Visual appeal (1-9)	3.1	3.2	-0.1

* Significant ($P = 0.05$)

^aSSS = Satellite selection strategy (F_2 selected in Target Environment, further selection of F_{3-5} at Breeding Station)

^bCSS = Control selection strategy (selected at Breeding Station from F_{2-5})

^c(1-9) = rating scale, where 1 is good and 9 is poor

1.5 DISCUSSION

In the target environment a yield response to the satellite selection strategy was expected *a priori*, but the magnitude of this response was not anticipated. A yield improvement of 11.9% relative to the control strategy in the target environment was achieved with selection of only the F_2 in the target environment (Table 1.3). To place the magnitude of the response in this study into perspective, long term annual yield gains in soybean breeding programs have generally been calculated as being between 1 to 2% (Wilcox, 2001; Ferrarotti, 2004; Singh *et al.*, 2004). The large effect of selection in the F_2 , when loci are highly heterozygous, strongly implicates additive gene action for the genes conditioning specific adaptation. The comparisons of individual line rankings at the two locations (Table 1.4) showed that it is possible to select lines at the breeding station (CSS) that would perform well at the satellite station and *vice versa*. Their good performance could conceivably be ascribed to general adaptation being carried forward to the F_5 . Nevertheless, the best line and the overwhelming majority of the better performing lines at

the satellite site were selected for specific adaptation to that site using the SSS. Whilst the lore that exclusive selection in an environment is the best way to achieve adaptation to that environment held true in this study at the breeding location, a third selection strategy (selection of all generations in the target environment) would be required to provide an estimate of the effectiveness of a satellite selection site relative to exclusive selection in a target environment.

One of the factors contributing towards the large relative effect of the satellite selection strategy was the dissimilar nature of the two selection environments (Table 1.1). It is possible, and indeed probable, that more subtle differences between environments may not elicit a response of the same magnitude. Selection of the parents in the trial was done specifically to include varieties that were adequately adapted to both localities. Not doing this could have amplified the difference between the strategies and skewed comparisons in a particular direction. In addition, the differing reactions between and within pedigrees indicates that there is variation amongst parents in genes for specific and general adaptation that could be exploited further. As an example, selection of the F₂ populations containing the male parent Prima produced lines which had specific adaptation to both F₂ selection environments. Responsive parents such as Prima are ideally suited to satellite selection programs. In contrast, selection within the population containing PAN 430 as a parent generated low yielding lines (particularly in the CSS) that did not demonstrate a consistent response pattern. The population containing Wilge as a parent produced a line (JV933) with exceptional general adaptation, being ranked highly at both localities (Table 1.4).

The difference in the mean effect of the selection strategies was smaller when measured at the breeding station than at the satellite site. This indicates that the subsequent selection (F₃-F₅) of the SSS lines at the breeding station improved their adaptation to that environment too, leading to a smaller (non-significant) difference between the strategies at that site. From this it is deduced that selection in more than one environment can improve general adaptation and reduces environmental sensitivity. This is consistent with the 'shuttle breeding' approach in wheat, referred to by Allard (1999). Use of a satellite selection site would thus not only improve specific adaptation to target sites but conceivably also improve adaptation to non-target environments (where subsequent selection is performed) thereby enhancing yield stability.

The two agronomic characteristics which differed significantly between the selection strategies were days to maturity and pod height (Table 1.5). In a study of maturity isolines of the genotype Clark, Zhang *et al.* (2001) reported the decrease in basal flower height with earliness. This causal and direct relationship between maturity and flower height (and ultimately pod height) would account for the significance of both characteristics. Based on prior knowledge of the maturity requirements for genotype adaptation to the two locations, the effect of SSS on maturity could have been predicted. Selection at the satellite site produced lines which, when tested, were on average 2.9 d earlier maturing than those selected exclusively at the breeding station. The extent to which maturity alone aided the yield adaptation to the target environment has not been quantified, but it can be assumed to have made some contribution. Individual early maturing lines (data not shown) selected in the CSS performed better than the mean at the target environment, which strengthens this argument. By simply selecting for earlier maturity at the breeding station, a level of general adaptation to the satellite site is achieved, which represents the historic methodology for cultivar development for Delmas in particular and the cool region in general.

Phenotypically, the two selection strategies produced lines of similar agronomic appeal. This is important since the technician that conducted the F₂ selections at the satellite location was given instructions on what criteria to select for, but had no experience in actually doing selections. Given this situation, it would not be unreasonable to expect the lines selected under these conditions to differ in their agronomic characteristics from the norm of the programme. However, a uniform selection pressure across strategies in the subsequent generations was effective in producing lines of a homogeneous visual appeal regardless of who selected the F₂. One of the benefits of utilizing a pedigree breeding system is that heritable characteristics such as lodging and shattering can be improved and manipulated through selection in the later generations. This allows for a greater freedom of using agronomists, technicians or farmers (as in the case of participatory breeding programmes) for selections at satellite sites without compromising the agronomic standards of the programme.

1.6 APPLICATION OF THIS RESEARCH

To demonstrate the ease with which this strategy could be implemented, a short account of how it has been applied in the PANNAR breeding programme follows. The satellite

selection breeding strategy takes advantage of existing structure (which has been put in place for multi-location testing of elite varieties) for the planting of segregating populations without requiring any additional resources.

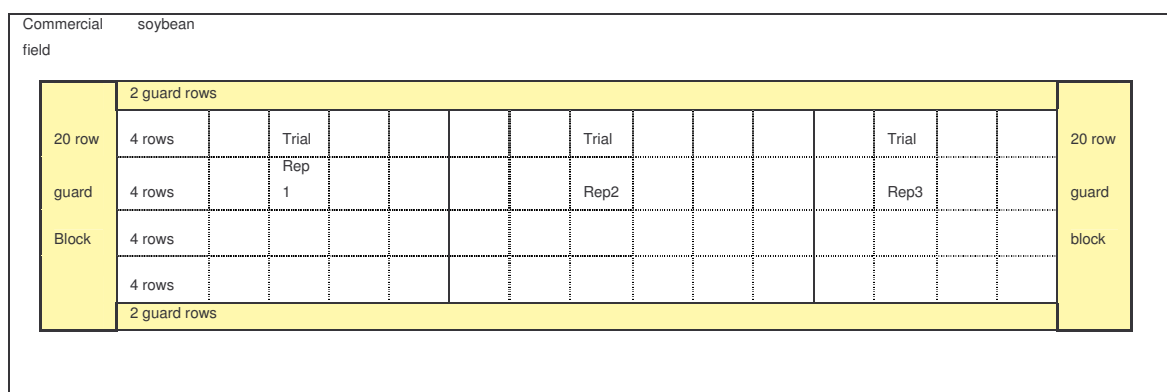
1.6.1 Multi-location testing structure

Elite variety testing takes place at 16 localities within the soybean production region. Some of the test sites are on existing research stations, but many are conducted on farms within commercial soybean fields. The exact sites vary from year to year, but some of the farmer co-operators have been assisting in the evaluation programme for over 20 y. The localities have been strategically chosen to get a good representation of the most important production regions. They also encompass a wide range of tillage systems (no-till, minimum till and convention tillage), row widths (0.3 to 0.9 m), soil types and herbicide programmes. The yield potentials vary dramatically between locations and over seasons, with the common range in trial mean between 1.0 and 4.5 t ha⁻¹, dependant on rainfall or irrigation. Planting date is typically at any time from the last week in October through to sometimes as late as the third week in December, if the trial follows wheat.

1.6.2 Layout of selection plots

The trial layout is fairly standard across sites: size 20 (4x5) row x column design using four row plots replicated three times (Fig. 1.2). Each trial thus uses 16 rows bordered on both sides by two guard rows, and 15 columns (or blocks) which would be bordered in the front and back by guard blocks. It is in these guard blocks of 20 rows that the satellite segregating populations are planted. The segregating populations are space planted at a plant intra-row spacing of 0.15 m; this means that each guard block can accommodate a population of 600 individuals. Each trial site therefore has the potential of accommodating up to two populations of 600 individuals or a single population of 1 200 if both fore and aft guard blocks are utilised.

Fig. 1.2 Segregating populations planted in the 20 row guard blocks flanking the elite variety statistical trials



1.6.3 Implementation of the satellite selection strategy

In the 2006/07 season, segregating F_2 populations were planted at 12 off-station trial locations for selection under farm conditions. All of these populations were also represented on at least one breeding station, where selection would continue in the conventional manner and effectively act as a control in the later evaluation of the success of the strategy. Two of the trial locations failed, so neither the trial nor the populations were harvested. For the remaining 10 populations, two were selected by the breeder and eight were selected by the trial technicians responsible for harvesting the adjoining trial. Twenty single plants were selected (selection intensity of 3.3%) from each population, labelled and returned to the Greytown breeding station for further processing. Both of the trial technicians involved in the selection agreed that selecting these 20 single plants made no significant difference to their effort required at that locality.

The selections made at the satellite sites were then re-included in the pedigree breeding programme as F_3 families at the breeding station, alongside their siblings selected only at the breeding stations. Colour coding of the breeding book entries (Fig. 1.3) was done to help distinguish the sources of the F_3 lines and to highlight families that originated from satellite selection sites. A more lenient selection for adaptation to the breeding station would be applied to these families compared to those that had been selected exclusively at the breeding station. It was theorized that by applying a lenient selection pressure for

adaptation in families where the primary selection location was other than the breeding station, fixing genes for specific adaptation to those satellite sites would be maximized. Priority was given to selection for agronomic attributes whilst the lines were advanced to homozygosity. These populations were not made up specifically with the satellite site in mind. Rather, they were made up to target one of the three major production regions (cool, moderate or hot), and then allocated to the most appropriate satellite site within that region. A future refinement of the strategy could be to make up populations based on their parents' performance at that locality *per se*. For some of the localities there would be up to 20 years of replicated trial data available, and although genotypes and cultivation methods or practices have changed over this period, it would be possible to make up parental combinations that would have specific adaptation to that locality.

Fig. 1.3 Excerpt from the breeder's book, detailing the colour coding of the families derived from satellite selection sites. F₂ selections made at the breeding station are coded in black, Delmas in blue and Groblersdal in red

F ₃ Row	F ₂ Source	F ₁ Source	Cross Code	Pedigree
07G72134	06G66142a	05GS035	JX750	JV1096/A430
07G72135	06G66143a	05GS035	JX750	JV1096/A430
07G72136	06G66145a	05GS035	JX750	JV1096/A430
07G72137	06GD66939a	05GS035	JX750	JV1096/A430
07G72138	06GD66940a	05GS035	JX750	JV1096/A430
07G72139	06GD66941a	05GS035	JX750	JV1096/A430
07G72149	Groblersdal10a	05GS035	JX750	JV1096/A430
07G72150	Groblersdal11a	05GS035	JX750	JV1096/A430
07G72151	Groblersdal12a	05GS035	JX750	JV1096/A430

True participatory breeding could or should involve farmer input (Ceccarelli and Grando, 2007). Although none of the co-operating farmers were involved in the selection process, the satellite selection strategy could potentially make use of interested farmers in the future. The satellite site strategy could be classified as a form of participatory plant breeding, an approach which has until now had more support in third world agriculture than success. Use of farmers for selection is not something that would be particularly practical in a commercial farming operation. Some farmers may well have an interest in

soybean improvement and practically all farmers have strong views on the perfect ideotype for their conditions, but few would have time during the short commercial harvesting window to consider getting involved. From a practical perspective it would be preferred to have the plant breeder (or technical staff responsible for the trial site) interact with the farmer during the season and make sure that his needs are accounted for in the selection process. The fact that only one generation is selected in the target environment means that each year a new population could be selected at that site, elevating the chances of success with this approach.

Final evaluation of the success of the systematic application of the satellite selection site strategy can only be made when the homozygous lines derived from the programme are tested in replicated trials across all 10 localities, using lines selected exclusively at the breeding station as a control. Initial indications after all F_3 families were selected alongside each other at the breeding station was that the different selection pressures at the various satellite sites did reveal variation in the populations which was different to that generated at the breeding station. For example, the families selected at Potchefstroom shattered considerably more than lines selected from the same population at Greytown. This may be more of a function of selection date rather than locality or selector. Lines selected at Winterton lodged significantly less on average than lines from the same population selected at the breeding station. Winterton is an irrigated high potential site. Whilst these observations are very provisional in nature, the early indications are that the systematic use of satellite selection sites has the potential to broaden the adaptation base of a breeding programme significantly. All breeding programmes require a testing structure, so the practical implementation of satellite selection sites should be universally possible.

1.7 CONCLUSION

This study showed that it is possible to secure a significant level of adaptation to a target site by a single generation (F_2) of selection in that environment. It also demonstrated that the utilization of a satellite breeding site could be an efficient and practical method of expanding the effectiveness of established breeding programmes. The two locations used in the main study are not important to the conclusion except for the magnitude of the differing cultivar reaction they evoke. Caution should therefore be exercised when extrapolating these results to other environments and the strategy in general to other

breeding programmes. This selection strategy is being expanded to include many additional localities, but has already been successfully employed by the author using the Greytown breeding station and a satellite site near Harare (Zimbabwe), leading to a cultivar registration in that country (PAN 806). Conditions for soybean growth and adaptation at Greytown and Harare are so dissimilar that it is not possible to make progress at the breeding station for either yield or agronomic traits (such as lodging and shattering) which must represent an extreme limit in the application of the strategy. Although pedigree breeding is not commonly employed in commercial soybean breeding, the use of a satellite selection site could be adapted to other breeding procedures including the early generation testing system described by Cooper (1990). The benefits of utilizing a satellite selection site would vary in magnitude depending on a number of factors, including the choice of parents and the dissimilarity of the two environments from each other.

One of the criticisms of an anonymous reviewer of the journal article based on this chapter (General appendix: Publication 1) was that the same person did not do the selection at both the satellite site and the breeding station, leading to confounding of the effects of the SSS and the effect of the selector. The criticism is valid, but since this represented the very first attempt at participatory breeding for the technician who was not yet skilled in selection, it is likely that the difference between the strategies demonstrated is a conservative estimate. The objective of the research was to develop a strategy that would improve on the *status quo* or the control strategy. It was not to compare the effectiveness of one selector with another, nor was it to do a full GxE analysis of the data. Accepting any bias that may be inherent in the data, the aim of using a satellite selection site was to acquire specific adaptation to a target environment. In the process of achieving this, it has also been shown that selection at more than one environment improves general adaptation, which is an important objective for any commercial programme.

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APPENDICES 1

Appendix 1.1 Test for homocedasticity of error variances.

Table A1.1 Analysis of variance, Greytown early planting date

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.32267	0.32267	4.50	
Rep.*Units* stratum					
Genotype	29	4.49889	0.15513	2.16	0.021
Residual	29	2.08057	0.07174		
Total	59	6.90213			

Table A1.2 Analysis of variance, Greytown late planting date

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.32380	0.32380	4.95	
Rep.*Units* stratum					
Genotype	29	8.46555	0.29192	4.46	<.001
Residual	29	1.89816	0.06545		
Total	59	10.68752			

Table A1.3 Analysis of variance, Delmas early planting date

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0010	0.0010	0.01	
Rep.*Units* stratum					
Genotype	29	25.9539	0.8950	4.85	<.001
Residual	29	5.3472	0.1844		
Total	59	31.3021			

Table A1.4 Analysis of variance, Delmas late planting date

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0413	0.0413	0.31	
Rep.*Units* stratum					
Genotype	29	10.0570	0.3468	2.60	0.006
Residual	29	3.8711	0.1335		
Total	59	13.9693			

F test for homocedasticity of the error variances:

For the two trials at Greytown:

$$F = s_1^2 / s_2^2 = 0.07174 / 0.06545 = 1.096 \text{ ns at 10\% probability}$$

For the two trials at Delmas:

$$F = s_1^2 / s_2^2 = 0.1844 / 0.1335 = 1.381 \text{ ns at 10\% probability}$$

Appendix 1.2 Pooled line performances at the Satellite site and breeding station

Table A1.5 Analysis of variance, pooled for Delmas

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	9.2983	3.0994	11.79	
Rep.*Units* stratum					
Genotype	29	22.3578	0.7710	2.93	<.001
Residual	87	22.8714	0.2629		
Total	119	54.5275			

Table A1.6 Analysis of variance pooled for Greytown

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	10.56643	3.52214	37.68	
Rep.*Units* stratum					
Genotype	29	8.81008	0.30380	3.25	<.001
Residual	87	8.13310	0.09348		
Total	119	27.50961			

CHAPTER 2

CROP MANAGEMENT CHALLENGES ASSOCIATED WITH SOYBEAN RUST

2.1 LITERATURE REVIEW: SOYBEAN RUST

2.1.1 Introduction

Soybean rust (*Phakopsora pachyrhizi* Sydow) was reported on soybeans in the Vryheid district of South Africa in February 2001 (Pretorius *et al.*, 2001), and later identified in several other parts of KwaZulu-Natal and Eastern Highveld production regions. Epidemics of soybean rust have since occurred every season to date (2008) and chemical control has become a standard commercial practice in the affected growing regions of the country. Shortly after rust was identified in neighbouring Zimbabwe in 1998, a soybean rust workshop (Smit, 1998) was convened in Potchefstroom, South Africa, to familiarize local researchers with the disease and develop a pre-emptive national soybean rust strategy. Through visits to Zimbabwe in the three year period between the first outbreak in Zimbabwe and the first reported outbreak in South Africa, many local researchers gained valuable experience in identifying the disease and managing the epidemics (Smit, 1999). Consequently, commercial losses in the first two seasons were far less than they could have been, as chemicals and protocols used in Zimbabwe were adopted until local research could support the soybean cropping industry. A soybean rust task team was established to coordinate research and refine a national strategy to combat the disease.

There are approximately 80 species of *Phakopsora* known worldwide (Hennen, 1996), of which six occur on legumes. Soybean rust is caused by two species, *P. pachyrhizi* and less commonly *P. meibomiae* (Arthur) Arthur. The latter species, commonly known as the cause of Latin American rust or Legume rust, is found in the western hemisphere and is not known to cause severe yield losses (Hartman *et al.*, 2005). The nomenclature history of these two species of rust is complex and their correct assignment in early reports, especially from Africa, remains uncertain (Hennen, 1996). The rust research reported on in this thesis is restricted exclusively to *P. pachyrhizi*, known commonly as Asian soybean rust, or simply soybean rust hereafter.

2.1.2 Historical background and geographical distribution

2.1.2.1 Eastern Hemisphere

Before 1992, soybean rust was known to cause significant losses in Asia and Australasia, inclusive of the following countries: Australia; India; Indonesia; Japan; Korea; Peoples Republic of China; Philippines; Taiwan, Thailand; Vietnam (Wang and Hartman, 1992).

2.1.2.2 Africa

Whilst the distribution of soybean rust in Africa before 1996 remains uncertain (given the problems with nomenclature), the following sequence of first reports (Levy, 2003) were confirmed: Uganda, Kenya and Rwanda, 1996; Zimbabwe and Zambia, 1998; Nigeria, 1999; Mozambique, 2000; South Africa, 2001.

2.1.2.3 Latin America

During 2001 *P. pachyrhizi* was detected in Paraguay (Morel *et al.*, 2004) and this was followed shortly by confirmation of presence in Argentina in 2002 (Rossi, 2004), Brazil and Bolivia in 2003 (Yorinori, 2004). Uruguay, also a significant soybean producing country, recorded soybean rust for the first time in 2004 (Stewart *et al.*, 2005).

2.1.2.4 USA

Rust was detected in Hawaii in 1994 (Killgore and Heu, 1994) which stimulated the convening of a workshop to discuss the potential threat that this held for the soybean crop in the USA. As correctly predicted by the delegates of this workshop (Sinclair and Hartman, 1996), soybean rust had the potential to threaten crops on mainland USA. In 2004, nine years later, Schneider *et al.* (2005) confirmed the presence of soybean rust in the USA. From detection in Louisiana in 2004, it spread to nine states in 2005, and was detected in 15 states in 2006 (Hartman, 2007).

2.1.3 Biology of soybean rust

2.1.3.1 Alternative hosts

Soybean rust is known to naturally infect 95 species from 42 genera of legumes (Hartman *et al.*, 2005), inclusive of important weed species like Kudzu vine (*Pueraria lobata*) and major crop species such as dry bean. Such a broad host range is unusual amongst rust pathogens which normally have a narrow host range (Hartman *et al.*, 2005). The significance of the numerous alternative host possibilities for soybean rust, is, that these may serve as an inoculum reservoir or a 'green bridge' from one soybean planting season to the next.

2.1.3.2 Symptoms of soybean rust

First symptoms of soybean rust could be described as small water soaked lesions which develop into grey, tan to dark brown, or reddish brown lesions particularly on the abaxial leaf surface (Sinclair and Hartman, 1999). The colour of the lesions is dependent on lesion age and interaction with the genotype. Lesions tend to be restricted by leaf veins and may reach 2-5mm in size. Red brown (RB) lesions with little sporulation indicates a semi-compatible reaction, whereas Tan lesions with much sporulation (Fig 2.1) indicates a fully compatible reaction. During the early stages of development, before sporulation (Fig 2.2), soybean rust may be confused with bacterial pustule (*Xanthomonas campestris* pv *glycines* (Nakano) Dye) (Sinclair and Hartman, 1999).

2.1.3.3 Epidemiology of soybean rust

The presence of a susceptible host, viable pathogen spores and suitable environmental conditions are requisite for the development of a soybean rust epidemic to occur. The optimum temperature for urediniospore germination ranges between 12 to 27°C, depending on the source of the research (Table 2.1). Spore germination is better in darkness, with light either inhibiting or delaying germination (Marchetti *et al.*, 1976). A further requirement for urediniospore germination is a period of leaf wetness. This period is accepted to be about 6 h when this occurs within the optimal temperature range (Tschanz and Shanmugasundaram, 1984). Hyphal penetration of the soybean leaf by the pathogen

occurs via an appressorium, which unlike many other rusts, penetrates directly through the cuticle into the epidermal cell of the leaf (Bonde and Peterson, 1995). Primary and secondary hyphae colonize the leaf mesophyll tissue, followed by uredinia formation and sporulation. The optimum temperature for uredinia formation is reported by Kochman (1979) to be 17°C (night) and 27°C (day). A telial stage is also known in soybean rust (Yeh *et al.*, 1982) but teliospore germination is thought to be rare (Bonde and Peterson, 1995). Shanmugasundaram (1999) summarized the sequence of events in the development of soybean rust (Table 2.2).



Fig. 2.1 Field symptoms of a fully susceptible (Tan) reaction, with profuse sporulation on the abaxial leaf surface



Fig. 2.2 First symptoms after infection and before sporulation may be difficult to diagnose as soybean rust

Table 2.1 Temperature requirement for urediniospore germination

Temperature °C		
Optimum	No germination	Reference
15-20	<5 and >33	Keogh (1974)
12-21	<9 and >28	Melching and Bromfield (1975)
15-25	<10 and >28.5	Marchetti <i>et al.</i> (1976)

Table 2.2 Summary of the sequence of events in the development of soybean rust (Shanmugasundaram, 1999)

Event	Time	Sequence
A urediniospore lands on the leaf surface	0	HPI ^a
Germ tube development	12	HPI
Appressorium cone formation	16	HPI
Penetration hyphae formed	16	HPI
Primary hyphae produced	18-20	HPI
Collapse of epidermal cell	24	HPI
Secondary hyphae formation	48-72	HPI
Mycelia development in mesophyll tissue	3	DPI ^b
Collapse of appressorium and penetration hyphae	4	DPI
Necrotic lesions appear on the leaf	6	DPI
Runner hyphae pass through mesophyll cells	7	DPI
Hyphae aggregate to form uredial primordia	9	DPI
Urediniospores mature	11-12	DPI

^aHPI = Hours post infection

^bDPI = Days post infection

2.1.4 Distribution and spread of soybean rust

There has not yet been a formal attempt to survey the distribution of soybean rust in South Africa; however, the reports of positive identification of soybean rust sent in by experienced researchers to the soybean rust task team secretary have been collated for the period 2001 to 2008 and presented in Table 2.3. The reports increased in frequency over the years surveyed as more scientists became involved in reporting. The lack of reports in 2001 to 2005 does not reflect less rust, only less reporting. In order to get an idea of the distribution of soybean rust, localities that have had one or more reports of soybean rust have been plotted on a rainfall map of South Africa (Fig. 2.3). The highest incidence of soybean rust reports coincides with the high rainfall regions east of the Drakensberg mountain range.

Table 2.3 A compilation of soybean rust reports made to the soybean rust task team

Date	Location	Reporter ^a	Date	Location	Reporter
8 Feb 2001	Vryheid	H. Oellerman	16 Feb 2006	Vryheid	M. Craven
6 Mar 2001	Howick	K. Horne	24 Feb 2006	Morgenzon	M. Craven
9 Mar 2001	Ahrens	F.J. Kloppers	27 Feb 2006	Amersfoot	W. van Wyk
14 Mar 2001	Greytown	J.A. Jarvie	3 Mar 2006	Winterton	M. Craven
-Mar 2001	Amersfoot	Un-confirmed	14 Mar 2006	Normandien	M. Craven
-Mar 2001	Ermelo	Un-confirmed	14 Mar 2006	Kinross	M. Craven
-Mar 2001	Piet Retief	Un-confirmed	15 Mar 2006	Kroonstad	F.J. Kloppers
4 Feb 2002	Cedara	E.D. Du Preez	30 Mar 2006	Kestell	M. Craven
15 Feb 2002	Amsterdam	J.L. Purchase	3 Apr 2006	Potchefstroom	M. Craven
15 Feb 2002	Greytown	J.A. Jarvie	24 Apr 2006	Bothaville	F.J. Kloppers
8 Jan 2003	Cedara	E.D. Du Preez	25 May 2006	Letsitele	J.A. Jarvie
8 Jan 2003	Karkloof	E.D. Du Preez	25 Jan 2007	Cedara	A. Liebenberg
14 Feb 2003	Greytown	J.A. Jarvie	26 Jan 2007	Piet Retief	M. Craven
26 Jan 2004	Cedara	E.D. Du Preez	31 Jan 2007	Greytown	F.J. Kloppers
26 Jan 2004	Karkloof	E.D. Du Preez	1 Feb 2007	Vryheid	M. Craven
16 Feb 2004	Greytown	F.J. Kloppers	1 Feb 2007	Morgenzon	M. Craven
8 Apr 2004	Ermelo	P. Kruger	12 Feb 2007	Merrivale	N.C. van Rij
3 Jan 2005	Cedara	E.D. Du Preez	22 Feb 2007	Normandien	M. Craven
11 Jan 2005	Karkloof	E.D. Du Preez	1 Mar 2007	Bergville	E.D. Du Preez
3 Feb 2005	Winterton	E.D. Du Preez	8 Mar 2007	Besters	E.D. Du Preez
3 Feb 2005	Weenen	E.D. Du Preez	25 Jan 2008	Cedara	N.C. van Rij
24 Feb 2005	Greytown	J.A. Jarvie	28 Jan 2008	Greytown	S. Tweer
9 Mar 2005	Winterton	N. Hackland	7 Feb 2008	Vryheid	M. Craven
1 Feb 2006	Karkloof	S. Tweer	7 Feb 2008	Baynesfield	P.M. Caldwell
1 Feb 2006	Cedara	S. Tweer	5 Mar 2008	Normandien	M. Craven
2 Feb 2006	Greytown	E.D. Du Preez	26 Mar 2008	Seven Oaks	J.A. Jarvie
3 Feb 2006	Sudwala Cave	Z.A. Pretorius	7 Apr 2008	Winterton	J.A. Jarvie
9 Feb 2006	Piet Retief	M. Craven	7 Apr 2008	Grobblersdal	J.A. Jarvie

^a Contact addresses detailed in Appendix 2.1

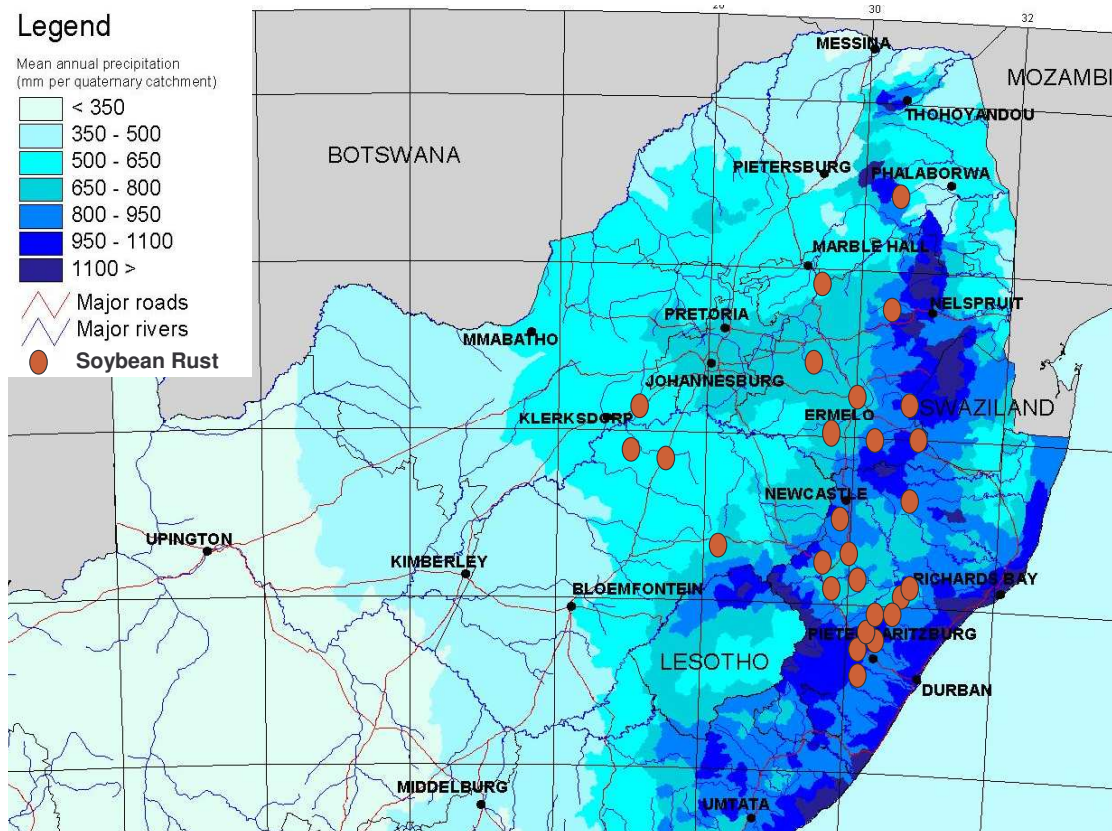


Fig. 2.3 Distribution of locations with one or more reports of soybean rust during the period 2001-2008, superimposed on the annual rainfall map of South Africa (Map source: Surface resources of South Africa, 1990)

During the 2006 season, reports of soybean rust were obtained atypically far west of the normal distribution, but mostly too late in the season (Table 2.3) to have a significant impact on yield. The collated reports are probably not ideally suited to make judgements on the progression of the disease, because the date of the report is not always a particularly good indication of the start of the epidemic. However, in seasons that had sufficient reports to substantiate a trend (2006 to 2008), first reports for the season generally started in the east and progressed westward. This may indicate a closer proximity to the inoculum source in the eastern production region, or simply that weather conditions favouring infection and development of symptoms occur earlier in the season in the east compared to the west.

There is no literature on how the soybean rust pathogen survives from one season to the next in South Africa; however, Caldwell and McLaren (2004) established that it required a live host and did not survive on soybean stubble. Since most of the production regions receive significant frosts in winter, the pathogen is presumed to over-winter in frost free areas within the country. Soybean rust epidemics in the KwaZulu-Natal midlands normally originate from a few clearly distinguishable foci (Fig. 2.4) within a field, which would imply that initial infections have been started by a low concentration of windborne urediniospores. Infections that have resulted from urediniospores generated within these foci are a lot more uniform, clearly a function of inoculum concentration around these foci.



Fig 2.4 A soybean field showing the initial focus point of infection which served as a source of inoculum for the surrounding fields (Photo source: F.J. Kloppers)

Pivonia and Yang (2004) used a model to predict the likelihood of year-round survival of *P. pachyrhizi* across the world based only on historical temperature and moisture data. Host availability or presence of an inoculum source was not considered. They found that conditions for the survival of *P. pachyrhizi* were very favourable all along the east and

southern coast of South Africa. Since this area does not coincide with the soybean production area, it is likely then that the soybean rust pathogen survives the winter in this area on the many possible alternative hosts. Pretorius *et al.* (2007) established that Kudzu vine was one of the alternate hosts of *P. pachyrhizi* that provided a green bridge in South Africa for the survival of the pathogen through winter in the frost-free areas. It is speculated that this area then provides the initial inoculum source each season for the inland areas that have summer conditions favourable for the development of soybean rust. The consistency with which the epidemics have occurred at Greytown since 2001 (Table 2.4) would tend to support the postulation that the source of urediniospores is, at the very least, regional and that local epidemics are not reliant on major weather phenomena for the deposition of urediniospores from the tropics of Africa.

Table 2.4 Date of the first soybean rust symptoms recorded in sentinel plots for eight consecutive seasons at Greytown

Year	Date of first rust symptoms at Greytown
2001	14 March ^a
2002	15 February
2003	14 February
2004	16 February
2005	24 February
2006	2 February
2007	31 January
2008	28 January

^a In the first season, no specific early planted sentinel plot had been planted

2.1.5 Effect of soybean rust on yield

There is a dearth of published information on the effects of soybean rust on soybean yields in South Africa. The experimental data relating to the effects of soybean rust on yield that has been published has demonstrated considerable variability over seasons and genotypes (Caldwell and McLaren, 2004; McLaren, 2008). McLaren (2008) evaluated all the South African commercial soybean genotypes over two seasons and concluded that there was no

tolerance of economic value amongst them. He also observed that the yield loss sustained in earlier maturing genotypes was lower than in the later maturing genotypes and ascribed this to a longer retention of leaf material after infection relative to their maturity. This observation confirmed the earlier work of Caldwell and McLaren (2004) who had come to a similar conclusion but had conducted their research on only one genotype per maturity class, leaving some doubt as to whether the effect was genotype specific or maturity group related. Initial indications from the research of Caldwell and McLaren (2004) showed that planting date did influence the yield loss, but two seasons' data was not sufficient to substantiate a trend. Soybean rust symptoms were more severe in the 0.45 m than in 0.9 m row spacing, and this was attributed to poorer fungicide penetration into the canopy (Caldwell and McLaren, 2004). McLaren (2008) found that disease severity, as measured by the area under the disease potential curve (AUDPC), was poorly correlated with yield loss%. Mean yield loss varied considerably between the two seasons (2003/04 and 2004/05) and genotype ranking for yield loss% was substantially different. The value of this research was to highlight the considerable variability of soybean rust epidemics over seasons and varied response of genotype. Jarvie and Shanahan (in press) have attempted to quantify yield loss and identify yield loss trends that could have significance in the management of soybean rust in South Africa.

2.1.6 Chemical control of soybean rust

Emergency registration of a number of chemicals (Du Preez and Caldwell, 2004) made it possible for farmers to control soybean rust epidemics in the first two seasons that it affected production in South Africa. Much debate in South African soybean workgroups revolved around the difference in rates used in Zimbabwe compared to the recommended chemical rates in South Africa. The fear existed that sub-optimal doses of chemical would promote the build up of pathogen resistance to the active ingredients that controlled soybean rust. With pathogen diversity and variability clearly demonstrated in host-pathogen relationships, this was a valid concern. Du Preez and Caldwell (2004) set out to evaluate the chemical control options in South Africa, which included the effective dosage rates, timing of application and frequency of applications. This research contributed towards a leaflet being published (Pretorius and McLaren, 2006) that made recommendations to soybean producers regarding control of soybean rust and included the registered chemicals (Table 2.5).

Table 2.5 Chemicals registered (November 2006) for the use of controlling soybean rust on soybeans in South Africa (Pretorius and McLaren, 2006)

Product name ^a	Active Ingredient	Company
Bayfidan 250DC	Triadimenol	Bayer
Capitan 250EW	Flusilazole	DuPont
D-Zole 250EC	Difenoconazole	Universal Crop Protection
Folicur 250EW	Tebuconazole	Bayer
Impact	Flutriafol	Cheminova
Lyric 250EW	Flusilazole	DuPont
Lyric C	Flusilazole/Carbendazim	DuPont
Pronto 250EC	Difenoconazole	Meridian Agritech
Punch C	Flusilazole/Carbendazim	DuPont
Punch Xtra	Flusilazole/Carbendazim	DuPont
Scope 250	Difenoconazole	Villa Crop Protection
Score 250EC	Difenoconazole	Syngenta
Shavit 25EC	Triadimenol	Makhteshim-Agan
Tebucure	Tebuconazole	Universal Crop Protection
Tristar EC	Triadimenol	Meridian Agritech

^aRegistered trade name

Du Preez and Caldwell (2004) established that effective chemical control varied in a range from 10 d (triforine) to 19 d (flusilazole/carbendazim), which supported the generalization that spray intervals should be no longer than 21 d apart, and that one to three sprays may be required. They also concluded that some chemicals (flusilazole/carbendazim) had limited curative action, whereas others such as azoxystrobin (not registered for use on soybean rust in South Africa) were only effective in preventative applications. This conclusion was very important to the national strategy used to control rust. If control was primarily preventative, then the timing of fungicide applications in the absence of symptoms would be crucial (Fig 2.5), a conclusion that was also reached by several other researchers (Miles *et al.*, 2003).



Fig 2.5 A commercial field of soybeans near Seven Oaks, KZN, showing dramatic defoliation where a fungicide swath had been accidentally missed

A reliable indicator of when to apply the first spray is required, bearing in mind that spraying too early would mean additional sprays, and spraying at first symptom would result in yield losses. As part of the national strategy to control soybean rust in South Africa, a series of 10 soybean indicator plots were planted throughout the production region, using early planting dates and genotypes which represented the extremes of maturity range for the country. These plots were not sprayed with fungicide and were monitored on a weekly basis from January through to April (Craven, 2008) for the presence of rust, both *in situ* and via leaf samples in the laboratory. These plots were used as sentinel crops to give producers advance warning of the presence and severity of the disease in an area. Producers were notified of the first presence of rust in their area via cellular phone SMS (text message) or alerts on farm radio programmes (Craven, 2008). The system of sentinel crops is currently also one of the methods being applied in the USA (Schonyers *et al.*, 2006) for the advance warning of the presence of the disease.

Systems that recommend spraying at predetermined soybean growth stages, for example at flower or at 60 days after planting (dap) as in Zimbabwe (Levy, 2004), do not take into consideration that the timing and severity of epidemics may have considerable seasonal variation. This could result in unnecessary spraying in some seasons. Hartman (2007), however, reported that there were occasional yield benefits to spraying fungicides in the absence of rust which may make this system both cost effective and simple to apply.

In 2005, a report from Washington State University (Feng *et al.*, 2005) claimed that Roundup (Glyphosate) herbicide had been found to have fungicidal action on *P. pachyrhizi* under laboratory conditions. Due to the popularity of RR soybean varieties in South Africa, Kloppers and Jarvie (unpublished data) performed a pilot study with sequential sprays of Roundup on an experimental RR variety to establish whether there was a need to pursue this avenue of research further. The preliminary results showed that pre-flower applications of Roundup had no effect on soybean rust severity, but post-flower applications visibly reduced the premature defoliation due to rust. Since Roundup used as a herbicide was primarily applied to soybeans at a pre-flower stage, it was felt that these findings would have little practical applicability and this line of research was not pursued further. The results of this pilot study were later confirmed by independent research conducted in the USA by Jurick *et al.* (2007). In their study, control of soybean rust by applications of Roundup at the R2 and R4 stage significantly improved yield over the untreated control, but the yield benefit and control of the disease was inferior to that of conventional fungicide (azoxystrobin) applications.

The combination of an efficient warning system and the effective application of fungicides have to a large extent averted significant financial losses as a result of soybean rust in South African soybean production. Whilst seasonal soybean rust epidemics will persist, and chemical control measures will continue to be necessary, further local research is still required to be conducted on the control of yield loss through genotypic means.

2.2 OBJECTIVE

Since soybean rust was first reported in South Africa during the 2000/01 season (Pretorius *et al.*, 2001), it has been present to a greater or lesser extent in the mid-altitude/high rainfall production region (mist belt) in all of the subsequent seasons to date (2008). Initially the emphasis of local research efforts concentrated on optimising chemical control programmes (Du Preez and Caldwell, 2004), which successfully limited commercial yield losses. With the short term crop security assured through the use of chemicals, the emphasis of research has turned to genetic control of the disease through the use of genotypes which resist or tolerate soybean rust.

In the seasons following the first report of soybean rust, several thousand genotypes were screened for their reaction to rust at the PANNAR research station at Greytown. The *P. pachyrhizi* race/s prevalent in South Africa caused symptoms on all the genotypes tested, including the set of resistance sources made available by the AVRDC (Asian vegetable research and development centre) and USDA (United States department of agriculture). This material included the sources of the four independent dominant genes (*Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*) that have been identified with race specific resistance. Hartman (1996) reported that the soybean rust pathogen had multiple virulence factors, which rendered the long term use of specific genes ineffective and questioned the value of this line of research. Consequently, breeding for tolerance has become an important objective in the Greytown breeding programme.

Tolerance implies susceptibility to the pathogen, and is quantified by the relative yielding ability of genotypes infected by rust. Relative yields are evaluated by comparisons of fungicide protected (sprayed) and unprotected plots (unsprayed) under field conditions in the presence of soybean rust. These evaluations are only of value if the rust epidemic is severe enough to cause significant yield loss and if the genotypes evaluated are well adapted to the environment in the absence of rust. The aim of this study is to analyse yield loss patterns that would assist in optimizing selection for tolerance in a breeding programme. This study investigates the interaction of genotype maturity, planting date and season with yield loss, and makes recommendations relevant to rust tolerance research.

2.3 MATERIAL AND METHODS

2.3.1 Genotypes

The criterion for genotype inclusion in these trials was commercial potential in the absence of rust. Genotypes included commercial cultivars, pre-commercial varieties and high yielding advanced lines in their final stages of testing. The genotypes evaluated were separated into two clusters of 20 entries each, based on their maturity. The first cluster included genotypes from maturity groups IV and V was called the MG45 cluster. The second cluster included genotypes from maturity groups VI to VIII and was referred to as the MG68 cluster. The primary objective of the clustering of genotypes was to evaluate the broad effect of genotype maturity on yield loss due to rust, without this being unduly biased by the effects of individual genotypes. The individual genotypes comprising the two clusters varied from season to season but the maturity group clustering remained constant over seasons. There were some common genotype entries between consecutive seasons, but only two entries were common to all three seasons.

2.3.2 Experimental design

The basic layout of the experiment was a factorial combination of two maturity clusters (MG) and two spray treatments (Treat), producing four treatment combinations which were randomised over three replications. The two levels of Treat were sprayed versus unsprayed, and the two MG clusters were MG45 and MG68. There were 20 genotypes (Vno) randomised in a 4 x 5 rectangular lattice within each MG cluster, thus the four treatment combinations were represented by four lattices (Appendix 2.2). These four lattices were repeated over two planting dates (PD) each season and the experiment repeated over three seasons (2002/03, 2003/04 and 2004/05), totalling 24 lattices or treatment combinations. The planting dates chosen (Table 2.6) were generally in the first week in November (Nov) and second week in December (Dec), which represented the extremes of the normal commercial planting season at Greytown.

Trials were hand planted at a seeding rate of 380 000 seed ha⁻¹. Each plot consisted of four rows of 4.4 m in length, spaced 0.9 m apart. The middle two rows were harvested with a

plot combine and yields were adjusted to 12.5% moisture. The sprayed lattices were sprayed before the first symptoms of soybean rust appeared in the trial, using the early planted sentinel plot warning system (Table 2.6). The sentinel plots were part of a national network (Craven, 2008) of early planted plots which provided advance warning for the need to spray. Spraying was done by knapsack using Punch C (Flusilazole/Carbendazim, SC 250/125 g l⁻¹) at the recommended rate of 400 ml ha⁻¹, and a second spray followed approximately 21 d later. In all seasons, soybean rust in the sprayed lattices was successfully controlled in the upper two thirds of the canopy for the duration of the growing cycle using this methodology.

Table 2.6 Soybean rust yield loss trial site details for Greytown, South Africa (S28°08'; E30° 37')

Season	Planting date		Annual	
	Nov	Dec	Rainfall (mm) ^a	Date of first rust ^b
2002/03	06.11.2002	11.12.2002	789.8	14/02/2003
2003/04	07.11.2003	08.12.2003	666.4	16/02/2004
2004/05	08.11.2004	10.12.2004	751.3	24/02/2005

^aThirty two year mean annual rainfall for Greytown = 832.6 mm

^bSoybean rust symptoms on the sentinel plots, used as an indicator of the earliest presence of rust symptoms in the area and a stimulus to start fungicide spraying

2.3.3 Statistical analysis

A number of different data analysis options were explored, including analysing the data as a split-plot in time using REML (Genstat version 10.2). After careful consideration, a simple factorial design analysed using the REML META analysis procedure (Genstat version 10.2) was deemed the most appropriate as it generated the same means as the split-plot in time analysis, but provided moderately conservative standard errors of differences (SEDs) for comparisons.

The factor MG comprised of twenty genotypes set out in a 4 x 5 lattice, which then formed part of the 3 x 2 x 2 x 2 (Season x PD x MG x Treat) factorial combination.

2.3.3.1 Statistical model 1

Analysis of the data was done using REML META analysis (Genstat Version 10.2), based on the following model:

Fixed = Season*PD*MG*Treat

Random = Rep/Block

Experiment = Lattice

[Statistical model 1]

Since the META analysis routine was used, a formal test of homogeneity of error variances of the individual lattices was not required as the error variance for each lattice was determined separately. The treatment factor MG was represented by 20 genotypes within each season, but because the genotypes constituting the MG clusters were not all common over the three seasons, Statistical model 1 was not resolved down to the level of genotype (Vno).

2.3.3.2 Statistical model 2

There were two genotypes that were common across all seasons and 12 genotypes that were common across two of the three seasons. Although the initial intention was not to assess reactions of specific genotypes to soybean rust infections, it was possible to resolve the treatment effects down to the level of Vno by using the following model with Vno nested within the interactions of the other treatment factors:

Fixed = (Season*PD*MG*Treat)/Vno

Random = Rep/Block

Experiment = Lattice

[Statistical model 2]

2.4 RESULTS AND DISCUSSION

2.4.1 Statistical model 1

This experiment was an auxiliary evaluation of the elite variety trial entries (PANNAR programme) for their reaction to soybean rust. For this reason the choice of genotypes in this experiment was not a random selection of genotypes to represent specific maturity groups, rather, they were purposefully chosen for their commercial potential and represent high yielding elite genotypes which were separated into two clusters based on maturity. Consequently MG and Vno (in Statistical model 2) are considered fixed effects.

2.4.1.1 Main effects

The Wald statistics for the main effects of Season, PD, Treat and MG were all significant (Table 2.7). The difference between the Treat effects (sprayed versus unsprayed) is ascribed to the negative influence of soybean rust on yield in the unsprayed treatment. This effect is termed yield loss, and for comparative purposes may also be expressed as a percentage of the unsprayed yield (yield loss %). The mean yield loss to soybean rust, over three seasons, two planting dates per season and 120 genotypes was 0.708 t ha^{-1} or 25% (Table 2.9) which was significant ($P = 0.05$).

Yields in the 2002/03 season (1.783 t ha^{-1}) were significantly ($P = 0.01$) lower than in 2003/04 (2.903 t ha^{-1}) or 2004/05 (2.767 t ha^{-1}). Yields in the Nov planting date were 0.236 t ha^{-1} higher (significant, $P = 0.05$) than the Dec planting date. Due to the temporal nature of planting date (PD) and Season, these factors could not be spatially randomised within the factorial design and any bias that is associated with that needs to be considered. All experiments over seasons have to deal with this issue. The mean yield difference between the two maturity group clusters was not significant (0.135 t ha^{-1}). Since there are significant interactions between the factors, the main effects are not of statistical interest.

**Table 2.7 Wald statistics for the REML META analysis using Statistical model 1:
Fixed terms = Season*PD*MG*Treat; Random = Rep/Block; Experiment = Lattice**

Fixed term	Wald Statistic	d.f	Wald/d.f	χ^2 pr
Season	4194.51	2	2097.25	<0.001
PD ^a	349.43	1	349.43	<0.001
Treat ^b	1108.10	1	1108.10	<0.001
MG ^c	92.55	1	92.55	<0.001
Season.PD	323.76	2	161.88	<0.001
Season.Treat	525.69	2	262.85	<0.001
PD.Treat	1.54	1	1.54	0.215
Season.MG	5.42	2	2.71	0.067
PD.MG	65.88	1	65.88	<0.001
Treat.MG	0.28	1	0.28	0.598
Season.PD.Treat	32.77	2	16.38	<0.001
Season.PD.MG	21.97	2	10.98	<0.001
Season.Treat.MG	29.77	2	14.89	<0.001
PD.Treat.MG	24.69	1	24.69	<0.001
Season.PD.Treat.MG	12.60	2	6.30	0.002

^aPD = Planting date

^bTreat = Fungicide spray treatment

^cMG = Maturity group cluster

2.4.1.2 First order interactions

The Wald statistic for the interaction between Season and PD was highly significant (Table 2.7). In the first two seasons (2002/03 and 2003/04) mean yields were significantly higher ($P = 0.01$) in the Nov planting date compared to Dec planting date. In the final season (2004/05), yields in the Dec planting date exceeded those of Nov by a significant margin ($P = 0.05$). Considering the bias that is potentially inherent in the main effects for PD and Season due to their temporal nature, no general inferences should be made from their interaction. The interactions between: PD and Treat; Season and MG; and Treat and MG generated non-significant Wald statistics. The MG45 cluster of genotypes had a

slightly smaller yield loss response (0.638 t ha^{-1} or 26.3%) to rust compared to MG68 (0.781 t ha^{-1} or 30.6%) when meaned over all planting dates and seasons, but the difference between the two was not significant. The interaction between PD and MG cluster was significant, as was the interaction between Season and Treat (Table 2.7).

2.4.1.3 Second order interactions

All three factor interactions were highly significant based on their Wald statistics (Table 2.7). Again, since the third order interaction was significant, the second order interactions are of lesser interest.

2.4.1.4 Third order interaction

The third order interaction in Statistical model 1 (Table 2.7) was highly significant (Wald statistic, $P = 0.002$). In 2002/03, there was no significant yield loss in the Nov planting date, and only the MG45 genotype cluster had a significant ($P = 0.05$) yield loss of 0.375 t ha^{-1} in the Dec planting date. In the following two seasons (2003/04 and 2004/05), all combinations of PD and MG presented significant ($P = 0.01$ and $P = 0.05$) yield losses as a result of soybean rust (Table 2.9).

With the exception of the 2002/03 season where the yield losses were similar (Table 2.9), the MG45 cluster was less affected by rust than the MG68 cluster of genotypes for the Nov planting date. Furthermore, the MG45 cluster consistently (all three seasons) had a lower yield loss in the Nov planting date compared to the Dec planting date. Yield loss trends in the MG68 cluster were less consistent.

Table 2.9 Mean effect of soybean rust on yield loss relative to a fungicide sprayed control at Greytown, at two planting dates over three consecutive seasons (2002/03, 2003/04 and 2004/05)

Season	Planting date ^a	Maturity cluster ^b	Yield (t ha ⁻¹)			Yield loss % ^d
			sprayed ^c	unsprayed	loss	
2002/03	Nov	MG45	2.179	2.079	0.100	4.6
		MG68	2.026	1.939	0.087	4.3
	Dec	MG45	1.591	1.218	0.373*	23.4
		MG68	1.727	1.506	0.221	12.8
		Mean	1.881	1.686	0.195*	10.4
2003/04	Nov	MG45	3.334	2.639	0.695**	20.9
		MG68	4.024	2.533	1.491**	37.1
	Dec	MG45	3.015	2.148	0.867**	28.8
		MG68	3.224	2.307	0.917**	28.4
		Mean	3.399	2.407	0.993**	29.2
2004/05	Nov	MG45	3.027	2.252	0.775**	25.6
		MG68	3.161	2.036	1.125**	35.6
	Dec	MG45	3.259	2.259	1.000**	30.7
		MG68	3.493	2.647	0.846**	24.2
		Mean	3.235	2.299	0.937**	29.0
All	Nov	MG45	2.847	2.323	0.524*	18.4
		MG68	3.070	2.170	0.900**	29.3
	Dec	MG45	2.622	1.875	0.747**	28.5
		MG68	2.815	2.153	0.662**	23.5
		Mean	2.839	2.130	0.708*	25.0

^aPlanting date: Nov = Early November, Dec = Early December

^bMaturity cluster: MG45 = 20 Maturity group IV and V genotypes; MG68 = 20 Maturity group VI to VIII genotypes

^csprayed = Two applications of Punch C @ 400 ml ha⁻¹

^dYield loss % = (sprayed yield - unsprayed yield)/sprayed yield x 100

* = Significant ($P = 0.05$); ** = Highly significant ($P = 0.01$)

2.4.2 Statistical model 2

In the analysis using Statistical model 2, treatment effects were resolved down to the level of Vno. Two genotypes (PAN 421R and PAN 520R) were common in all seasons, 12 genotypes common in two seasons and the rest only present in a single season, resulting in an unbalanced analysis. The REML META analysis routine is capable of analysing such unbalanced datasets, but only generated comparable means for the highest order interaction.

The interaction of genotype (Vno) with the other factors in the experiment (Table 2.8) was highly significant (Wald statistic, $P = 0.001$). The two genotypes that were common in all three seasons were maturity group IV and maturity group V genotypes that had been categorised in the MG45 cluster. There were no genotypes common to all seasons which were from the MG68 cluster. Yield losses to soybean rust, as measured by the difference between Treat levels in the genotypes PAN 421R and PAN 520R, were lower in the Nov planting date compared to Dec planting date in each of the seasons, with one exception (Table 2.9). In the 2003/04 season, PAN 421R yielded more in the unsprayed treatment than the sprayed treatment, which could be ascribed to experimental error and spatial separation of the plots at the Vno level. The design of the trial was perhaps not optimal for evaluating yield loss of **individual** genotypes because the spray treatment was imposed at the lattice level on each of the MG clusters. As a consequence the trial design maximized the precision in the comparison of the sprayed and unsprayed treatments at the MG cluster level which would lead to less precise comparisons at the Vno level.

Within the MG45 cluster, and in addition to PAN 421R and PAN 520R, two genotypes (PAN 494 and PAN 564) were common to the 2002/03 and 2003/04 seasons and three genotypes (X48R104, PAN 535R and JV1118) were common to the 2003/04 and 2004/05 seasons. Yield losses of these common genotypes were graphed (Fig 2.6) to confirm the general trends of MG45 established in the analysis of Statistical model 1. In Fig 2.6, the individual genotypes have been arranged on the x-axis in order of increasing maturity (data not shown) and the two planting dates graphed separately. A general trend (which is perhaps more pronounced in the Nov planting date) that yield loss % increases with increasing genotype maturity was evident. This trend was not evident in the 2002/03 season, but was pronounced in 2003/04 and 2004/05. Whilst general trends associated with

genotype maturity were evident, the extent of seasonal variation and genotypic variation was apparent in Fig 2.6. PAN 421R had an individual response that appeared to be either unassociated with maturity, or alternatively, atypical of its maturity classification.

Table 2.8 Wald statistics for the REML META analysis using Statistical model 2: Fixed terms = (Season*PD*MG*Treat)/Vno; Random = Rep/Block; Experiment = Lattice

Fixed term	Wald statistic	d.f.	Wald/d.f.	χ^2 pr
Season	7506.48	2	3753.24	<0.001
PD ^a	743.17	1	743.17	<0.001
MG ^b	34.81	1	34.81	<0.001
Treat ^c	1861.36	1	1861.36	<0.001
Season.PD	537.80	2	268.90	<0.001
Season.MG	0.11	2	0.05	0.948
PD.MG	209.49	1	209.49	<0.001
Season.Treat	993.10	2	496.55	<0.001
PD.Treat	5.47	1	5.47	0.019
MG.Treat	0.33	1	0.33	0.567
Season.PD.MG	28.00	2	14.00	<0.001
Season.PD.Treat	53.49	2	26.75	<0.001
Season.MG.Treat	38.79	2	19.40	<0.001
PD.MG.Treat	40.79	1	40.79	<0.001
Season.PD.MG.Treat	21.23	2	10.61	<0.001
Season.PD.MG.Treat.Vno ^d	1781.75	452	3.94	<0.001

^aPD = Planting date

^bMG = Maturity group cluster

^cTreat = Fungicide spray treatment

^dVno = Genotype

Table 2.9 Yield loss to soybean rust in two genotypes, as measured by the difference between sprayed and unsprayed treatments. Trials were conducted at Greytown over three seasons (2002/03, 2003/04, 2004/05) using two planting dates per season

Season	Planting date ^a	Genotype	Yield (t ha ⁻¹)			Yield loss % ^c
			sprayed ^b	unsprayed	loss	
2002/03	Nov	PAN 421R	2.303	2.068	0.235	10.2
		PAN 520R	1.948	1.842	0.106	5.4
	Dec	PAN 421R	1.473	1.072	0.401	27.2
		PAN 520R	1.405	1.032	0.373	26.5
2003/04	Nov	PAN 421R	2.666	2.917	-0.251	-9.4
		PAN 520R	2.954	2.676	0.278	9.4
	Dec	PAN 421R	2.850	2.626	0.224	7.9
		PAN 520R	2.493	1.624	0.869**	34.9
2004/05	Nov	PAN 421R	2.844	2.588	0.256	9.0
		PAN 520R	3.100	2.576	0.524*	16.9
	Dec	PAN 421R	3.686	2.633	1.053**	28.6
		PAN 520R	3.267	2.454	0.813**	24.9

^aPlanting date: Nov = Early November; Dec = Early December

^bsprayed = two applications of Punch C @ 400 ml ha⁻¹

^cYield loss % = (sprayed yield-unsprayed yield)/sprayed yield x 100

* = Significant ($P = 0.05$)

** = Highly significant ($P = 0.01$)

Within the MG68 cluster, there were five genotypes (PAN 660, PAN 1564, PAN 626, PAN 854 and PAN 809) common to 2002/03 and 2003/04 and two genotypes (JV1134 and PAN 737R) common to 2003/04 and 2004/05. Yield loss % of these common genotypes were graphed (Fig 2.7) to confirm MG68 trends established in Statistical analysis 1. In Fig 2.7, the individual genotypes have been arranged on the x-axis in order of increasing maturity (data not shown) and the two planting dates graphed separately. As with the MG45 genotypes, the yield loss % in the 2002/03 season was different to 2003/04 and 2004/05 in magnitude and trend. A seasonal trend that yield loss increased with increased maturity was evident in the Nov planting date for the 2003/04 and 2004/05 seasons

(Fig 2.7). In the Dec planting date, this trend was less well defined for all seasons and more individual genotype deviation from the seasonal trend was evident.

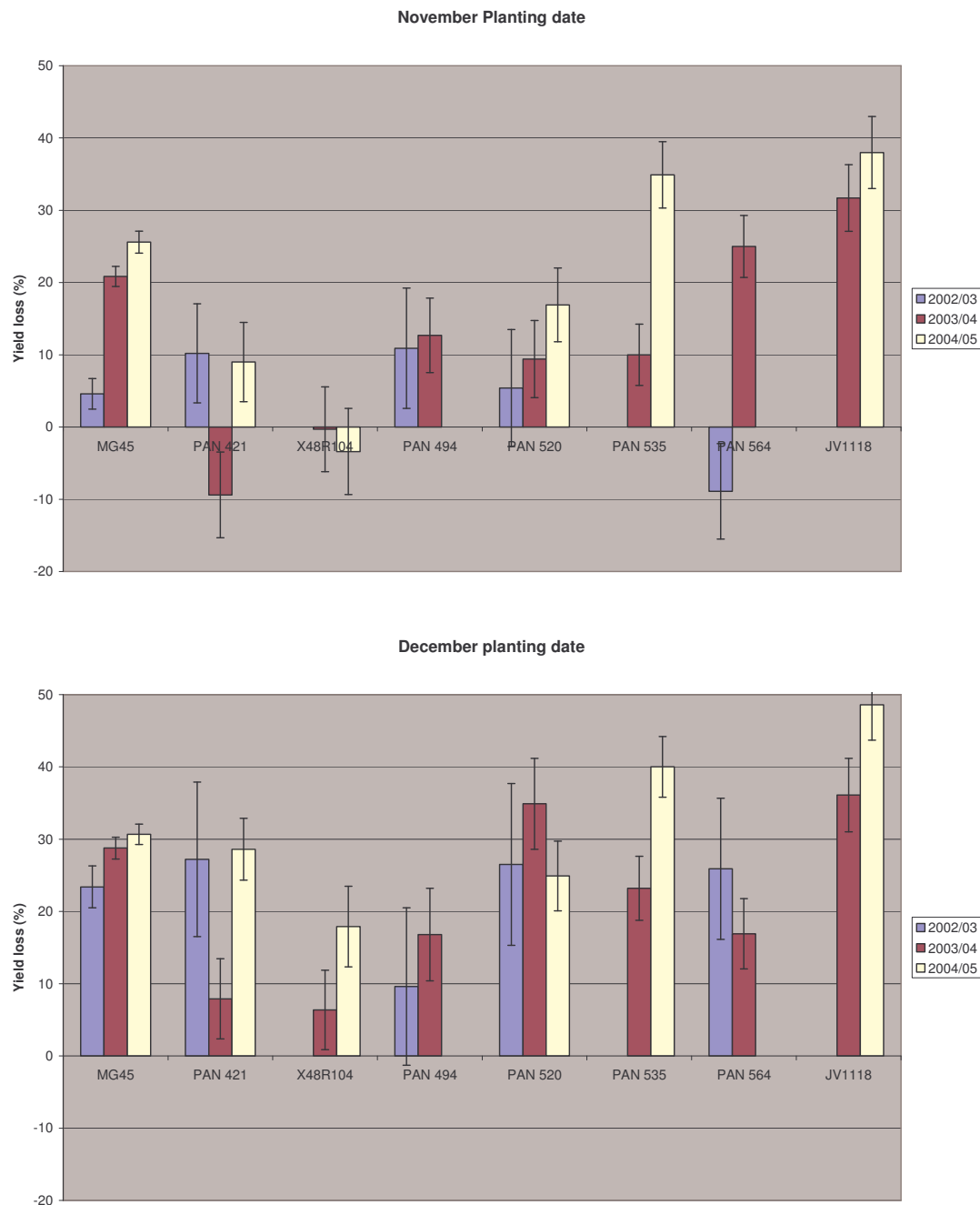


Fig 2.6 Percentage yield loss of individual genotypes compared to the MG45 genotype cluster mean for two planting dates and three seasons (2002/03, 2003/04 and 2004/05). The vertical line at the top of each bar represents the average SE (\pm) expressed as a % of the unsprayed mean

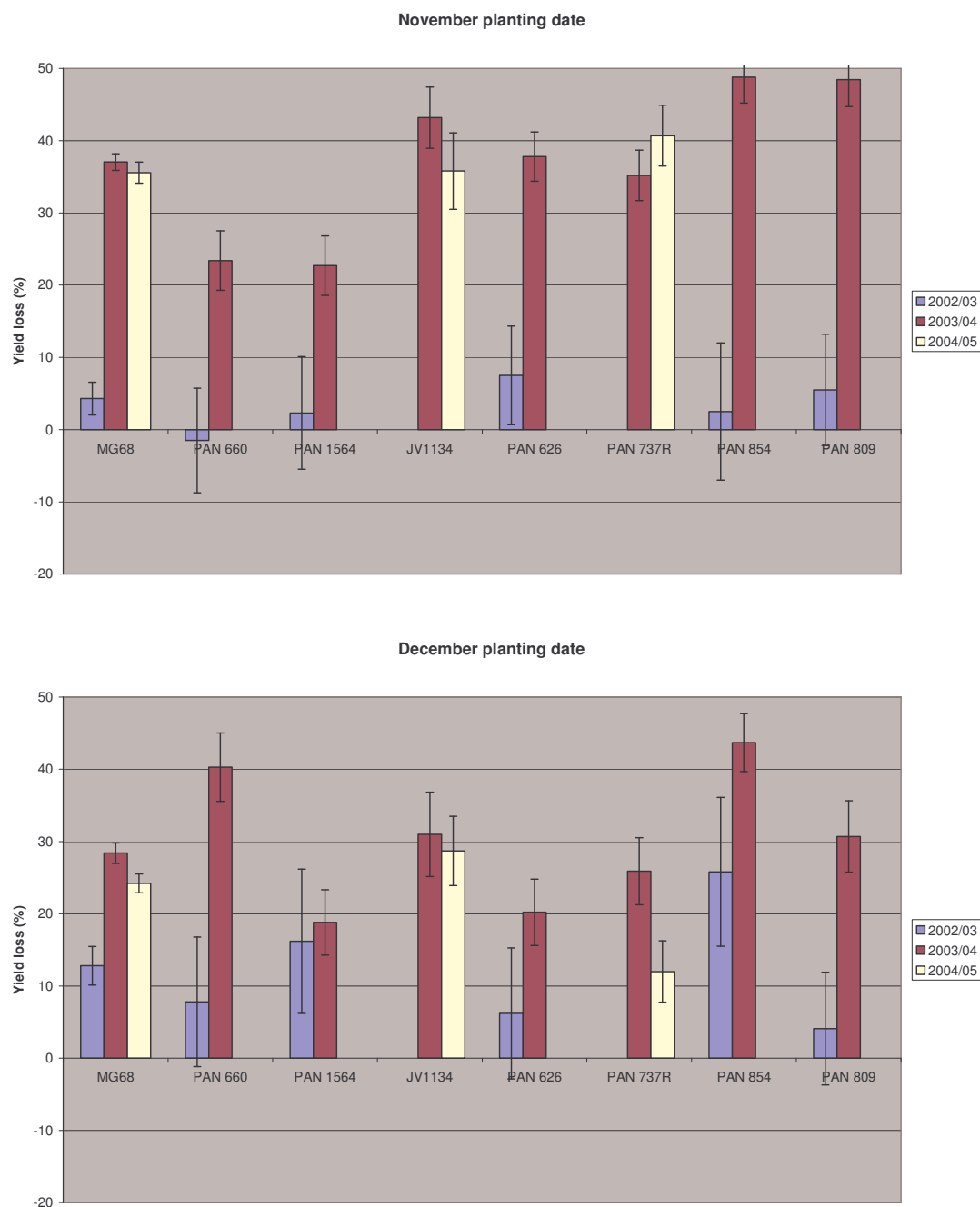


Fig 2.7 Percentage yield loss of individual genotypes compared to the MG68 genotype cluster mean for two planting dates and three seasons (2002/03, 2003/04 and 2004/05). The vertical line at the top of each bar represents the average SE (\pm) expressed as a % of the unsprayed mean

2.5 DISCUSSION

There is a need to quantify the effects of soybean rust on yield, and this is often done using a small number of genotype and environment combinations. In this study the main effect of Treat (sprayed versus unsprayed), derived from 120 genotypes from two PD per season and three consecutive seasons, quantifies the yield loss to soybean rust at Greytown at 0.708 t ha⁻¹ or 24%. The mean loss over three seasons is moderated by the fact that yield loss in the 2002/03 season was substantially less (10.4%) than in 2003/04 (29.2%) and 2004/05 (29.0%). Accounting for the lower yield loss in the first season of the experiment is difficult as there are many factors that could play a role in yield loss. Observations from the sentinel plots would indicate that the first incidence of rust was detected at roughly the same time for each of the seasons in this experiment (Table 2.6). Planting dates of the experiment were similar over the seasons, and the rainfall patterns (Appendix 2.3) in the latter part of the three seasons (March and April) were distinctly similar. Del Ponte *et al.* (2006) showed that cumulative rainfall in the period after initial rust detection was positively correlated to disease severity. By extrapolation then, seasons with similar initial rust detection dates and similar rainfall patterns are likely to have similar epidemics. With there being no obvious basis for the reduced yield loss in 2002/03, it is only possible to speculate on the possible causative factors. Since 2002/03 was only the third season of soybean rust at Greytown since initial detection in 2001, it is conceivable that the initial starting inoculum pressure was lower for this season than the following two, resulting in a less severe infection. Yield losses in the following two seasons were similar (0.993 t ha⁻¹ and 0.937 t ha⁻¹) averaging 29.1% loss (Table 2.9).

The use of a large number of genotypes within the MG clusters successfully dilutes the effects of individual genotypes and should give a representative indication of the effect of maturity on yield loss. The yield loss reaction (Fig 2.6 and Fig 2.7) of some individual genotypes may be atypical of their maturity classification, which implies that many genotypes are required to substantiate a trend. The poor discrimination between MG clusters in Statistical analysis 1 was not anticipated, given that it was based on the reactions of 20 genotypes and considering the crucial role physiological maturity of the host plays in rust infection and development. Whilst the large number of genotypes included in the trial gives the yield loss values substantial credibility, it has not improved the resolution of response to soybean rust as determined by MG. Broad maturity clustering

has been successfully used in genotype evaluation for yield adaptation to different environments, hence the use of the same clustering in this experiment. The reduced response to MG suggests that perhaps the maturity clusters were too broad to accurately and separately reflect interactions with maturity group without overlap in trends. The more distinct trends evident in the analysis of individual genotypes (Statistical model 2) would tend to support this suggestion.

In Statistical model 1 there were significant interactions between MG, PD and Treat (Table 2.9). The MG45 genotypes planted in Nov were the least affected by yield loss (0.524 t ha^{-1} or 18.4%) as a result of soybean rust when compared to other combinations of MG and PD, which varied from 23.5% to 29.3% (Table 2.9). Analysis of the individual genotypes in Statistical model 2 supported this general trend (Fig 2.6), which strengthens the argument made by Caldwell and McLaren (2004) and McLaren (2008) that planting early maturing genotypes in combination with early planting dates could reduce the risk of yield loss to soybean rust. A likely explanation is that the reduced yield loss incurred by planting the MG45 genotypes in Nov arose from the partial escape of the rust epidemics. Planting maturity group IV or V genotypes in Greytown in an attempt to escape yield loss would be a strategy fraught with risks for commercial production. The mean yield loss due to soybean rust for the MG45 genotype cluster ranged between 4.6 and 25.6% over the three seasons for Nov PD (Table 2.9). At the low end of the range in yield loss (4.6%), commercial growers may find it financially viable not to spray their crop with fungicide. However, considering that chemical control is primarily preventative (Du Preez and Caldwell, 2004), commitment to a spray or no-spray strategy would need to be made by the grower before there is any indication of the severity of the impending rust epidemic. With the threat of yield losses as much as 25% (at the high end of the range) possible, the risk of this strategy would be economically prohibitive. Furthermore, in South Africa later maturing genotypes (MG68) are typically planted at early planting dates to make full use of the extended season. Any reduced yield loss to soybean rust attained by planting MG45 genotypes early (Nov) would need to be offset by their lower yield potential at this planting date.

Although the general trends displayed in Fig 2.6 and Fig 2.7 relied on comparisons of subsets of genotypes, it was evident that across the range of individual maturities tested yield loss to soybean rust increased with increase in genotype maturity regardless of

planting date. This has important commercial consequences and requires further verification using a fully representative set of genotypes across all levels of MG, PD and season.

2.6 CONCLUSION

Whilst it has been argued that early planting date in combination with early maturity is unlikely to be a reliable method of escaping rust for a commercial producer, the effect of late planting date has application in rust research. High yield losses are more consistently incurred with late planting dates. For rust tolerance research, it is important to ensure that all genotypes (regardless of maturity) are exposed to significant infections every season, and by planting late this can be consistently obtained.

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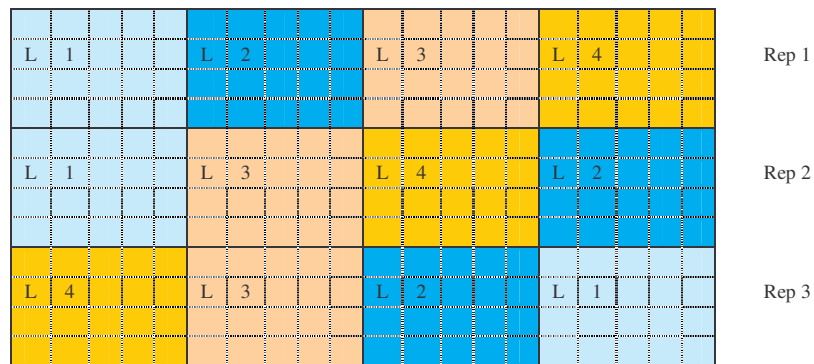
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APPENDICES 2

**Appendix 2.1 Contact addresses of soybean rust epidemic reporters listed in
Table 2.3**

Reporter^a	Email contact address
H. Oellerman	heinz.oellermann@pioneer.com
K. Horne	cropgro@saol.com
F.J. Kloppers	rikus.kloppers@pannar.co.za
J.A. Jarvie	antony.jarvie@pannar.co.za
E.D. Du Preez	eve.dunlop@pannar.co.za
J.L. Purchase	john@agbiz.co.za
P. Kruger	KrugerP@arc.agric.za
N. Hackland	nigel.hackland@basf.com
S. Tweer	stephanie.tweer@pannar.co.za
Z.A. Pretorius	pretorza.sci@mail.uovs.ac.za
M. Craven	CravenM@arc.agric.za]
W. van Wyk	zenzele@netactive.co.za
A. Liebenberg	liebenberga@arc.agric.za
N.C. van Rij	neil.vanrij@dae.kzntl.gov.za
P.M. Caldwell	CaldwellP@ukzn.ac.za

Appendix 2.2 Field layout for yield loss trials



Key:

	Season	PD	MG Cluster	Treat
L1=lattice 1	2002/03	Nov	MG45	Unsprayed
L2=lattice 2	2002/03	Nov	MG68	Unsprayed
L3=lattice 3	2002/03	Nov	MG45	Sprayed
L4=lattice 4	2002/03	Nov	MG68	Sprayed

Fig. A 2.1 Example of the field layout for one planting date and one season

Appendix 2.3 Rainfall records for three seasons

Rainfall 2002/03

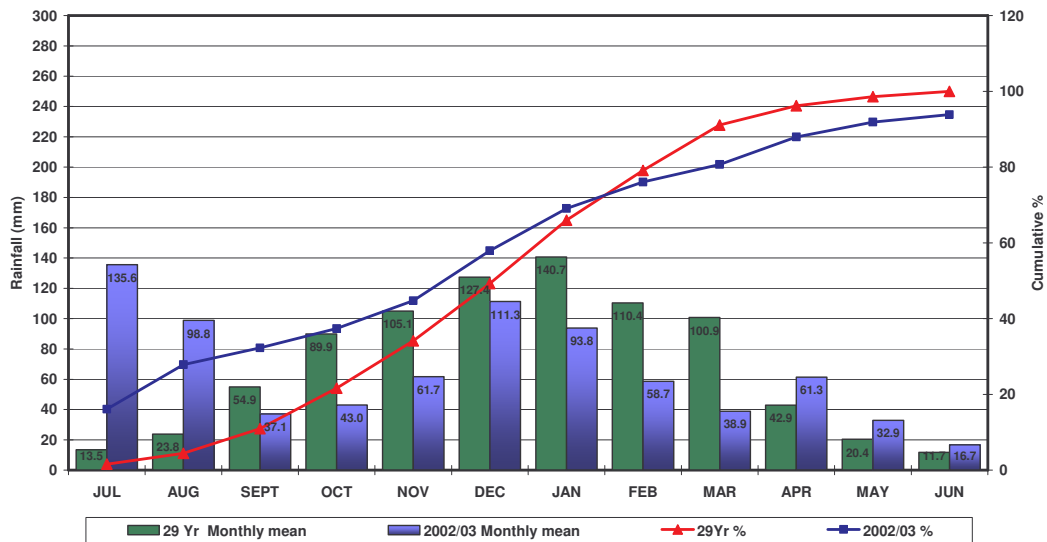


Fig. A2.2 Rainfall data for Greytown research station during 2002/03 (Source: A. Skelton, 2008)

Rainfall 2003/04

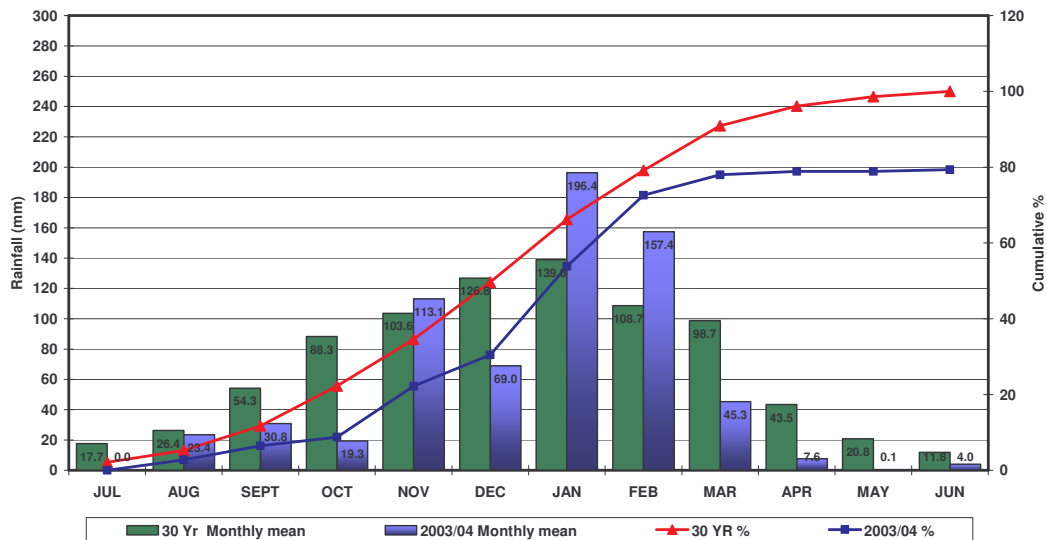


Fig. A2.3 Rainfall data for Greytown research station during 2003/04 (Source: A. Skelton, 2008)

Rainfall 2004/05

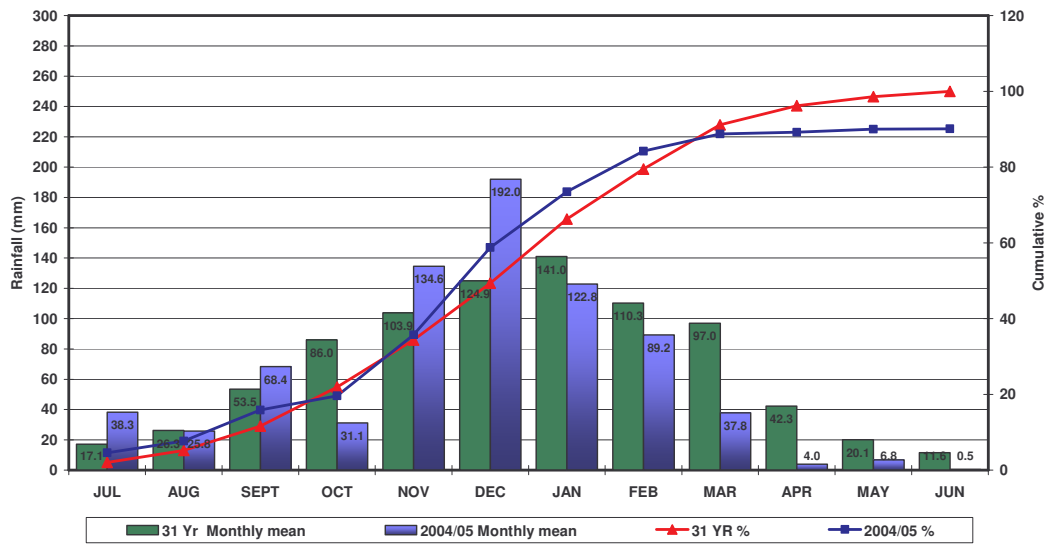


Fig. A2.4 Rainfall data for Greytown research station during 2004/05 (Source: A. Skelton, 2008)

CHAPTER 3

BREEDING CHALLENGES ASSOCIATED WITH SOYBEAN RUST

3.1 LITERATURE REVIEW: BREEDING AGAINST SOYBEAN RUST

3.1.1 Resistance

3.1.1.1 Resistance of non-host species

In rust species that enter the plant via the stomata, it has been postulated that two responses are involved in the infection process. The first is germ tube adherence to the host in order to recognize the second stimulus: the striking of the lip of the stomatal guard cell by the germ tube. This is known as ‘germ tube orientation’ which induces appressoria formation. Koch and Hoppe (1988) established that germ tube adherence was also required for *P. pachyrhizi*, a non-stomata entering rust. ‘Germ tube orientation’ was not required for appressoria formation in *P. pachyrhizi*, which was more frequent and less dependant on specific stimuli than stomata entering rusts. Even non-host species were able to stimulate *P. pachyrhizi* to form appressoria. A further stimulus (the precise nature of which is unknown) is required to trigger the development of penetration hyphae from the appressoria. The inability of non-host plants to trigger this development appears to be the most critical factor conditioning their resistance (Koch and Hoppe, 1988).

3.1.1.2 Resistance in wild perennial relatives

Nearly 300 accessions of wild perennial soybeans have been evaluated as potential sources of resistance to soybean rust (Hartman *et al.*, 1992). Accessions of *G. tomentella* were found to have good levels of resistance but utilization of this in soybean breeding has been hampered by differences in ploidy between *G. max* and *G. tomentella*. The first fertile lines from an amphidiploid ($2n=118$) hybrid of *G. max* ($2n=40$) x *G. tomentella* ($2n=78$) were produced by Singh *et al.* (1993), which paved the way for the introgression of wild perennial *Glycine* spp. genes into domesticated soybean. Further research (Patzoldt *et al.*, 2007) showed that the soybean rust resistance found in *G. tomentella* was expressed in the hybrid clones of *G. max* x *G. tomentella* but was subsequently lost during the repeated backcrosses that were required to recover fertility. Although sources of resistance from

wild *Glycine* spp. have not yet been utilized in soybean breeding, it would appear that they still remain a potential useful source in the future.

3.1.1.3 Screening for resistance

From the early 1960s through to the 1990s, much of the soybean rust research focused on resistance. Tschanz reported that he and his co-workers at the AVRDC had, over the years, screened more than 9000 accessions (Tschanz *et al.*, 1983) for resistance to soybean rust. Hartwig (1996) reported to have evaluated 1675 germplasm lines adapted to the southern USA for resistance to soybean rust in Taiwan. From this early screening work, it was clear that various levels of specific resistance, partial resistance and tolerance to soybean rust all occurred in soybean germplasm.

One of the recent objectives of the USDA soybean rust research programme has been to evaluate the USDA germplasm collection for resistance (Miles *et al.*, 2004). A set of 174 soybean genotypes, inclusive of the most important parental germplasm and the most promising sources of resistance, were screened against field populations of *P. pachyrhizi* in Brazil, China, Paraguay and Thailand (Miles *et al.*, 2004). The programme at PANNAR also participated in this evaluation, where soybean rust symptoms on this set of germplasm were recorded in the 2002/03 and 2003/04 seasons at Greytown. No lines were found to be resistant at all locations. With the threat of soybean rust looming in the USA at that time, the search for resistance intensified further, eventually involving the screening of a total of 16595 accessions in the Fort Detrick containment facility (Hartman, 2007). Once rust was finally detected in the USA, a subset of these accessions were tested in many locations in the USA in the 2006 and 2007 seasons (Hartman, 2007) and some have further been used as parents in crosses.

3.1.1.4 Soybean maturity and disease rating

Under field conditions, early maturing genotypes will have a higher disease rating earlier in the season than the equivalent later maturing genotype. The rate of rust development in these lines is also higher than that of later maturing lines, and if a correction for host maturity is not made, erroneous conclusions from field data will result (Hartman, 1996). To correct for maturity, relative life time (RLT) is calculated as the proportion of the life

cycle of the genotype completed relative to the complete life time (time from planting to harvest) of the genotype. Only rust severity ratings at comparable RLTs can be compared, which makes a single simple field severity rating meaningless unless all genotypes are of a similar maturity. McLaren (2008) showed that disease severity, as measured by the area under the disease potential curve (AUDPC), was poorly correlated with yield loss. For this reason, disease severity ratings or measurements are seldom used as a measure of resistance

3.1.2 Physiological specialization of the pathogen and specific resistance in the host

McLean and Blyth (1980) reported the first evidence of the presence of physiological races in *P. pachyrhizi* on soybean genotypes in Australia. Race 1 was virulent on Wills and avirulent on PI 200492. Race 2 was virulent on both varieties. Subsequent to this, considerable variation in isolate virulence (collected from the same field, as well as isolates from geographically distant regions) has been shown to occur (Hartman *et al.*, 2005). Three infection types have been described: the Tan lesion is a fully susceptible reaction; the resistant RB reaction is a red-brown lesion with no or few sporulating uredinia; and the absence of any macroscopic symptoms is immunity (Wang and Hartman, 1992). Eleven genotypes were used as a differential set to determine the physiological races of 42 purified *P. pachyrhizi* isolates by Wang and Hartman (1992), and based on the infection type they were able to identify nine races. The data suggested that the rust races studied were complex and that they possessed multiple virulence genes for compatibility on many of the differential cultivars. Bromfield (1984) reported on a *P. pachyrhizi* race that had three virulence genes, more than were necessary to overcome host resistance. More recent research (Hartman *et al.*, 2005) indicates that field pathogen populations are often mixtures of many races which may induce mixed infection types in the host. This is not uncommon in rust pathogens, as was shown to be the case with common bean rust (*Uromyces appendiculatus*) where the more tropical locations (including South Africa) were found to induce greater race variability than more temperate climates (Jochua *et al.*, 2008). It is not known how many races are commonly found in South African soybean fields, but since mixed infection types on the same plant have been observed (Fig. 3.1), at least two races must be present. Variability in race virulence is also known to occur. In inoculation studies conducted under controlled conditions, researchers reported that recent isolates collected

from southern Africa and South America were significantly more virulent than Asian isolates collected in the 1970s (Hartman *et al.*, 2004). Interestingly, the most virulent isolate they reported was collected in Zimbabwe.



Fig. 3.1 Mixed infection types on the same leaf, RB (resistant) and Tan (susceptible), taken from a genotype (JRS1832) carrying the *Rpp*₃ gene from Ankur (photo source: S. Tweer)

The specific resistance gene in PI 200492 was given the designation *Rpp*₁ (Bromfield and Hartwig, 1980), and since then three other independent dominant genes have been named: *Rpp*₂ (Bromfield *et al.*, 1980); *Rpp*₃ (Hartwig and Bromfield, 1983); *Rpp*₄ (Hartwig, 1986). Bernard (1995) developed a series of isolines of Williams 82 (Table 3.1) which contained the dominant *Rpp* genes, whilst Hartwig (1996) released a 'Forrest' type line (D86-8286) which contained the *Rpp*₄ gene.

In Brazil, where the Rpp₁ and Rpp₃ genes are ineffective and Rpp₂ and Rpp₄ currently confer resistance, Neto (2007) reported that many ‘new’ (un-named) gene sources of resistance have been discovered. These were tested for allelism to Rpp₂ and Rpp₄, and of the 26 sources reported, 23 were found to be at different loci to Rpp₂ and Rpp₄. One of these sources of resistance was conditioned by a single recessive gene (Neto, 2007) from the variety Abura, and this has been incorporated in a variety (BR01-18437) destined for release in 2008. Neto (2007) also reported the preliminary findings that stacking Rpp₂ and Rpp₄ in a single genotype had no additive advantage in the expression of resistance.

Table 3.1 Rust resistant isolines of Williams 82, containing the Rpp gene series (Bernard, 1995)

Isoline designation	Donor variety name	Donor PI ^a number	Gene transferred
L85-2378	Komata	200492	Rpp ₁
L85-1752	Unknown	230970	Rpp ₂
	Ankur	462312	Rpp ₃
L87-0482	Bing Nan Dou	459025	Rpp ₄

^aPI = Plant introduction

The presence of multiple virulence genes in the pathogen population and the lack of multiple resistance genes in the host provides the soybean rust pathogen with a competitive advantage. The deployment of specific single genes for resistance is thus unlikely to be a successful strategy. As an example of gene failure, Hartman *et al.* (2005) quoted the examples cited by Bromfield, where the Rpp₁, Rpp₂ and Rpp₃ lost their effectiveness in the field within 10 years of exposure. In Taiwan, Shanmugasundaram *et al.* (2004) quoted examples of Tainung 3, Tainung 4 and Kaohsiung 3 (all cultivars containing Rpp₁) becoming susceptible within a few years of release. PI 230970 and PI 230971 were identified as being resistant in Taiwan, and these were subsequently used as parents in crosses to generate a number of resistant lines (AGS 181, AGS 182, AGS 183, AGS 229, AGS 233, AGS 240, AGS 244, and AGS 247). So too were the resistances of these genotypes short lived. Following that, new sources of resistance were identified in PI 459024, PI 459025 (Rpp₄) and PI 339871 (*G. soja*) but have all since been defeated

(Hartman *et al.*, 2005; Shanmugasundaram *et al.*, 2004). In Brazil, Yorinori (2004) had a similar experience with germplasm that had shown resistance in 2002 being susceptible in 2003.

The use of gene pyramiding and gene rotation is also unlikely to offer a stable solution because the pathogen retains unnecessary virulence genes at a high frequency in its population (Tschanz, 1987). In addition, resistance associated with the RB infection type is a semi-compatible host-pathogen reaction, which generally allows pathogen reproduction and has not been shown to significantly affect epidemic development (Tschanz, 1987).

3.1.3 Partial resistance

Partial or rate reducing resistance to soybean rust has been documented in soybean (Wang and Hartman, 1992), but it has not been widely employed because of complexities in assessment. Plants or genotypes maturing at different times cannot be compared to each other in the field because of the different environmental conditions that they are exposed to at similar growth stages. Ontogenic effects can be partially corrected for by regressing relative life time (RLT) on the log transformation of rust severity. The slopes of these graphs can be compared to identify the ‘slow rusting’ genotypes. Collecting the data required to generate these graphs is laborious and cannot be conducted on a large number of genotypes, limiting its practical application. Hartman *et al.* (2005) suggested that measuring the latent period would help to identify genotypes with a long latent period and hence a slower rate of rust development. The difficulties associated with identifying partial resistance and the lack of durability of specific resistance genes has led to the suggested use of tolerance as a breeding remedy for soybean rust.

3.1.4 Tolerance

3.1.4.1 Yield loss

Tolerance implies some degree of susceptibility, and can be defined as the relative ability of a genotype to yield under stress from rust (Wang and Hartman, 1992). Tolerance is traditionally assessed by comparing yields of paired plots of fungicide protected versus unprotected plots. The percentage yield loss between fungicide protected and unprotected

plots is not necessarily correlated to rust susceptibility ratings or to rust development rates (Hartman *et al.*, 2005) and may be linked to other stress tolerance mechanisms. Significant variation in tolerance levels exist in soybean, which could be exploited by breeders. From work conducted at the AVRDC in Taiwan, Hartman (1996) demonstrated yield losses of 12 genotypes ranging between 29-85%. Based on reduced pustule numbers, the two lines that had the smallest yield losses (29% and 31%) could conceivably have had some form of partial resistance. This, when compared to a possible 85%, appears to be significant but in reality is still far too high for practical benefit on a commercial scale. In more recent research conducted in Brazil (Neto, 2007), minor genes have contributed towards tolerance in the genotype EMGOPA 313, with yield losses in the order of magnitude where fungicide spraying would still be financially attractive. McLaren (2008) evaluated commercial soybean cultivars in South Africa during the 2003/04 and 2004/05 seasons and came to the conclusion that there was not sufficient tolerance to yield loss available in the commercial genotypes to be of significant economic value.

Tolerance is a characteristic that can only be evaluated in the target environment while under rust stress, as it implies a measure of adaptation to that environment. Tolerance is of little value unless the genotype is high yielding in that environment and it maintains yield stability despite rust infections. Selecting for yield stability in the presence of rust is not an easy task (Hartman *et al.*, 2005), since over and above the normal GxE interaction that breeders have to contend with for adaptation, seasonal variation in severity and timing of rust epidemics is superimposed.

3.1.4.2 Yield stability

Several stability statistics have been evaluated for the simultaneous selection of yield and yield stability in soybean (Dashiell *et al.*, 1994). Since yield and yield stability are both relevant when assessing tolerance to soybean rust, these statistics could be used to evaluate genotypic performance. Whilst yield is normally the primary consideration, a consistent performance is also valuable to a producer, who may be willing to sacrifice some yield in order to achieve a stable yield over seasons (Kang, 2002). Two concepts in yield stability have been defined: static and dynamic.

Static or biological stability (Becker and Léon, 1988) occurs where the performance of a genotype is unchanged regardless of the environment. Static stability is characterised by a low variance over environments but it implies little or no response to inputs. From a breeding perspective it is the type of stability that could be associated with a major disease resistance gene, and a parameter such as coefficient of variation (CV %) (Francis and Kannenburg, 1978) could be used to quantify it.

Dynamic or agronomic stability (Becker and Léon, 1988) describes a measure of performance in which a genotype responds to improved environmental conditions. According to Becker and Léon (1988), all stability procedures based on quantifying GxE interaction effects belong to the dynamic concept of stability. Numerous methods have been developed to analyse agronomic stability, with no one method the best for all situations. A commonly used method in the past has been regression analysis, introduced by Yates and Cochran (1938) and subsequently modified by Finlay and Wilkinson (1963) and Eberhart and Russell (1966), where stability was quantified by the regression coefficient (b_i). Deviation mean square from regression (S^2_{di}) (Eberhart and Russell, 1966) may also be used as a measure of dynamic stability and is most valuable where there are large numbers of genotypes and environments and a good range in environmental means (Sneller *et al.*, 1997). The usefulness of regression approaches is fundamentally dependant on the assumption that genotypes respond linearly to the environments (Flores *et al.*, 1998). Other methods also used include the ecovalence statistic (W_i) proposed by Wricke (1962), which was subsequently modified by Shukla (1972) and called stability variance (σ_i^2). The ecovalence statistic is based on the Sums of Squares (SS) of the interaction effects and measures the contribution of genotype to the GxE interaction. A genotype with a low W_i is considered stable. Lin and Binns (1988) introduced the superiority measure (P_i), which is defined as the distance mean square between the genotype response and the maximum response, averaged over all environments. Multivariate methods that have been applied as measures of stability include the AMMI model (Gauch and Zobel, 1996), Principal coordinate analysis (PCA) and Component analysis (CA) (DeLacy *et al.*, 1996). Non-parametric measures of stability such as rank correlations (Spearman's rank correlation, KETRANK (Ketata *et al.*, 1989), FOXRANK (Fox *et al.*, 1990)) give an indication in change in rank or crossover interaction between seasons. Unlike the parametric measures, these stability statistics require no assumptions about data distribution and homogeneity of variances.

3.2 OBJECTIVE

Much of the soybean breeding research around the world on soybean rust has been focused on the search for resistance (Miles *et al.*, 2004; Tichagwa, 2004; Neto, 2007), with research programmes literally screening thousands of cultivars, germplasm lines and accessions in the search for novel sources of resistance. Considerable effort has been made to characterize these sources by comparing them to known genes (Monteros *et al.*, 2007; Ribeiro *et al.*, 2007; Silva *et al.*, 2008) and developing markers to assist in their incorporation into adapted germplasm (Hyten *et al.*, 2007; Monteros *et al.*, 2007; Neto, 2007; Boerma, 2008). There have, however, been numerous documented failures of specific resistance genes in the past (Shanmugasundaram *et al.*, 2004; Yorinori, 2004; Hartman *et al.*, 2005; Silva *et al.*, 2008), yet breeders and researchers persevere with this line of research in the hope that a new source of resistance will prove to be more stable.

Marker technology is available (Neto, 2007; Boerma, 2008; Silva *et al.*, 2008) to pyramid major soybean rust resistance genes into a single genotype; however, there is a school of thought (Tschanz, 1987) that this too is unlikely to be a stable solution because the pathogen retains unnecessary virulence genes at a high frequency in its population.

The protracted process of assessing tolerance, compounded by the restrictions of working with a polygenic character, has probably impacted negatively on the popularity of tolerance as a breeding strategy. For a genotype to be considered tolerant it is required not only to have a good yield in the presence of rust, but to be able to maintain yield stability over a range of rust stress conditions. The use of highly tolerant genotypes does provide a durable, but admittedly currently elusive, solution to soybean rust. This study sets out to add to the knowledge base of soybean rust tolerance research. Genotypes identified with putative tolerance, were evaluated over three seasons for tolerance to yield loss in the presence of soybean rust. The stability of this tolerance is assessed over seasons and discussions follow on how best to interpret this data.

3.3 MATERIAL AND METHODS

3.3.1 Genotypes

3.3.1.1 Pre-selection of genotypes for tolerance research

Pre-selection of candidate genotypes for tolerance research was done by splitting the single row unreplicated plots from the PANNAR germplasm and line collection into sprayed and unsprayed subplots. The second half of each 5 m row formed the sprayed subplot, which was sprayed with Punch C (Flusilazole/Carbendazim) at the recommended rate of 400 ml ha⁻¹ using a knapsack sprayer. This commenced at the first signs of flowering of the earliest genotype, and was repeated at 21 d intervals until the last genotype reached harvest maturity. Plots of the germplasm collection had been set out roughly in order of maturity in the field, which aided both the spraying and the evaluation. Note was made of all genotypes that had little or no difference in harvest maturity date between the sprayed and unsprayed subplots. Where soybean rust had caused severe defoliation and premature senescence, the difference in maturation between sprayed and unsprayed subplots was a week or more. In genotypes relatively unaffected by soybean rust, the difference between sprayed and unsprayed subplots was not visually apparent.

Difference in seed size between the sprayed and unsprayed subplots was also recorded. Where soybean rust causes defoliation or stress late in the reproductive period, yield loss is primarily via seed size reduction. Whilst difference in seed size between sprayed and unsprayed plots has been put forward as an efficient and simple technique for evaluating tolerance (Shanmugasundaram, 1999; Tichagwa, 2004), it was found to be somewhat variable in this study (likely as a result of small sample size). Genotypes, in which the difference in both maturation and seed size suggested tolerance, were included in the next phase of evaluation.

The lines identified with putative tolerance were then evaluated in more detail the following season. Field observations of flowering date (R1), first pustule date, pustule type, followed by an assessment of rust severity within three strata of the plant canopy and finally physiological maturity (R8) date (Fehr *et al.*, 1971) were noted. This was done firstly to expressly exclude genotypes that had specific resistance gene pustule reactions

(RB) from the group that would be evaluated for tolerance. Secondly, if genotypes exhibited indications of partial resistance that could be contributing towards the tolerance, it would be possible to identify some of these mechanisms using the data collected (Appendix 3.1). Based on two seasons of pre-selection, 14 genotypes were included for the evaluation of soybean rust tolerance over the next three seasons.

3.3.1.2 Genotypes used for the evaluation of tolerance

One susceptible non-tolerant control (PAN 875, a cultivar commercially released in Zimbabwe) and a resistant control (UFV 3, an old germplasm line out of Brazil) showing a RB pustule reaction were included, along with 12 genotypes presenting Tan pustule reaction types (susceptible) that had exhibited some level of tolerance in the two seasons of pre-screening. Two genotypes, Cordell (Hartwig and Young, 1990) and Delsoy 4900 (Anand, 1991), displayed initial indications of slow rusting. JV781 and JV783 were suspected of having a long latent period and JV762 of having a low infection rate.

3.3.2 Experimental design

The trials were planted at Greytown during the 2003/04, 2004/05 and 2005/06 seasons (Year) at planting dates that could be considered 'late', ensuring that all maturities received significant exposure to soybean rust (Table 3.2). The trials were arranged in a split plot design, with the whole plot factor being genotype (Genotype), which was split for fungicide spray treatment (Treat). The sprayed treatment subplots were protected with applications of Punch C (400 ml ha^{-1}) at 21 d intervals starting from the date of first rust symptoms in the sentinel plots (Table 3.2), effectively excluding soybean rust symptoms for the entire season. At the time of spraying there were no symptoms of soybean rust in the trial plots. The sentinel plots (described in section 2.1.6) were part of a national network (Craven, 2008) of early planted plots which provided a timely warning for the need to spray. The subplots consisted of four 4.4 m long rows, with an inter-row spacing of 0.9 m. The centre two rows of the subplots were harvested with a plot combine, and the yields were converted to t ha^{-1} at a moisture content of 12.5%.

Table 3.2 Planting date and site details for the rust tolerance split plot trials at Greytown, South Africa (S28° 08'; E30° 37')

Season	Planting date	Rainfall (mm) ^a	Date of first rust ^b
2003/04	08/12/2003	666.4	16/02/2004
2004/05	09/12/2004	751.3	24/02/2005
2005/06	30/11/2005	954.8	2/02/2006

^aThirty two year mean annual rainfall for Greytown = 832.6 mm

^bSoybean rust symptoms on the sentinel plots, used as an indicator of the earliest presence of rust symptoms in the area

3.3.3 Statistical analysis

The data was analysed utilising the REML META analysis routine in Genstat (Version 10.2) using the following model:

Fixed = Year*Genotype*Treat

Random = Rep/Whole plot/Subplot

Experiment = Year

[Statistical model 3]

Least significant differences were calculated using t-values appropriate to the degrees of freedom and average standard errors of the differences of means determined by Genstat.

3.3.3.1 Yield loss

To evaluate tolerance to soybean rust, yields of unsprayed subplots were compared to the yields of sprayed subplots and yield loss % was calculated for each genotype using the following formulae:

Yield loss = sprayed yield-unsprayed yield [Equation 1]

Yield loss % = (Yield loss)/sprayed yield x 100 [Equation 2]

3.3.3.1 Correlations

A non-parametric test of the seasonal variation in genotypic ranking of yield loss %, sprayed yield and unsprayed yield was conducted by calculating Spearman's rank correlation coefficients between seasons. The correlations of mean yields over seasons were also calculated after the means had been normalized (Appendix 3.2) for seasonal error variance and scale (seasonal mean).

3.3.3.2 Superiority measure

The Lin and Binns (1988) superiority measure (P_i) was calculated on the sprayed and unsprayed yields using the formula:

$$P_i = \sum_{j=1}^n (X_{ij} - M_j)^2 / (2n) \quad [\text{Equation 3}]$$

where n is the number of seasons, X_{ij} is the i th genotype yield in the j th season, and M_j is the maximum yield response in the j th season. From this equation, the most consistently superior genotype has the lowest P_i value. P_{iu} was calculated on the unsprayed yields of all 14 genotypes using the highest unsprayed yield each season as the maximum. The corresponding superiority measure determined on the sprayed yields, P_{is} , was primarily calculated to determine the change in superiority (ΔP_i) brought about by soybean rust stress using the formula:

$$\Delta P_i = P_{is} - P_{iu} \quad [\text{Equation 4}]$$

3.3.3.3 Ecovalence

Phenotypic stability measured by using the ecovalence statistic (W_i) developed by Wricke (1962) was calculated for unsprayed yield (W_{iu}) and sprayed yield (W_{is}) using the formula:

$$W_i = \sum_{j=1}^n (X_{ij} - X_{i.} - X_{.j} + X_{..})^2 \quad [\text{Equation 5}]$$

where n is the number of seasons, X_{ij} is the i th genotype yield in the j th season, $X_{i.}$ is the mean of the i th genotype across n seasons, $X_{.j}$ is the mean of all genotypes in the j th season, and $X_{..}$ is the grand mean over n seasons. The most stable genotypes have the lowest W_i . The change in the ecovalence statistic attributed to soybean rust (ΔW_i) was calculated using the formula:

$$\Delta W_i = W_{is} - W_{iu} \quad [\text{Equation 6}]$$

3.3.3.4 $W_i P_i$

The $W_i P_i$ statistic is calculated as the distance of the coordinate in the biplot of W_{iu} and P_{iu} from the origin of the graph. $W_i P_i$ is thus the hypotenuse of a right angle triangle with two sides equal to W_{iu} and P_{iu} . The square of the hypotenuse is equal to the sum of the squares of the two opposite sides, therefore:

$$W_i P_i = \sqrt{W_{iu}^2 + P_{iu}^2} \quad [\text{Equation 7}]$$

3.4 RESULTS

3.4.1 Yield

The main effects of Year, Genotype and Treat were all highly significant (Table 3.3). The interaction between Genotype and Year and Genotype and Treatment were significant, while the interaction between Year and Treat was not. The interaction between Year, Genotype and Treat was non-significant. Yields of the sprayed subplot treatment measured the yield potential of the genotype in the absence of rust. The mean of all genotypes gives an indication of the yield potential of the season. Due to lower than normal rainfall (Table 3.2), the yields (Appendix 3.3) and yield loss % (Table 3.4) were lowest for the 2003/04 season. Moderate soybean rust pressure was present in all three seasons, which can be judged by the yield loss of the susceptible control, ranging from 17.7 to 27.9% (Table 3.4).

The main effect of Treat or the mean yield loss of all entries over the three seasons was 0.362 t ha⁻¹ or 11.7%, which when compared to the susceptible control mean of 23.1% indicates appreciable levels of tolerance within the trial (Table 3.4). The lowest mean yield loss over all three seasons was measured on UFV 3, the resistant control variety. Six other genotypes (Cordell, JX270-2, JV861, JV860, Delsoy 4900 and JV783) had mean yield losses of less than 10%, in some cases with appreciable seasonal variability (Table 3.4).

Table 3.3 Wald Statistics for the REML META analysis of yield loss data using the following model: Fixed terms = Year*Genotype*Treat; Random = Rep/Whole plot/Subplot; Experiment = Experiment

Fixed term	Wald Statistic	d.f	F statistic	d.d.f	F pr
Year	56.80	2	28.22	107.5	<0.001
Genotype	365.72	13	28.13	156.8	<0.001
Treat ^a	98.33	1	98.33	156.8	<0.001
Year.Genotype	227.05	26	8.64	139.5	<0.001
Year.Treat	4.62	2	2.29	107.5	0.106
Genotype.Treat	23.86	13	1.84	156.8	0.042
Year.Genotype.Treat	13.11	26	0.50	139.5	0.980

^aTreat = Fungicide spray treatment

Table 3.4 Yield loss (t ha⁻¹) and yield loss % over three seasons (2003/04, 2004/05 and 2005/06) at Greytown, as a result of soybean rust

	3 y mean yield (t ha ⁻¹)			Yield loss % ^c			
	No spray	Spray ^a	Loss ^b	3 y Mean	2003/04	2004/05	2005/06
Cordell	1.879	1.995	0.116	5.8	9.6	5.8	2.1
Delsoy 4900	2.394	2.645	0.251	9.5	-1.4	8.9	19.3
JV762	2.608	3.030	0.422*	13.9	16.2	9.1	17.5
JV780	2.981	3.501	0.520**	14.9	14.8	12.4	17.2
JV781	2.683	3.144	0.461**	14.7	10.2	18.9	15.3
JV783	3.118	3.453	0.335*	9.7	5.8	11.5	11.1
JV860	3.043	3.337	0.294	8.8	-1.2	20.9	6.3
JV861	3.070	3.340	0.270	8.1	8.2	6.4	9.3
JV870	2.789	3.255	0.466**	14.3	16.3	12.4	14.4
JX270-2	2.998	3.214	0.216	6.7	3.7	6.0	10.2
PAN 494	2.561	2.867	0.306*	10.7	5.7	6.8	20.3
PAN 589	2.973	3.457	0.484**	14.0	5.7	16.2	18.0
PAN 875	2.686	3.494	0.808**	23.1	27.9	25.3	17.7
UFV 3	2.421	2.536	0.115	4.5	0.6	-3.3	15.2
Mean	2.729	3.091	0.362	11.7	9.0	11.6	14.1

^aSpray = Punch C (400 ml ha⁻¹) sprayed at first symptoms in the sentinel plot and repeated at 21 d intervals

^bLoss = Sprayed yield – Unsprayed yield (t ha⁻¹)

^cYield loss % = Loss/Unsprayed Yield x 100

Difference in genotypic means: LSD (0.05) = 0.304 t ha⁻¹

LSD (0.01) = 0.424 t ha⁻¹

Difference in spray treatment means: LSD (0.05) = 0.478 t ha⁻¹

* = Significant ($P = 0.05$)

** = Highly significant ($P = 0.01$)

3.4.2 Correlations

Spearman's rank correlation coefficients were calculated to determine the correlation between the rankings of the genotypes for each of the three seasons for: yield loss % (Table 3.5); unsprayed yield (Table 3.6); sprayed yield (Table 3.7). The correlation of normalized means for unsprayed yield (Table 3.6) and sprayed yield (Table 3.7) were also determined. The Spearman's rank correlation coefficient gives a measure of the crossover

interaction (qualitative interaction) occurring in genotypic ranking between seasons, with the lower the coefficient (r_s), the greater the crossover between seasons. The correlation coefficient for normalized mean yield (r_{nm}) gives an indication of non-crossover interaction, or quantitative interaction between seasons, with the lower r_{nm} values indicating poor correlation of normalised REML mean yield between seasons.

Table 3.5 Spearman's rank correlations between seasons for ranking based on yield loss % due to soybean rust at Greytown

	2003/04	2004/05	2005/06
2003/04	1		
2004/05	0.367	1	
2005/06	0.033	0.244	1

Very weak correlations in genotype ranking for yield loss % between seasons were found (Table 3.5). For unsprayed yield, the genotype ranking and normalized mean yields for 2003/04 and 2005/06 seasons were significantly correlated, but the 2004/05 season was dissimilar to the other two seasons (Table 3.6). Sprayed yield was the most consistent over seasons, with moderate rank correlations and significant normalized mean yield correlations between 2005/06 season and the preceding two seasons (Table 3.7). The correlations (r_s and r_{nm}) between sprayed and unsprayed yield within a season was significant for all seasons (Table 3.8).

Table 3.6 Correlations between three seasons of unsprayed yield in the presence of soybean rust at Greytown, with Spearman's rank correlation below the diagonal and normalized mean yield correlation above the diagonal

	2003/04	2004/05	2005/06
2003/04	1	0.0352	0.606*
2004/05	-0.103	1	0.240
2005/06	0.648*	0.011	1

Table 3.7 Correlation matrix for sprayed yield at Greytown, with Spearman's rank correlation below and normalized mean yield correlation above the diagonal

	2003/04	2004/05	2005/06
2003/04	1	0.435	0.673**
2004/05	0.169	1	0.631*
2005/06	0.481	0.490	1

Table 3.8 Correlations between sprayed and unsprayed treatments for three seasons at Greytown

	Correlation	
	Spearman's rank	Normalized mean yield
2003/04	0.701**	0.879**
2004/05	0.626*	0.786**
2005/06	0.916**	0.957**

3.4.3 Stability measures

The P_i superiority measure was calculated using the mean square difference of each genotype from the maximum yield in each season. A derivative of the P_i statistic, P_{iu} , was calculated on unsprayed yield. The lowest P_{iu} indicates the least variation from the maximum yield and the best general adaptation in unsprayed conditions. Genotypes were arranged (Table 3.9) according to their superiority under soybean rust stress, from most superior (JV783) to least superior (Cordell).

Table 3.9 Mean stability measures P_i and W_i over three seasons (2003/04, 2004/05 and 2005/06) for yield of 14 genotypes grown in the presence of soybean rust at Greytown

Genotype	Superiority measure ^a			Ecovalence ^b			$W_i P_i$	
	P_{iu}	P_{is}	ΔP_i	W_{iu}	W_{is}	ΔW_i	$W_i P_i$ ^c	Rank
JV783	0.026	0.034	0.007	0.090	0.096	0.006	0.094	2
JV861	0.044	0.137	0.092	0.251	0.399	0.148	0.255	6
JV860	0.054	0.072	0.018	0.291	0.027	-0.263	0.296	7
JX270-2	0.057	0.170	0.113	0.138	0.152	0.014	0.149	4
JV780	0.068	0.036	-0.032	0.041	0.014	-0.027	0.079	1
PAN 589	0.069	0.047	-0.022	0.188	0.404	0.216	0.200	5
JV870	0.138	0.122	-0.017	0.022	0.001	-0.021	0.140	3
JV781	0.227	0.272	0.046	0.303	0.318	0.015	0.378	8
PAN 875	0.239	0.023	-0.217	0.387	0.132	-0.255	0.455	9
JV762	0.349	0.339	-0.010	0.451	0.312	-0.139	0.571	11
PAN 494	0.376	0.470	0.095	0.478	0.278	-0.200	0.608	12
Delsoy 4900	0.432	0.591	0.159	0.284	0.216	-0.068	0.517	10
UFV 3	0.552	0.777	0.225	0.752	0.506	-0.246	0.933	13
Cordell	1.036	1.558	0.522	0.111	0.209	0.098	1.042	14
S.D. ^d	0.335	0.407	0.256	0.192	0.152	0.147	0.125	

^a Superiority measure, calculated on unsprayed yields (P_{iu}), sprayed yields (P_{is}) and change in superiority due to rust ($\Delta P_i = P_{is} - P_{iu}$)

^b Ecovalence = Wricke's ecovalence, calculated on unsprayed yield (W_{iu}), sprayed yields (W_{is}) and change in W_i due to soybean rust ($\Delta W_i = W_{is} - W_{iu}$)

^c $W_i P_i = \sqrt{W_{iu}^2 + P_{iu}^2}$

^d Standard deviation

A biplot of P_{iu} and P_{is} (Fig. 3.2) plots the effect of soybean rust on the genotype superiority, where the closer the coordinate is to the diagonal, the less the genotype superiority is affected by soybean rust. The scale of the biplot was chosen to ensure good separation of the genotypes; consequently two genotypes (Cordell with a coordinate of 1.036: 1.558 and UFV 3 with a coordinate of 0.552: 0.777) were not plotted. The biplot has been subdivided into quartiles based on the median value, with quartile A containing

genotypes insensitive to rust but inferior yielding in the absence of rust. Quartile B contains genotypes that are both rust sensitive and inferior yielding, while quartile C contains genotypes that are rust insensitive and superior in yield. The control genotype PAN 875 is the only genotype in quartile D, which is classed as rust sensitive but superior yielding in the absence of rust.

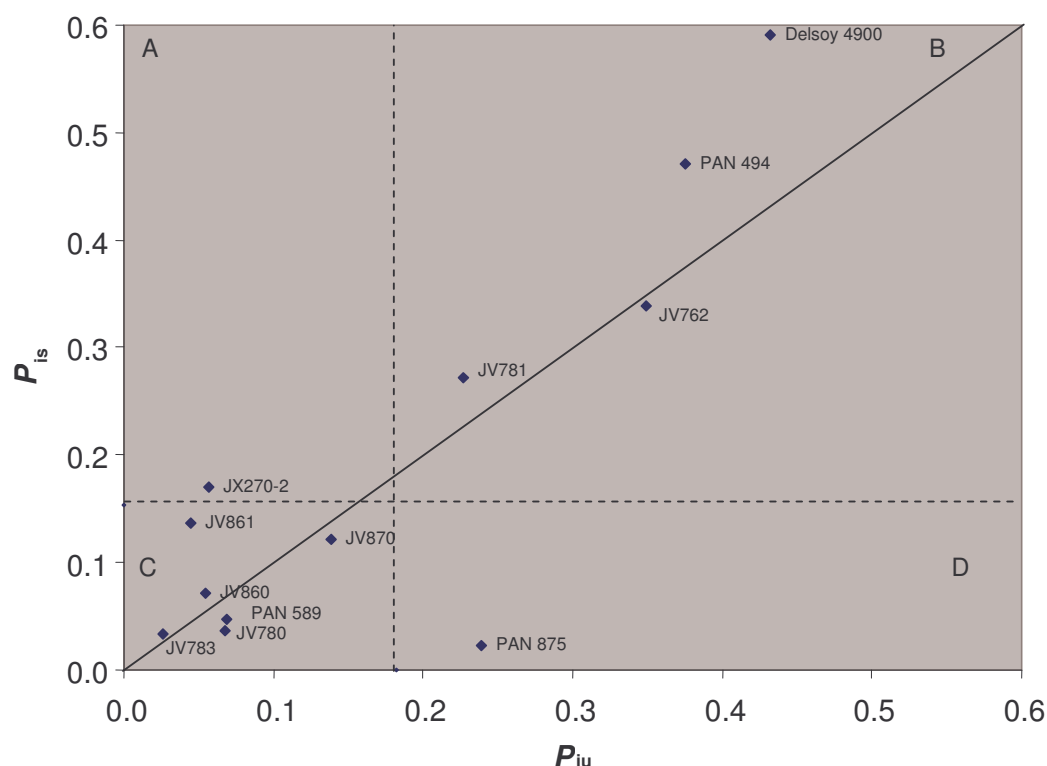


Fig. 3.2 Biplot of superiority measures for unsprayed yield (P_{iu}) versus sprayed yield (P_{is}), subdivided into quartiles: Quartile A = Rust insensitive but inferior yield; Quartile B = Rust sensitive and inferior yield; Quartile C = Rust insensitive and superior yield; Quartile D = Rust sensitive but superior yield

The ecovalence statistic was determined for sprayed yield (W_{is}) and unsprayed yield (W_{iu}) and the change in stability (ΔW_i) as a result of soybean rust as the difference between W_{is} and W_{iu} . A small W_i value indicates reduced interaction between genotype and environment and the smaller the value the greater the genotypic stability over seasons. The difference between W_{is} and W_{iu} (ΔW_i) quantifies the extent to which the yield stability of genotypes changes under soybean rust pressure. It is commonly accepted that yield instability

increases with crops under stress (Cattivelli *et al.*, 2008), and the ΔW_i statistic would give a good indication of which genotypes are being stressed the most by exposure to rust. A biplot of W_{is} versus W_{iu} (Fig. 3.3) demonstrates the relationship between the two statistics for each genotype, where genotypes that plotted close to the diagonal have similar yield stabilities under rust and rust free conditions. The scale of the biplot was chosen to apportion equal weights to W_{is} and W_{iu} and to ensure good separation of the genotypes. As a consequence genotype UFV 3 with a coordinate of 0.752: 0.506 has not been plotted.

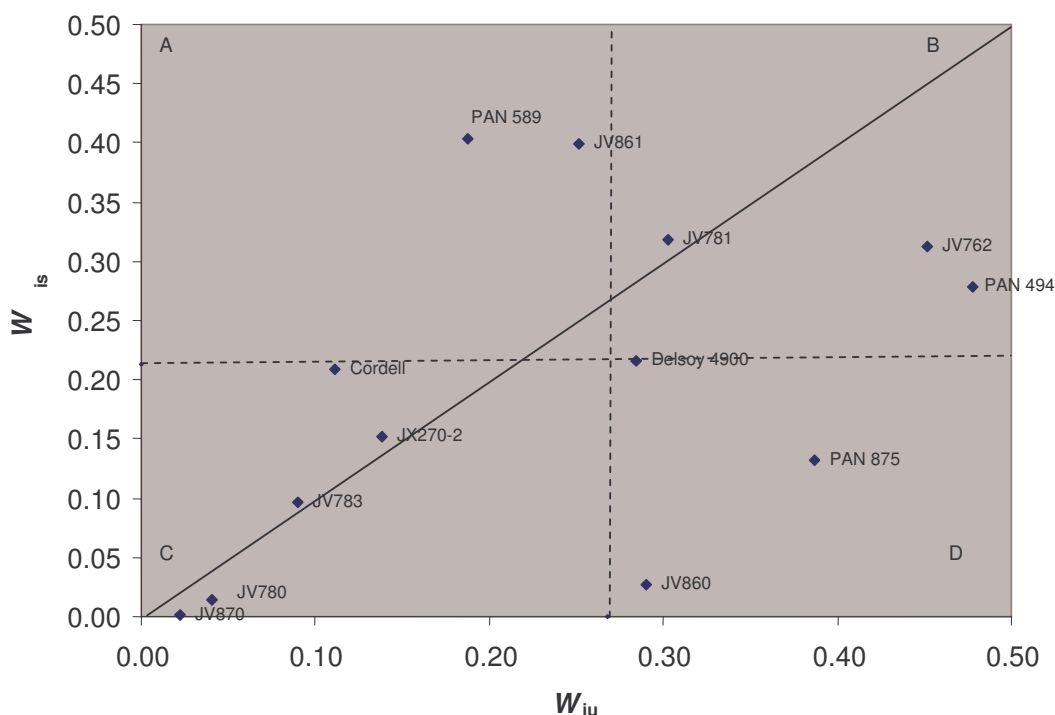


Fig 3.3 Ecovalence biplot of unsprayed yields (W_{iu}) versus sprayed yields (W_{is}), subdivided into yield stability quartiles: Quartile A = Unstable sprayed becoming stable under rust infection; Quartile B = Consistently unstable; Quartile C = Consistently stable; Quartile D = Stable sprayed becoming unstable under rust stress

The biplot is divided into stability quartiles using the median values. Genotypes in quartile A are unstable under rust free conditions, yet becoming more stable under rust infection. Quartile B contains consistently unstable genotypes, while the genotypes in quartile C are consistently stable regardless of soybean rust. Quartile D contains genotypes (such as

PAN 875, the susceptible control) that are stable under sprayed conditions but are unstable under rust stress conditions.

The relationship between W_{iu} and P_{iu} was explored by plotting the two statistics against each other, in an attempt to ‘fill up the dark zones’ (Flores *et al.*, 1998) left by each individual statistic. The pattern that emerged from this biplot (Fig. 3.4) was that the genotypes were predominantly distributed in two quartiles. Quartile B contained rust sensitive genotypes that had low and unstable yields under soybean rust pressure. Quartile C contained tolerant genotypes that were both stable and consistently high yielding under rust infections.

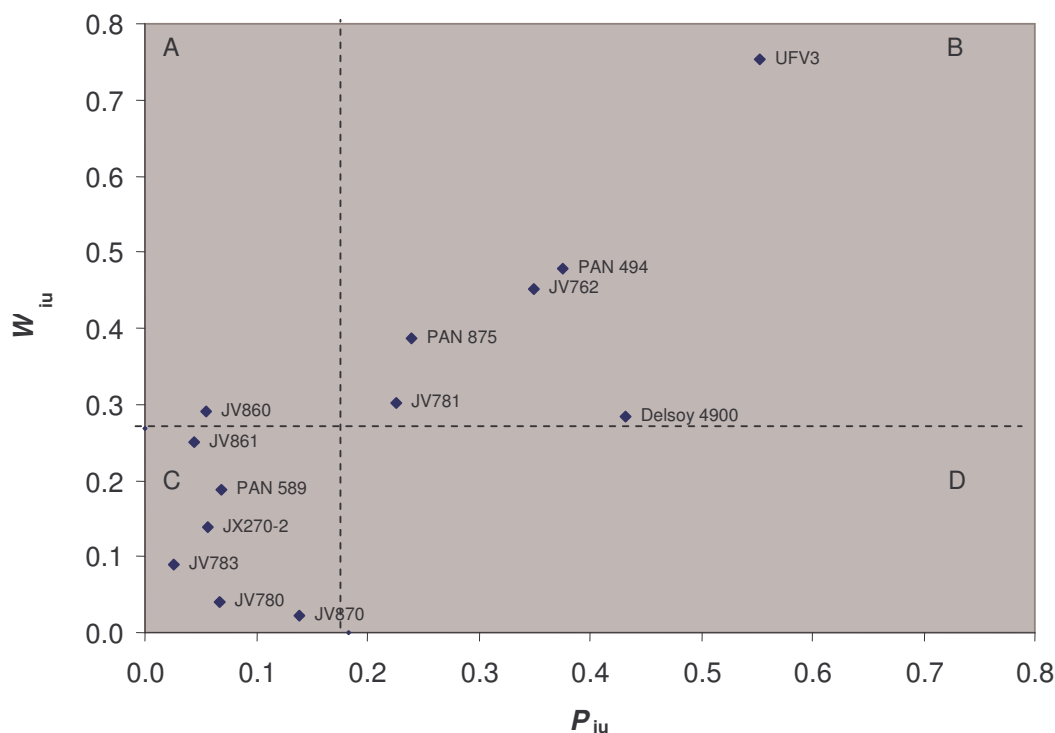


Fig. 3.4 Tolerance to soybean rust, as determined by the biplot of superiority measure (P_{iu}) and ecovalence (W_{iu}) measured under rust stress conditions, where soybean genotypes are defined by the quartiles into which they plot: Quartile A = superior yielding but unstable; Quartile B = inferior and unstable yielding; Quartile C = superior and stable yielding (Tolerant); Quartile D = inferior yielding but stable

3.5 DISCUSSION

It is re-emphasized that the entries in this trial had been pre-selected to include genotypes that were susceptible to infection but tolerant to the effects of soybean rust, and any genotypes showing RB specific gene resistance (Fig 3.5) were excluded. The rationale was that the tolerance to yield loss selected with this methodology would be durable and not reliant on a race-specific reaction that could be defeated.



Fig. 3.5 RB reaction type on the genotype UFV 3, inoculated in the glasshouse with a spore suspension of 24×10^3 spores ml^{-1}

The soybean rust resistant control (UFV 3) was only included in the trial to provide a benchmark for tolerance. The lowest mean yield loss (4.5%) was recorded on the resistant control variety UFV 3, which, when considering its resistant RB pustule reaction is not unexpected. Interestingly, in one season (2005/06) it had a yield loss not dissimilar to the trial mean, indicating that there is also variability in seasonal yield loss to contend with

when using specific resistance. Subsequent glasshouse inoculation of UFV 3 (data not shown) with a mixed field isolate has confirmed the continued presence of the RB pustule reaction (Fig 3.5): sparse sporulation and low rate of infection associated with specific resistance. This verifies that the yield loss in the last season was not as a consequence of defeated resistance.

3.5.1 Traditional measures of tolerance

Depending on how tolerance is defined, any one of a number of the candidates could be chosen as being the most tolerant genotype. Excluding the resistant control, the lowest mean yield loss % (5.8%) was achieved by Cordell, although it was also the lowest yielding by a significant margin ($P = 0.01$). Subsequent to these trials, glasshouse inoculations have shown that Cordell has a measure of partial resistance and does not allow sporulation as prolifically as the other tolerant genotypes. The objective of using tolerance in preference to race specific resistance to restrict yield loss due to soybean rust is to have a genetic mechanism that is stable and durable. Due to the limited number of seasons evaluated, this study cannot make conclusions regarding durability, but it does highlight the seasonal variability associated with the expression of tolerance when assessed using yield loss % as the measure. From the weak Spearman's correlation in ranking between seasons for yield loss % (Table 3.5), it would appear that progress in selecting for tolerance will be slow when using this index as a selection criterion. The yield loss % index is calculated using sprayed and unsprayed yield. It is possible that the physiological mechanisms driving yield and yield stability under rust and rust free conditions are different for some genotypes and not for others, resulting in the increased variability of the index over and above the individual variate components of the index. To improve progress in tolerance breeding, more efficient measure of tolerance need to be explored.

3.5.2 Exploring improved measures of tolerance

3.5.2.1 Unsprayed yield

There is a school of thought that would support the concept that the highest yielding genotype under rust stress is the most tolerant. In this scenario JV783 would be the most tolerant genotype, and would out perform five out of the remaining 13 genotypes even if

they were sprayed. Genotype ranking for unsprayed yield was significantly correlated between seasons in two of the three seasons (Table 3.6), suggesting that it may be a more reliable index than yield loss % to base the characterization of tolerance on. In spite of being less variable than yield loss %, seasonal variation in unsprayed yield is still of consequence. The precise source of the seasonal variability in unsprayed yield is unclear, but it is likely to be related to uneven rust inoculum pressure and to the timing of the epidemics relative to genotype maturity. It is also possible that the actual biochemical or physiological nature of the tolerance mechanism could be moderated by the environment. As a measure to improve the uniformity of rust epidemics in the field, inoculations or the use of spreader-rows in the trials could be considered. This may reduce the variability associated with severity (and timing) of infection so that the variability remaining can be attributed largely to post infection mechanisms within the host. If the timing of the soybean rust epidemics relative to the developmental stage of the soybean genotype is crucial in determining the yield, then temporal replication of genotypes grouped according to maturity is required. Sequential planting of trials would expose each genotype to a number of environments within a single season, and multi-season data would improve confidence in the characterization of tolerance. Unsprayed yield on its own is, however, an unsatisfactory measure of tolerance because it has no reference to the potential yield that could be attained in that environment if rust was controlled and provides no measure of variability.

3.5.2.2 Superiority measure

The superiority measure proposed by Lin and Binns (1988) uses a maximum yield to set the upper boundary in each environment, and generates a mean square statistic that measures deviations from this maximum yield. The most tolerant genotype as selected by the P_{iu} superiority measure was JV783 (Table 3.9), which had also been selected as being the highest yielding in the presence of rust. Genotypic ranking of P_{iu} was highly correlated ($r_s = 0.991^{***}$) with unsprayed yield, confirming that it is a variance statistic strongly driven by performance (Flores *et al.*, 1998; Alberts, 2004). Importantly, P_{iu} as a rust tolerance statistic discriminated against Cordell, UFV3 and Delsoy 4900 because of poor yield adaptation, which yield loss % (Table 3.4) as a tolerance index was incapable of doing. All three had low mean yield losses, but should not be considered tolerant because of poor unsprayed yields.

Calculation of the superiority measure based on sprayed yields (P_{is}) was done to further note the change in superiority of genotypes from sprayed to unsprayed conditions. In the biplot of P_{is} vs P_{iu} (Fig. 3.2), most of the genotypes (with the obvious exception of PAN 875) plotted close to the diagonal. This indicates a close relationship between P_{is} and P_{iu} , which is further illustrated by the highly significant rank correlation ($r_s = 0.736^{**}$) between the genotypic ranking of P_{iu} and P_{is} . This is likely to be as a consequence of the genotypes included in the trial being pre-selected for tolerance to soybean rust, resulting in their ranking for superiority under sprayed and unsprayed conditions being similar. The biplot correctly categorizes PAN 875 (the susceptible control) as being high yielding but sensitive to rust.

3.5.2.3 Ecovalence

The ecovalence statistic W_i measures the interaction variance over seasons and is considered a measure of specific stability, whilst P_i is considered a measure of general superiority (Lin and Binns, 1988). Plotting of W_{iu} against W_{is} (Fig 3.3) raised interesting observations related to the variance of genotypes under rust stress. It was expected that most genotypes would tend to be more variable and less yield stable under unsprayed conditions than under sprayed conditions. This was shown to be generally the case, with the exception of three genotypes (Cordell, PAN 589 and JV861) that plotted appreciably above the diagonal (Fig 3.3). The significance of the relationship between rust stress and yield stability for these three genotypes has not been determined, but they display the sort of reaction that might be elicited by genotypes with sensitivity to the sprayed chemical. Yield suppression resulting from sensitivity to the sprayed chemical may be mistaken for tolerance where yield loss % is used as a tolerance index, since the sprayed yields would be reduced relative to the unsprayed yields. Chemical yield suppression is expected to be accompanied by an increase in variance, so it is possible that it could be detected with biplots of W_{iu} vs W_{is} . None of the three genotypes involved have, however, been evaluated for sensitivity to Flusilazole/Carbendazim.

In Fig. 3.3 (W_{iu} vs W_{is}), quartile C has been classified to contain the genotypes that are consistently stable. The genotypes that plot close to the diagonal have similar levels of stability under rust stress and rust free conditions, and could conceivably use the same non-

additive mechanisms to achieve this stability. Cordell plotted in quartile C, but had a slightly larger ΔW_i (Table 3.9) than the other genotypes in this quartile. In the pre-screening exercise, it was established that this genotype had a degree of partial resistance to soybean rust, which would mean that the genetic control of stability under sprayed and unsprayed conditions is likely to be different for this genotype at least. There was a significant correlation ($r_s = 0.609^*$) between the genotypic ranking of W_{iu} and W_{is} , however, less significant than between P_{iu} and P_{is} .

3.5.2.4 Combined statistic

In a study on yield stability in wheat, Purchase *et al.* (2000) found that the ecovalence statistic of Wricke (W_i) ranked genotypes in a significantly similar manner to the AMMI stability value (ASV), but differently to the Lin and Binns (1988) P_i statistic. Their conclusion was that P_i ranked genotypes more similarly to a performance measure than to a stability measure. By inference then, the plotting of W_{iu} against P_{iu} holds prospects of selecting genotypes simultaneously for consistency of yield performance (P_{iu}) and yield stability (W_{iu}) under soybean rust stress conditions. P_i is generated from the mean performance of a genotype relative to the maximum performance in each environment, and is considered a good measure of the variance of general adaptation or the consistency of superior performance. W_i is generated from the non-additive interaction effects between genotype and environment and is thought of as a measure of specific stability. The two statistics are thus complimentary to each other, and could be used in combination to detect tolerance to soybean rust which would conform to the strictest definition of soybean rust tolerance: consistent superior yield and high levels of stability in the presence of rust.

Plotting W_{iu} against P_{iu} (Fig. 3.4) produced two distinct groupings of genotypes: the more stable, higher yielding rust tolerant group, clustered close to the origin of the graph in quartile C; the lower yielding less stable, less tolerant group, in quartile B. It is possible from the biplot to select the most tolerant group of genotypes, but it is not possible to rank them for tolerance from the biplot. The algebraic calculation of the distance from the origin of the biplot to the coordinate would provide a single statistic ($W_i P_i$) to facilitate this.

Purchase *et al.* (2000) used the same principle to develop the ASV statistic from the biplot of IPCA1 and IPCA2, except that the IPCA components were weighted by their

proportional contribution to the interaction sum of squares. Weighting of W_i and P_i would be unnecessary in the case of the W_iP_i statistic, since yield and yield stability are equally important in the strictest definition of tolerance. This is likely the first application of these statistics, using either a W_i vs P_i biplot or a single combined statistic (W_iP_i), to characterise genotypes for soybean rust tolerance.

3.5.3 The relationship between indicators of tolerance and rust-free yield

The objective of tolerance breeding should be to develop a genotype that would yield consistently well despite soybean rust infection. A further requirement would be that the genotype should be high yielding under low rust pressure, or in the absence of the disease altogether. Due to the highly significant correlations between unsprayed and sprayed yield within each season (Table 3.8) and P_{iu} with P_{is} and W_{iu} with W_{is} , it is concluded that selection for low P_{iu} or low $W_{iu}P_{iu}$ may also indirectly select for high yield and yield stability in the absence of rust. Care should be taken in extrapolating this conclusion to other situations, as it is again emphasized that the genotypes evaluated in this study were a highly select group which had had two seasons of pre-selection for tolerance before inclusion in this trial. Further, the effectiveness of P_i and W_i in discriminating tolerance levels and the relationship between unsprayed and sprayed yields may change with increasing levels of yield stress induced by soybean rust. Tschanz *et al.* (1983) reported that sprayed and unsprayed yields were not correlated in their field trials in Taiwan. Conditions in their trials were distinctly more severe than those experienced in this experiment (up to 90.2% yield loss), and therefore the lack of agreement between the two data sets is perhaps not surprising.

3.6 CONCLUSION

The P_{iu} statistic appears to be a highly suitable measure of the consistency of genotype performance in the presence of rust, as it combines unsprayed yield variability relative to an achievable maximum yield in a single parameter. Compared to the current norm of using yield loss % for determining tolerance, the calculation of P_{iu} would involve fewer resources as it does not require a full split-plot fungicide trial to generate the data. Genotypes could be evaluated under rust pressure, using a single **sprayed** genotype as a benchmark for the maximum yield at each location. The optimum control genotype for

each environment may be chosen, without the necessity of having the same control over all environments (Lin and Binns, 1988). The W_{iu} statistic is complementary to P_{iu} and may also be derived without the need of a full split plot design.

The combination of W_{iu} and P_{iu} in a biplot or as a combined statistic $W_{iu}P_{iu}$, successfully identified the highest yielding, most stable genotypes and therefore the most tolerant genotypes in the presence of moderate levels of soybean rust stress. JV780, JV783, JV870, JX270-2, PAN 589 and JV861 (Table 3.9 and Fig 3.4) have the potential to be used as tolerant parents in the next cycle of breeding and selection. Given the current yields, grain price and spraying costs, the level of tolerance demonstrated is unlikely to be sufficient for a commercial producer to plant these genotypes without spraying at all. The level of tolerance exhibited could potentially make the timing of the spraying less critical and possibly reduce the number of sprays required in a season. The benefits of this level of tolerance have not yet been demonstrated because until now, there have been no suitable genotypes released for this sort of research to be conducted.

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APPENDICES 3

Appendix 3.1 Data from soybean rust pre-screening trials

Genotype	MG ^a	Reprod ^b Days	Latent ^c Days	Latent ^d %	Exposure ^e Days	Pustule Type	Rust % within canopy strata ^f			Overall rust
							Lower	Mid	Upper	
PAN 494	IV	56	29	52	27	Tan	60	40	30	43.3
JX270-2	V	56	31	55	25	Tan	30	20	10	20.0
JV781	V	56	43	77	13	Tan	40	10	10	20.0
Delsoy 4900	V	57	31	54	26	Tan	40	15	5	20.0
JV870	V	56	31	55	25	Tan	20	10	10	13.3
JV861	VI	56	31	55	25	Tan	40	20	10	23.3
JV783	VI	58	45	78	13	Tan	30	10	5	15.0
PAN 589	VI	59	32	54	27	Tan	50	30	30	36.7
JV780	VI	55	30	55	25	Tan	40	20	20	26.7
JV860	VII	55	30	55	25	Tan	40	20	10	23.3
Cordell	VII	58	24	41	34	Tan	40	20	10	23.3
JV762	VIII	67	37	55	30	Tan	20	10	5	11.7
PAN 875	VIII	53	26	49	27	Tan	40	20	10	23.3
UFV 3	VIII	67	37	55	30	RB	20	10	5	11.7

^aMG = Maturity group

^bReprod days = number of days from flower to physiological maturity

^cLatent days = number of days from flower to first pustule

^dLatent % = Latent days/Reprod days x 100

^eExposure days = Reprod days – latent days

^fRust % = visual estimate of leaf area covered with pustule, taken in the lower, mid and upper thirds of the canopy, and meaned to give an overall %

Notes:

Latent period

The latent period is normally defined as the time between infection and the first symptom. In this case, the actual infection date is not known, but the assumption is made that inoculum is present in the field and that the genotypes are all post flower when the

conditions in the field are favourable for infection to take place. Whether or not this is the case, it is probably appropriate to correct for RLT and where possible, make comparisons within maturity groups. Latent periods under ideal conditions in a glasshouse range between 6 to 12 d, which indicates that the data presented is likely to be inflated by a post flower period before infection.

Exposure

The 'Exposure days' is simply the number of days post first pustule in which the rust has to cause yield loss. Where the number of days of exposure is low, essentially the genotype is escaping the effects of soybean rust. If it is as a result of a long latent period, then this is a source of partial resistance that can be exploited further. If it is as a result of short maturity, this is not necessarily a stable mechanism over all planting dates and seasons despite being effective.

Stratified canopy

Soybean rust normally develops at the base of the canopy, where conditions are most favourable, and moves up the canopy as the disease progresses over time. The idea of measuring rust infection in three strata within the canopy is to assess not only the severity of leaf area loss, but also to judge the disease progression. For this to be of any value, all the genotypes need to be assessed at the same stage of development (R7 in this case). Genotypes with low severity measurements in the upper canopy, may be expressing slow rusting. Due to the sensitivity of the measurements to RLT, this is not a highly regarded technique.

Appendix 3.2 Normalisation of data means

Normalisation of sprayed and unsprayed mean yields before the calculation of correlation coefficients was computed according to the following formula:

$$\text{Normalised data mean} = (\text{data mean yield} - \text{general trial mean})/\text{SE}$$

Appendix 3.3 Seasonal yield loss at Greytown

Table A3.2 Yield loss in 2003/04 season at Greytown, as a result of soybean rust

	Yield (t ha ⁻¹)			Yield
	No spray	Spray	Loss	Loss%
Cordell	1.659	1.835	0.176	9.6
Delsoy 4900	2.693	2.655	-0.038	-1.4
JV762	2.244	2.678	0.434	16.2
JV780	2.721	3.195	0.474	14.8
JV781	3.022	3.367	0.345	10.2
JV783	2.869	3.047	0.178	5.8
JV860	3.064	3.029	-0.035	-1.2
JV861	3.241	3.530	0.289	8.2
JV870	2.559	3.056	0.497	16.3
JX270-2	3.184	3.306	0.122	3.7
PAN 494	2.644	2.804	0.160	5.7
PAN 589	2.755	2.922	0.167	5.7
PAN 875	2.235	3.101	0.866**	27.9
UFV 3	1.780	1.791	0.011	0.6
Mean	2.619	2.880	0.260	9.0

Difference in genotypic means: LSD (0.05) = 0.500 t ha⁻¹

LSD (0.01) = 0.676 t ha⁻¹

Difference in spray treatment means: LSD (0.05) = 0.280 t ha⁻¹

Table A3.3 Yield loss (t ha^{-1}) in 2004/05 season at Greytown, as a result of soybean rust

	Yield (t ha^{-1})			Yield
	No spray	Spray	Loss	Loss%
Cordell	2.116	2.247	0.131	5.8
Delsoy 4900	2.026	2.225	0.199	8.9
JV762	3.122	3.434	0.312	9.1
JV780	3.082	3.518	0.436	12.4
JV781	2.448	3.018	0.570*	18.9
JV783	2.979	3.368	0.389	11.5
JV860	2.580	3.262	0.682**	20.9
JV861	2.638	2.817	0.179	6.4
JV870	2.829	3.229	0.400	12.4
JX270-2	2.758	2.935	0.177	6.0
PAN 494	2.890	3.101	0.211	6.8
PAN 589	2.702	3.226	0.524*	16.2
PAN 875	2.496	3.341	0.845**	25.3
UFV 3	3.058	2.959	-0.099	-3.3
Mean	2.695	3.049	0.354*	11.6

Difference in genotypic means: LSD (0.05) = 0.500 t ha^{-1}

LSD (0.01) = 0.676 t ha^{-1}

Difference in spray treatment means: LSD (0.05) = 0.280 t ha^{-1}

Table A3.4 Yield loss (t ha^{-1}) in 2005/06 season at Greytown, as a result of soybean rust

	Yield (t ha^{-1})			Yield
	No spray	Spray	Loss	Loss%
Cordell	1.863	1.902	0.039	2.1
Delsoy 4900	2.464	3.054	0.590*	19.3
JV762	2.458	2.978	0.520*	17.5
JV780	3.140	3.791	0.651*	17.2
JV781	2.581	3.047	0.466	15.3
JV783	3.507	3.943	0.436	11.1
JV860	3.486	3.720	0.234	6.3
JV861	3.331	3.674	0.343	9.3
JV870	2.979	3.482	0.503*	14.4
JX270-2	3.053	3.401	0.348	10.2
PAN 494	2.149	2.696	0.547*	20.3
PAN 589	3.463	4.223	0.760**	18.0
PAN 875	3.326	4.041	0.715**	17.7
UFV 3	2.424	2.858	0.434	15.2
Mean	2.873	3.344	0.470*	14.1

Difference in genotypic means: LSD (0.05) = 0.500 t ha^{-1}

LSD (0.01) = 0.676 t ha^{-1}

Difference in spray treatment means: LSD (0.05) = 0.280 t ha^{-1}

CHAPTER 4

GENERAL OVERVIEW AND FINAL CONCLUSIONS

4.1 DISCUSSION

The need for soybean breeding to continue in South Africa due to the unique environmental conditions peculiar to local agriculture was established in the introduction to this thesis. Furthermore, the threat of patented traits restricting the free flow of germplasm has become a reality, and programmes / countries that do not have access to these traits or genotypes containing these traits face the threat of becoming marginalized. For soybean production in the country to remain competitive in the global market, local breeding programmes need to be efficient and effective.

Breeding strategies dictate the long term success and efficiency of a programme and most often follow tried and tested strategies. In the case of South African programmes, these strategies would generally have been established elsewhere in the world where the factors affecting programmes may have been different. Almost all procedures carried out in a programme will have a consequence, some benign, others potentially important. Some procedures are dictated by convenience and the effect may not be fully appreciated in the short term. An example of this is a case alluded to earlier in this thesis. The shuttle breeding approach in the CIMMYT wheat breeding programme (Braun *et al.*, 1996) had originally been implemented to speed up the breeding process but the consequence of this change in strategy was the effective selection for wide adaptation. In the PANNAR programme, selection within the segregating generations is left to the end of the harvest season to give preference to trial harvesting and data collection. The long term consequence of this decision (dictated primarily by convenience) is that resistance to shattering is a characteristic trait in all the commercial varieties bred in the programme. In a similar vein, the segregating generations are generally planted late (after the main trial programme is complete) also as a matter of convenience. The long term effect of this is less positive, as the later planting date restricts vegetative growth and selection for lodging resistance is less efficient than it could be.

Decisions on programme strategy have long term effects on breeding programme effectiveness and efficiency, and deserve continual assessment of their validity. As

variables in the programme change, strategies need to be re-evaluated. So too, where possible, strategies that were based on global norms need to be assessed under local conditions and customs.

4.1.1 Pedigree breeding

Soybean breeders have to a large extent adopted SSD as the default breeding method over the more labour intensive pedigree breeding method (Boerma and Cooper, 1975; Snape and Riggs, 1975). Contrary to soybean breeding convention, the PANNAR programme has continued to use the pedigree breeding approach. The move to mechanisation and the increased cost of manual labour that have historically influenced the change to SSD in other parts of the world have not had the same influence in Africa. Work in this thesis has highlighted two distinct benefits that are to be gained from the continued implementation of a pedigree breeding strategy.

Manjit Kang (2002) states: “I expect that there would be a greater emphasis on participatory plant breeding, which involves scientists, farmers, consumers, extension personnel, industry and others, in the future”. Despite Kang’s predictions, mainstream plant breeders have not embraced participatory breeding approaches for a number of valid reasons. There are probably very few farmers on the land that do not have an opinion (valid or not) on the perfect crop ideotype for his or her conditions. The problem with the participatory approach is that an opinion alone is inadequate; it also requires the participant to have an understanding of basic plant breeding principles and biometrical techniques to be successful. Further, the mechanics of the breeding process needs to be managed to ensure that a result is achieved at the end of the process. The classical participatory approach takes the control and any semblance of predictability of an end result out of the hands of the plant breeder, and places a number of logistical problems in the way of achieving them. The satellite section site strategy as proposed in Chapter 1 could be employed in a participatory approach without the loss of control over the end result, but requires a pedigree breeding system for effective implementation.

Single seed descent is greatly dependent on the yield in the first trial evaluation season being representative of genotypic potential. The success of SSD is to a significant extent reliant on evaluating large numbers of genotypes and therefore equally reliant on high

selection intensities. With the large seasonal variation in genotypic ranking displayed in the soybean rust trials in Chapter 3, regardless of the method of rust tolerance assessment, a single season's evaluation remains a high risk strategy. For this reason, the pedigree breeding approach may be more suited to breeding for soybean rust tolerance. With the pedigree approach, families are repeatedly exposed, visually evaluated and selected under soybean rust pressure which is subject to seasonal variation in timing and severity for a number of generations.

The assessment of yield loss to soybean rust conducted by McLaren (2008) indicated that South African commercial genotypes selected for yield in the absence of rust exhibited no useful tolerance when evaluated in the presence of rust. The chances of achieving a commercially viable level of tolerance by simply evaluating homozygous lines in the presence of rust is low given the lack of reported success around the world. Clearly, this presents a strong case for the implementation of pedigree breeding for rust tolerance that utilizes repeated exposure and selection throughout the segregating generations.

4.1.2 Adaptation strategies and dealing with GxE interaction

Plant breeding efforts are generally considered to be hindered by significant GxE interactions; however, if the data are analysed wisely there may be opportunities that could be exploited by appropriate breeding strategies (Annicchiarico, 2002). In Chapter 1, the control strategy was a broad adaptation strategy, where lines were generated at the breeding station in Greytown and tested for adaptation at another locality, in this case Delmas. This has historically been the strategy followed in the programme for adaptation to all the major production regions (Cool, Moderate and Hot) and has had a measure of success in the past based on broad adaptation. Implementation of the satellite selection strategy provides an opportunity to take advantage of the GxE interaction by developing lines with specific adaptation to different regions by selecting segregating populations in those regions. The yield advantage is brought about by the change in breeding selection strategy from broad to specific adaptation and by the change from conventional to participatory breeding.

Kang (2002) suggested that where genotype x planting date interaction exists, more replications and more planting dates are needed to improve precision. An increase in the

precision of soybean rust tolerance breeding can be brought about by taking cognisance of this interaction with planting date and adjusting the programme strategy to accommodate this. Selecting for soybean rust tolerance in the segregating generations needs to be conducted at a 'late' planting date to minimise the possibility of escapes, considering that no replication in the early selection generations is normally possible. At the later stages of evaluation, Kang's suggestion of more replications and more planting dates may be implemented.

Poor correlation in genotype ranking for yield loss over seasons in Chapter 3 resulted in inconsistent classifications of soybean rust tolerance using traditional methods. Since conditions that generate year to year variation in genotypic ranking cannot be predicted in advance of the season, there is no breeding strategy that can take advantage of this GxE interaction. Stability analysis can, however, quantify this and assist in the selection of genotypes with the highest yield stability. The development of the novel W_iP_i statistic allows for the simultaneous selection of consistency of yield performance and specific stability. Using the W_iP_i statistic to select best performing genotypes combines a broad adaptation strategy for yield performance and a specific adaptation strategy for yield stability. Further investigation is required to see whether this concept has application in other areas of plant breeding.

4.1.3 Genotypes

The primary objective of any breeding programme is the output of genotypes. A thesis of this nature would be incomplete without comment on genotypes even if the objective of the research was primarily to optimise strategies, not to directly breed genotypes.

Three genotypes (JV933, JV919 and JV910) with good general adaptation were identified in the satellite selection study in Chapter 1. These genotypes all ranked within the top 10 yields at both the satellite site and the breeding station. Of these, JV933 had exceptional adaptation to both sites and could be considered as a parental source for general adaptation in future breeding projects. As parents in combination with Forrest, Wilge and PAN 556 each contributed three out of the top 10 lines at each of the testing sites, indicating that they have a large number of yield genes and should be considered for further use as parents. Prima as a parent on the other hand, produced progeny lines with a high mean

performance in their selection environment, indicating good specific adaptation to both localities. Prima would also have great value as a parent in future breeding projects.

In Chapter 2, the intention was to assess trends in yield loss as a result of soybean rust. This study used a large number of genotypes in order to reduce the possibility of any single genotype having a major influence on the trends. From the graphs (Fig 2.6) it is clear that PAN 421R had an atypical reaction relative to its maturity, and probably should not be included in future soybean rust trials if few genotypes are evaluated.

In Chapter 3, the biplot of W_{iu} versus P_{iu} (Fig 3.4) identified the most tolerant genotypes to soybean rust. Whilst progress has been made in selecting for tolerance, the genotypes JV780, JV783, JV870, JX270-2, PAN 589 and JV861 are at best moderately tolerant, because fungicide application would still be required under South African conditions to prevent economic yield loss. These genotypes could be used in further research to establish the value of moderate levels of tolerance in a spraying programme. It has not been established whether moderate tolerance would affect the number and timing of fungicide spray operations for commercial soybean growers. None of these genotypes were bred from crosses made intentionally for soybean rust tolerance, so it is expected that further improvements in tolerance levels are possible through inter-crossing these lines. While cultivar registration would not be considered on any of these genotypes, they have potential as parental germplasm. A further application of these tolerant genotypes would be to combine them with single gene sources of resistance. Specific resistance genes (Monteros *et al.*, 2007; Neto, 2007; Boerma, 2008) could be backcrossed into soybean rust tolerant backgrounds using marker technology. This may provide high yielding resistant genotypes that would be buffered by tolerance against the risk of large yield losses should the specific genes be defeated during any season.

4.2 FINAL CONCLUSION

The findings of this collation of studies on improving the efficiencies of this particular breeding programme are important for the competitiveness of this programme in the South African context. The recent termination of the national soybean breeding efforts in 2007 elevates the importance of this commercial programme to that of national interest, as the responsibility for providing suitable genotypes for the entire country is placed in the hands

of few. Further growth in soybean production in South Africa is anticipated in the near future as the bio-fuels industry gears up for processing more than 1×10^6 t soybean grain for oil and high protein feed. These developments add to the importance of the conclusions of this study in the local context. In a global context, it is possible with the necessary care to extrapolate aspects of the research conducted in this study to other programmes. The most important results of this research which have potential application in other programmes can be summarized as follows:

- Application of satellite selection of F_2 generations to the participatory breeding concept
- Use of the pedigree breeding system in breeding for rust tolerance
- Need for increased number of planting dates and replications in soybean rust tolerance screening
- Use of the superiority measure P_i for rust tolerance evaluation diminishes the need for labour intensive split-plot trials
- Evaluation of rust tolerance using the combined statistic (W_iP_i) for simultaneous selection of consistency of superior yield performance and yield stability

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GENERAL APPENDICES

General appendix: Publication 1

Investigating the use of satellite selection sites in soybean (*Glycine max* (L.) Merr.) breeding

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Investigating the use of satellite selection sites in soybean (*Glycine max* (L.) Merr.) breeding

A satellite selection site is a decentralized selection environment that could be employed to expand the influence of existing breeding programmes to additional target environments. This study was designed to establish whether it would be possible to achieve site-specific adaptation in soybean (*Glycine max* (L.) Merr.) through the selection of only a single generation (F_2) at a satellite breeding site. Five soybean populations were subjected to two pedigree selection strategies, and the lines generated were evaluated in trials at both selection localities. The control selection strategy (CSS) entailed selection exclusively at the breeding station (Greytown) from F_2 to F_5 generations. The satellite selection strategy (SSS) involved selection of the F_2 in the target environment (Delmas), followed by the F_3 to F_5 generations at the breeding station (Greytown). In yield trials at the satellite location, lines developed through the SSS performed best, averaging 11.9% higher yield than CSS lines. The results indicate that a single generation (F_2) of selection in the target environment is effective in fixing significant yield adaptation to that environment. The study shows that breeders can use satellite breeding sites to extend the influence of their programmes with very little additional effort.

Keywords: yield; decentralized strategy; site-specific adaptation;

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Introduction

Plant breeding lore for developing genotypes for a specific environment would advocate selection in that environment (Falconer, 1981). In practice, it is not always logistically possible to maintain a full breeding programme in all target environments. The norm for soybean (*Glycine max* (L.) Merr.) breeding would be to select the segregating generations in one environment (usually at the main breeding station) and the lines generated would then be tested in multiple target environments (Simmonds, 1991; Orf, 2004). This strategy is generally effective in selecting superior genotypes if the genotypic reaction in the target environment is similar to the genotypic reaction in the selection environment. However, if there is a significant genotype by environment interaction, this practice is unlikely to be the most effective method for developing varieties for that specific target environment.

Financial considerations may prevent the establishment and maintenance of separate breeding stations for all target environments, but may still allow the operation of satellite selection sites. A satellite selection site could be established at any facility (such as a remote testing site with little or no infrastructure) that would allow for effective selection of a segregating population. The breeding activities at a satellite selection site should be restricted to the minimum required to achieve a significant adaptation to that site; however, this has not yet been quantified. This study was designed to establish whether it is possible to secure site-specific adaptation to a target site by a single generation of selection in that environment.

Material and methods

Breeding strategy

The data used in this study was generated in the soybean breeding programme of PANNAR, a private seed company headquartered in Greytown, KZN, Republic of South

Africa. The breeding station is located at Greytown (Table 1), which has a moderate climate allowing the evaluation of a wide range of maturity groups, IV through to VIII (Smit & de Beer, 1994; 1995). The limited size of the total South African soybean production area, however, does not justify a second breeding station. The target environment chosen for the establishment of a satellite selection site was Delmas, Mpumalanga, located within an important soybean production area. Unlike at the breeding station, genotypic adaptation at the satellite site is more specific and is restricted to maturity groups IV and V (Smit & de Beer, 1994; 1995). A conventional pedigree breeding procedure was used in the study, where visual selection was carried out up to the F₅ generation. Five populations were generated in 1996, all utilizing Forrest (Hartwig & Epps, 1973) as a female parent. Five male parents were chosen from a diverse range of well adapted privately bred genotypes. All six parents had good yield performance records at both localities (Smit & de Beer, 1994; 1995). The female parent Forrest had white flowers and all the male parents had purple flowers. Confirmation of the F₁ was done using flower colour as a marker. Each of the five F₂ populations was split in two equal quantities containing 900 seeds, in order to impose the two selection strategies. In the control selection strategy (CSS), all segregating generations were selected at the breeding station by the breeder. In the satellite selection strategy (SSS), F₂ plant selections were made at the satellite site in the target environment by a trial technician, after which all subsequent generations (F₃ through to F₅) were selected at the breeding station by the breeder. All breeding generations were space planted (0.15 m intra-row, 0.9 m inter-row) to enhance selection efficiency. Thirty F₂ plants from all of the five F₂ populations at each of the selection sites were selected. Pedigree selection was followed through to the F₅ generation, where three of the best lines from each population and for each selection strategy were individually bulked for testing in the F₆ generation. A selection index that considered all

important agronomic criteria at harvest (including, but not limited to: lodging; shattering; pod height; green stem) in a single rating was used to determine the three best lines per population. In cases where the index alone failed to discriminate the three best lines, progeny row yields were used to aid selection.

Evaluation of breeding lines

The effect of the two selection strategies (15 lines per selection strategy, represented by three $F_{4,6}$ lines from each of five different pedigrees) on yield adaptation was evaluated in trials set out in a randomized block design with two replications, planted in both the target environment and at the breeding station. The trials were planted early in November and repeated again at both locations at a later planting date in the same season (Table 1). The net plot size was two rows of 4.4 m, planted at an inter-row spacing of 0.9 m and seeded at a plant population of 380000 plants ha^{-1} . Plots were harvested with a plot combine and the plot grain yields were converted to kg ha^{-1} at 12.5% moisture.

Statistical analysis

After F tests showed homocedasticity of the error variances derived from the analyses of the individual planting dates, the data from the two planting dates at each of the locations were pooled. A three factor ANOVA was performed using Genstat version 4.2 to estimate the main effects of selection strategy, pedigree, trial location and their interactions. Least significant differences ($P = 0.05$) based on t -values were determined by Genstat.

Results

Mean yield

The effect of using the satellite selection site to improve adaptation to the target environment can be quantified by comparing yields of the 15 lines developed using SSS with those of the 15 CSS lines (Table 2). The positive yield difference between SSS and CSS, as measured in the trials conducted in the target environment, represents the beneficial effects of applying a single generation (F_2) of selection in the target environment. Although there was some variation in the effect of the two selection strategies across pedigrees, the mean difference between SSS and CSS of $+306 \text{ kg ha}^{-1}$ was significant ($P = 0.05$). In contrast, the negative yield difference between SSS and CSS, as measured in the trials conducted at the breeding station, represents the detrimental effects that a single generation of selection at the satellite site had on yield adaptation to the breeding station location. Again, the effect of selection strategy varied across pedigrees, with the mean effect of -164 kg ha^{-1} not significant ($P = 0.05$).

Standardizing the effect of selection strategy by expressing it as a percentage of the control strategy, allows for the comparison of these responses across the two trial environments. The mean standardized effect of the SSS in the target environment was 11.9%, compared to -5.3% in the breeding station environment. In the target environment yield trials, the difference in the mean effect between the two selection strategies is attributable solely to selection of the F_2 in the target environment. In contrast, in the breeding station yield trials, the difference in the mean effect of selection strategies resulted from the negative effect of selecting the F_2 in the target environment not being completely moderated by the positive effects of selection at the breeding station in the later filial generations ($-F_2 + [F_3 \text{ to } F_5]$).

Individual line performance

Although three individual lines selected using the CSS were well ranked at the satellite site (Table 3), the frequency of SSS lines within the top 10 rankings (7/10) far exceeded those of the CSS, which demonstrates the effectiveness of the SSS strategy. All three CSS lines that ranked within the top 10 (JV933; JV942; JV910) at the satellite site, were also well ranked at the breeding station. In contrast at the breeding station, the domination of the CSS strategy was less well defined based on individual line rankings. There were two individual SSS lines (JV919 and JV951) that ranked within the top 10 at both locations.

Discussion

In the target environment a yield response to the satellite selection strategy was expected *a priori*, but the magnitude of this response was not predicted. A yield improvement of 11.9% relative to the control strategy in the target environment was achieved with selection of only the F₂ in the target environment (Table 2). To place the magnitude of the response in this study into perspective, long term annual yield gains in soybean breeding programmes have generally been calculated as being between 1-2% (Wilcox, 2001; Ferrarotti, 2004; Singh *et al.*, 2004). The large effect of selection in the F₂, when loci are highly heterozygous, strongly implicates additive gene action for the genes conditioning specific adaptation. The comparisons of individual line rankings at the two locations (Table 3) showed that it is possible to select lines at the breeding station (CSS) that would perform well at the satellite station and *vice versa*. Their good performance could conceivably be ascribed to general adaptation. Nevertheless, the best line and the overwhelming majority of the better performing lines at the satellite site were lines

selected for specific adaptation to that site using the SSS. Whilst the lore that exclusive selection in an environment is the best way to achieve adaptation to that environment held true in this study at the breeding location, a third selection strategy (selection of all generations in the target environment) would be required to provide an estimate of the relative effectiveness of a single generation versus exclusive selection in a target environment.

One of the factors contributing towards the large relative effect of the satellite selection strategy was the dissimilar nature of the two selection environments (Table 1). It is possible, and indeed probable, that more subtle differences between environments may not elicit a response of the same magnitude. Parents adequately adapted to both localities, were specifically used in this investigation. Not doing this could have amplified the difference between the strategies and skewed comparisons in a particular direction. In addition, the differing reactions between pedigrees indicate that there is variation amongst parents in genes for specific and general adaptation that could be exploited further. As an example, selection of the F_2 populations derived from the male parent Prima produced lines which had specific adaptation to either F_2 selection environments. Responsive parents such as Prima are ideally suited to satellite selection programmes. In contrast, selection within the population containing PAN430 as a parent generated low yielding lines (particularly in the CSS) that did not demonstrate a consistent response pattern.

The difference in the mean effect of the selection strategies was smaller when measured at the breeding station than at the satellite site. This indicates that the subsequent selection (F_3 – F_5) of the SSS lines at the breeding station improved their adaptation to that environment too, leading to a smaller (non-significant) difference between the strategies at that site. From this it is deduced that selection in more than one environment could improve general adaptation and reduce environmental sensitivity. This is consistent with

the ‘shuttle breeding’ approach in wheat, referred to by Allard (1999). Use of a satellite selection site would thus not only improve specific adaptation to target sites but conceivably also improve adaptation to non-target environments (where subsequent selection is performed), thereby enhancing yield stability.

Conclusions

This study showed conclusively that it is possible to secure a significant level of adaptation to a target site by a single generation (F_2) of selection in that environment. It also demonstrated that the utilization of a satellite breeding site could be an efficient and practical method of expanding the effectiveness of established breeding programmes. The two locations used in the study are not important to the conclusion *per se* other than for the magnitude of the differing cultivar reaction they evoke. Caution should still be exercised when extrapolating these results to other environments and the strategy in general to other breeding programmes. Although pedigree breeding is not commonly employed in commercial soybean breeding, the use of a satellite selection site could be adapted to other breeding procedures including the early generation testing system described by Cooper (1990). The benefits of utilizing a satellite selection site would vary in magnitude depending on a number of factors, including the choice of parents and how dissimilar the two environments are from each other. The original aim of using a satellite selection site was to acquire specific adaptation to a target environment. However, in the process it has been shown that selection at more than one environment also improves general adaptation, which is an important consideration for any commercial programme.

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Table 1 Selection site details.

	Breeding station (Greytown)	Target environment (Delmas)
Altitude (masl)	1012	1580
Latitude (°S)	29°04'57''	26°08'75''
Longitude (°E)	30°36'12''	28°42'74''
Annual rainfall (mm)	776	562
Crop rotation	Maize/soybean	Maize/soybean/maize/sunflower
Normal soybean planting season	1 November – 15 December	1 – 30 November
Trial planting dates:	5.11.2001	2.11.2001
	8.12.2001	27.11.2001
Best suited soybean maturity group	Mid VI	Late IV

Table 2 Analysis of the effect of selection strategy on yield performance of soybean lines.

Location	Pedigree [†]	Selection strategy		Effect of SSS	Standardized
		SSS [‡]	CSS [§]	(SSS-CSS)	effect of SSS
		Yield (kg ha ⁻¹)			% [¶]
Target	Forrest/PAN430	2596	2310	286	12.4
Environment	Forrest/PAN556	3033	2684	349	13.0
(Delmas)	Forrest/Hennops	2626	2602	24	0.9
	Forrest/Prima	3169	2446	723*	29.6
	Forrest/Wilge	2922	2770	152	5.4
	Mean[#]	2869	2563	306*	11.9
Breeding	Forrest/PAN430	3023	2834	189	6.7
Station	Forrest/PAN556	3075	3021	54	1.7
(Greytown)	Forrest/Hennops	2855	3065	-210	-6.9
	Forrest/Prima	2683	3128	-445*	-14.2
	Forrest/Wilge	2920	3327	-350	-10.5
	Mean[#]	2911	3075	-164	-5.3

* Significant ($P = 0.05$)

[†]Each pedigree is represented by the mean yield of three individual lines

[‡]SSS = Satellite selection strategy, F₂ selected in target environment, further selection of F₃₋₅ at breeding station

[§]CSS = Control selection strategy, selected at breeding station from F₂₋₅

[¶]% = Difference between the selection strategies, expressed as a percentage of the yield of CSS

[#]Mean = Mean derived from 15 lines x 2 reps x 2 planting dates, SED = 0.1073

Table 3 Yield ranking of individual lines tested at the satellite site and the breeding station, pooled over planting dates.

Satellite site (Delmas)				Breeding station (Greytown)			
Rank	Selection strategy	Line Code	Pedigree	Rank	Selection strategy	Line code	Pedigree
1	SSS [†]	JV963	Forrest/Prima	1	CSS	JV930	Forrest/Wilge
2	SSS	JV951	Forrest/Wilge	2	CSS	JV933	Forrest/Wilge
3	CSS [‡]	JV933	Forrest/Wilge	3	SSS	JV917	Forrest/PAN556
4	SSS	JV916	Forrest/PAN556	4	CSS	JV910	Forrest/PAN556
5	SSS	JV919	Forrest/PAN556	5	CSS	JV936	Forrest/Prima
6	SSS	JV952	Forrest/Wilge	6	SSS	JV980	Forrest/Hennops
7	CSS	JV942	Forrest/Hennops	7	CSS	JV943	Forrest/Hennops
8	CSS	JV910	Forrest/PAN556	8	SSS	JV919	Forrest/PAN556
9	SSS	JV967	Forrest/Prima	9	SSS	JV994	Forrest/PAN430
10	SSS	JV983	Forrest/Hennops	10	SSS	JV951	Forrest/Wilge
11	SSS	JV966	Forrest/Prima	11	CSS	JV942	Forrest/Hennops
12	SSS	JV997	Forrest/PAN430	12	SSS	JV996	Forrest/PAN430
13	CSS	JV945	Forrest/PAN430	13	SSS	JV953	Forrest/Wilge
14	SSS	JV981	Forrest/Hennops	14	CSS	JV911	Forrest/PAN556
15	CSS	JV911	Forrest/PAN556	15	CSS	JV938	Forrest/Prima
16	SSS	JV996	Forrest/PAN430	16	CSS	JV935	Forrest/Prima
17	CSS	JV930	Forrest/Wilge	17	CSS	JV928	Forrest/Wilge
18	SSS	JV917	Forrest/PAN556	18	CSS	JV948	Forrest/PAN430
19	CSS	JV939	Forrest/Hennops	19	SSS	JV981	Forrest/Hennops
20	CSS	JV938	Forrest/Prima	20	CSS	JV939	Forrest/Hennops
21	CSS	JV935	Forrest/Prima	21	SSS	JV967	Forrest/Prima
22	CSS	JV936	Forrest/Prima	22	SSS	JV997	Forrest/PAN430
23	CSS	JV948	Forrest/PAN430	23	SSS	JV916	Forrest/PAN556
24	CSS	JV928	Forrest/Wilge	24	CSS	JV947	Forrest/PAN430
25	CSS	JV943	Forrest/Hennops	25	CSS	JV945	Forrest/PAN430
26	CSS	JV912	Forrest/PAN556	26	SSS	JV966	Forrest/Prima
27	SSS	JV994	Forrest/PAN430	27	CSS	JV912	Forrest/PAN556
28	SSS	JV980	Forrest/Hennops	28	SSS	JV952	Forrest/Wilge
29	SSS	JV953	Forrest/Wilge	29	SSS	JV963	Forrest/Prima
30	CSS	JV947	Forrest/PAN430	30	SSS	JV983	Forrest/Hennops

[†]SSS = Satellite selection strategy, F₂ selected in target environment, further selection of F_{3.5} at breeding station

[‡]CSS = Control selection strategy, selected at breeding station from F_{2.5}

General appendix: Publication 2

A review of soybean rust from a South African perspective

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This review article describes the nature of the soybean rust pathogen, its interaction with the soybean host and documents some of the history of soybean rust in South Africa. Soybean rust has affected soybean cropping in parts of South Africa since 2001. The disease causes leaf lesions, which may progress to premature defoliation and ultimately result in grain yield loss in susceptible soybean genotypes. Chemical control measures have been successfully employed to limit commercial yield losses in South Africa; however, controlling the effects of this disease through host resistance or tolerance mechanisms remains a long-term goal.

Soybean rust, caused by the fungus *Phakopsora pachyrhizi* Sydow, was reported on soybeans (*Glycine max* L. Merr) in the Vryheid district of South Africa in February 2001,¹ and later identified in several other parts of KwaZulu-Natal (KZN) and Eastern Highveld production regions. Epidemics of soybean rust have since occurred in these areas every season to date (2008) and chemical control has become a standard commercial practice in the affected growing regions. Shortly after rust was identified in neighbouring Zimbabwe in 1998, a soybean rust workshop² was convened in Potchefstroom, South Africa, and a soybean rust task team was established to familiarise local researchers with the disease and

develop a pre-emptive national soybean rust strategy. Through visits to Zimbabwe in the three-year period between the first outbreak in Zimbabwe and the first reported outbreak in South Africa, many local researchers gained valuable experience in identifying the disease and managing the epidemics.³ Consequently, commercial losses in the first two seasons were far less than they could have been, as chemicals and protocols used in Zimbabwe were adopted until local research could support the soybean cropping industry.

The pathogen

There are approximately 80 species of *Phakopsora* known worldwide,⁴ of which six occur on legumes. Soybean rust is caused by two species, *P. pachyrhizi* and less commonly *P. meibomiae* (Arthur) Arthur. The latter species (*P. meibomiae*), commonly known as the cause of Latin American rust or Legume rust, is found in the western hemisphere and is not known to cause severe yield losses.⁵ The nomenclature history of these two species of rust is complex and their correct assignment in early reports, especially from Africa, remains uncertain.⁴ The subject of this review is restricted exclusively to *P. pachyrhizi*, the cause of the disease known commonly as Asian soybean rust, or simply soybean rust hereafter.

Global distribution

Before 1992, soybean rust was known to cause significant losses in Asia and Australasia, inclusive of the following countries: Australia; India; Indonesia; Japan; Korea; Peoples Republic of China; Philippines; Taiwan, Thailand; Vietnam.⁶ Not much was documented about the distribution of soybean rust in Africa before 1996 (given the problems with nomenclature); however, the following sequence of first reports⁷ were confirmed: Uganda, Kenya and Rwanda, 1996; Zimbabwe and Zambia, 1998; Nigeria,

1999; Mozambique, 2000; South Africa, 2001. During 2001 *P. pachyrhizi* was detected in Paraguay⁸ and this was followed shortly by confirmation of its presence in Argentina in 2002,⁹ Brazil and Bolivia in 2003.¹⁰ Uruguay, also a significant soybean producing country, recorded soybean rust for the first time in 2004.¹¹ Soybean rust was detected in Hawaii in 1994¹² which stimulated the convening of a workshop to discuss the potential threat that this held for the soybean crop in the USA. As correctly predicted by the delegates of this workshop,¹³ soybean rust had the potential to threaten crops on mainland USA. In 2004, nine years later, Schneider *et al.*¹⁴ confirmed the presence of soybean rust in the USA. From detection in Louisiana in 2004, it spread to nine states by 2005, and was detected in 15 states in 2006.¹⁵

Alternative hosts

The soybean rust pathogen is known to naturally infect 95 species from 42 genera of legumes, inclusive of important weed species like Kudzu vine (*Pueraria lobata*) and major crop species such as common bean (*Phaseolus vulgaris*).⁵ Such a broad host range is unusual amongst rust pathogens⁵ which normally have a narrow host range. The significance of the numerous alternative host possibilities for the soybean rust pathogen is that these may serve as an inoculum reservoir or a 'green bridge' from one soybean planting season to the next.

Epidemiology of soybean rust

The presence of a susceptible host, viable pathogen spores and suitable environmental conditions are requisites for the development of a soybean rust epidemic. The optimum temperature for urediniospore germination ranges between 12 to 27°C, depending on the source of the research.^{16,17,18} Urediniospore germination is greater in

darkness, with light either inhibiting or delaying germination.¹⁸ A further requirement for urediniospore germination is a period of leaf wetness. This period is considered to be about 6 h when this occurs within the optimal temperature range.¹⁹ The optimum temperature for uredinia formation is reported by Kochman²⁰ to be 17°C (night) and 27°C (day). Uredinia form on the leaves nine days post infection (DPI) under these conditions, with the urediniospores maturing 2 to 3 d later.²¹

Symptoms of soybean rust

First symptoms of soybean rust could be described as small water soaked lesions which develop into grey, tan to dark brown, or reddish brown lesions (uredinia) particularly on the abaxial leaf surface.²² The colour of the lesion is dependent on lesion age and interaction with the host genotype.⁶ Red brown (RB) lesions with little sporulation indicates a semi-compatible reaction, whereas tan lesions with much sporulation (Fig. 1) indicates a fully compatible reaction. During the early stages of development, before sporulation, soybean rust may be confused with bacterial pustule disease [*Xanthomonas campestris* pv *glycines* (Nakano) Dye].²² Soybean rust symptoms generally occur first on the leaves at the base of the plant and progress up the canopy as the disease severity increases. Increased lesion density leads to leaf yellowing and ultimately premature leaf senescence, resulting in yield losses primarily through reduced grain size.²³

Effect of soybean rust on yield

There is a dearth of published information on the effects of soybean rust on soybean yields in South Africa. Researchers that have published data relating to the effects of soybean rust on yield have recorded considerable variability over seasons and genotypes.^{24,25} McLaren²⁵ evaluated all the commercial soybean genotypes over two

seasons and concluded that there was no tolerance of economic value amongst them. He also observed that the yield loss sustained in shorter maturity genotypes was lower than the longer maturity genotypes. This confirmed the earlier work of Caldwell and McLaren²⁴ who had come to a similar conclusion but had conducted their research on only one genotype per maturity class, leaving some doubt as to whether the effect was genotype specific or maturity group related.

Initial indications from the research of Caldwell and McLaren²⁴ showed that planting date did influence the yield loss, but their two seasons' data were not sufficient to substantiate a trend. Soybean rust symptoms were more severe in the 0.45 m than in 0.90 m row spacing, and this was attributed to poorer fungicide penetration into the canopy.²⁴ McLaren²⁵ found that disease severity, as measured by the area under the disease progress curve (AUDPC), was poorly correlated to yield loss%. Mean yield loss for 2003/04 season was 31.1% or 1.68 t ha⁻¹ and in 2004/05 season it was a devastating 60.9% or 3.4 t ha⁻¹. Genotype ranking for yield loss% between the two seasons was substantially different, highlighting the considerable variability of soybean rust epidemics over seasons and the difficulty in selecting for improved genotypic response.

Distribution and spread of soybean rust in South Africa

There has not yet been a formal attempt to survey the distribution of soybean rust in South Africa; however, the reports of positive identification of soybean rust sent in by members of the soybean rust task team have been collated for the period 2001-2008 (Table 1). The reports increased in frequency over the years surveyed, likely as a result of more scientists becoming involved in reporting rather than an increase in disease incidence. The distribution of locations with one or more soybean rust reports have been plotted on a rainfall map of South Africa (Fig. 2). The area with the highest incidence of

soybean rust reports coincides with the high rainfall region east of the Drakensberg mountain range. Del Ponte *et al.*²⁶ showed that cumulative rainfall in the period after initial rust detection was positively correlated to disease severity, which probably accounts for the similarity in the rainfall and soybean rust distribution patterns. During the 2006 season, reports of soybean rust were obtained atypically far west of the normal distribution, but mostly too late in the season (Table 1) to have a significant impact on yield.

The collated reports are probably not ideally suited to making judgements on the progression of the disease, because the date of the report is not always a good indication of the start of the epidemic. However, in seasons that had sufficient reports to substantiate a trend (2006-2008), first reports for the season generally started in the east and progressed westward. While this may indicate a closer proximity to the inoculum source in the east of the production region, weather conditions favouring infection and development of symptoms may simply occur earlier in the season in the east compared to the west.

There is no literature on how the soybean rust pathogen survives from one season to the next in South Africa; however, Caldwell and McLaren²⁴ established that it required a live host and did not survive on soybean stubble. Since most of the soybean production regions receive significant frosts in winter, the pathogen is presumed to over-winter in frost-free areas within the country. Soybean rust epidemics in the KZN midlands normally originate from a few clearly distinguishable foci within a field, which would infer that initial infections have been started by a low concentration of windborne urediniospores. Infections that have resulted from urediniospores generated from within these foci, are a lot more uniform, clearly a function of inoculum concentration around these foci.

Pivonia and Yang²⁷ used a mathematical model to predict the likelihood of year-round survival of *P. pachyrhizi* across the world based only on historical temperature and moisture data. Host availability and presence of an inoculum source were not considered.

They found that conditions for the survival of *P. pachyrhizi* were very favourable all along the east and southern coasts of South Africa. Since this area does not coincide with the soybean production area, it is likely then that the soybean rust pathogen survives the winter in this area on the many possible alternative hosts. Pretorius *et al.*²⁸ established that Kudzu vine (*Pueraria lobata*) was one of the alternate hosts of *P. pachyrhizi* that provided a green bridge in South Africa for the survival of the pathogen through winter in the frost-free areas. It is speculated that this frost-free area then provides the initial inoculum source each season for the inland areas that have summer conditions favourable for the development of soybean rust. The consistency with which the epidemics have occurred since 2001 (Table 1) would tend to support the postulation that the source of urediniospores is, at the very least, regional and that local epidemics are not reliant on major weather phenomena for the deposition of urediniospores from the tropics of Africa.

Chemical control

Emergency registration of a number of chemicals made it possible for farmers to control epidemics during the first two seasons that soybean rust affected production in South Africa.²⁹ Much debate in South African soybean workgroups revolved around the difference in rates used in Zimbabwe compared to the recommended chemical rates in South Africa. The fear existed that sub-optimal doses of chemical would promote the build up of pathogen resistance to the active ingredients that controlled soybean rust. With pathogen diversity and variability clearly demonstrated in host-pathogen relationships,⁵ this was a valid concern. Du Preez and Calwell²⁹ evaluated effective dosage rates, timing of application and frequency of applications. This research contributed towards a leaflet being published³⁰ that made recommendations to soybean producers regarding control of soybean rust and included the registered chemicals. Du Preez and Caldwell²⁹ established

that effective chemical control varied in a time range from 10 d (triforine) to 19 d (flusilazole/carbendazim), which supported the generalisation that spray intervals should be no longer than 21 d apart, and that between one and three sprays may be required. They also concluded that some chemicals (flusilazole/carbendazim) had limited curative action, whereas others (azoxystrobin) were only effective in preventative applications. This conclusion was very important to the national strategy used to control rust. If control was primarily preventative, then the timing of fungicide applications in the absence of symptoms would be crucial, a conclusion that was also reached by several other researchers.³¹ A reliable indicator of first spray was required, since spraying too early would mean unnecessary additional sprays, and spraying at first symptom would result in yield losses. As part of the national strategy to control soybean rust in South Africa, a series of 10 soybean indicator plots were planted throughout the production region, using early planting dates and genotypes which represented the extremes of maturity range for the country. These plots were not sprayed with fungicide and were monitored on a weekly basis from January through to April³² for the presence of rust, both *in situ* and via leaf samples in the laboratory. These plots were used as sentinel plots to give producers advance warning of the presence and severity of the disease in an area. Producers were notified of the first presence of soybean rust in their area via cell phone SMS or alerts on farm radio programmes.³² The system of sentinel crops is currently also one of the methods being applied in the USA³³ for the advance warning of the presence of the disease. Systems that recommend spraying at predetermined soybean growth stages, for example at flower or at 60 days after planting (dap) as in Zimbabwe,³⁴ do not take into consideration that the timing and severity of epidemics may have considerable seasonal variation. This could result in unnecessary spraying in some seasons. Hartman,¹⁵ however, reported that there

were occasional yield benefits to spraying fungicides in the absence of rust which may make this system both cost effective and simple to apply.

In 2005, a report from Washington State University³⁵ claimed that Roundup herbicide (glyphosate) had been found to have fungicidal action on *P. pachyrhizi* under laboratory conditions. Due to the popularity of Roundup Ready (RR) soybean genotypes in South Africa, Kloppers and Jarvie (unpublished data) performed a pilot study with sequential sprays of Roundup on an experimental RR genotype to establish whether there was a need to pursue this avenue of research further. The preliminary results showed that pre-flower applications of Roundup had no effect on soybean rust severity, but post-flower applications visibly reduced the premature defoliation due to rust. Since Roundup when used as a herbicide is primarily applied to soybeans at a pre-flower stage, it was felt that these findings would have little practical applicability and this line of research was not pursued further. The results of this pilot study were later confirmed by independent research conducted in the USA by Jurick and co-workers.³⁶ In their study, control of soybean rust by applications of Roundup at the R2 and R4 stage significantly improved yield over the untreated control, but the yield benefit and control of the disease was inferior to that of conventional fungicide (azoxystrobin) applications.

Resistance

Screening for resistance. From the early 1960s through to the 1990s, much of the soybean rust research focused on resistance. Tschanz³⁷ reported that he and his co-workers at the AVRDC (Asian Vegetable Research and Development Centre) had, over the years, screened more than 9000 accessions for resistance to soybean rust. Hartwig³⁸ reported to have evaluated 1675 germplasm lines adapted to the southern USA for resistance to soybean rust in Taiwan. From this early screening work, it was clear that various levels of

specific resistance, partial resistance and tolerance to soybean rust all occurred in soybean germplasm. One of the recent objectives of the USDA-ARS soybean rust research programme has been to evaluate the USDA germplasm collection for resistance. A set of 174 soybean genotypes, inclusive of the most important parental germplasm and the most promising sources of resistance, were screened against field populations of *P. pachyrhizi* in Brazil, China, Paraguay and Thailand.³⁹ South Africa also participated in this evaluation, where soybean rust symptoms on this set of germplasm were recorded in the 2002/03 and 2003/04 seasons at Greytown, KZN. No lines were found to be resistant at all locations. With the threat of soybean rust looming in the USA at that time, the search for resistance intensified further, eventually involving the screening of 16595 accessions in the Fort Detrick containment facility.¹⁵

Under field conditions, early maturing soybean genotypes will have a higher disease rating earlier in the season than the equivalent later maturing genotype. The rate of rust development in these genotypes is also higher than that of later maturing genotypes, and if a correction for host maturity is not made, erroneous conclusions from field data will result.⁴⁰ To correct for maturity, relative life time (RLT) is calculated as the proportion of the life cycle completed relative to the complete life time (time from planting to harvest) of the genotype. Only rust severity ratings at comparable RLTs can be compared, which makes a single simple field severity rating meaningless unless all genotypes are of a similar maturity. McLaren²⁵ showed that disease severity, as measured by the area under the disease progress curve (AUDPC), was poorly correlated with yield loss. For this reason, disease severity ratings are seldom used as a measure of resistance.

Specific resistance in soybean. Mclean and Byth⁴¹ presented the first evidence of physiological races in *P. pachyrhizi* on soybean genotypes in Australia. Race 1 was virulent on Wills and avirulent on PI 200492. Race 2 was virulent on both varieties.

Subsequent to this, considerable variation in isolate virulence (collected from the same field, as well as isolates from geographically distant regions) has been shown to occur.⁵ Three infection types have been described: the Tan lesion is a fully susceptible reaction; the resistant RB reaction is a red-brown lesion with no or few sporulating uredenia; and the absence of any macroscopic symptoms is immunity.⁶ Eleven genotypes were used as a differential set to determine the physiological races of 42 purified *P. pachyrhizi* isolates by Wang and Hartman,⁶ and based on the infection type they were able to identify nine races. The data suggested that the pathogen races studied were complex and that they possessed multiple virulence genes for compatibility on many of the differential cultivars. Bromfield⁴² reported on a *P. pachyrhizi* race that had three virulence genes, more than were necessary to overcome host resistance. More recent research⁵ indicates that field pathogen populations are often mixtures of many races which may induce mixed infection types in the host. This is not uncommon in rust pathogens, as was shown to be the case with bean rust (*Uromyces appendiculatus*) where the more tropical locations (like South Africa) were found to induce greater race variability than more temperate climates.⁴³ It is not known how many races are commonly found in South African soybean fields, but since mixed infection types on the same plant have been observed, at least two races must be present. Variability in race virulence is also known to occur. In inoculation studies conducted under controlled conditions, researchers reported that recent isolates collected from southern Africa and South America were significantly more virulent than Asian isolates collected in the 1970s.⁴⁴ The most virulent isolate they reported was collected in Zimbabwe.

The specific resistance gene in PI 200492 was given the designation Rpp₁,⁴⁵ and since then three other independent dominant genes have been named: Rpp₂,⁴⁶ Rpp₃,⁴⁷ Rpp₄.⁴⁸ In Brazil, where the Rpp₁ and Rpp₃ genes are ineffective and Rpp₂ and Rpp₄

currently confer resistance, Neto⁴⁹ reported that many ‘new’ (unnamed) gene sources of resistance have been discovered. These were tested for allelism to Rpp₂ and Rpp₄, and of the 26 sources reported, 23 were found to be at different loci to Rpp₂ and Rpp₄. One of these sources of resistance was conditioned by a single recessive gene⁴⁹ from the variety Abura, and this has been incorporated in a variety (BR01-18437) destined for release in Brazil during 2008. Neto⁴⁹ also reported the preliminary findings that stacking Rpp₂ and Rpp₄ in a single genotype had no additive advantage in the expression of resistance.

The presence of multiple virulence genes in the pathogen population and the lack of multiple resistance genes in the host provides the soybean rust pathogen with a competitive advantage. The deployment of specific single genes for resistance is thus unlikely to be a successful strategy. As an example of gene failure, Hartman *et al.*⁵ quoted the examples cited by Bromfield, where the Rpp₁, Rpp₂ and Rpp₃ lost their effectiveness in the field within 10 years of exposure. In Taiwan, Shanmugasudaram *et al.*⁵⁰ quoted examples of Tainung 3, Tainung 4 and Kaohsiung 3 (all cultivars containing Rpp₁) becoming susceptible within a few years of release. Genotypes PI 230970 and PI 230971 were identified as being resistant in Taiwan, and these were subsequently used as parents in crosses to generate a number of resistant lines (AGS 181, AGS 182, AGS 183, AGS 229, AGS 233, AGS 240, AGS 244, AGS 247). So too were the resistances of these lines short lived. Following that, new sources of resistance were identified in PI 459024, PI 459025 (Rpp₄) and PI 339871 (*G. soja*) but have all since been defeated.^{5,50} In Brazil, Yorinori¹⁰ had a similar experience with germplasm that had shown resistance in 2002 being susceptible in 2003.

The use of gene pyramiding and gene rotation is also unlikely to be a stable solution because the pathogen retains unnecessary virulence genes at a high frequency in its population.⁵¹ In addition, resistance associated with the RB infection type is a semi-

compatible host-pathogen reaction, which generally allows pathogen reproduction and has not been shown to significantly affect epidemic development.⁵¹

Partial resistance. Partial or rate reducing resistance to soybean rust has been documented in soybean,⁵¹ but it has not been widely employed because of complexities in assessment. Plants or genotypes maturing at different times cannot be compared to each other in the field because of the different environmental conditions that they are exposed to at similar growth stages. Physiological differences can be partially corrected for by regressing relative life time (RLT) on the log transformation of rust severity. The slopes of these graphs can be compared to identify the ‘slow rusting’ genotypes. Collecting the data required to generate these graphs is laborious and cannot be conducted on a large number of genotypes, limiting its practical application. Hartman *et al.*⁵ suggested that measuring the latent period would help identify genotypes with a long latent period and hence a slower rate of rust development. The difficulties associated with identifying partial resistance and the ineffectiveness of specific resistance genes has led to the suggested use of tolerance as a breeding remedy for soybean rust.

Tolerance. Tolerance implies susceptibility, and can be defined as the relative ability of a genotype to yield under stress from rust.⁶ Tolerance is a characteristic that can only be evaluated in the target environment while under rust stress, as it implies a measure of genotypic adaptation to that environment. Tolerance is of little value unless the genotype is high yielding in that environment and it maintains yield stability despite rust infections. Selecting for yield stability in the presence of rust is not an easy task⁵ since over and above the normal genotype x environment interaction that breeders have to contend with for adaptation, seasonal variation in severity and timing of rust epidemics is superimposed. Whilst yield is normally the primary consideration, a consistent performance is also valuable to a producer, who may be willing to sacrifice some yield in

order to achieve a stable yield over seasons.⁵² Tolerance is traditionally assessed by comparing yields of paired plots of fungicide protected versus unprotected plots. The percentage yield loss between fungicide protected and unprotected plots is not necessarily correlated to rust susceptibility ratings or to rust development rates⁵ and may be linked to other stress tolerance mechanisms. Significant variation in tolerance levels exist in soybean, which could be exploited by breeders. From work conducted at the AVRDC in Taiwan, Hartman⁴⁰ demonstrated yield losses of 12 genotypes ranging between 29 to 85%. Based on reduced pustule numbers, the two genotypes that had the smallest yield losses (29% and 31%) could conceivably have had some form of partial resistance. This, when compared to a possible 85%, appears to be significant but in reality is still far too high for practical benefit on a commercial scale. In more recent research conducted in Brazil,⁴⁹ minor genes have contributed towards tolerance in the genotype EMGOPA 313, with yield losses in the order of magnitude where fungicide spraying would still be financially attractive.

Conclusion

High levels of tolerance or sustainable rust resistance in South African genotypes is not imminent, which means that for the foreseeable future control of soybean rust by a combination of chemical and cultural means will need to continue. An efficient warning system and effective fungicides have been instrumental in averting potentially large financial losses to producers. Whilst seasonal soybean rust epidemics will persist and control measures will continue to be required, the soybean rust crisis in South African soybean production is largely over as a result of the efforts of forward thinking policy makers and pro-active researchers.

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Table 1. A compilation of soybean rust reports made to the soybean rust task team.

Date	Location	Reporter	Date	Location	Reporter
8 Feb 2001	Vryheid	H. Oellerman	16 Feb 2006	Vryheid	M. Craven
6 Mar 2001	Howick	K. Horne	24 Feb 2006	Morgenzon	M. Craven
9 Mar 2001	Ahrens	F.J. Kloppers	27 Feb 2006	Amersfoot	W. van Wyk
14 Mar 2001	Greytown	J.A. Jarvie	3 Mar 2006	Winterton	M. Craven
-Mar 2001	Amersfoot	Un-confirmed	14 Mar 2006	Normandien	M. Craven
-Mar 2001	Ermelo	Un-confirmed	14 Mar 2006	Kinross	M. Craven
-Mar 2001	Piet Retief	Un-confirmed	15 Mar 2006	Kroonstad	F.J. Kloppers
4 Feb 2002	Cedara	E.D. Du Preez	30 Mar 2006	Kestell	M. Craven
15 Feb 2002	Amsterdam	J.L. Purchase	3 Apr 2006	Potchefstroom	M. Craven
15 Feb 2002	Greytown	J.A. Jarvie	24 Apr 2006	Bothaville	F.J. Kloppers
8 Jan 2003	Cedara	E.D. Du Preez	25 May 2006	Letsitele	J.A. Jarvie
8 Jan 2003	Karkloof	E.D. Du Preez	25 Jan 2007	Cedara	A. Liebenberg
14 Feb 2003	Greytown	J.A. Jarvie	26 Jan 2007	Piet Retief	M. Craven
26 Jan 2004	Cedara	E.D. Du Preez	31 Jan 2007	Greytown	F.J. Kloppers
26 Jan 2004	Karkloof	E.D. Du Preez	1 Feb 2007	Vryheid	M. Craven
16 Feb 2004	Greytown	F.J. Kloppers	1 Feb 2007	Morgenzon	M. Craven
8 Apr 2004	Ermelo	P. Kruger	12 Feb 2007	Merrivale	N.C. van Rij
3 Jan 2005	Cedara	E.D. Du Preez	22 Feb 2007	Normandien	M. Craven
11 Jan 2005	Karkloof	E.D. Du Preez	1 Mar 2007	Bergville	E.D. Du Preez
3 Feb 2005	Winterton	E.D. Du Preez	8 Mar 2007	Besters	E.D. Du Preez
3 Feb 2005	Weenen	E.D. Du Preez	25 Jan 2008	Cedara	N.C. van Rij
24 Feb 2005	Greytown	J.A. Jarvie	28 Jan 2008	Greytown	S. Tweer
9 Mar 2005	Winterton	N. Hackland	7 Feb 2008	Vryheid	M. Craven
1 Feb 2006	Karkloof	S. Tweer	7 Feb 2008	Baynesfield	P.M. Caldwell
1 Feb 2006	Cedara	S. Tweer	5 Mar 2008	Normandien	M. Craven
2 Feb 2006	Greytown	E.D. Du Preez	26 Mar 2008	Seven Oaks	J.A. Jarvie
3 Feb 2006	Sudwala Cave	Z.A. Pretorius	7 Apr 2008	Winterton	J.A. Jarvie
9 Feb 2006	Piet Retief	M. Craven	7 Apr 2008	Groblersdal	J.A. Jarvie



Fig. 1. Tan sporulation of soybean rust on the lower leaf surface of a susceptible soybean genotype.

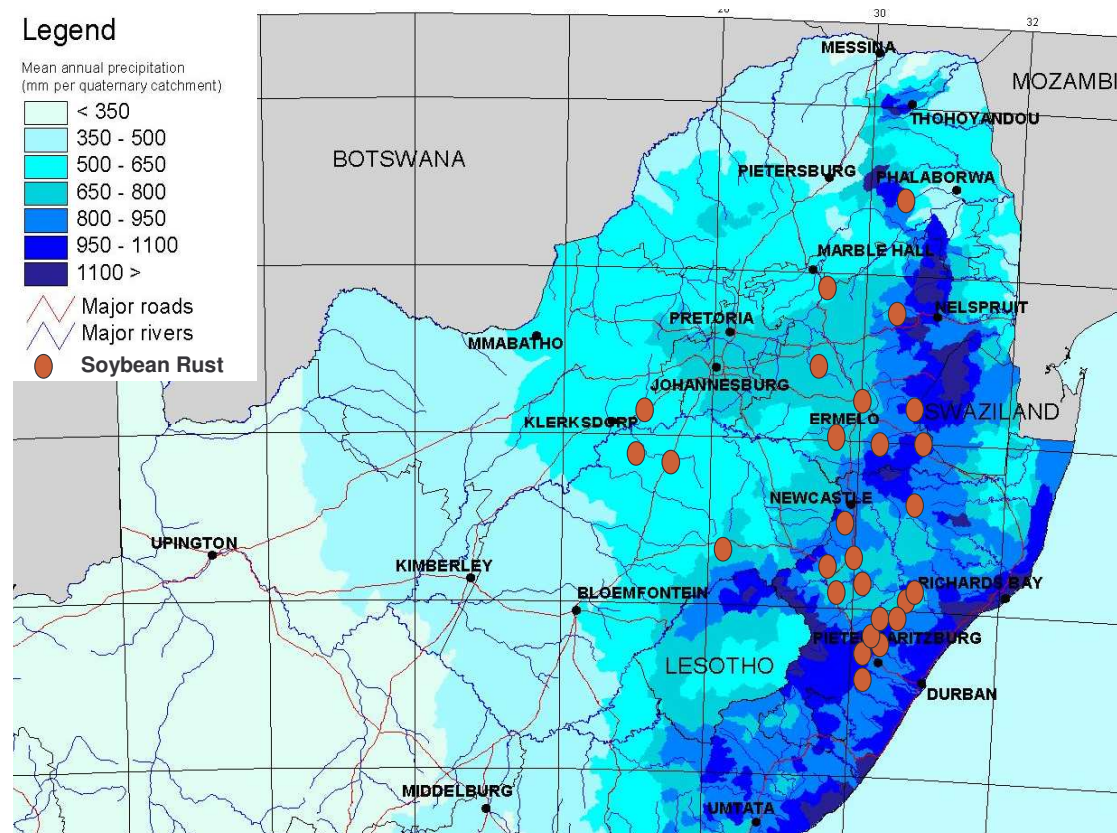


Fig. 2. Distribution of locations with one or more reports of soybean rust during the period 2001-2008, superimposed on the annual rainfall map of South Africa (Source: Surface resources of South Africa, 1990).

General appendix: Publication 3

Soybean yield loss trends associated with soybean rust in South Africa

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(prepared and formatted for submission to Crop Protection)

Abstract

Assessing yield loss patterns associated with genotype maturity and planting date could assist in establishing an efficient soybean rust tolerance breeding programme. Forty genotypes representing two maturity group clusters (MG IV-V and VI-VIII) were evaluated for yield loss to soybean rust at two planting dates over three seasons at Greytown, RSA. Mean yield loss to soybean rust combined over all maturity groups, planting dates and seasons, established by comparing sprayed with unsprayed treatments, was 0.708 t ha⁻¹ or 24%. The lowest yield losses were incurred when the early MG cluster of genotypes were planted in November. There was enough seasonal variability in this loss to prevent it from being adopted as a strategy to commercially avoid losses to soybean rust. Conversely, the highest yield losses were consistently incurred in the late planting date (December), which has important implications for rust tolerance screening research.

Key words: Phakopsora pachyrhizi; tolerance; maturity; planting date; avoidance

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1. Introduction

Since soybean rust (*Phakopsora pachyrhizi*) was first reported in South Africa during the 2000/01 season (Pretorius et al., 2001), it has been present to a greater or lesser extent in the mid-altitude high rainfall soybean (*Glycine max* (L.) Merr.) production region (mist belt) in all of the subsequent seasons to date (2008). Initially the emphasis of local research efforts concentrated on optimising chemical control programmes (Du Preez and Caldwell, 2004), which successfully limited commercial yield losses. With the short term crop security assured through the use of chemicals, the emphasis of research has turned to genetic control of the disease through the use of genotypes which resist or tolerate soybean rust.

In the seasons following the first report of soybean rust, several thousand genotypes were screened for their reaction to soybean rust at the PANNAR research station at Greytown. The *P. pachyrhizi* race/s prevalent in South Africa caused symptoms on all the genotypes tested, including the set of resistance sources made available by the AVRDC and USDA. This material included the sources of the four independent dominant genes (*Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*) that have been identified with race specific resistance. Hartman (1996) reported that the soybean rust pathogen had multiple virulence factors, which rendered the long term use of specific genes ineffective and questioned the value of this line of research. Consequently, breeding for tolerance has become an important objective in this breeding programme. Tolerance is traditionally quantified by the relative yielding ability of genotypes infected by rust. Relative yields are evaluated by comparisons of fungicide protected (sprayed) and unprotected plots (unsprayed) under field conditions

in the presence of soybean rust. These evaluations are only of value if the rust epidemic is severe enough to cause significant yield loss and if the genotypes evaluated are well adapted to the environment in the absence of rust. The aim of this study is to analyse yield loss patterns that would assist in optimizing selection for tolerance in a breeding programme. This study investigates the interaction of different genotype maturity groups, planting date and season with yield loss, and makes recommendations relevant to rust tolerance research.

2. Materials and methods

2.1. Genotypes

The criterion for genotype inclusion in these trials was commercial potential in the absence of rust. Genotypes included commercial cultivars, pre-commercial varieties and high yielding advanced lines in their final stages of testing. The genotypes evaluated were separated into two clusters of 20 entries each, based on their maturity. The first cluster, including genotypes from maturity groups IV and V were collectively called the MG45 cluster. The second cluster included genotypes from maturity groups VI to VIII, and was referred to as the MG68 cluster. The primary objective of this clustering of the genotypes was to evaluate the effect of maturity group on yield loss due to rust, without this being confounded by the effects of individual genotypes. The individual genotypes making up the two clusters varied from season to season but the maturity groups making up the clusters remained constant over seasons. There were common genotype entries between consecutive seasons, but only two entries were common to all three seasons.

2.2. Layout

The basic layout of the experiment was a factorial combination of two maturity clusters (MG) and two spray treatments (Treat), which were randomised over three replications. The two Treat combinations were sprayed versus unsprayed, and the two MG clusters were MG45 and MG68. The entries within these MG clusters consisted of 20 genotypes (Vno) randomised in a 4 x 5 rectangular lattice, thus the four treatment combinations were represented by four lattices. The four lattices were repeated at two planting dates (PD) each season and the experiment repeated over three seasons (2002/03, 2003/04 and 2004/05), totalling 24 lattices or treatment combinations. The planting dates chosen (Table 1) were generally in the first week in November (Nov) and second week in December (Dec), which represented the extremes of the normal commercial planting season at Greytown.

Trials were hand planted at a seeding rate of 380 000 seed ha⁻¹. Each plot consisted of four rows of 4.4 m in length, spaced 0.9 m apart between rows. The middle two rows were harvested with a plot combine and yields were adjusted to 12.5% moisture. The sprayed lattices were sprayed before the first symptoms of soybean rust appeared in the trial, using the sentinel plot warning system (Table 1). The sentinel plots were part of a national network (Craven, 2008) of early planted plots which provided advance warning for the need to spray. Spraying was done by knapsack using Punch C (Flusilazole/Carbendazim, SC 250/125 g l⁻¹) at the recommended rate of 400 ml ha⁻¹, and a second spray followed approximately 21 d later. In all seasons, rust in the sprayed lattices was successfully controlled in the upper two thirds of the canopy for the duration of the growing cycle using this methodology. Yield losses were calculated as sprayed minus unsprayed yield and expressed as a percentage of the sprayed yields.

[Table 1]

2.3. Analysis

A number of different data analysis options were explored, including analysing the experiment as a split plot in time. After careful consideration, a simple factorial design was deemed the most appropriate, as it generated the same means as the split plot in time analysis, but provided moderately conservative standard errors for comparisons. The factor MG comprised of twenty genotypes set out in a 4 x 5 lattice, which then formed part of the 3 x 2 x 2 x 2 (Season x PD x MG x Treat) factorial combination.

2.3.1. Statistical model 1

Analysis of the data was done using REML META analysis (Genstat Version 10.2), using the following model:

Fixed = Season*PD*MG*Treat

Random = Rep/Block

Experiment = Lattice

[Statistical model 1]

Since the META analysis routine was used, a formal test of homogeneity of error variances of the individual lattices was not required as the error variance for each lattice was determined separately. The treatment factor MG was represented by 20 genotypes (Vno) within each season, but because the genotypes constituting the MG clusters varied over the three seasons, Statistical model 1 was not resolved down to the level of Vno.

2.3.2. Statistical model 2

There were two genotypes that were common across all seasons and 12 genotypes that were common across two seasons. Although the initial intention was not to consider the reactions of individual genotypes, it was possible to resolve the treatment effects down to the level of Vno by using the following model with Vno nested within the interactions of the other treatments:

Fixed = (Season*PD*MG*Treat)/Vno;

Random = Rep/Block

Experiment = Lattice

[Statistical model 2]

3. Results

3.1. Statistical model 1

The choice of genotypes in this experiment was not a random selection of genotypes to represent specific maturity groups; rather, they were purposefully chosen for their commercial potential and represent high yielding elite genotypes which were separated into two clusters based on maturity. As a consequence MG and Vno (in Statistical model 2) are fixed effects and cannot be considered random.

The Wald statistics for the main effects of Season, PD, Treat and MG were all significant (Table 2). The difference between the Treat effects (sprayed versus unsprayed) is ascribed to the negative influence of soybean rust on yield in the unsprayed treatment. This effect is termed yield loss, and for comparative purposes may also be expressed as a

percentage of the unsprayed yield (yield loss %). The main effects of Treat, and therefore yield loss to soybean rust, over a period of three years and taking two planting dates per season into account and covering the reactions of 120 genotype entries was 0.708 t ha⁻¹ or 25% (Table 3) which was significant ($P = 0.05$).

[Table 2]

Yields in the 2002/03 season (1.783 t ha⁻¹) were significantly ($P = 0.01$) lower than in 2003/04 (2.903 t ha⁻¹) or 2004/05 (2.767 t ha⁻¹). Yields in the Nov planting date were 0.236 t ha⁻¹ higher (significant, $P = 0.05$) than the Dec planting date. The mean yield difference between the two maturity group clusters was not significant (0.135 t ha⁻¹). Since there are significant interaction effects between the factors, the main effects are not of particular interest.

The Wald statistic for the interaction between Season and PD was highly significant (Table 2). In the first two seasons (2002/03 and 2003/04) mean yields were significantly higher ($P = 0.01$) in the Nov planting date compared to Dec planting date. In the final season (2004/05), yields in the Dec planting date exceeded those of Nov by a significant margin ($P = 0.05$). Considering the bias that is potentially inherent in the main effects for PD and Season due to their temporal nature, no general inferences should be made from their interaction. The interactions between: PD and Treat; Season and MG; Treat and MG generated non-significant Wald statistics. The MG45 cluster of genotypes had a slightly smaller yield loss response (0.638 t ha⁻¹ or 26.3%) to rust compared to MG68 (0.781 t ha⁻¹ or 30.6%) when meaned over all planting dates and seasons, but the difference between the two was not significant. The interaction between PD and MG cluster was significant (Table 2), as was the interaction between Season and Treat. All three factor

interactions were highly significant based on their Wald statistics (Table 2). Again, since the third order interaction was significant, the second order interactions are of lesser interest.

The third order interaction in Statistical model 1 (Table 2) was highly significant (Wald statistic, $P = 0.002$). In 2002/03, there was no significant yield loss in the Nov planting date, and only the MG45 genotype cluster had a significant ($P = 0.05$) yield loss of 0.375 t ha^{-1} in the Dec planting date. In the following two seasons (2003/04 and 2004/05), all combinations of PD and MG presented significant ($P = 0.01$ and $P = 0.05$) yield losses as a result of soybean rust (Table 3). With the exception of the 2002/03 season where the yield losses were similar (Table 3), the MG45 cluster was less affected by rust than the MG68 cluster of genotypes for the Nov planting date. Further, the MG45 cluster consistently (all three seasons) had a lower yield loss in the Nov planting date compared to the Dec planting date. Yield loss trends in the MG68 cluster were less consistent.

[Table 3]

3.2. Statistical model 2

In the analysis using Statistical model 2, treatment effects were resolved down to the level of Vno. Two genotypes (PAN 421R and PAN 520R) were common in all seasons, 12 genotypes common in two seasons and the rest only present in a single season, resulting in an unbalanced analysis. The REML META analysis routine is capable of analysing such unbalanced datasets, but could only generate comparable means for the highest order interaction. The interaction of genotype (Vno) with the other factors in the experiment (Table 4) was highly significant (Wald statistic, $P = 0.001$). The two genotypes that were

common in all three seasons were MG IV and MG V genotypes that had been categorised in the MG45 cluster. There were no genotypes common to all seasons which were from the MG68 cluster. Yield losses to soybean rust, as measured by the difference between Treat effects in the genotypes PAN 421R and PAN 520R, were lower in the Nov planting date compared to Dec planting date in each of the seasons, with one exception (Table 5). In the 2003/04 season, PAN 421R yielded more in the unsprayed treatment than the sprayed treatment, which could be ascribed to experimental error and spatial separation of the plots at the Vno level. The design of the trial was perhaps not optimal for evaluating yield loss of individual genotypes because the spray treatment was imposed at the lattice level on each of the MG clusters. As a consequence the trial design maximized the precision in the comparison of the sprayed and unsprayed treatments at the MG cluster level which would lead to less precise comparisons at the Vno level.

Within the MG45 cluster, and in addition to PAN 421R and PAN 520R, two genotypes (PAN 494 and PAN 564) were common to the 2002/03 and 2003/04 seasons and three genotypes (X48R104, PAN 535R and JV1118) were common to the 2003/04 and 2004/05 seasons. Yield losses of these common genotypes were graphed (Fig 1) to confirm MG45 trends established in the analysis of Statistical model 1. In Fig 1, the individual genotypes have been arranged on the axis in order of increasing maturity (data not shown) and the two planting dates graphed separately. A general trend (which is perhaps more pronounced in the Nov planting date) that yield loss % increases with increasing genotype maturity was evident. This trend was not evident in the 2002/03 season, but was pronounced in 2003/04 and 2004/05. Whilst general trends associated with genotype maturity were evident, the extent of seasonal variation and genotypic variation was apparent in Fig 1. PAN 421R had an individual response that appeared to be either unassociated with maturity, or alternatively atypical of its maturity classification.

Within the MG68 cluster, there were five genotypes (PAN 660, PAN 1564, PAN 626, PAN 854 and PAN 809) common to 2002/03 and 2003/04 and two genotypes (JV1134 and PAN 737R) common to 2003/04 and 2004/05. Yield loss % of these common genotypes were graphed (Fig 2) to confirm MG68 trends established in Statistical analysis 1. In Fig 2, the individual genotypes have been arranged on the axis in order of increasing maturity (data not shown) and the two planting dates graphed separately. As with the MG45 genotypes, the yield loss % in the 2002/03 season was different to 2003/04 and 2004/05 in magnitude and trend. A seasonal trend that yield loss increased with increased maturity was evident in the Nov planting date for the 2003/04 and 2004/05 seasons (Fig 2). In the Dec planting date, this trend was less well defined for all seasons and more individual genotype deviation from the seasonal trend was evident.

4. Discussion

There is a need to quantify the effects of soybean rust on yield, and this is often done using a small number of genotype and environment combinations. In this study the main effect of Treat (sprayed versus unsprayed), derived from 120 genotypes from two PD per season and three consecutive seasons, quantifies the yield loss to soybean rust at Greytown at 0.708 t ha^{-1} or 24%. The mean loss over three seasons is moderated by the fact that yield loss in the 2002/03 season was substantially less (10.4%) than in 2003/04 (29.2%) and 2004/05 (29.0%). Accounting for the lower yield loss in the first season of the experiment is difficult as there are many factors that could play a role in yield loss. Observations from the sentinel plots would indicate that the first presence of rust was detected at roughly the same time for each of the seasons in this experiment (Table 1). Planting dates of the experiment were similar over the seasons, and the rainfall patterns (rainfall distributions not shown) in the latter part of the three seasons (March and April)

were distinctly similar. Del Ponte et al. (2006) showed that cumulative rainfall in the period after initial rust detection was positively correlated to disease severity. By extrapolation then, seasons with similar initial rust detection dates and similar rainfall patterns are likely to have similar epidemics. With there being no obvious basis for the reduced yield loss in 2002/03, it is only possible to speculate on the possible causative factors. Since 2002/03 was only the third season of soybean rust at Greytown since initial detection in 2001, it is conceivable that the initial starting inoculum pressure was lower for this season than the following two, resulting in a less severe epidemic. Yield losses in the following two seasons were similar (0.993 t ha^{-1} and 0.937 t ha^{-1}) averaging 29.1% loss (Table 3).

The use of a large number of genotypes within the MG clusters successfully dilutes the effects of individual genotypes and should give a representative indication of the effect of maturity on yield loss. The yield loss reaction (Fig 1 and Fig 2) of some individual genotypes may be atypical of their maturity classification, which implies that many genotypes are required to substantiate a trend. The poor discrimination of between MG clusters in Statistical analysis 1 was unanticipated, given that it was based on the reactions of 20 genotypes and considering the crucial role physiological maturity of the host plays in rust infection and development. Whilst the large number of genotypes included in the trial gives the yield loss values substantial credibility, it has not improved the resolution of response to soybean rust as determined by MG. The reduced response to MG suggests that perhaps the maturity clusters were too broad to accurately and separately reflect interactions with maturity group without overlap in trends. The more distinct trends evident in the analysis of individual genotypes (Statistical model 2) would tend to support this suggestion.

There were significant interactions in Statistical model 1 between MG, PD and Treat (Table 3). The MG45 genotypes planted in Nov were the least affected by yield loss (0.524 t ha^{-1} or 18.4%) as a result of soybean rust when compared to other combinations of MG and PD, which varied from 23.5% to 29.3% (Table 3). Analysis of the individual genotypes in Statistical model 2 supported this general trend (Fig 1), which strengthens the argument made by Caldwell and McLaren (2004) and McLaren (2008) that planting early maturing genotypes in combination with early planting dates could reduce the risk of yield loss to soybean rust. A likely explanation is that the reduced yield loss incurred by planting the MG45 genotypes in Nov arose from the partial escape of the rust epidemics. Planting maturity group IV or V genotypes in Greytown in an attempt to escape yield loss would be a strategy fraught with risks for commercial production. The mean yield loss due to soybean rust for the MG45 genotype cluster ranged between 4.6 and 25.6% over the three seasons for Nov PD (Table 3). At the low end of the range in yield loss (4.6%), producers may find it financially viable not to spray their crop with fungicide. However, considering that chemical control is primarily preventative (Du Preez and Caldwell, 2004), commitment to a spray or no-spray strategy would need to be made by the producer before there is any indication of the severity of the impending rust epidemic. With the threat of yield losses as high as 25% (at the high end of the range) possible, the risk of this strategy would be economically prohibitive. Furthermore, in South Africa later maturing genotypes (MG68) are typically planted at early planting dates to make full use of the extended season. Any reduced yield loss to soybean rust attained by planting MG45 genotypes early (Nov) would need to be offset by their lower yield potential at this planting date.

Although the general trends displayed in Fig 1 and Fig 2 relied on comparisons of subsets of genotypes, it was evident that across the range of individual maturities tested yield loss to soybean rust increased with increase in genotype maturity regardless of

planting date. This has important commercial consequences and requires further verification using a fully representative set of genotypes across all levels of MG, PD and season.

5. Conclusion

Whilst it has been argued that planting date in combination with maturity group is unlikely to be a reliable method of escaping rust for a commercial producer, the converse effect of planting date has application in rust research. The data shows that high yield losses are consistently incurred with late planting dates. This knowledge can be applied in soybean rust research to ensure maximum reactions and minimise the incidence of escapes. For rust tolerance research, it is important to ensure that all genotypes (regardless of maturity) are exposed to severe epidemics every season, and by planting late this can be consistently obtained.

Acknowledgments

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6. References

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Table 1

Soybean rust yield loss trial site details for Greytown, South Africa (S28° 08'; E30° 37')

Season	Planting date		Annual	
	Nov	Dec	Rainfall (mm) ^a	Date of first rust ^b
2002/03	06.11.2002	11.12.2002	789.8	14/02/2003
2003/04	07.11.2003	08.12.2003	666.4	16/02/2004
2004/05	08.11.2004	10.12.2004	751.3	24/02/2005

^aThirty two year mean annual rainfall = 832.6 mm^bSoybean rust symptoms on the sentinel plots, used as an indicator of the earliest presence of rust symptoms in the area and a stimulus to start fungicide spraying

Table 2

Wald statistics for the REML META analysis, Statistical model 1: Fixed terms = Season*PD*MG*Treat; Random = Rep/Block; Experiment = Lattice

Fixed term	Wald Statistic	d.f	Wald/d.f	chi pr
Season	4194.51	2	2097.25	<0.001
PD ^a	349.43	1	349.43	<0.001
Treat ^b	1108.10	1	1108.10	<0.001
MG ^c	92.55	1	92.55	<0.001
Season.PD	323.76	2	161.88	<0.001
Season.Treat	525.69	2	262.85	<0.001
PD.Treat	1.54	1	1.54	0.215
Season.MG	5.42	2	2.71	0.067
PD.MG	65.88	1	65.88	<0.001
Treat.MG	0.28	1	0.28	0.598
Season.PD.Treat	32.77	2	16.38	<0.001
Season.PD.MG	21.97	2	10.98	<0.001
Season.Treat.MG	29.77	2	14.89	<0.001
PD.Treat.MG	24.69	1	24.69	<0.001
Season.PD.Treat.MG	12.60	2	6.30	0.002

^aPD = Planting date

^bTreat = Fungicide spray treatment

^cMG = Maturity group cluster

Table 3

Mean effect of soybean rust on yield loss relative to a fungicide sprayed control at Greytown, at two planting dates over three consecutive seasons (2002/03, 2003/04 and 2004/05)

Season	Planting Date	Maturity Cluster	Yield (t ha ⁻¹)			Yield Loss %
			Sprayed	Unsprayed	Loss	
2002/03	Nov	MG45	2.179	2.079	0.100	4.6
		MG68	2.026	1.939	0.087	4.3
	Dec	MG45	1.591	1.218	0.373*	23.4
		MG68	1.727	1.506	0.221	12.8
		Mean	1.881	1.686	0.195*	10.4
2003/04	Nov	MG45	3.334	2.639	0.695**	20.9
		MG68	4.024	2.533	1.491**	37.1
	Dec	MG45	3.015	2.148	0.867**	28.8
		MG68	3.224	2.307	0.917**	28.4
		Mean	3.399	2.407	0.993**	29.2
2004/05	Nov	MG45	3.027	2.252	0.775**	25.6
		MG68	3.161	2.036	1.125**	35.6
	Dec	MG45	3.259	2.259	1.000**	30.7
		MG68	3.493	2.647	0.846**	24.2
		Mean	3.235	2.299	0.937**	29.0
All	Nov	MG45	2.847	2.323	0.524*	18.4
		MG68	3.070	2.170	0.900**	29.3
	Dec	MG45	2.622	1.875	0.747**	28.5
		MG68	2.815	2.153	0.662**	23.5
		Mean	2.839	2.130	0.708*	25.0

Planting date: Nov = Early November, Dec = Early December

Treatment: Unsprayed; Sprayed = 2 x Punch C @ 400 ml ha⁻¹

Maturity cluster: MG45 = 20 MG IV and V genotypes

MG68 = 20 MG VI to VIII genotypes

%Yield Loss = (sprayed yield-unsprayed yield)/sprayed yield x 100

* = Significant ($P = 0.05$)

** = Highly significant ($P = 0.01$)

Table 4

Wald statistics for the REML META analysis using Statistical model 2: Fixed terms = (Season*PD*MG*Treat)/Vno; Random = Rep/Block; Experiment = Lattice

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Season	7506.48	2	3753.24	<0.001
PD ^a	743.17	1	743.17	<0.001
MG ^b	34.81	1	34.81	<0.001
Treat ^c	1861.36	1	1861.36	<0.001
Season.PD	537.80	2	268.90	<0.001
Season.MG	0.11	2	0.05	0.948
PD.MG	209.49	1	209.49	<0.001
Season.Treat	993.10	2	496.55	<0.001
PD.Treat	5.47	1	5.47	0.019
MG.Treat	0.33	1	0.33	0.567
Season.PD.MG	28.00	2	14.00	<0.001
Season.PD.Treat	53.49	2	26.75	<0.001
Season.MG.Treat	38.79	2	19.40	<0.001
PD.MG.Treat	40.79	1	40.79	<0.001
Season.PD.MG.Treat	21.23	2	10.61	<0.001
Season.PD.MG.Treat.Vno ^d	1781.75	452	3.94	<0.001

^aPD = Planting date

^bMG = Maturity group cluster

^cTreat = Fungicide spray treatment

^dVno = Genotype

Table 5

Yield loss to soybean rust in two genotypes, as measured by the difference between sprayed and unsprayed treatments. Trials were conducted at Greytown over three seasons (2002/03, 2003/04, 2004/05) using two planting dates per season

Season	Planting		Yield (t ha ⁻¹)			Yield
	date ^a	Genotype	sprayed ^b	unsprayed	loss	loss % ^c
2002/03	Nov	PAN 421R	2.303	2.068	0.235	10.2
		PAN 520R	1.948	1.842	0.106	5.4
	Dec	PAN 421R	1.473	1.072	0.401	27.2
		PAN 520R	1.405	1.032	0.373	26.5
2003/04	Nov	PAN 421R	2.666	2.917	-0.251	-9.4
		PAN 520R	2.954	2.676	0.278	9.4
	Dec	PAN 421R	2.850	2.626	0.224	7.9
		PAN 520R	2.493	1.624	0.869**	34.9
2004/05	Nov	PAN 421R	2.844	2.588	0.256	9.0
		PAN 520R	3.100	2.576	0.524*	16.9
	Dec	PAN 421R	3.686	2.633	1.053**	28.6
		PAN 520R	3.267	2.454	0.813**	24.9

^aPlanting date: Nov = Early November; Dec = Early December

^bsprayed = two applications of Punch C @ 400 ml ha⁻¹

^cYield loss % = (sprayed yield-unsprayed yield)/sprayed yield x 100

* = Significant ($P = 0.05$)

** = Highly significant ($P = 0.01$)

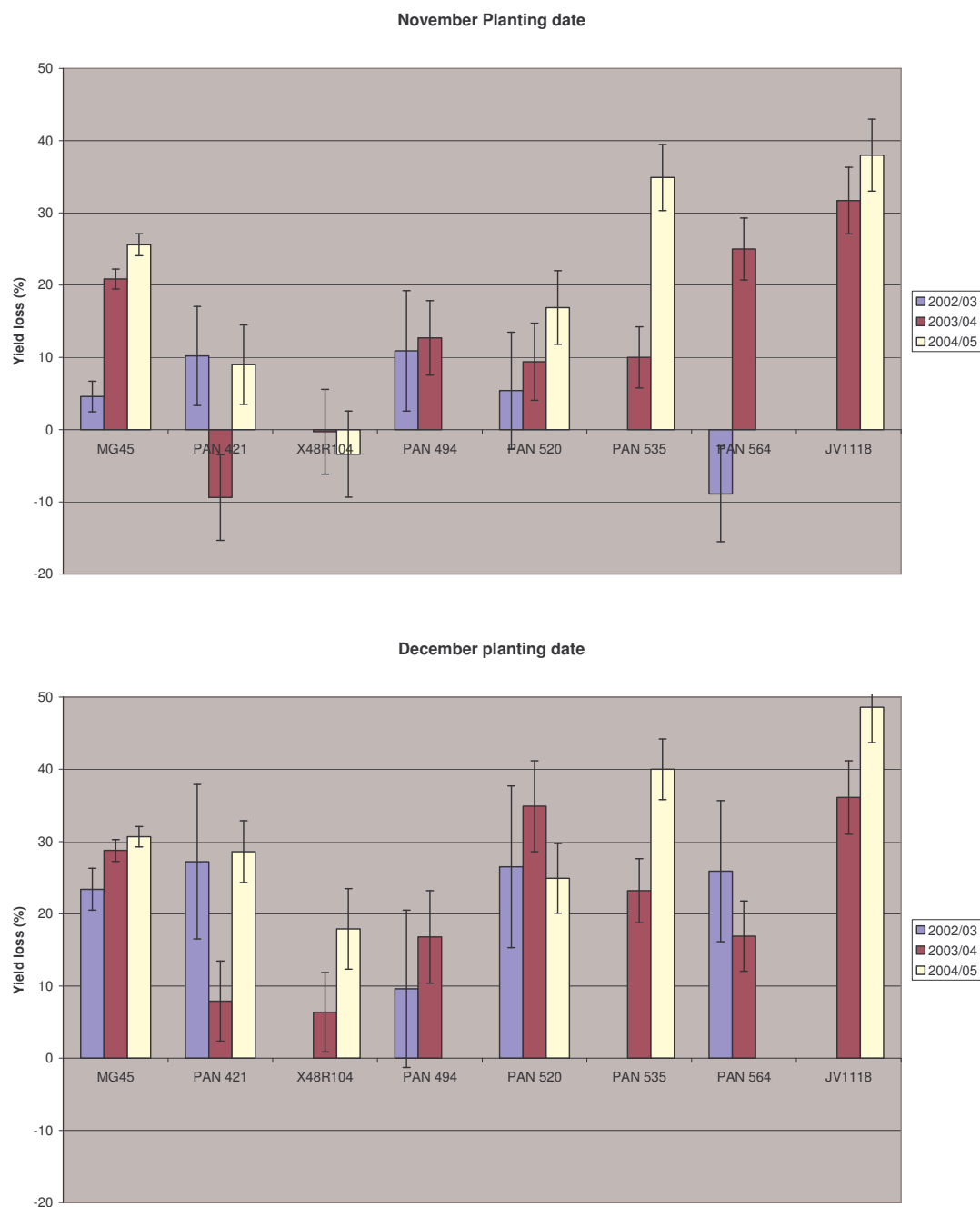


Fig 1 Percentage yield loss of individual genotypes compared to the MG45 genotype cluster mean for two planting dates and three seasons (2002/03, 2003/04 and 2004/05). The vertical line at the top of the bar represents the average SE expressed as a % of the unsprayed mean

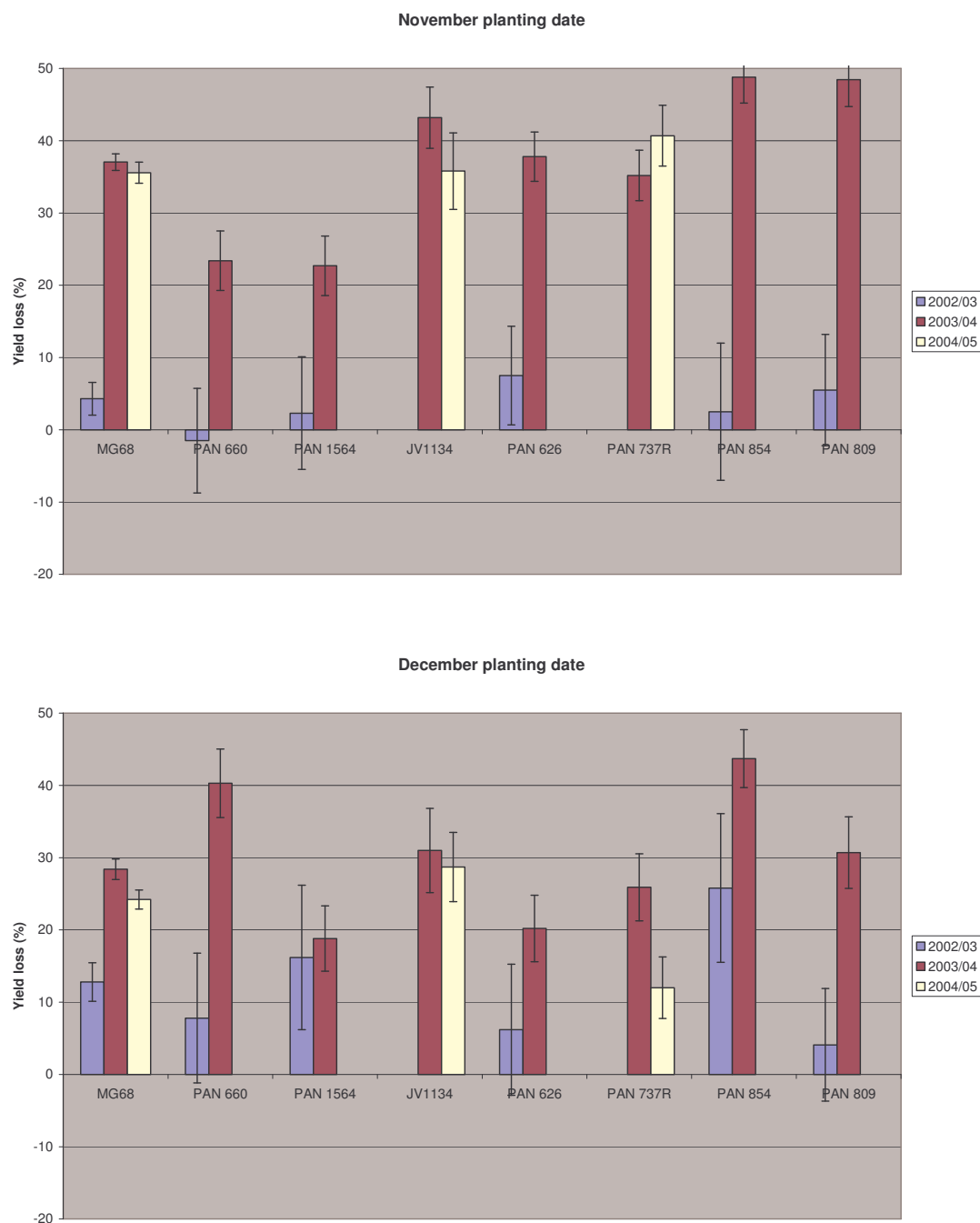


Fig 2. Percentage yield loss of individual genotypes compared to the MG68 genotype cluster mean for two planting dates and three seasons (2002/03, 2003/04 and 2004/05). The vertical line at the top of the bar represents the average SE expressed as a % of the unsprayed mean

General appendix: Publication 4

Assessing tolerance to soybean rust in selected genotypes

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Abstract

Breeding for tolerance to soybean rust has long been regarded as a more durable approach than the use of specific resistance genes; however, very little progress in tolerance breeding has been documented. Conventional methodology for assessing tolerance uses the yield loss % index which has been shown to produce highly variable results over seasons, which likely accounts for the lack of success. This study has used the superiority measure (P_i) of Lin and Binns and the ecovalence statistic (W_i) of Wricke in a biplot to identify the most tolerant genotypes. A novel statistic (W_iP_i) has been generated from this biplot which facilitates the simultaneous selection for general performance and yield stability of genotypes under rust stress.

Key words: superiority measure; ecovalence; stress; yield stability; resistance

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1. Introduction

Much of the soybean (*Glycine max* (L.) Merr.) breeding research around the world on soybean rust (*Phakopsora pachyrhizi*) has been focused on the search for resistance (Neto, 2007; Miles et al., 2004; Tichagwa, 2004), with programmes literally screening thousands of cultivars, germplasm lines and accessions in the search for novel sources of resistance. Considerable effort has been made to characterize these sources by comparing them to known genes (Monteros et al., 2007; Ribeiro et al., 2007; Silva et al., 2008) and developing markers to assist in their incorporation into adapted germplasm (Hyten et al., 2007; Monteros et al., 2007; Neto, 2007; Boerma, 2008). There have, however, been numerous documented failures of specific resistance genes in the past (Shanmugasundaram et al., 2004; Yorinori, 2004; Hartman et al., 2005; Silva et al., 2008), yet breeders and researchers persevere with this line of research in the hope that a new source of resistance will prove to be more stable. Marker technology is available (Neto, 2007; Boerma, 2008; Silva et al., 2008) to pyramid major soybean rust resistance genes into a single genotype; however, there is a school of thought (Tschanz, 1987) that this too is unlikely to be a stable solution because the pathogen retains unnecessary virulence genes at a high frequency in its population.

The use of highly tolerant genotypes does provide a durable but admittedly currently elusive, solution to soybean rust. The protracted process of assessing tolerance and the historically poor correlation of results over seasons (Shanmugasundaram, 1999; McLaren, 2008) has probably impacted negatively on its popularity as a breeding strategy. This study sets out to illustrate the seasonal variability of conventional evaluations of tolerance, and to offer a novel method of assessing tolerance to soybean rust.

2. Materials and methods

2.1. Genotypes

2.1.1. Pre-selection of genotypes for tolerance research

Pre-selection of candidate genotypes for tolerance research was done by splitting the single row unreplicated plots from the PANNAR germplasm and line collection into sprayed and unsprayed subplots. The second half of each 5 m row formed the sprayed subplot, and was sprayed with Punch C (Flusilazole/Carbendazim) at the recommended rate of 400 ml ha⁻¹ using a knapsack. This commenced at the first signs of flowering of the earliest genotype, and was repeated at 21 d intervals until the last genotype reached harvest maturity. Plots of the germplasm collection had been set out roughly in order of maturity in the field, which aided both the spraying and the evaluation. Note was made of all genotypes that had little or no difference in harvest maturity date between the sprayed and unsprayed subplots. Where soybean rust had caused severe defoliation and premature senescence, the difference in maturation between sprayed and unsprayed subplots was a week or more. In genotypes relatively unaffected by soybean rust, the difference between sprayed and unsprayed subplots was not visually apparent. Difference in seed size between the sprayed and unsprayed subplots was also recorded. Where soybean rust causes defoliation or stress late in the reproductive period, yield loss is primarily via seed size reduction. Whilst difference in seed size between sprayed and unsprayed plots has been put forward as an efficient and simple technique for evaluating tolerance (Shanmugasundaram, 1999; Tichagwa, 2004), it was found to be somewhat variable in this study (likely as a

result of small sample size). Genotypes, in which the difference in both maturation and seed size suggested tolerance, were included in the next phase of evaluation.

The lines identified with putative tolerance were then evaluated in more detail the following season. Field observations of flowering date (R1), first pustule date, pustule type, followed by an assessment of rust severity within three strata of the plant canopy and finally physiological maturity (R8) date (Fehr et al., 1971) were noted. This was done firstly to expressly exclude genotypes that had specific resistance gene pustule reactions (RB) from the group that would be evaluated for tolerance. Secondly, if genotypes exhibited indications of partial resistance that could be contributing towards the tolerance, it would be possible to identify some of these mechanisms using the data collected. Based on two seasons of pre-selection, 14 genotypes were included for the evaluation of soybean rust tolerance over the next three seasons.

2.1.2. Genotypes used for the evaluation of tolerance

One susceptible non-tolerant control (PAN 875, a cultivar commercially released in Zimbabwe) and a resistant control (UFV 3, an old germplasm line out of Brazil) showing a RB pustule reaction were included, along with 12 genotypes presenting Tan pustule reaction types (susceptible) that had exhibited some level of tolerance in the two seasons of pre-screening. Amongst the 12 tolerant genotypes: Cordell (Hartwig and Young, 1990) and Delsoy 4900 (Anand, 1991) showed preliminary indications of slow rusting; JV781 and JV783 of having a long latent period; and JV762 of having a low infection rating.

2.2. Trial design

The trials were planted at Greytown (South Africa) during the 2003/04, 2004/05 and 2005/06 seasons at planting dates that could be considered 'late', ensuring that all maturities received significant exposure to soybean rust (Table 1). The trials were arranged in a split-plot design, with the whole plot factor being genotype, which was split for sprayed or unsprayed treatment. The sprayed treatment subplots were protected with sprays of Punch C (Flusilazole/Carbendazim, SC 250/125 g l⁻¹) at the recommended rate of 400 ml ha⁻¹ at 21 d intervals starting from the date of first rust symptoms in the sentinel plots (Table 1), effectively excluding soybean rust for the entire season. At the time of spraying there were no symptoms of soybean rust in the trial plots. The sentinel plots were part of a national network (Craven, 2008) of early planted plots which provided a timely warning for the need to spray. The subplots consisted of four 4.4 m long rows, with an inter-row spacing of 0.9 m. The centre two rows of the subplots were harvested with a plot combine, and the yields were converted to t ha⁻¹ at a moisture content of 12.5%.

[Table 1]

2.3. Statistical analysis

The data was analysed utilising the REML META analysis routine in Genstat (Version 10.2) using the following model:

Fixed = Year*Genotype*Treat

Random = Rep/Whole plot/Subplot

Experiment = Year

Least significant differences were calculated using t-values appropriate to the degrees of freedom and average standard errors of the differences of means determined by Genstat.

2.3.1 Yield loss

To evaluate tolerance to soybean rust, yields of unsprayed subplots were compared to the yields of sprayed subplots and yield loss % was calculated for each genotype using the following formulae:

$$\text{Yield loss} = \text{sprayed yield} - \text{unsprayed yield} \quad [\text{Equation 1}]$$

$$\text{Yield loss \%} = (\text{Yield loss}) / \text{sprayed yield} \times 100 \quad [\text{Equation 2}]$$

2.3.2 Correlations

A non-parametric test of the seasonal variation in genotypic ranking of yield loss %, sprayed yield and unsprayed yield was conducted by calculating Spearman's rank correlation coefficients between seasons.

2.3.3 Superiority measure

The Lin and Binns (1988) superiority measure (P_i) was calculated on the sprayed and unsprayed yields using the formula:

$$P_i = \sum_{j=1}^n (X_{ij} - M_j)^2 / (2n) \quad [\text{Equation 3}]$$

where n is the number of seasons, X_{ij} is the i th genotype yield in the j th season, and M_j is the maximum yield response in the j th season. From this equation, the most consistently

superior genotype has the lowest P_i value. P_{iu} was calculated on the unsprayed yields of all 14 genotypes using the highest unsprayed yield each season as the maximum. The corresponding superiority measure determined on the sprayed yields, P_{is} , was primarily calculated to determine the change in superiority (ΔP_i) brought about by soybean rust stress using the formula:

$$\Delta P_i = P_{is} - P_{iu} \quad [\text{Equation 4}]$$

2.3.4 Ecovalence

Phenotypic stability measured by using the ecovalence statistic (W_i) developed by Wricke (1962) was calculated for unsprayed yield (W_{iu}) and sprayed yield (W_{is}) using the formula:

$$W_i = \sum_{j=1}^n (X_{ij} - X_{i.} - X_{.j} + X_{..})^2 \quad [\text{Equation 5}]$$

where n is the number of seasons, X_{ij} is the i th genotype yield in the j th season, $X_{i.}$ is the mean of the i th genotype across n seasons, $X_{.j}$ is the mean of all genotypes in the j th season, and $X_{..}$ is the grand mean over n seasons. The most stable genotypes have the lowest W_i . The change in the ecovalence statistic attributed to soybean rust (ΔW_i) was calculated using the formula:

$$\Delta W_i = W_{is} - W_{iu} \quad [\text{Equation 6}]$$

2.3.5 $W_i P_i$

The $W_i P_i$ statistic is calculated as the distance of the coordinate in the biplot of W_{iu} and P_{iu} from the origin of the graph. $W_i P_i$ is thus the hypotenuse of a right angle triangle

with two sides equal to W_{iu} and P_{iu} . The square of the hypotenuse is equal to the sum of the squares of the two opposite sides, therefore:

$$W_i P_i = \sqrt{W_{iu}^2 + P_{iu}^2} \quad [\text{Equation 7}]$$

3. Results

3.1. Yield loss %

Using conventional methodology to evaluate tolerance to soybean rust, yields of unsprayed subplots were compared to the yields of sprayed subplots and yield loss % was calculated for each genotype in each season and over seasons (Table 2). Yields of the sprayed subplot treatment measured the yield potential of the genotype in the absence of rust. The mean of all genotypes gives an indication of the yield potential of the season. Due to lower than normal rainfall (Table 1), the yields and yield loss % were lowest for the 2003/04 season. Moderate soybean rust pressure was present in all three seasons, which can be judged by the yield loss of the susceptible control, ranging from 17.7 to 27.9% (Table 2). The mean yield loss of all entries over the three seasons was 11.7%, which when compared to the susceptible control mean of 23.1% indicates appreciable levels of tolerance within the trial. The lowest mean yield loss over all three seasons was measured on UFV 3, the resistant control variety. Six other genotypes, Cordell, JX270-2, JV861, JV860, Delsoy 4900 and JV783, had mean three year mean yield losses of less than 10%. The mean yield loss % for each genotype over three seasons is presented in Table 3, along with the yield loss % in each individual season to illustrate the seasonal variability.

[Table 2]

Spearman's rank correlation was calculated to determine the correlation between the rankings of the genotypes for each of the three seasons for yield loss % and unsprayed yield (Table 3). The rank correlation coefficient gives a measure of the crossover interaction (qualitative interaction) occurring in genotypic ranking between seasons. Very weak correlations in genotype ranking for yield loss % between seasons were found. The rank correlations between sprayed and unsprayed yield (Table 4) within a season was significant for all seasons.

[Tables 3-4]

3.3. *Stability measures*

The P_i superiority measure was calculated using the mean square difference of each genotype from the maximum yield in each season. P_{iu} was calculated on the unsprayed yields of all 14 genotypes using the highest unsprayed yield each season as the maximum. The corresponding superiority measure determined on the sprayed yields (P_{is}) was primarily calculated to detect the change in superiority (ΔP_i) brought about by soybean rust stress. Genotypes were arranged (Table 5) according to their superiority under rust stress, with the smallest P_{iu} indicating the smallest variation from the maximum yield and the best general adaptation in unsprayed conditions.

[Table 5]

A biplot of P_{iu} and P_{is} (Fig. 1) graphically shows effect of soybean rust on the superiority measure. The scale of the biplot was chosen to ensure good separation of the genotypes, consequently two genotypes (Cordell with a coordinate of 1.036; 1.558 and UFV 3 with a coordinate of 0.552; 0,777) were not plotted. The biplot has been subdivided into quartiles based on the median value, with quartile A containing genotypes insensitive to rust but inferior yielding in the absence of rust. Quartile B contains genotypes that are both rust sensitive and inferior yielding, while quartile C contains genotypes that are rust insensitive and superior in yield. The control genotype PAN 875 is the only genotype in quartile D, which is classed as rust sensitive but superior yielding in the absence of rust.

[Fig. 1]

The ecovalence statistic was calculated on unsprayed (W_{iu}) and on sprayed (W_{is}) yield, and the change in genotypic stability (ΔW_i) as a result of soybean rust as the difference between the two statistics (Table 5). A small W_i value indicates reduced interaction between genotype and environment and the smaller the value the greater the genotypic stability over seasons. The difference between W_{iu} and W_{is} (ΔW_i) shows the extent to which the yield stability of genotypes change under soybean rust pressure. It is commonly accepted that yield instability increases with crops under stress (Cattivelli et al., 2008), and the ΔW_i statistic would give a good indication of which genotypes are being stressed the most by exposure to rust. A biplot of W_{iu} versus W_{is} (Fig. 2) demonstrates the relationship between the two statistics for each genotype, where genotypes that plotted close to the diagonal have similar yield stabilities under rust and rust free conditions. The scale of the biplot was chosen to apportion equal weights to W_{iu} and W_{is} and to ensure good separation of the genotypes. As a consequence UFV 3 with a coordinate of 0.752; 0.506

has not been plotted. The biplot is divided into stability quartiles using the median values. Genotypes in quartile A are unstable under rust free conditions, yet becoming more stable under rust infection. Quartile B contains consistently unstable genotypes, while the genotypes in quartile C are consistently stable regardless of soybean rust. Quartile D contains genotypes (such as PAN 875, the susceptible control) that are stable under sprayed conditions but are unstable under rust stress conditions.

[Fig. 2]

The relationship between W_{iu} and P_{iu} was explored by plotting the two statistics against each other, in an attempt to ‘fill up the dark zones’ (Flores et al., 1998) left by each individual statistic. The pattern that emerged from this biplot (Fig. 3) was that the genotypes were predominantly distributed in two quartiles. Quartile B contained rust sensitive genotypes that had low and unstable yields under soybean rust pressure. Quartile C contained tolerant genotypes that were both stable and consistently high yielding under rust infections.

[Fig. 3]

4. Discussion

It is re-emphasized that the entries in this trial had been pre-selected to include genotypes that were susceptible to infection but tolerant to the effects of soybean rust, and any genotypes showing specific gene resistance (RB) were excluded. The rationale was that the tolerance to yield loss selected using this methodology would be durable and not

reliant on a race-specific reaction that could be defeated. The objective of using tolerance in preference to resistance to restrict yield loss due to soybean rust is to have a genetic mechanism that is stable and durable. Due to the limited number of seasons evaluated, this study cannot make inferences regarding durability, but it does highlight the seasonal variability associated with the expression of tolerance when assessed using yield loss %. From the weak correlation in ranking between seasons for yield loss %, it would appear that progress in selecting for tolerance will be slow when using this as a selection criterion. The components of the tolerance index used (yield loss %) are sprayed and unsprayed yield. It is possible that the physiological mechanisms driving yield and yield stability under rust and rust free conditions are different, resulting in the increased variability of the index over and above the components of the index. To improve progress in tolerance breeding, more efficient measures of tolerance need to be explored.

4.1. Exploring improved measures of tolerance

4.1.1. Unsprayed yield

There is a school of thought that would support the concept that the highest yielding genotype under rust stress is the most tolerant. In this scenario JV783 would be the most tolerant genotype, and would out perform five out of the remaining 13 genotypes even if they were sprayed. Genotype ranking for unsprayed yield was significantly correlated between two of the three seasons (Table 3), suggesting that it may be a more reliable index than yield loss % to base the characterization of tolerance on. The precise source of the seasonal variability in unsprayed yield is unclear, but it is likely to be related to uneven rust inoculum pressure and to the timing of the epidemics relative to genotype

maturity. It is also possible that the actual biochemical or morphological nature of the tolerance mechanism could be moderated by the seasonal environments. As a measure to improve the uniformity of rust epidemics in the field, inoculations or the use of spreader-rows in the trials could be considered. This may reduce the variability associated with severity (and timing) of infection so that the variability remaining can be attributed largely to post infection mechanisms within the host. If the timing of the soybean rust epidemics relative to the developmental stage of the soybean genotype is crucial in determining the yield, then temporal replication of genotypes grouped according to maturity is required. Sequential planting of trials would expose each genotype to a number of environments within a single season, and multi-season data would lend confidence to the characterization of tolerance. Unsprayed yield on its own is an unsatisfactory measure of tolerance because it has no reference to the potential yield that could be attained in that environment if rust was controlled and provides no measure of the variability.

4.1.2. Superiority measure

The superiority measure proposed by Lin and Binns (1988) uses a maximum yield to set the upper boundary in each environment, and generates a mean square statistic that measures deviations from this maximum yield. The most tolerant genotype as selected by the P_{iu} superiority measure was JV783 (Table 5), which had also been selected as being the highest yielding in the presence of rust. Genotypic ranking of P_{iu} was highly correlated ($r_s = 0.991^{***}$) with unsprayed yield, confirming that it is a statistic strongly driven by yield (Flores et al. 1998; Alberts, 2004). Importantly, P_{iu} as a rust tolerance statistic discriminated against Cordell, UFV3 and Delsoy 4900 because of poor yield adaptation, which yield loss % (Table 2) was incapable of doing. All three had low mean yield losses,

but should not be considered tolerant because of poor unsprayed yields. Calculation of the superiority measure on sprayed yields (P_{is}) was done to further note the change in superiority of genotypes from sprayed to unsprayed conditions. In the biplot of P_{is} vs P_{iu} (Fig. 1), most of the genotypes (with the obvious exception of PAN 875) plotted close to the diagonal. This indicates a close relationship between P_{is} and P_{iu} , which is further illustrated by the highly significant rank correlation ($r_s = 0.736^{**}$) between the genotypic ranking of P_{iu} and P_{is} . The biplot correctly categorizes PAN 875 (the susceptible control) as being high yielding but sensitive to rust.

4.1.3. *Ecovalence*

The ecovalence statistic W_i measures the interaction variance over seasons and is considered a measure of specific stability, whilst P_i is considered a measure of general superiority (Lin and Binns, 1988). Plotting of W_{iu} against W_{is} (Fig. 2) raised interesting observations related to the variance of genotypes under rust stress. It was expected that most genotypes would tend to be more variable and less yield stable under unsprayed conditions and this was shown to be generally the case, with the exception of three distinct outliers that plotted appreciably above the diagonal. The significance of the relationship between rust stress and yield stability for these three genotypes (Cordell, PAN 589 and JV861) has not been determined, but they display the sort of reaction that might be elicited by genotypes with sensitivity to the sprayed chemical. Yield suppression resulting from sensitivity to the sprayed chemical may be mistaken for tolerance where yield loss % is used as a tolerance index, since the sprayed yields would be reduced relative to the unsprayed yields. Yield suppression is expected to be accompanied by an increase in variance, so it is possible that it could be detected with biplots of W_{iu} vs W_{is} . None of the

three genotypes involved have, however, been evaluated for phytotoxicity to Flusilazole/Carbendazim. In Fig. 2, quartile C has been classified to contain the genotypes that are consistently stable. The genotypes that plot close to the diagonal have similar levels of stability under rust stress and rust free conditions, and could conceivably use the same non additive mechanisms to achieve this stability. Cordell plotted in quartile C, but had a slightly larger ΔW_i than the other genotypes in this quartile. In the pre-screening exercise, it was established that this genotype had a degree of partial resistance to soybean rust, which would mean that the genetic control of stability under sprayed and unsprayed conditions is likely to be different for this genotype at least. There was a significant correlation ($r_s = 0.609^*$) between the ranking of genotypes using W_{iu} versus W_{is} , however, less significant than with P_{iu} vs P_{is} .

4.1.4. Combined statistic

In a study on yield stability in wheat, Purchase et al. (2000) found that the ecovalence statistic of Wricke (W_i) ranked genotypes in a significantly similar manner to the AMMI stability value (ASV), but differently to Lin and Binns (P_i). Their conclusion was that P_i was more of a performance measure than a stability measure. By inference then, the plotting of W_{iu} against P_{iu} holds prospects of selecting genotypes simultaneously for yield performance (P_{iu}) and yield stability (W_{iu}) under soybean rust stress conditions. P_i is generated from the additive main effects of genotype, and is considered a good variance measure for general adaptation/superiority. W_i is generated from the non-additive interaction between genotype and environment and is thought of as a measure of specific stability. The two statistics are thus complimentary to each other, and could be used in combination to detect tolerance to rust which would conform to the strictest definition of

tolerance: superior yield and high levels of stability in the presence of rust. Plotting W_{iu} against P_{iu} (Fig. 3) produced two distinct groupings of genotypes: the more stable, higher yielding rust tolerant group, clustered close to the origin of the graph in quartile C; the lower yielding less stable, less tolerant group, in quartile B. It is possible from the biplot to select the most tolerant group of genotypes; however, it is not possible to rank them for tolerance from the biplot. The algebraic calculation of the distance from the origin of the biplot to the coordinate would provide a single statistic to facilitate this. Purchase et al. (2000) used the same principle to develop the ASV statistic from the biplot of IPCA1 and IPCA2, except that the IPCA components were weighted by their proportional contribution to the interaction sum of squares. Weighting of W_i and P_i would be unnecessary in the case of the W_iP_i statistic, since yield and yield stability are equally important in our definition of tolerance. To our knowledge, this is the first application of these statistics using either a W_i vs P_i biplot or a single combined statistic (W_iP_i) to characterise genotypes for soybean rust tolerance.

4.1.5. The relationship between indicators of tolerance and rust-free yield

The objective of tolerance breeding should be to have a genotype that would yield consistently well despite soybean rust infection. A further requirement would be that the genotype should be high yielding under low rust pressure, or in the absence of the disease altogether. Due to the highly significant correlations between unsprayed and sprayed yield within each season (Table 5) and P_{iu} with P_{is} and W_{iu} with W_{is} , it is concluded that selection for low P_{iu} or low $W_{iu}P_{iu}$ may also indirectly select for high yield and yield stability in the absence of rust. Care should be taken in extrapolating this conclusion to other situations, as it must be emphasized that the genotypes evaluated in this study were a

highly select group which had had two seasons of pre-selection for tolerance before inclusion in this trial. Further, the effectiveness of P_i and W_i in discriminating tolerance levels and the relationship between unsprayed and sprayed yields may change with increasing levels of yield stress induced by soybean rust. Tschanz et al. (1983) reported that sprayed and unsprayed yields were not correlated in their field trials in Taiwan. Conditions in their trials were distinctly more severe than those experienced in this experiment (up to 90.2% yield loss), and therefore the lack of agreement between the two data sets is perhaps not surprising.

5. Conclusion

The P_{iu} statistic appears to be a highly suitable measure of genotype performance in the presence of rust, as it combines unsprayed yield variability relative to an achievable maximum yield in a single parameter. Compared to the current norm of using yield loss % for determining tolerance, the calculation of P_{iu} would involve fewer resources as it does not require a full split-plot fungicide trial to generate the data. Genotypes could be evaluated under rust pressure, using a single sprayed genotype as a benchmark for the maximum yield at each location. The optimum control genotype for each environment may be chosen, without the necessity of having the same control over all environments (Lin and Binns, 1988). The W_{iu} statistic is complementary to P_{iu} and may also be derived without the need of split plot data. The combination of W_{iu} and P_{iu} in a biplot or as a combined statistic $W_{iu}P_{iu}$, successfully identifies the highest yielding, most consistently stable genotypes in the presence of moderate levels of soybean rust stress.

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Table 1

Planting date and site details for the rust tolerance split plot trials at Greytown, South Africa (S28° 08'; E30° 37')

Season	Planting date	Rainfall (mm) ^a	Date of first rust ^b
2003/04	08/12/2003	666.4	16/02/2004
2004/05	09/12/2004	751.3	24/02/2005
2005/06	30/11/2005	954.8	2/02/2006

^aThirty two year mean annual rainfall for Greytown = 832.6 mm

^bSoybean rust symptoms on the sentinel plots, used as an indicator of the earliest presence of rust symptoms in the area

Table 2

Yield loss (t ha^{-1}) and yield loss % over three seasons (2003/04; 2004/05; 2005/06) at Greytown, as a result of soybean rust

	3 y mean yield (t ha^{-1})			yield loss % ^c			
	No spray	Spray ^a	Loss ^b	3 y Mean	2003/04	2004/05	2005/06
Cordell	1.879	1.995	0.116	5.8	9.6	5.8	2.1
Delsoy 4900	2.394	2.645	0.251	9.5	-1.4	8.9	19.3
JV762	2.608	3.030	0.422*	13.9	16.2	9.1	17.5
JV780	2.981	3.501	0.520**	14.9	14.8	12.4	17.2
JV781	2.683	3.144	0.461**	14.7	10.2	18.9	15.3
JV783	3.118	3.453	0.335*	9.7	5.8	11.5	11.1
JV860	3.043	3.337	0.294	8.8	-1.2	20.9	6.3
JV861	3.070	3.340	0.270	8.1	8.2	6.4	9.3
JV870	2.789	3.255	0.466**	14.3	16.3	12.4	14.4
JX270-2	2.998	3.214	0.216	6.7	3.7	6.0	10.2
PAN 494	2.561	2.867	0.306*	10.7	5.7	6.8	20.3
PAN 589	2.973	3.457	0.484**	14.0	5.7	16.2	18.0
PAN 875	2.686	3.494	0.808**	23.1	27.9	25.3	17.7
UFV 3	2.421	2.536	0.115	4.5	0.6	-3.3	15.2
Mean	2.729	3.091	0.362	11.7	9.0	11.6	14.1

^aSpray = Punch C (400 ml ha^{-1}) sprayed at first symptoms in the sentinel plot and repeated at 21 d intervals

^bLoss = Sprayed yield – Unsprayed yield (t ha^{-1})

^cyield loss % = Loss/Unsprayed Yield x 100

Difference in genotypic means: LSD (0.05) = 0.304 t ha^{-1} ; LSD (0.01) = 0.424 t ha^{-1}

Difference in spray treatment means: LSD (0.05) = 0.478 t ha^{-1}

Table 3

Spearman's rank correlations between seasons for yield loss % due to soybean rust above the diagonal and unsprayed yield below the diagonal

	2003/04	2004/05	2005/06
2003/04		0.0367	0.033
2004/05	-0.103		0.244
2005/06	0.648*	0.011	

Table 4

Rank correlations between sprayed and unsprayed treatments for three seasons at
Greytown

	Spearman rank correlation
2003/04	0.701**
2004/05	0.626*
2005/06	0.916**

Table 5

Stability measures P_i and W_i determined on three seasons (2003/04; 2004/05; and 2005/06) of yield data generated at Greytown in the presence of soybean rust, with genotypes ordered according to P_{iu} and ranked according to W_iP_i

Genotype	Superiority measure ^a			Ecovalence ^b			W_iP_i	Rank
	P_{iu}	P_{is}	ΔP_i	W_{iu}	W_{is}	ΔW_i	W_iP_i ^c	
JV783	0.026	0.034	0.007	0.090	0.096	0.006	0.094	2
JV861	0.044	0.137	0.092	0.251	0.399	0.148	0.255	6
JV860	0.054	0.072	0.018	0.291	0.027	-0.263	0.296	7
JX270-2	0.057	0.170	0.113	0.138	0.152	0.014	0.149	4
JV780	0.068	0.036	-0.032	0.041	0.014	-0.027	0.079	1
PAN 589	0.069	0.047	-0.022	0.188	0.404	0.216	0.200	5
JV870	0.138	0.122	-0.017	0.022	0.001	-0.021	0.140	3
JV781	0.227	0.272	0.046	0.303	0.318	0.015	0.378	8
PAN 875	0.239	0.023	-0.217	0.387	0.132	-0.255	0.455	9
JV762	0.349	0.339	-0.010	0.451	0.312	-0.139	0.571	11
PAN 494	0.376	0.470	0.095	0.478	0.278	-0.200	0.608	12
Delsoy 4900	0.432	0.591	0.159	0.284	0.216	-0.068	0.517	10
UFV 3	0.552	0.777	0.225	0.752	0.506	-0.246	0.933	13
Cordell	1.036	1.558	0.522	0.111	0.209	0.098	1.042	14
S.D. ^d	0.335	0.407	0.256	0.192	0.152	0.147	0.125	

^a Superiority measure, calculated on unsprayed yields (P_{iu}), sprayed yields (P_{is}) and change in superiority due to rust ($\Delta P_i = P_{is} - P_{iu}$)

^b Ecovalence = Wricke's ecovalence, calculated on unsprayed yield (W_{iu}), sprayed yields (W_{is}) and change in W_i due to soybean rust ($\Delta W_i = W_{is} - W_{iu}$)

^c $W_iP_i = \sqrt{W_{iu}^2 + P_{iu}^2}$

^d Standard deviation

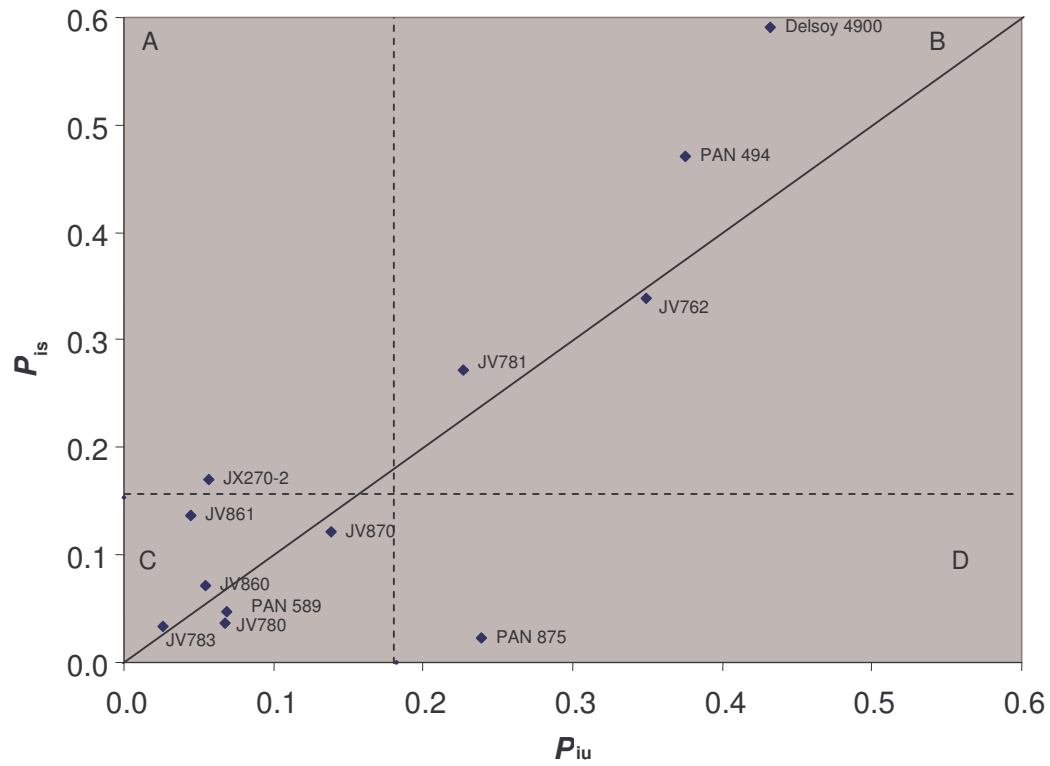


Fig. 1. Biplot of superiority measures for unsprayed yield (P_{iu}) versus sprayed yield (P_{is}), subdivided into quartiles: Quartile A = Insensitive to rust but inferior yield; Quartile B = Rust sensitive and inferior yield; Quartile C = Insensitive to rust and superior yield; Quartile D = Rust sensitive but superior yield.

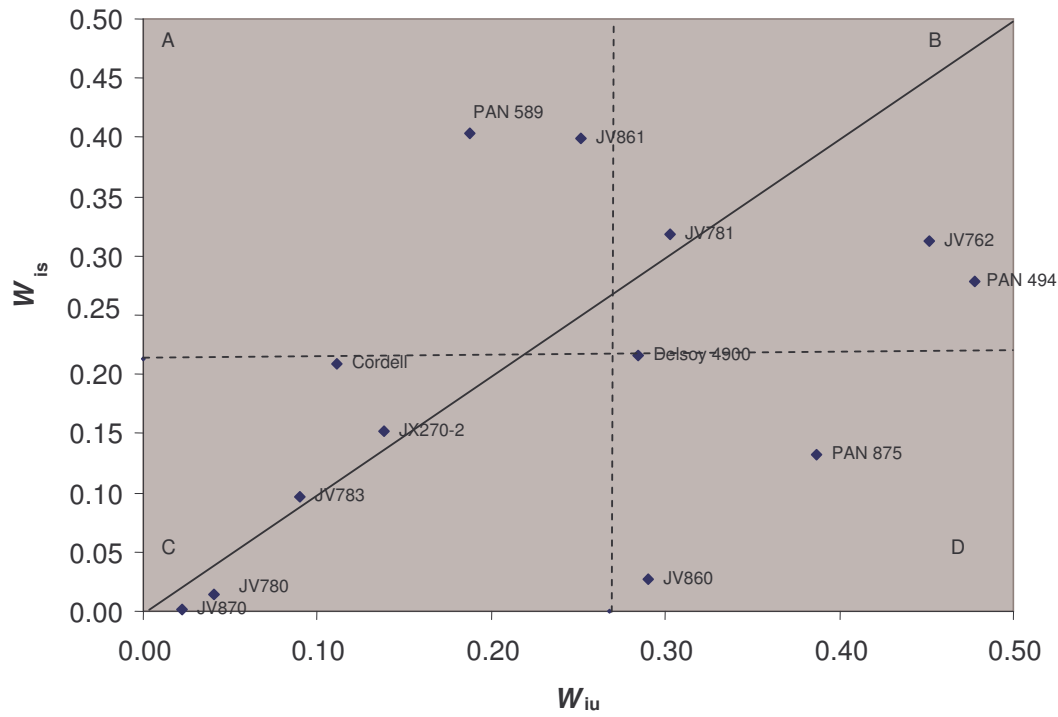


Fig. 2. Ecovalence biplot of unsprayed yields (W_{iu}) versus sprayed yields (W_{is}), subdivided into yield stability quartiles: Quartile A = Unstable sprayed becoming stable under rust infection; Quartile B = Consistently unstable; Quartile C = Consistently stable; Quartile D = Stable sprayed becoming unstable under rust stress.

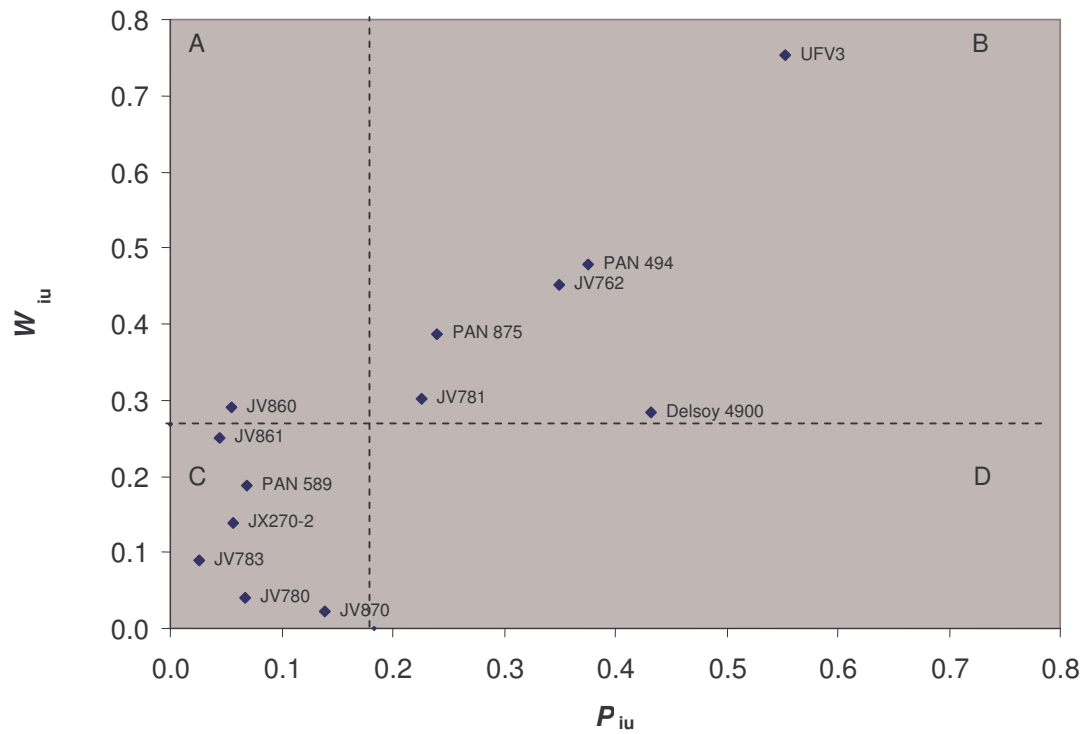


Fig. 3. Tolerance to soybean rust, as determined by the biplot of superiority measure (P_{iu}) and ecovalence (W_{iu}) measured under rust stress conditions, where soybean genotypes are defined by the quartiles into which they plot: Quartile A = superior yielding but unstable; Quartile B = inferior and unstable yielding; Quartile C = superior and stable yielding (Tolerant); Quartile D = inferior yielding but stable.